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ORIGINAL ARTICLE

Lymphoma Predisposing Gene in an Extended Family: CD70 Signaling Defect

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Abstract

Genome-wide sequencing studies in pediatric cancer cohorts indicate that about 10% of patients have germline mutations within cancer predisposition genes. Within this group, primary immune deficiencies take the priority regarding the vulnerability of the patients to infectious agents and the difficulties of cancer management. On the other hand, early recognition of these diseases may offer specific targeted therapies and hematopoietic stem cell transplantation as an option. Besides therapeutic benefits, early diagnosis will provide genetic counseling for the family members. Within this context, an extended family with multiple consanguineous marriages and affected individuals, who presented with combined immune deficiency (CID) and/or Hodgkin lymphoma phenotype, were examined by exome sequencing. A pathogenic homozygous missense *CD70* variation was detected (NM_001252.5:c332C>T) in concordance with *CD70* phenotype and familial segregation was confirmed. *CD70* variations in patients with CID and malignancy have very rarely been reported. This paper reports extended family with multiple affected members with CID and malignancy carrying a missense *CD70* variation, and reviews the rare cases reported in the literature. Primary immune deficiencies appear to be a potential cause for pediatric cancers. Better focusing on these inborn disorders to prevent or make an early diagnosis of malignant transformation and reduce mortalities is important.

Keywords *CD70* · immune deficiency · lymphoma · EBV · malignancies

Introduction

The last decade has seen an enormous increase in research on genetic susceptibility to childhood cancer, which has led to many important insights. Germline components of cancer predisposition showed that up to 10% of children and adolescents with cancer are associated with inherited mutations. Identification of germline alterations is very important for

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testing the family members at risk and surveillance. A variety of genetic disorders lead to increased risk for cancer development including Down syndrome, RASopathies, chromosome breakage syndromes, and primary immune deficiencies [1].

Up to date several genes (such as *ITK* [2–4], *SH2D1A* [5], *MAGT1* [6–8], *CTPS1* [9], *CD27* [10], *CD70* [11–13], *CORO1A* [14, 15], *RASGRP1* [15–17], *TNFRSF9* [18], *STK4* [19, 20], *CARMIL2* [21–23], and *PRKCD* [24, 25]) were found to be associated with immunodeficiency and predisposition to EBV-induced lymphoma [26–29]. Recently, an autosomal recessive combined immunodeficiency (CID) phenotype associated with EBV-induced malignancy has been described as a result of rare *CD70* gene variations [11–13]. CD70 is a member of tumor necrosis factor superfamily (TNFS) and the ligand for CD27 [30, 31]. Co-activation of CD27 and CD70 regulates the survival, function, and differentiation of T, B, NK, and plasma cells [32–36]. In the CID patients and mouse models, disruption of CD27-CD70 showed impaired memory responses against different viruses [37, 38]. Moreover, co-activation of CD27-CD70 on B cells triggers plasma cell formation and increased IgG production [39]. *CD70* expression is not restricted to healthy activated T and B cells but also is found to be expressed in different hematological cancers including lymphomas [40], chronic lymphocytic leukemia (CLL) [41], and multiple myelomas (MM) [42]. Here, we describe a homozygous *CD70* mutation in a family with many affected individuals with immunodeficiency and malignancies.

Patients and Methods

Patients

Hereby, we present a family with multiple consanguineous marriages and individuals affected with lymphoma and/or immune deficiency (Fig. 1 and Table 1). Two siblings diagnosed with Hodgkin's lymphoma (HL) and whose parents had a consanguineous marriage were referred to our center for further evaluation and treatment.

The first sibling (V-1) was diagnosed with stage IIIsB Hodgkin lymphoma of nodular sclerosing histopathology, at 18 months of age in the local university hospital in the Northeastern part of Turkey. Treatment had been initiated with two courses of chemotherapy consisting of vincristine, etoposide, prednisone, and doxorubicin (OEPA), as per the Euronet-Pediatric Hodgkin Lymphoma-C1 protocol after which a partial remission was attained and he was referred to our center. Treatment continued with the same protocol, with four additional courses of cyclophosphamide, vincristine, procarbazine, prednisone (COPP) chemotherapy. He experienced a relapse right after the sixth course. He received three courses of chemotherapy consisting of ifosfamide, etoposide, carboplatinum (ICE) with a complete response followed by high dose chemotherapy and autologous stem cell transplantation. He had a second relapse after 3 months, was treated with targeted therapy (antiCD30 monoclonal antibody-brentuximab) and ICE, and he achieved complete response. Treatment continued with radiotherapy. He is under follow-up for 52 months since diagnosis of HL and with no evidence of disease. Due to recurrent infections and history of consanguineous marriage in the family, the immunological evaluation revealed a severe CD4 T cell lymphopenia, which was thought to be compatible with combined immune deficiency (Table 1). Allogeneic hematopoetic stem cell transplantation has been performed when a fully matched unrelated donor was found (Table 2).

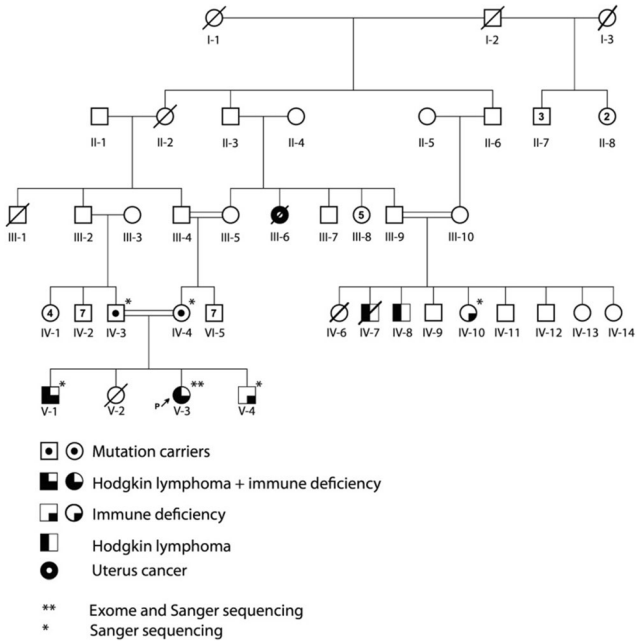

Fig. 1 Five generations of the pedigree with the index V-3. Indicated numbers within circles and squares are number of individuals, who were reported not having any clinical phenotypes

The second sibling (V-3), at 3 years of age, was diagnosed with stage IIIsB Hodgkin lymphoma of mix cellular histopathology in the local university hospital in the Northeastern part of Turkey and was referred to our center for further evaluation and treatment at the same time as her brother who had experienced a relapse of Hodgkin lymphoma (V-1). Treatment was initiated with two courses of chemotherapy consisting of cyclophosphamide, vincristine, procarbazine, prednisone/doxorubicin, bleomycin, vinblastine (COPP/ABV) chemotherapy, as per our institutional protocol [43], after which a partial remission was attained. Treatment continued with the same protocol, with four additional courses of COPP/ABV chemotherapy. She experienced a relapse right after the sixth course. She received three courses of chemotherapy consisting of ifosfamide, etoposide, and carboplatinum (ICE) with a complete response followed by high dose chemotherapy and

Table 1 Clinical characteristics of individuals with immunodeficiency and/or lymphoma

	V1	V2	V3	V4	IV6	IV7	IV8	IV10
Age	5 years	40 days	6 years	8 years	3 months	20 years	17 years	14 years
Age of diagnosis	1.5 years	-	3 years	6 years	-	15 years	12 years	14 years
Cancer	Hodgkin lymphoma stage IIIsB	-	Hodgkin lymphoma stage IIIsB	-	-	Hodgkin lympho-ma	Hodgkin lymphoma	-
Immunodeficiency	Low T cells and hypogammaglobulinemia	?	Low T cells and hypogammaglobulinemia	Decreased CD4/CD8 ratio	-	-	-	Decreased CD4/CD8 ratio, hypogammaglobulinemia
Infections	EBV	-	EBV	EBV	-	NA	NA	HSV1 encephalitis, recurrent pneumonia, chronic lung disease
Treatments at *initial diagnosis, **at relapse, ***forCID	**2 courses of OEPA/4 courses of COPP, **ICE brentuximab autologous HSCT radiotherapy, ***allogeneic HSCT at $5^{9/12}$ years of age		*6 courses of COPP/ABV, **ICE autologous HSCT ***allogenic HSCT at 6 years of age	***Rituximab and allogenic HSCT at age 8				
Last status	Alive	Ex	Alive	Alive	Ex	Ex	Alive	Alive
ANS/μl	1950 [2300–7000/μl]	NA	3553 [2300–7000/μl]	3900 [2300–7000/μl]	NA	NA	7600	NA
ALS/μl	2960 [1000–11,000/μl]	NA	2550 [1000–11,000/μl]	3200 [1000–11,000/μl]	NA	NA	2000 [1000–11,00-0/μl]	NA
PLT 10^3/μl	195 [150–450]	NA	281 [150–450]	301 [150–450]	NA	NA	300 [150–450]	300 [150–450]
CD45	96% [90–100] #2841	NA	96% [90–100] #2448	88% [70–99] #2816	NA	NA	NA	98% [80–99]
CD3	60% [53–75] #1776 [1400–3700]	NA	63% [53–75] #1606 [1400–3700]	69% [57–86] #2208 [1200–2600]	NA	NA	NA	68% [50–75]
CD4	*6% [32–51* *#178* [700–2200]	NA	*16%* [32–51] *#408* [700–2200]	*28%* [29–57] #896 [650–1500]	NA	NA	NA	26%[25–55]
CD8	*54%* [14–30] *#1600* [490–1300]	NA	*44%* [14–30] #1122 [490–1300]	40% [13–47] *#1280* [370–1100]	NA	NA	NA	*38%* [15–35]
CD19	18% [16–35] #532 [390–1400]	NA	28% [16–35] #714 [390–1400]	11% [3.5–15.5] #352 [270–860]	NA	NA	NA	*28%* [9–25]
CD3-CD16+CD56+	*16.4%* [3–15] #485 [130–720]	NA	5% [3–15] #128 [130–720]	*3.7%* [4.5–30] #118 [100–720]	NA	NA	NA	NA
CD3+CD16+CD56+	0.1%	NA	3.4%	0.5%	NA	NA	NA	1%
CD3-HLA-DR+	21%	NA	28%	11%	NA	NA	NA	NA
CD3+HLA-DR+	27%	NA	25.6%	33%	NA	NA	NA	NA
IgG mg/dl	*546 [745–1804]*	NA	*255 [745–1804]*	725 [*764–2134*]	NA	NA	NA	*584* [987–1958]
IgM mg/dl	130 [78–261]	NA	99 [78–261]	*45* [69–387]	NA	NA	NA	*27.3* [83–282]
IgA mg/dl	*24 [57–282]*	NA	*10 [57–282]*	*12* [78–383]	NA	NA	NA	*26* [96–465]

EBV, Ebstein-Barr virus; *PLT*, platelet; *ANC*, absolute neutrophil count; *ALC*, absolute lymphocyte count, *HSCT*; hematopoietic stem cell transplantation; *ICE*, ifosfamide, etoposide, carboplatin; *COPP*, cyclophosphamide, vincristine, procarbazine, prednisone; *OEPA*, vincristine, etoposide, prednisone, doxorubicin; *COPP/ABV*, cyclophosphamide, vincristine, procarbazine, prednisone/doxorubicin, bleomycin, vinblastine. Abnormal values are given in italics. Respective age reference values are given in brackets and # represents absolute cell counts

Table 2 Transplant-related data of the three patients who underwent hematopoietic stem cell transplant

	V1	V3	V4
Age at HSCT	5.5 years	6 years	8 years
Donor source	10/10 matched unrelated	10/10 mother	10/10 mother
Conditioning regimen	Treosulfan/fludarabin	Treosulfan/fludarabin	Treosulfan/fludarabin
GvHD prophylaxis	CsA + MMF + Post-Tx CyC	CsA + Post-Tx CyC	CsA
CD34+ stem cell dose	5.7×10^6/kg	5×10^6/kg	5.4×10^6/kg
Engrafment days	Neutrophil: 12	Neutrophil: 13	Neutrophil: 11
	Lymphocyte: 18	Lymphocyte: 21	Lymphocyte: 10
	Thrombocyte: 10	Thrombocyte: 12	Thrombocyte: 11
Post-tx complications	None	Grade 2 chronic skin GvHD	Grade 3 acute skin and liver GvHD
Follow-up time after transplant (tr)	3 months	11 months	17 months
Last laboratory investigations after tr.	At 3rd month	At 9th month	At 15th month
T cell chimerism	100%	100%	100%
IgG	840 mg/dl	692 mg/dl	583 mg/dl
IgA	54 mg/dl	48 mg/dl	26 mg/dl
IgM	92 mg/dl	110 mg/dl	121 mg/dl
CD3%	93	59	68
CD4%	5	29	19
CD8%	80	22	46
CD19%	0.2	19	5
CD16+56%	4	11	16
Ongoing treatments	Prophylactic antimicrobials-CsA	Prophylactic antimicrobials-prednisolon (for 2 months, now tapering)*	Prophylactic antimicrobials-CsA prednisolon (for 10 months, now tapering)

*Cyclosporin A was given for 9 months as GvHD prophylaxis and was stopped. After 2 months, GvHD recurred and responded to prednisolone, cyclosporin A was not given again. 2 mg/kg/day prednisolon was started as initial dose; if there is a response after 2 weeks, the dose was tapered by 10–25% every 2 weeks according to maintenance of response

autologous stem cell transplantation. Due to severe recurrent infections and history of consanguineous marriage in the family, combined immunodeficiency was suspected and she underwent an allogeneic transplantation from the mother, who was a full match donor, 29 months after termination of treatment for HL. Fludarabine and treosulfan were used as conditioning regimen; lymphocyte engraftment was achieved at day 21 post-transplant. Full donor chimerism in both total and T cell was achieved. Cyclosporin A and post-transplant cyclophosphamide were used for graft versus host (GvHD) prophylaxis. Grade 2 chronic skin GvHD was observed at 9 months post-transplant, which responded to steroids (which is tapered) and post-transplant cyclophosphamide. Her Karnofsky/Lansky performance score is 100. She is under follow-up for 48 months since diagnosis of HL and with no evidence of disease.

On family history, another sibling (V-2) had died of infection at 40 days of age. Soon after the two siblings' (V-1 and V-3) referral to our center, a 14-year-old female relative (IV-10) was referred to our center with encephalitis due to HSV1 infection and recurrent pneumonia. The family history revealed that the siblings of the patient (IV-10) were previously diagnosed with HL (IV-7, diagnosed at age 15 and died at 20, and IV-8, diagnosed at age 12, currently in remission at age 17). Paternal aunt of this patient (who was also a maternal aunt of V-1 and V-3) has been diagnosed with endometrium cancer (III-6).

Due to the diagnosis of HL in two siblings (V-1 and V-3) born to consanguineous parents, family history of sibling and cousins with severe infections and malignancies, and recurrent infections, an association with EBV was suspected, and the level of EBV DNA levels at admission to our center was found to be significantly elevated (V-1 43,900 copies/ml; V-3 14,067 copies/ml). The EBV DNA levels were further elevated at relapse (V-1 185761; V-3 309,242 copies/ml). An inherited immunodeficiency was also suspected and both siblings were evaluated for immunodeficiency including complete blood counts; flow cytometry of peripheral blood for CD3, CD4, CD8, CD45, CD19, and CD16; HLA-DR

counts; and IgG, IgA, and IgM levels (Table 1). Both had severe CD4 T cell lymphopenia and low immunoglobulin levels and were diagnosed with combined immunodeficiency (Table 1). In both siblings, immunoglobulin replacement and prophylactic antibiotics were initiated. In both patients EBV DNA levels were negative (values below 100 copies/ml were considered normal) after allogeneic stem cell transplantation.

Index patients' parents and the 6-year-old brother (V-4) who had cervical lymphadenomegalies were also evaluated. The brother (V-4) was also found to have elevated EBV DNA levels (47,566 copies/ml) and was also diagnosed with combined immune deficiency (Table 1). There was no malignancy in the excisional biopsy of the enlarged lymph nodes. He underwent an allogeneic transplantation from the mother, who was a full match donor, 15 months after diagnosis of CID. Fludarabine-treosulfan was used as conditioning regimen. Cyclosporin A was given for graft versus host (GVHD) prophlaxis. He achieved lymphocyte engraftment on day 10 with donor chimerism of 100%, respectively. He developed grade 2 acute GVHD with skin and liver involvement in the second month of transplantation, which responded to steroids. The GvHD recurred at 11 months post-transplant and responded to steroids. He is clinically well for 12 months since allogeneic transplantation with Karnofsky/Lansky performance score of 100. His EBV DNA levels were also negative after the allogeneic SCT.

The first sibling (V1) diagnosed with CID and HL, underwent allogeneic stem cell transplantation from the matched unrelated donor 51 months after diagnosis and 37 months after the termination of treatment for relapsed HL. He did not experience a further relapse before the transplantation. Fludarabine-treosulfan was used as conditioning regimen. Cyclosporin A and post-transplant cyclophosphamide were given as graft versus host (GVHD) prophlaxis. He achieved lymphocyte engraftment on day 14 with full donor chimerism on the first month of transplantation. Now, he is at the third month after transplantation and there is no evidence of GvHD. All three siblings are clinically well with IVIG replacement and prophylactic antibiotics, V-1 for 52 months, V-3 for 48 months since diagnosis of HL, and V-4 for 27 months since diagnosis of CID. After transplantation, IVIG replacement was given to all three patients for three to four times when the IgG level was < 400 mg/dl. Transplant-related data of the three patients who underwent allogeneic hematopoietic stem cell transplantation are presented in Table 2.

All analyses were performed after the approval of Istanbul University Medical Faculty Ethical Board; written and oral informed consents were taken from the family members or legal representatives.

Exome Sequencing

Peripheral blood of the patients at remission and healthy individuals were obtained (denoted with asterisk (* or **) in pedigree in Fig. 1) and DNA was isolated with column-based method of QIAamp DNA Blood Mini kit according to the manufacturer's instructions. Quantity and purity of DNA samples were assessed with spectrophotometry.

A whole exome sequencing (WES) approach was performed by using Agilent SureSelect Human All ExonV6 kit for exome capturing. Illumina's raw data was aligned to hg19 reference genome with BWA and SAMtools to generate final BAM file and variant list. For the selection of candidate genes, whole variant list was filtered, where intronic and synonymous variant were left out. Remaining variants were prioritized according to their MAF (< 0.01, reported by 1000Genomes, ExAC, and gnomAD). For the clinical interpretation of candidate variants OMIM, ClinVar's known reports (benign/pathogenic/uncertain), PolyPhen's and SIFT's predictions, and conservation scores (CADD) were evaluated. Sanger sequencing was used to validate and familial segregation analysis was performed for the candidate variations.

Kappa Deleting Excision Circle Analysis

Kappa deleting excision circle (KREC) analysis was performed to homozygous mutation carriers (V-1, V-3, and V-4), heterozygous parents (IV-3 and IV-4), and age-matched healthy controls. All KREC analyses were performed during remission and after autologous bone marrow transplantations for V-1 and V-3. KREC copy numbers were detected by quantitative real-time PCR (qRT-PCR) assay on a LC96 real-time PCR System (Roche USA). TRAC gene was used as housekeeping gene. Primers, probes, and PCR conditions were previously described [44].

Results

Variants revealed by exome sequencing of index (V-3) were filtered in the following order: exonic and splice sites, non-synonymous, homozygous, and finally to variants with MAF < 0.01 and all identified candidate variants were given in Supplementary Table 1. Among the genes, which are known to be associated with immunodeficiency and predisposition to EBV-associated lymphoma, we have identified a homozygous missense variation in the *CD70* and a non-synonymous, heterozygous exonic variant in *ITK* with MAF < 0.01 (rs17054374), which is reported in ClinVar as having conflicting interpretation (uncertain significance and benign).

Additional homozygous variations were also identified in *COL5A3, UACA*, and *USP17L10* genes which are predicted as disease causing, probably damaging, and deleterious

according to 3 different prediction tools (Mutation Taster, PolyPhen and SIFT). There is no ClinVar or OMIM phenotype data for those genes. Germline *UACA* loss-of-function variations were previously reported in one study in colorectal cancer [45], *COL5A3* variants were reported as genetic risk factor for autism spectrum disorder [46], and *USP17L10* gene variation was classified as variant unknown significance (VUS). *CD70* variation is the strongest candidate among the others fulfilling the clinical presentation that was previously reported and suited recessive inheritance pattern. This missense variation (NM_001252.5:c332C>T) was previously identified (rs1378830614) with a frequency of 7.95577e−06 (2/251390, heterozygous in two individuals) and presented in gnomAD database. No previous association was reported about the functional impact or clinical outcome of the variation. Familial segregation analysis confirmed all siblings as homozygous and parents as heterozygous for the *CD70* mutation (Fig. 2a). Mother's (IV-4) cousin (IV-10) was also homozygous for this mutation.

In silico prediction tools predict as probably damaging (Polyphen) and deleterious (SIFT) and the position is widely conserved between the species. This nucleotide is highly conserved (CADD scores of 25.8) and its amino acid is located in the extra cytoplasmic TNF domain of CD70 protein that is the region that CD70 interacts with CD27 (Fig. 3a–c).

KREC analysis carried out in affected homozygous patients (V-1, V-3, and V-4), heterozygous parents (IV-3 and IV-4), and healthy controls. The results showed that patients with immune deficiency and EBV-associated HL (V-1 and V-3) showed much higher KREC copies (197,893 copies and 168,269 copies, respectively) compared with heterozygous parents (IV-3: 22,000 copies and IV-4: 25,900 copies) and healthy controls childhood average count 29,900 copies (max count 98,000 copies) and adult average count 5290 (max count 29,100 copies) (Fig. 2b). V-4 who did not develop HL but only had immune deficiency had KREC copy numbers (35,832 copies) comparable with parents and healthy controls.

Discussion

Increased risk of cancer development in immune deficient patients has been acknowledged previously [47–52]. A study conducted on 3658 primary immunodeficiency patients in the USIDNET cohort reported a cancer incidence of 4.7%, of which 48% were diagnosed with lymphoma, 15% skin cancer, 8% genitourinary cancer, 8% gastrointestinal, 6% breast cancer, 5% endocrine cancer, 4% head and neck cancer, 3% lung cancer, 1% bone cancer, and 2% cancers with unspecified origin [51, 52].

Several primary immune deficiencies have high predisposition to malignancies, especially to EBV-associated lymphoproliferative disorders due to the impaired anti-viral response. Germline mutations in genes *SH2D1A*, *ITK*, *MAGT1*, *CORO1A*, *CD27*, *CD70*, *CTPS1*, *TNFRSF9*, *STK4*, *CARMIL2 PRKCD*, and *RASGRP1* are associated with a high incidence of EBV-associated lymphomas [26–29].

CD27 and CD70 are TNF family members and function as homodimer type I and homotrimer type II membrane proteins, respectively [30]. CD70-CD27 pathway is very important for immune surveillance of B cells and T cell immune function. *CD27* is highly expressed in T cells and some B cells and acts as co-stimulatory molecule of T cell activation (Fig. 3b) [35, 53]. *CD70* is expressed only in a small fraction of B cells. *CD70*'s expression is highly upregulated on activated B cells and EBV-infected B cells. When CD27 on T cells or CD70 on B cells have defects and impair the CD27-CD70 axis, antiviral response of T cells toward EBV-infected cells is impaired which in turn leads to lymphoproliferative disorder [13]. All of the previously reported mutation sites, including our report, are located in the extracellular domain of CD70, which might explain the association with immune deficiency and impaired response to EBV (Fig. 3a).

Up to date six cases with CID and/or EBV-related Hodgkin's lymphoma with *CD70* mutation have been reported. Caorsi et al. reported *CD70* mutation in a case with EBV-

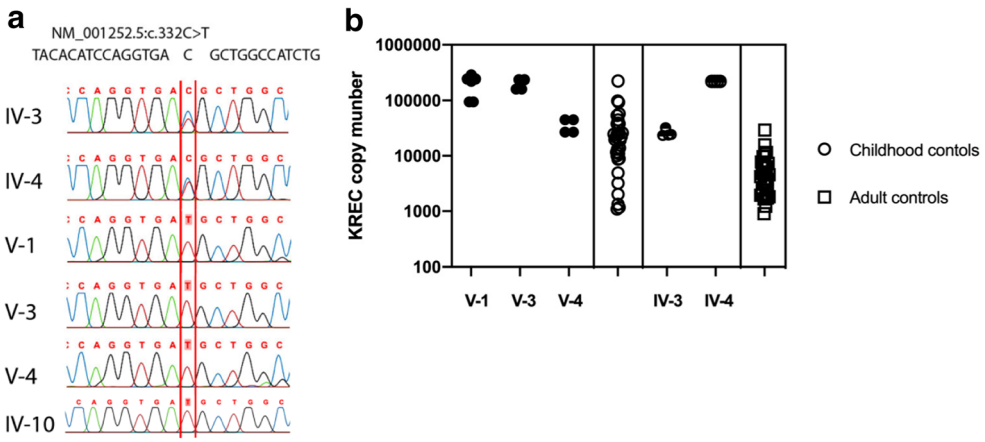

Fig. 2 **a** Sanger validation of NM_001252.5:c.332C>T mutation. **b** KREC copy numbers in homozygous mutant, heterozygous and homozygous wild type individuals. Each patient's sample was studied in technical replicates and the results were compared with the age-matched controls both for childhood and adults

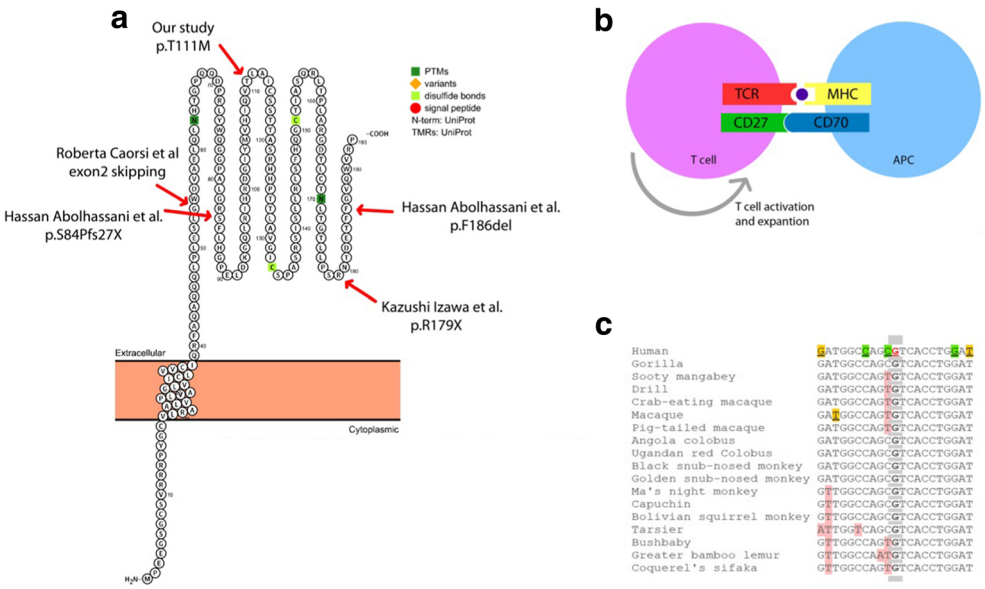

Fig. 3 **a** Schematic view of CD70 protein and previously reported mutations, **b** schematic view of T cell and antigen presenting cell's (APC) interaction. **c** NM_ 001252.5:c332C conservation across vertebrae (base G with gray underline)

positive recurrent infections and without leukemia or lymphoma development [12]. Abolhassani et al. reported two separate families with two different *CD70* variations: in the first family, patients with *CD70* mutations had presented with viral encephalitis or immune deficiency or EBV-associated HL, and in the second family, two patients were diagnosed with immune deficiency and EBV-positive HL [11]. Izawa et al. also reported a case with immune deficiency and EBV-positive HL [13].

In the present study, four members of a family were identified with CD70 homozygous mutations; two had combined immunodeficiency, high titers of EBV DNA, and HL (V-1)(V-3); one was diagnosed with immune dysregulation (V-4); and one who had encephalitis and recurrent infections (IV-10) is being investigated for immunodeficiency. Apart from our case (V-10), only one patient with CD70 deficiency is reported with viral encephalitis although the causative agent is not known in the latter [11]. The patients described till now are either presented with recurrent infections including severe viral infections suggesting a combined immune deficiency or with immune dysregulation and especially malignancy. The susceptibility to EBV is remarkable, whereas varicella pneumonia and herpes simplex encephalitis were also noted [11]. Laboratory evaluations of the patients in the literature varied from normal to decreased T cells, decreased T and B cells, and/or abnormal antibody responses. So far, we do not have data suggesting dysgammaglobulinemia following EBV infections like in X-linked lymphoproliferative syndrome [11–13].

All reported CD70 mutated patients who developed lymphoma had specifically Hodgkin lymphoma. Approximately 40% of Hodgkin lymphomas are associated with EBV infections with the predominance of mixed cellularity subtype [54]. EBV is thought to serve as an anti-apoptotic functionary to provide the escape of BCR-negative Hodgkin-Reed-Sternberg cells from the immune surveillance [55]. The origins of Hodgkin lymphoma are not fully elucidated and the preference for the HL in the case of germline *CD70* mutations may be an interesting matter to focus. CD70 may play a role specifically in these BCR-negative Hodgkin-Reed-Sternberg cells.

Kappa deleting excision circles are DNA segments generated in B cells during their maturation in the bone marrow. These segments are unable to replicate and consequently are diluted following cell divisions. Thus, KRECs can be used as a diagnostic tool in evaluating new lymphocyte output [56]. Until now, KREC measures were not reported in lymphoma patients. In this study, we found higher KREC levels in homozygous CD70 deficient patients who developed lymphoma. Interestingly, the other affected patient without lymphoma diagnosis showed KREC levels similar to healthy controls and heterozygous carriers. Kappa deleting excision circles occur during T cell–independent phase of B cell development in the bone marrow and are generated during the maturation from large pre B II cells to small pre B II cells [56]. Izawa et al. suggested that CD70 expressed on B cells might act as a signaler in the case of abnormal B cell proliferation to T cells. Additionally, somatic *CD70* mutations are found in B cell lymphomas, especially diffuse large B cells and Burkitt's lymphoma [57–59]. Thus, we may speculate that CD70 deficiency may result in abnormal B cell development following EBV encountering and leading to development of lymphoma. The normal KREC levels in patient V-4 may support this hypothesis. On the other hand, the samples were drawn following autologous transplantation for V-1 and V-3, which may also reflect the reconstitution of B cells. Nevertheless, this finding needs further investigations and is weak due to lack of inadequate number of patient data.

Exome sequencing has an advantage in identifying disease-causing gene(s) especially in a heterogeneous disease group such as immune deficiency and cancer predisposition syndromes with long and growing list of genes. In this study, exome sequencing has enabled us to identify the *CD70* mutation in multiple family members with immunodeficiency and malignancies. We were able to identify the homozygous mutation in three siblings and the heterozygous mutation in both parents and a homozygous mutation in a distant cousin. The identification of this mutation has also implications for surveillance of other family members. Unfortunately, despite many communications, the second family consented for the analysis of only the child who had had life-threatening infection (encephalitis); they are further referred for clinical surveillance in the center in their local setting.

Although autologous stem cell transplantation is a known treatment option in recurrent Hodgkin lymphoma, in patients with immune deficiency, allogeneic stem cell transplantation is recommended. Thus, consanguineous marriages, history of recurrent infections in the family, or family history of HL should alert the physician for immune deficiency. Two of the patients in this study (V1 and V3) had undergone autologous stem cell transplantation for relapsed Hodgkin Lymphoma prior to the diagnosis of immune deficiency and *CD70* mutation.

In conclusion, primary immune deficiency patients are predisposed to malignancies, especially lymphomas [60]. *CD70* mutation has rarely been reported in CID and/or Hodgkin lymphoma. In families with consanguineous marriages, with family history of recurrent or severe infections, or family history of HL, especially if multiple members are affected, individuals should be investigated for EBV DNA levels, immune deficiency, and *CD70* mutation.

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Authors' Contributions M. Sayitoglu and R. Kebudi supervised the study. All authors contributed to the data collection. K. Khodzhaev and Y. Erbilgin directed the data analysis and family analysis. Y. Y. Ng, D. Altındirek, and O. H. Ng performed and analyzed the KREC study and provided molecular biology expertise. R. Kebudi and S. B. Bay provided clinical oncology expertise. A. Kaya and A. Kıykım provided clinical immunology expertise. F Çipe Erol and B Adaklı Aksoy provided allogeneic transplantation expertise, patient material, and clinical data for the study. F. Sen Zengin provided the patient material and data in Erzurum. Data analyses of molecular findings were performed by K. Khodzhaev, S. Firtina, and Y. Erbilgin. Data interpretation was carried out by R. Kebudi, M. Sayitoglu, K. Khodzhaev, S. B. Bay, and A. Kiykim. All authors contributed to manuscript preparation. M. Sayitoglu and R. Kebudi reviewed the manuscript. All authors made the final approval of the manuscript.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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