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ORIGINAL ARTICLE

Clinical, Immunological, and Genetic Findings in Iranian Patients with MHC-II Deficiency: Confirmation of c.162delG *RFXANK* Founder Mutation in the Iranian Population

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Abstract

Purpose Major histocompatibility complex class II (MHC-II) deficiency is a rare inborn error of immunity (IEI). Impaired antigen presentation to CD4 + T cells results in combined immunodeficiency (CID). Patients typically present with severe respiratory and gastrointestinal tract infections at early ages. Hematopoietic stem cell transplantation (HSCT) is the only curative therapy.

Methods We describe the clinical, immunologic, and genetic features of eighteen unrelated Iranian patients with MHC-II deficiency.

Results Consanguinity was present in all affected families. The median age at the initial presentation was 5.5 months (range 7 days to 18 years). The main symptoms included failure to thrive, persistent diarrhea, and pneumonia. Autoimmune and neurologic features were also documented in about one-third of the patients, respectively. Thirteen patients carried *RFXANK* gene mutations, two carried *RFX5* gene mutations, and three carried a *RFXAP* gene mutation. Six patients shared the same *RFXANK* founder mutation (c.162delG); limited to the Iranian population and dated to approximately 1296 years ago. Four of the patients underwent HSCT; three of them are alive. On the other hand, nine of the fourteen patients who did not undergo HSCT had a poor prognosis and died.

Conclusion MHC-II deficiency is not rare in Iran, with a high rate of consanguinity. It should be considered in the differential diagnosis of CID at any age. With the limited access to HSCT and its variable results in MHC-II deficiency, implementing genetic counseling and family planning for the affected families are mandatory. We are better determined to study the c.162delG *RFXANK* heterozygous mutation frequency in the Iranian population.

Keywords CD4 + lymphocytopenia · Hematopoietic stem cell transplantation · Inborn error of immunity · MHC-II deficiency · *RFX5* gene · *RFXANK* gene · *RFXAP* gene · Founder effect

Abbreviations

ARD	Ankyrin repeat domain
APCs	Antigen-presenting cells
ANCA	Antineutrophil cytoplasmic antibodies
AIHA	Autoimmune hemolytic anemia
BCG	Bacillus Calmette–Guerin
BAL	Bronchoalveolar lavage
BMSC	Bone marrow stem cells
CSF	Cerebrospinal fluid
CADD	Combined annotation-dependent depletion
CID	Combined immunodeficiency
CT	Computed tomography
CMV	Cytomegalovirus
DTH	Delayed-type hypersensitivity
DBD	DNA-binding domain
ENT	Ear, nose, and throat
FTT	Failure to thrive

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HSCT	Hematopoietic stem cell transplantation
HSV	Herpes simplex virus
iVDPV	Immunodeficiency-associated vaccine–derived poliovirus
IEI	Inborn errors of immunity
JIA	Juvenile idiopathic arthritis
MHC-II	Major histocompatibility complex class II
MRI	Magnetic resonance imaging
MRD	Matched-related donor
MAF	Minor allele frequency
MMRD	Mismatched-related donor
MRCA	Most recent common ancestor
MSC	Mutation significance cutoff
NDD	Neurodevelopmental delay
NLH	Nodular lymphoid hyperplasia
NTD	N-terminal domain
NLS	Nuclear localization signal
OPV	Oral poliovirus vaccine
PBSC	Peripheral blood stem cells
PEST	Activation domain rich in acidic amino acids (P, E, S, and T)
PHA	Phytohemagglutinin
SCID	Severe combined immunodeficiency
TCR	T cell receptor
VAPP	Vaccine-associated paralytic poliomyelitis
VDPV2	Vaccine-derived poliovirus type 2

Introduction

Major histocompatibility complex class II (MHC-II) molecules are transmembrane glycoproteins that specialized for the presentation of peptides to the T cell receptor (TCR); thus, they have key roles in the maturation of CD4 + T lymphocytes in the thymus as well as CD4 + T cell–dependent immune responses in the periphery [1–3]. Three different human MHC-II isotypes (HLA-DR, HLA-DP, and HLA-DQ) are expressed over antigen-presenting cells (APCs) such as dendritic cells, macrophages, and B lymphocytes [3, 4]. MHC-II deficiency is a rare inborn error of immunity (IEI) first described in the 1980s [5–8]. It is defined by the lack of MHC-II expression on APC cells and the absence of CD4 + T cell–dependent immune responses. Moreover, perturbation of central and peripheral immune tolerance mechanisms, specifically the defects in the maturation and function of thymic epithelial cells (TEC), underlies immune dysregulation in MHC-II deficiency [9]. The disease is caused by mutations in four distinct genes (*CIITA*, *RFXANK*, *RFXAP*, and *RFX5*) encoding trans-acting regulatory factors required to transcribe MHC-II genes and MHC-II expression [3, 4, 10, 11].

The resulting combined immunodeficiency (CID) leads to severe and recurrent respiratory and gastrointestinal infections. In most patients, infections start within the first year of life, but some have milder courses of disease and are diagnosed later, at ages of up to 15 years [12, 13]. Laboratory study typically shows CD4 + lymphocytopenia in the presence of normal total T cell numbers. Absence or very low HLA-DR expression on immune cells and abnormal antigen-specific cellular and humoral responses are also seen [4, 14]. MHC-II deficiency has a dismal outcome. However, allogeneic hematopoietic stem cell transplantation (HSCT) is the only available curative treatment with variable success.

MHC-II deficiency is rare; most patients are reported from North Africa [15–20]. The remaining patients are occasionally reported from other nationalities [21–25]. We present the clinical, immunological, and genetic features of eighteen unrelated Iranian patients with MHC-II deficiency. Six of them have the same *RFXANK* mutation, and we investigated and proved the possibility of a founder effect.

Materials and Methods

We report on 18 unrelated Iranian MHC-II-deficient patients in this case series. Thirteen patients are new, and five have been reported previously [26–30]. Informed consent for participating in the study was obtained from the patients or their parents. The Children's Medical Center Institutional Ethics Committee, affiliated with the Tehran University of Medical Sciences, approved the study. We thoroughly reviewed patient medical records and collected detailed demographic and clinical data: gender, age at presentation, diagnosis, family history, clinical presentation, diagnostic workup, management, and outcome. Clinical data from previously published cases have been compiled from the pertinent articles.

Clinical whole exome sequencing (WES) was performed on patient samples, as reported previously [31]. Exome capture was performed with the Sure Select Human All Exon 50 Mb kit (Agilent Technologies). Paired-end sequencing was performed on a HiSeq 2000 (Illumina), generating 100-base reads. Bi-directional sequence reads were assembled and aligned to the human genome build GRCh38/hg19.

Downstream processing and variant calling were performed with the genome analysis toolkit, SAMtools, and Picard. Substitution and InDel calls were made with GATK Unified Genotyper. All variants were annotated using an annotation software system that was developed in-house. All the variants have been documented by Sanger sequencing.

Common haplotype analysis on *RFXANK* variant (c.126delG) was conducted to prove the founder effect of c.126delG in Iranian patients. Haplotype analysis was determined by identifying shared regions of homozygous

variants from each affected individual with the homozygous c.126delG variant at chr19: 191,941,08.

Results

Epidemiologic Features

We investigated eighteen patients from unrelated families with MHC-II deficiency with varying clinical and immunological phenotypes. All the patients were of Iranian origin, born to consanguineous families. The median age at the initial presentation was 5.5 months (range 7 days to 18 years). The median age at immunodeficiency diagnosis and the genetic confirmation was 12 months and 25 months, respectively. Six of the 18 patients had another sibling who had died with similar symptoms.

Clinical Characteristics

The patients' clinical features are summarized in Table 1 (the detailed clinical data is presented in the supplementary Table S1).

All the patients received attenuated oral poliovirus vaccine (OPV) and Bacillus Calmette–Guerin (BCG) at birth. All the patients suffered from several manifestations before

Table 1 Summary of clinical characteristics of the included patients

Symptoms		Percent (numbers)
Failure to thrive		83.3% (15/18)
Gastrointestinal	Total	77.7% (14/18)
	Persistent diarrhea	66.6% (12/18)
	Persistent oral candidiasis	38.8% (7/18)
	Cryptosporidiosis	22.2% (4/18)
	CMV colitis	22.2% (4/18)
	Liver disease/cholangitis	16.6% (3/18)
	Poliovirus shedding	5.5% (1/18)
Pulmonary	Pneumonia	77.7% (14/18)
Upper respiratory tract	Total	33.33% (6/18)
	Otitis media	33.3% (6/18)
	Sinusitis	11.1% (2/18)
Severe viral infection	Total	66.6% (12/18)
	CMV	38.8% (7/18)
	COVID-19	16.6% (3/18)
	iVDPV	5.5% (1/18)
	Norovirus	5.5% (1/18)
Other infections	BCG lymphadenitis	11.1% (2/18)
	Mucormycosis	5.5% (1/18)
Autoimmunity	Total	33.33% (6/18)
	Autoimmune hemolytic anemia (AIHA)	16.6% (3/18)
	Juvenile idiopathic arthritis (JIA)	11.1% (2/18)
	Autoimmune enteropathy	5.5% (1/18)
Neurologic involvement	Total	38.8% (7/18)
	Neurodevelopmental delay (NDD)	16.6% (3/18)
	Hypotonia	11.1% (2/18)
	Chorea athetosis	5.5% (1/18)
	Ataxia	5.5% (1/18)
	Encephalopathy	5.5% (1/18)
Allergy	Total	50% (9/18)
	Food allergy	5.5% (1/18)
	Asthma	33.33% (6/18)
	Atopic dermatitis	22.22% (4/18)
	Drug allergy	11.1% (2/18)

BCG, Bacille Calmette–Guerin; *CMV*, cytomegalovirus; *EBV*, Epstein–Barr virus; *iVDPV*, immunodeficiency-associated vaccine–derived poliovirus; *JIA*, juvenile idiopathic arthritis

they came to our attention. Failure to thrive (FTT) was present in fifteen (83.3%) patients.

Respiratory Tract Infections

Recurrent pneumonia was reported in 14 patients (77.7%) and occurred before 1 year of age in 10 patients (55.5%). Three patients (P2, P5, and P15) developed COVID-19 pneumonia after SARS-CoV-2. P2 developed cardiomyopathy during COVID-19, which was finally resolved. P5 died after COVID-19 complications. Bronchoalveolar lavage (BAL) was positive for CMV infection in P6 and *Acinetobacter* spp. in P5.

Six patients (33.33%) had recurrent acute otitis media that four of which began before the age of 2 years and required a trans-tympanic drain in P9. Chronic sinusitis was documented in 2 patients (P10, P11). P1 died from sinus mucormycosis with brain invasion.

Gastrointestinal Manifestations

Fourteen patients (77.7%) had gastrointestinal manifestations. Persistent diarrhea (66.7%) was a common gastrointestinal problem. A histologic study of intestinal mucosa was performed in six patients. This study revealed autoimmune enteropathy in one patient (P2), nodular lymphoid hyperplasia in two (P11, P17), and granulation tissue in the colon in another (P5) patient. Seven patients (38.8%) displayed chronic oral candidiasis complicated by esophageal involvement in P11. Four patients were diagnosed with CMV colitis (P1, P2, P5, and P8); two of whom (P1, P2) developed bowel perforation. Four patients (P1, P2, P8, and P11) had recurrent diarrhea caused by *C. parvum*. In P11, cryptosporidium infection resulted in sclerosing cholangitis and hepatorenal syndrome. Recurrent diarrhea caused by norovirus was observed in one patient (P2) and persisted until death. Hepatic abnormalities were not frequent, and three patients had hepatomegaly and/or high serum levels of liver transaminases (P6, P8, and P11).

Other Infections

Two patients (P7, P17) had BCG lymphadenitis that resolved without complication.

Blood cultures were positive for *Pseudomonas aeruginosa* (P2) and *Enterobacter cloacae* (P5). P4 had CMV viremia without definitive organ involvement, and P13 had CMV viremia with brain involvement. One patient (P10) had recurrent UTI caused by *Klebsiella pneumonia*.

P18 developed vaccine-associated paralytic poliomyelitis (VAPP) at 7 months. He excreted vaccine-derived poliovirus type 2 (VDPV2).

Other Manifestations

Allergy was observed in 50% of the patients. Six patients had asthma, and four (P5, P8, P9, and P16) had atopic dermatitis. An allergic cutaneous drug reaction was observed in two patients (P6 after fluconazole and P8 after cefixime).

Autoimmune hemolytic anemia (AIHA) occurred in three patients (P1, P3, and P10) at ages 7, 2.5, and 36 years, respectively. P1 and P10 were treated with oral prednisolone and azathioprine, and P3 was treated with prednisolone and cyclosporine. Two other patients (P14, P4) had peripheral neutropenia with no detectable autoantibodies. Two patients (P8, P17) had a clinical diagnosis of juvenile idiopathic arthritis (JIA).

Neurologic complications unrelated to poliovirus occurred in seven patients. Neurodevelopmental delay (NDD) and hypotonia were observed in two patients (P12 and P16) with normal brain imaging. In addition to NDD, lower limb spasticity and axial hypotonia during infancy were observed in P13. Brain computed tomography (CT) scan revealed thalamus and basal ganglia calcification, probably caused by CMV infection. P14 patient had ataxic gait and lower limb muscle weakness at 2 years of age with normal brain magnetic resonance imaging (MRI). P1 presented with chorea athetosis at age 10 years. Brain imaging showed brain atrophy and mild ventriculomegaly. P7 experienced encephalopathy without documented infection; his symptoms improved after antibiotics, corticosteroid, and IVIG treatment. P15 had dysmorphic features, mild hypotonia, and brain atrophy in brain MRI. There were no enteroviruses or HSV found in the cerebrospinal fluid of any of the six patients who had neurological problems.

Immunologic Findings

All patients displayed a total absence of MHC class II (HLA-DR) molecule expression on B cells except two patients (P13, P17). There were low absolute CD4 + T cell counts in 13 of the 18 patients (72.2%). CD4 + T cell counts were below 500 cells/μL for ten patients (55.5%) between the ages of 4 months and 32 years. CD8 + T cell counts were low in three patients (16.6%) and high in another six (33.3%). All the patients, even those with normal T cell CD4 + count, had a low CD4:CD8 ratio.

Natural killer cell (CD3–CD16 + /CD56 +) counts were low in 3 of the 18 (16.6%) patients tested. In vitro proliferative responses of T cells to mitogen phytohemagglutinin (PHA) were normal for all patients, but no proliferation in response to antigens or delayed type hypersensitivity (DTH)

to tetanus toxoid was observed in 11 of 12 tested patients. B cell immunity was also profoundly impaired. Twelve of the 18 patients studied (66.6%) had normal numbers of circulating B lymphocytes, whereas the other 6 (33.3%) had low circulating B lymphocytes. Before the initiation of IgG substitution therapy, 12 of 16 patients (75%) had low levels of IgG, nine had low levels of IgA (56.3%), and 10 of 15 had low levels of IgM (66.6%). Two patients (P5, P17) had high baseline IgG levels at 2 and 3.5 years, respectively.

No specific antibodies against pneumococcal or protein vaccines could be detected in the 7 of 11 (63.6%) patients tested. Immunologic data are summarized in Table 2 (the detailed immunologic data is presented in the supplementary Table S2).

Genetic Results

We performed WES for the fourteen patients. Sanger sequencing confirmed the variants. All the parents were heterozygous.

Seven different mutations of the *RFXANK* gene have been characterized in thirteen unrelated patients (Table 3, Fig. 1a). The *RFXANK* variants are compared to the homozygous coding missense and predicted loss-of-function *RFXANK* mutations taken from GnomAD (Fig. 1b). Our reported variants are private and have combined annotation-dependent depletion (CADD) scores above the mutation significance cutoff (MSC) of 21.4 [32].

These include a deletion leading to a frameshift (P1–P6), three nonsense mutations (P9, P12, P13), one missense mutation (P8), and two splice-site mutations (P7, P10, P11). All the mutations affect the integrity of the ankyrin repeat domain (ARD), which is essential for the function of RFXANK [33, 34].

The c.162delG is a frameshift deletion documented in six unrelated patients. This mutation leads to an early termination codon, leaving a truncated protein. This is a novel mutation not reported in other populations and seems limited to the Iranian population.

The occurrence of homozygosity for the c.126delG mutation in these six kindreds of Iranian origin strongly suggested a founder effect. An analysis of the WES data showed that the index cases homozygous for the c.126delG mutation share a common homozygous haplotype around *RFXANK*, encompassing 2.7 Mbp corresponding to 83 SNVs (Fig. 2). The ESTIAGE method [35] estimated the age of the most recent common ancestor (MRCA) of the six patients at 48 generations [95% confidence interval (26–91)]. Assuming a generation time of 27 years [36], the MRCA of these patients with the c.126delG mutation would have lived about 1296 (702–2457) years ago.

The missense mutation p.Asp121His has been reported previously, resulting in the loss of the RFXANK function [24]. Two novel splice-site mutations are reported here. Splice-site mutation c.438 + 5G > A documented in two patients (P10, P11) seems responsible for a milder form of the disease. Three different *RFXAP* gene mutations have been identified (Table 3, Fig. 1c). These include two frameshift mutations (P15, P16) resulting from deletion and a nonsense mutation (P14). All these mutations lead to the synthesis of truncated proteins lacking the C-terminal

Table 2 Summary of immunologic characteristics of the patients

Patient lab test	Low % (patients/tested patients)	Normal or high % (patients/tested patients)
Lymphopenia	50% (8/16)	50% (8/16)
CD4 + T lymphocyte subset	72.2% (13/18)	27.7% (5/18)
CD8 + T lymphocyte subset	16.6% (3/18)	83.3 (15/18)
CD4:CD8 ratio	100% (16/16)	0%
B lymphocyte subset	33.3% (6/18)	66.6% (12/18)
NK lymphocyte subset	16.6% (3/18)	83.3% (15/18)
HLA-DR expression on monocytes	85.7% (12/14)	14.2% (2/14)
T cell proliferation in response to PHA	0%	100% (5/5)
T cell proliferation in response to antigens	80% (4/5)	20% (1/5)
DTH to tetanus toxoid	100% (7/7)	0%
IgG levels	75% (12/16)	25% (4/16)
IgA levels	56.2% (9/16)	43.7% (7/16)
IgM levels	66.6% (10/15)	33.3% (5/15)
IgE levels	16.6% (2/12)	83.3% (10/12)
Specific antibodies against tetanus/diphtheria toxoids	63.6% (7/11)	36.3% (4/11)

DTH, delayed type hypersensitivity; *PHA*, phytohemagglutinin

Table 3 Genetic findings of the patients

	Gene	Ref number	Exon	Mutation	Parents	Effect	Protein change	Literature reports
P1	*RFXANK*	ENST00000303088.9	Exon3	c.162delG	Het	A frame shift deletion mutation	p.Asp55fsX13	This study
P2	*RFXANK*	ENST00000303088.9	Exon3	c.162delG	Het	A frame shift deletion mutation	p.Asp55fsX13	This study
P3	*RFXANK*	ENST00000303088.9	Exon3	c.162delG	Het	A frame shift deletion mutation	p.Asp55fsX13	This study
P4	*RFXANK*	ENST00000303088.9	Exon3	c.162delG	Het	A frame shift deletion mutation	p.Asp55fsX13	Ref 26
P5	*RFXANK*	ENST00000303088.9	Exon3	c.162delG	Het	A frame shift deletion mutation	p.Asp55fsX13	This study
P6	*RFXANK*	ENST00000303088.9	Exon3	c.162delG	Het	A frame shift deletion mutation	p.Asp55fsX13	This study
P7	*RFXANK*	ENST00000303088.9	Intron5	c.338-1G > C (IVS5-1G > C)	Het	It affects a nucleotide within the consensus splice site of the intron		This study
P8	*RFXANK*	ENST00000303088.9	Exon6	c.361G > C	Het	A point mutation leading to an amino acid change	p.Asp121His	This study Ref 24
P9	*RFXANK*	ENST00000303088.9	Exon6	c.390G > A	Het	A point mutation leading to a premature stop codon	p.Trp130X	Ref 27
P10	*RFXANK*	ENST00000303088.9	Intron6	c.438 + 5G > A (IVS6 + 5G > A)	Het	It affects a nucleotide within the consensus splice site of the intron		This study
P11	*RFXANK*	ENST00000303088.9	Intron6	c.438 + 5G > A (IVS6 + 5G > A)	Het	It affects a nucleotide within the consensus splice site of the intron		Ref 30
P12	*RFXANK*	ENST00000303088.9	Exon7	c.563G > A	Het	A point mutation leading to a premature stop codon	p.Trp188X	Ref 28
P13	*RFXANK*	ENST00000303088.9	Exon9	c.634C > T	Het	A point mutation leading to a premature stop codon	p.Arg212X	This study Ref 24
P14	*RFXAP*	ENST00000255476.3	Exon1	c.163C > T	Het	A point mutation leading to a premature stop codon	p.Gln55X	This study
P15	*RFXAP*	ENST00000255476.3	Exon1	c.350delA	Het	A frame shift deletion mutation leading to a premature stop codon	p.His117fsX21	This study
P16	*RFXAP*	ENST00000255476.3	Exon1	c.467delA	Het	A frame shift deletion mutation leading to a premature stop codon	p.Gln156fsX28	This study
P17	*RFX5*	ENST00000452671.7	Exon6	c. 433G > C	Het	A point mutation leading to an amino acid change	p.Asp145His	This study
P18	*RFX5*	ENST00000452671.7	Exon10	c.1480dupC	Het	An insertion mutation leading to several amino acid changes	p.Gln494	Ref 29

glutamine-rich domain, which is known to be necessary for the RFX complex formation [37]. Two mutations of the *RFX5* gene have been identified (Table 3, Fig. 1d). These include a missense (P17) and a nucleotide duplication (P18). The missense mutation lies in the DNA-binding domain (DBD) and is novel. The DBD of RFX5 is essential for association with RFXANK and RFXAP, correct assembly of the RFX complex in solution, and specific

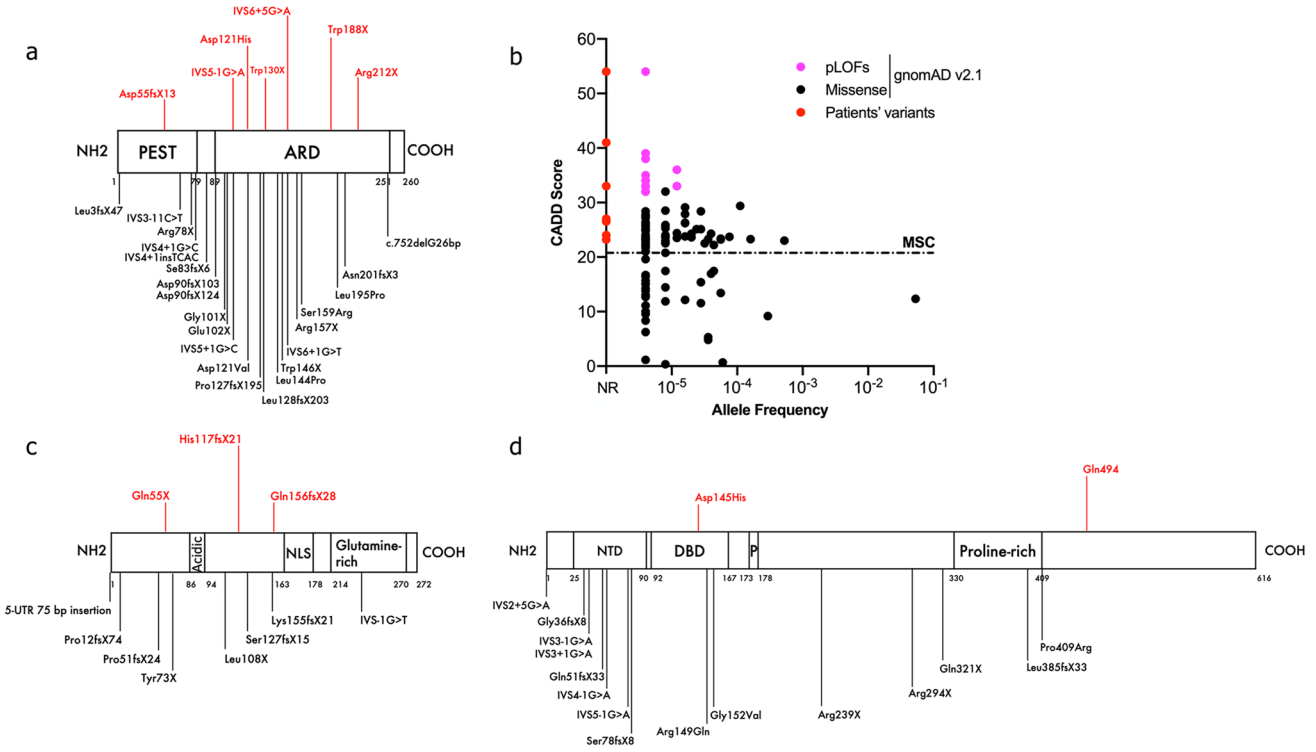

Fig. 1 MHC-II deficiency in the 18 Iranian patients. **a** Schematic representation of the RFXANK protein. ARD, ankyrin-repeat domain; PEST, activation domain rich in acidic amino acids. **b** Population genetics of *RFXANK*. The minor allele frequency (MAF) and CADD scores for all non-synonymous variants reported in the gnomAD database are shown. Seven homozygous variants found in our cohort are also shown. **c** Schematic representation of the RFXAP protein. NLS, nuclear localization signal. **d** Schematic representation of the RFX5 protein. NTD, N-terminal domain; DBD, DNA-binding domain; P, PxLPxI/L motif. The previously reported variants are indicated in black, while those of our patients are indicated in red

binding to the XX-box target site [38, 39]. The duplication mutation is an insertion leading to several amino acid changes, modifying the protein structure.

Outcome and Treatment

HSCT In our series, four (P3, P6, P14, and P16) of the 18 patients (23.5%) underwent HSCT (Fig. 3). The indications for HSCT are mostly related to clinical status (age and infection state). The median age at HSCT was 1.5 years (range 1–5 years). Three of the four patients who received HSCT (75%) are alive. All the patients received reduced intensity conditioning (RIC). The RIC regimen consisted of a combination of intravenous (IV) fludarabine (30 mg/m^2) administered for five consecutive days (days 8 to 4), IV melphalan (70 mg/m^2) for two consecutive days (days 3 and 2), and IV horse anti-thymocyte globulin (10 mg/kg) for four consecutive days (days 4 to 1). IV cyclosporine A (CSA) (1.5 mg/kg daily is used on day 1; then 3 mg/kg from day + 7 in PBSC to day + 11 in BMSC) and IV methylprednisolone (1 mg/kg/day (day + 5 to + 7), then 0.5 mg/kg/day by day + 14) are used as GVHD prophylaxis.

The patients received grafts from a matched-related donor (MRD, P16), HLA-identical sibling (P3), one-locus mismatched-related donor (MMRD, P14), and unrelated full-matched donor (P6). Primary engraftment occurred in all patients, but two experienced secondary graft failure (P6, P16); P6 underwent the second HSCT from the same donor, and she died after 1 month due to transplant-related complications (sepsis). P16 is alive, but re-transplantation is challenging because of her severe neurologic impairment. P3 and P14 developed full donor chimerism and doing well clinically.

The Natural History and Outcome of Patients Who Did Not Undergo HSCT Fourteen patients did not undergo HSCT (Table S3, Fig. 3). The clinical course was unfavorable in these patients, with the progression of infectious complications to death in nine patients (64.2%; range: 11 months to 15 years; mean age at death: 33 months). P1 died from severe mucormycosis infection. P2 died from peritonitis due to terminal ileum perforation after a norovirus infection. P11 had a clinical course like other patients in early childhood but displayed milder symptoms. The frequency of infection

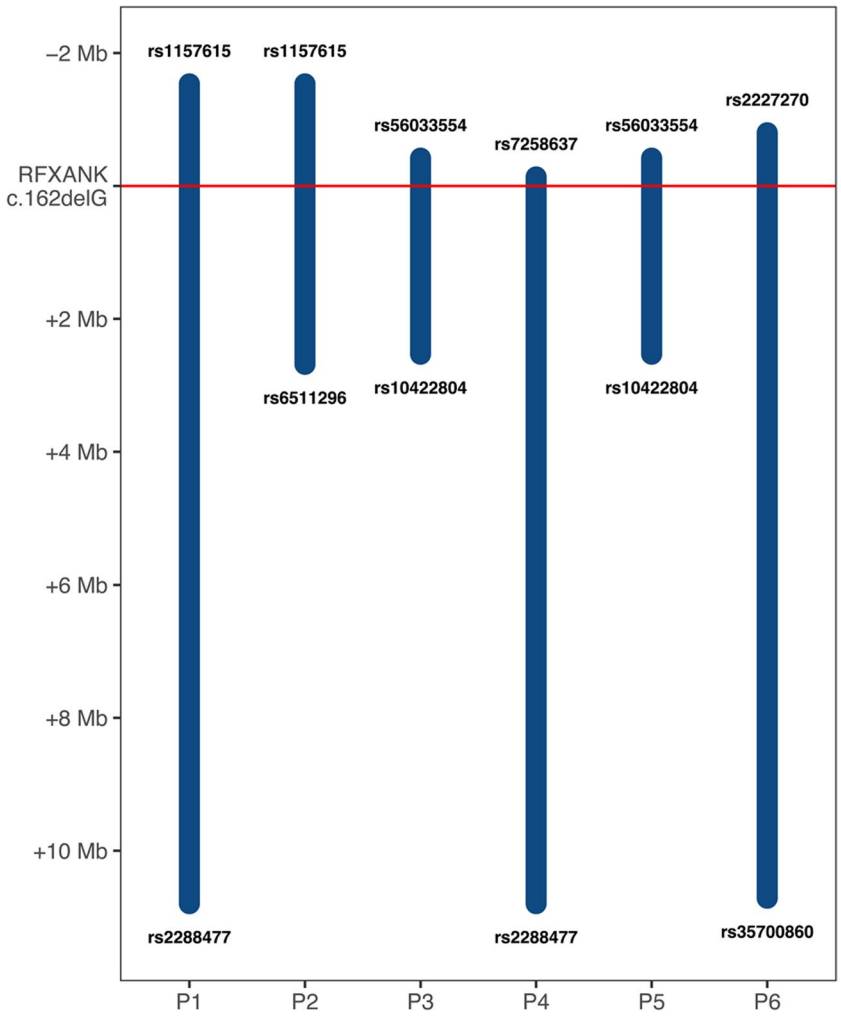

Fig. 2 Shared haplotype around the *RFXANK* c.162delG mutation for the six carriers. The distance from the mutation is represented on the *Y*-axis. Long continuous stretches of homozygosity were observed around the gene, consistent with its recessive mode of inheritance, and haplotypes were unambiguously derived from genotypes. The dbsnp reference numbers of the first (top) and last (bottom) unambiguous variants within the haplotype are reported for each carrier

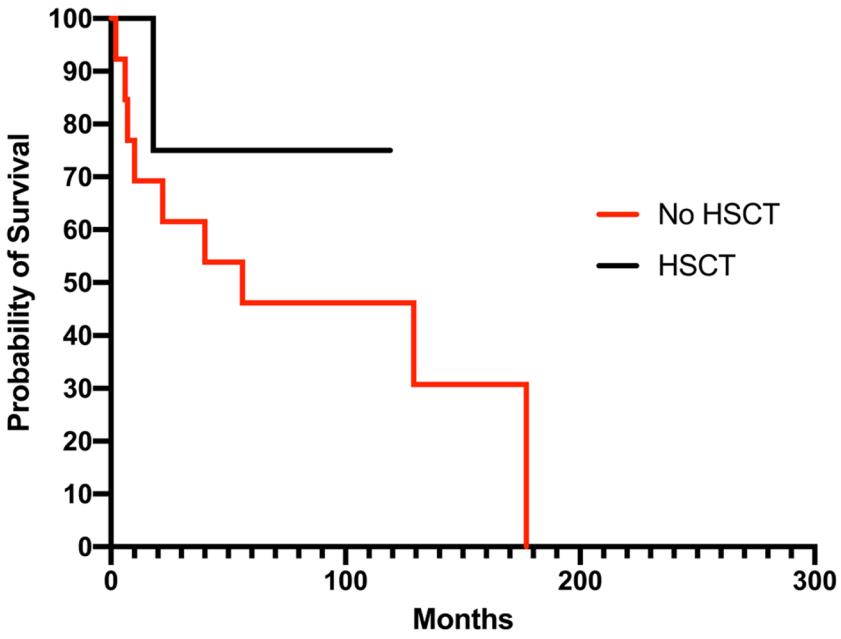

Fig. 3 Five-year overall survival curves (Kaplan–Meier) for patients who underwent HSCT and those who did not undergo HSCT

in this patient increased with age. Immunologic investigations revealed no significant differences from other patients. He died at 15 years of age because of hepatorenal failure due to cryptosporidiosis.

P12 died from severe pneumonia. P18 had a severe progressive respiratory failure due to VAPP. Other patients (P7, P13, and P15) died from undefined systemic inflammatory response syndrome (SIRS).

Five patients who did not undergo HSCT (35.7%) are still alive at the time of this report at a median age of 10 years (range: 6–42 years).

P4 has had recurrent diarrhea and chronic bronchopneumonia since age four and was diagnosed at 4.5. He is now 9 years old.

P8 presented with chronic diarrhea, upper respiratory infections, and oral candidiasis since early infancy and was diagnosed with MHC-II deficiency at the age of 2 years. She had recurrent diarrhea caused by *C. parvum* and CMV colitis during follow-up. She developed JIA for 8 years. He is now 10 years old.

P9 has had symptoms since the 6 months of life and was diagnosed with MHC-II deficiency at 9 months. She presented recurrent upper and lower respiratory infections (otitis media with effusion leading to ventilation tube insertion) and persistent diarrhea. She is 6 years old and has FTT, short stature, and hypothyroidism.

P10 has had symptoms since the age of 18 years and was diagnosed with MHC-II deficiency at the age of 37 years. Her symptoms worsen after the age of 35 years, with an increase in the frequency and severity of infections. She had AIHA and positive antineutrophil cytoplasmic antibodies (ANCA), requiring azathioprine treatment at the age of 36 years. She also has recurrent UTI (*K. pneumonia*), recurrent upper respiratory infections, and chronic bronchopneumonia. She is still alive on supportive care (IgG treatment and antibiotic prophylaxis with trimethoprim-sulfamethoxazole). P17 presented with recurrent pneumonia since 2 months and was diagnosed at 12 months. At the age of 2 years, he presented with JIA. He is 12 years old.

Discussion

We describe the clinical, immunologic, and genetic features of eighteen unrelated patients with MHC-II deficiency from Iran. In most of our patients, clinical signs and outcomes were similar to those reported in other groups of patients [4, 15, 16, 22, 23, 40].

The clinical features mainly included FTT and bacterial and severe viral infections in the respiratory and gastrointestinal systems. Viral infections are especially associated with poor outcomes, with patients dying during childhood [22, 41]. In our series, poliovirus, SARS-CoV-2, and norovirus infections are also associated with poor prognosis. One of our patients developed VAPP after OPV inoculation at birth. It is better to monitor these patients for virus shedding in the stool at the time of diagnosis and regularly after that [42, 43]. Chronic infection with *C. parvum* is associated with sclerosing cholangitis in some patients [15, 22]. Indeed, hepatic involvement appears to be associated with early mortality [15].

Allergy was observed in 50% of our patients. The prevalence of allergies in our patients was higher than in the general population (10–12%) or even other CIDs (20%) in general [44, 45]. MHC-II deficiency might be implicated in driving allergic immune dysregulation. The presence of autoimmunity in one-third of our patients highlights the role of MCH-II proteins in immune tolerance [9, 46]. MHC-II deficiency also causes a complex disruption of mucosal immunity, presenting with inflammatory intestinal manifestations [47]. Thus, MHC-II deficiency should be considered a differential diagnosis in early-onset inflammatory bowel diseases. Neurologic problems were documented in one-third of our patients, consistent with previous studies that reported neurologic involvement, mainly in patients with *RFXANK* mutation [15, 22, 48]. Some of these findings could be attributed to viral infections, but the exact mechanisms of the neurologic phenotypes are unclear.

In this study, most patients displayed a reduced absolute CD4 + T cell count and absent HLA-DR expression on B lymphocytes and monocytes. The CD4 + T cell counts were normal in 27.7% of the patients, while they were functionally defective in all tested patients. Despite normal CD4 + T cells, these patients had clinical courses like others. In our study, 33.3% of patients had low B cell numbers. In other cohorts, 21 to 24% of the patients had fewer circulating B cells than expected [15, 49]. The reasons for this variability in immunologic phenotype in this setting are unknown. Genetic and environmental factors other than the level of HLA-DR expression level may explain the variable clinical expression [13, 15]. We could not exclude the presence of revertant mosaicism as the underlying cause.

We documented *RFXANK*, *RFXAP*, and *RFX5* mutations in Iranian patients with MHC-II deficiency. Despite such genetic heterogeneity, these patients had a similar clinical presentation. About 72% of the patients had a *RFXANK* mutation, consistent with international observations. Six patients had the same homozygous c.162delG frameshift deletion in the *RFXANK* gene limited to the Iranian population. A founder effect was demonstrated about 1296 years ago. Also, we demonstrated the novel splice-site mutation (c.438 + 5G > A) in two patients with a milder form of the disease. Late onset of symptoms and milder course have been reported previously with other splicing *RFXANK* mutations [12].

HSCT is the only curative treatment for this IEI. However, HSCT had a limited success rate in these patients, not exceeding 50% [23, 41, 50–53]. Residual host immunity in MHC-II deficiency is sufficient to cause rejection [23, 50, 54, 55]. Alternatively, donor antigen-presenting cells could present donor antigens to recipient T cells leading to graft rejection.

Recent studies have shown an improved survival of up to 94% [49]. Multiple factors have contributed to this improvement, including the age at HSCT of less than 2 years, a meticulous graft selection strategy, improved graft manipulation methods, better supportive care, vigilant infection surveillance, and more effective antimicrobial therapies [49, 53, 54].

As with other studies, MHC-II-deficient patients undergoing HSCT seem to have persistently lower numbers of CD4 + T cells with a moderate decrease in naive CD4 + T cells. This finding is consistent with impaired thymic maturation due to defective MHC-II expression on thymic epithelia [9]. Despite post-HSCT CD4 + T cell lymphopenia, these patients display a normalization of antigen-specific T cell stimulation and antibody production in response to immunization antigens [15, 49].

In conclusion, MHC-II deficiency is not rare in Iran; it should be considered in the differential diagnosis of CID at any age. With the limited access to HSCT and its variable results in MHC-II deficiency, implementing genetic counseling and family planning for the affected families are mandatory. We have identified and dated a founder event responsible for the *RFXANK* c.162delG frameshift deletion limited to the Iranian population.

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Data Availability The NGS data have been submitted to GenBank; the accession numbers are pending.

Declarations

Ethics Approval The Children's Medical Center Institutional Ethics Committee, affiliated with the Tehran University of Medical Sciences, approved the study.

Consent to Participate/Consent for Publication Informed consent for participating in the study and publication of data was obtained from the patients or their parents.

Competing Interests The authors declare no competing interests.

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