CASE REPORT
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Scales of *Magt1* Gene: Novel Mutations, Different Presentations

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ABSTRACT

Loss-of-function mutations in *magnesium transporter 1 (MAGT1)* gene cause X-linked magnesium deficiency with Epstein–Barr virus (EBV) infection and neoplasm (X-MEN), a disease with quite diverse clinical and immunological consequences. The phenotypic characteristics of the initially described patients included CD4+ T cell lymphopenia, immune deficiency, EBV viremia, and EBV-related lymphoproliferative disease. To date, a total of 25 patients have been reported. The spectrum of the MAGT1 defect ranges from other viral infections (HSV, VZV, CMV, MCV) and sinopulmonary bacterial infections, autoimmune diseases, non-EBV driven lymphoproliferative disease, Castleman disease, HHV8+ Kaposi's sarcoma, vasculitis (Kawasaki) to glycosylation defects in new patients. Here, we report 2 patients from two different families with novel *MAGT1* mutations and different clinical features. The first patient presented with B cell lymphoma and low IgM level without recurrent infections. The second patient presented with recurrent upper respiratory tract infections, Kawasaki-like disease, hypogammaglobulinemia, and T cell lymphopenia. X-MEN disease is the first phenotype identified due to *MAGT1* mutation. The identification of new mutations and atypical presentations will clarify whether there is a relationship between the genotype and the phenotype and the characteristics of the disease.

Keywords: Epstein-barr virus infections; MagT1 protein

INTRODUCTION

Ebstein-Barr virus (EBV) is a gamma herpes virus that infects and persists in >90% of the global population. It is the first described oncogenic virus, associated with seven different malignancies up till now.[1] EBV only infects humans and remains latent in B lymphocytes. While primary infection with EBV typically occurs in childhood as an asymptomatic or mild infection, infections in adolescence or adulthood generally lead to symptomatic EBV infections-

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infectious mononucleosis.[2,3] Both innate and adaptive immunity are important for protection from infection. Especially, NK and CD8+ T cells play an important role.

In patients with underlying primary immunodeficiency, EBV may lead to severe immune dysregulation manifesting as fatal mononucleosis, Hodgkin and Non-Hodgkin lymphoma, lymphoproliferative disease (LPD), lymphomatoid granulomatosis, hemophagocytic lymphohistiocytosis (HLH), and dysgammaglobulinemia.[4] Since the first description of familial inheritance in 1975, several combined immunodeficiencies (CIDs) leading to a particular susceptibility to EBV infection and the development of EBV-driven diseases have been identified.[5-9] Those genetic defects include mutations in *SH2D1A*, *ITK*, *MAGT1*, *CTPS1*, *CD27*, *CD70*, *CORO1A*,and *RASGRP1*.

One of these defects, *MAGT1* mutations revealed the importance of magnesium in the control of EBV by T and NK cells. The *MAGT1* gene is a 70-kb gene with 10 exons encoding for a 335 amino acid protein, located on chromosome Xq21.1.[10] It encodes the transmembrane protein MAGT1, magnesium-specific transporter protein. During T cell activation, MAGT1 mediated Mg^{+2} flux is essential for T cell signal coordination. This flux triggers TCR-gated calcium (Ca^{+2}) flux leading to intracytoplasmic signaling. Thus, loss of function of this gene leads to impaired T cell function. XMEN disease (X-linked immunodeficiency with magnesium defect, EBV infection, and neoplasia) is a disease secondary to ion channelopathy of magnesium.[11] As another result, a chronic decrease in the baseline levels of intracellular free magnesium causes reduced expression of the activator receptor NKG2D on NK cells and CD8 T cells which has important functions in the cytolytic control of EBV-infected cells and tumor surveillance.[12] XMEN is the first PID associated with decreased NKG2D expression.

Here, we report 2 patients from two different families with novel *MAGT1* mutations; one patient presented with neoplasia and the other with recurrent fever and mild infections.

PATIENT'S REPORTS

Written informed consents were obtained from the parents of these two patients. This study was supported by TUBITAK Priority Areas R & D Project Support Program (1003) (Project Id:315S125)

Patient 1

A five-year-old boy was referred to our clinic due to refractory Non-Hodgkin lymphoma and positive EBV serology. He was born to non-consanguineous parents and had no history of infections or hospitalizations until age4. At age 4, he developed left submandibular lymphadenopathy and an excisional biopsy revealed diffuse large B cell lymphoma (DLBCL). Although many chemotherapy regimens (FAB LMB 96 protocol group B, 3 cycles RICE, salvage regime rituximab and ibrutinib, bendamustine, rituximab and ibrutinib, lenalidomide, and nivolumab) were given, his lymphoma could not be controlled. He was referred to our clinic with the suspicion of primary immunodeficiency leading to increased susceptibility to EBV infection after his 2nd relapse.

On his physical exam, only left submandibular lymphadenopathy (8x5x8cmin diameter) and bilateral axillary micro-lymphadenopathies were detected. In the immunological evaluation performed while receiving chemotherapy; severe lymphopenia (total lymphocyte counts: 140/mm^3), low IgM levels, according to healthy Turkish children age references[13] a significant decrease in B and NK cells, reversed CD4/CD8 ratio, and low TREC levels were detected. Neither plasma EBV PCR value nor EBER in the biopsy specimen was positive. Only plasma EBV VCA IgG was positive. (Table 1) His lymphoma, which was resistant to chemotherapy and which had progressed with frequent relapses, was thought to have developed based on primary immunodeficiency. Therefore, a genetic study was carried out.

A novel hemizygote mutation in the *MAGT1* gene [C 936 C>G (p.T312=) (p.Thr312=)] was identified by next-generation sequencing (NGS) analyses. According to the analysis by Mutation Taster and Human Splicing Finder, one of the in-silico evaluation tools, it was highly pathogenic because it affects the mRNA cutting. His mother and sister were also screened for this *MAGT1* mutation and his mother was found as a carrier while his sister was unaffected. Expression of the natural killer (NK) stimulatory receptor 'natural-killer group 2, member D' (NKG2D) in cytotoxic CD8 T lymphocytes was decreased as identified by flow cytometry supporting the diagnosis of X-MEN disease. Trimethoprim-sulfamethoxazole, acyclovir

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prophylaxes, and intravenous IgG (IVIG) were started. HSCT from his fully matched sister was planned after lymphoma treatment. Unfortunately, the relapsed lymphoma could not be taken under control with any chemotherapy regimen. The tumor mass of 8x8x8cm in the left cervical region (Figure 1) made significant pressure on the trachea. The patient had a seizure due to electrolyte imbalances (hypernatremia and hypokalemia) and died after 15 days of intubation.

Table 1. The clinical, laboratory features and genetic results of the patients

Clinical features	**Patient 1 (5y) Refractory and aggressive Non-Hodgkin Lymphoma**		**Patient 2 (15 mo) Recurrent upper and lower respiratory infections Kawasaki disease**	
Laboratory		**Age references**		**Age references**
WBC (mm^3)	90	5500-14500	15890	5500-14500
Hb (g/dL)	10.5	11.5-15	12.2	11-14
Thrombocyte (mm^3)	10000	150000-450000	290000	150000-450000
ANC (mm^3)	0	1500-8000	10170	1500-8000
AEC (mm^3)	0	0-700	230	0-700
AMC (mm^3)	0	300-1000	900	300-1000
ALC (mm^3)	60	2000-8000	4180	2000-8000
IgG (mg/dL)	1170	745-1804	337	574-974
IgA (mg/dL)	107	57-282	6,5	25-62
IgM (mg/dL)	29	78-261	83	58-138
Total IgE (IU/L)	nd	0-150	<17	0-65
EBV VCA Ig G	+	-	+	-
EBV PCR (copy/mL)	<153	<153	380-631	<153
Immunological workup		Age-matched references [ref.13]		Age-matched references [ref.13]
CD3+CD16/CD56- cells, % (mm^3)	95 (85)	55-79 (1900-3600)	42 (1755)	51-77 (1300-6500)
CD3+CD4+ T cells, % (mm^3)	67 (60)	26-49(600-2000)	22 (919)	29-55 (700-4500)
CD3+CD8+ T cells % (mm^3)	28 (25)	9-35 (300-1300)	16 (668)	15-33 (400-3200)
CD4+CD45RA+ T cells % (mm^3)	9 (54)	20-41 (500-6600)	22 (202)	19-49 (500-4200)
CD19+ B cells % (mm^3)	1 (9)	11-31 (300-1200)	25 (1045)	17-41 (500-3600)
CD3-CD16+CD56+ NK cells % (mm^3)	2 (18)	5-28 (200-1200)	17 (710)	4-15 (200-1300)
CD3+ γδ cells % (mm^3)	3	<5	4	<5
CD4+CD45RA+CD31+ % (mm^3)	10 (5)	55-74 (544-1516)	69 (2880)	57-79 (646-2284)
CD4/CD8 ratio	2.3	2	1.37	2
NKG2D (%)	21	-	2.95	-
Lymphocyte activation response to PHA				
CD3+CD25+	97	43-97	47	43-97
CD3+CD69+	87	45-100	45	45-100
Lymphocyte activation response to anti-CD3				
CD4+CD25+	69		27	
CD4+CD69+	71		25	
Mutation analysis	A novel hemizygous mutation in *MAGT1* gene C 936 C>G (p. T312) (p. Thr312)		A novel hemizygous mutation in *MAGT1* gene C 667 dup A, p. (Met223Asnfs*19)	

ANC: Absolute neutrophil counts, AEC: Absolute eosinophil counts AMC: Absolute monocyte counts,
ALC: Absolute lymphocyte counts

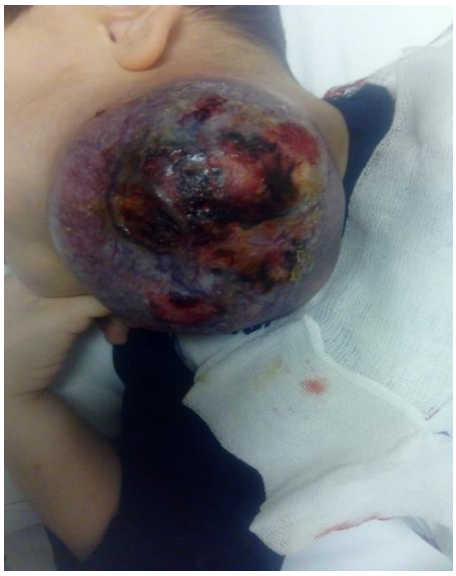

Figure 1. Left cervical tumor mass of P1

Patient 2

A 15-month-old boy was referred to our pediatric immunology department because of finding low IgG levels while performing investigations for malnutrition. He was the first and the only child of non-consanguineous parents. He had two bronchiolitis episodes requiring hospitalization at 2 months of age. On his physical examination, the only pathological finding was his curved ears.

On laboratory studies; IgG: 337 mg/dL (574-974) and IgA: 6.5 mg/dL (25-62) levels were low for age references. IgM: 83 mg/dL (58-138) level was normal. Biochemical tests were within normal ranges. Peripheral lymphocyte analyses revealed CD3+ T cell lymphopenia and CD4+ cell lymphopenia. CD40 and CD40L expressions were normal.

On the follow-up 2 months later, he had a persistent fever but no infectious agents were identified. He was hospitalized in ICU due to Kawasaki disease at 17 months and received immunoglobulin treatment. He had frequent upper respiratory tract infections afterward. Hypogammaglobulinemia was progressive and decreased T and T-helper cell ratios were persistent. Trimethoprim-sulfamethoxazole prophylaxis and IVIG were started reducing the frequency of infections.

Because of recurrent infections, progressive hypogammaglobulinemia, and T cell lymphopenia, genetic analyses were planned. A novel hemizygote mutation in the *MAGT1* gene [C 667 dup A, p.(Met223Asnfs*19)] was identified by NGS analyses. His parents were screened for this mutation and his mother was identified as a carrier. Donor screening is still ongoing. He only had plasma EBV PCR positivity once and it regressed spontaneously.

DISCUSSION

MAGT1 mutations were first described in 2011 by Li et al in 2 brothers with recurrent infections, chronic Epstein–Barr viremia, and low $CD4^+$ T-cell counts.[14] To the best of our knowledge, 25 male patients are reported until now.[14-23] Later in 2014, the same team described 7 X-MEN patients ranging from 3 to45-years of age. Our patients are the youngest patients diagnosed in the literature. Different than our patients, all the patients with lymphoma (4/7) were diagnosed postpubertally, and 2 of them had sequential EBV associated lymphomas.[15]

There is a wide range of presentations in X-MEN disease. Neurological symptoms (multifocal leukoencephalopathy),[15] autoimmune disorders,[17]and Kaposisarcoma[18] are some of them. Also, infections including molluscum contagiosum and herpes viruses (HSV, VZV, CMV) were previously reported.[15,17,19,20] None of our patients presented with these clinical features. Similar to the patient reported by Reynold et al,[20] one of our patients (P2) was diagnosed with Kawasaki disease. To the best of our knowledge, our patient is the second patient presenting with Kawasaki disease. Kawasaki disease association with X-MEN disease will be clarified as more reports appear.

In 2019 Klinken et al, reported 2 cases of X-MEN disease with novel mutations and summarized the clinical features of previously reported MAGT1 deficient patients.[21] Interestingly, while one of their patients presented with classical features, the second patient presented with a novel phenotype consisting of CNS vasculitis, HHV-8 negative multicentric Castleman disease, and severe molluscum contagiosum. They also reported one successful HSCT.

The results of the glycoproteomic analysis of 23 patients with MAGT1defectson T lymphocytes have recently been reported. This study showed XMEN disease as a congenital disorder of glycosylation. In their study, recurrent ear and sinopulmonary infections were the most common presentations, surprisingly

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pericardial and/or pulmonary effusions and even EBV-negative malignancies were rare clinical manifestations.[23]

Due to the rarity of this condition, there isn't a consensus about its treatment. Magnesium supplementation is controversial and there is limited information about HSCT. As far as we know, there are seven transplanted patients so far,[15,17,21,22] and 4 of them died because of transplant-related complications. Bleeding tendency is also reported among these patients.[15,22] IVIG replacement and antibiotic prophylaxis should be considered as supportive treatments. In the light of all the available information, both of our patients received IVIG replacement and antibiotic prophylaxis while none of them received magnesium supplementation.

In our opinion, patients with hypogammaglobulinemia, positive serology, and/ or PCR for EBV and lymphoproliferative diseases (benign or malignant), should be assessed for underlying primary immunodeficiencies even in the absence of a history of consanguinity. Both MAGT1 genotype and epigenetic features influence the clinical presentation, and further studies are necessary to explain this situation. As there is a wide range of presentations, genetic counseling should be used for diagnosis. Male patients with recurrent infections, Epstein–Barr viremia, and $CD4^+$ T lymphopenia should specifically be evaluated for X-MEN disease.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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