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Intermittent X-linked thrombocytopenia with a novel WAS gene mutation

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Abstract

X-linked thrombocytopenia (XLT) is caused by mutations in the *WAS* gene and characterized by thrombocytopenia with minimal or no immunodeficiency. Patients with XLT usually exhibit persistent thrombocytopenia, and intermittent thrombocytopenia has been described only in two families. Here, we report a patient with intermittent XLT carrying a novel missense mutation (Ala56Thr). He showed residual expression of Wiskott-Aldrich syndrome protein in the lymphocytes and platelets. There appeared to be an association between normal platelet numbers and a post infectious state. Our findings further support the importance of analysis of Wiskott-Aldrich syndrome protein in male patients who exhibit fluctuating courses of thrombocytopenia.

INTRODUCTION

Wiskott-Aldrich syndrome protein (WASp) is an important regulator of the actin cytoskeleton and is constitutively expressed in all nonerythroid hematopoietic cells [1]. Mutations in the *WAS* gene result in either a loss or gain of protein function. The former leads to classical Wiskott-Aldrich syndrome (WAS) or isolated X-linked thrombocytopenia (XLT) [2,3], whereas the latter leads to a distinct disease, congenital X-linked neutropenia [4]. XLT is a milder phenotype of classical WAS that is characterized by persistent thrombocytopenia with minimal or no signs of eczema and immunodeficiency [1]. Notarangelo et al. reported 2 families in which affected males with unique amino acid substitutions in the *WAS* gene exhibited intermittent thrombocytopenia in the absence of other clinical features [5]. This rare condition is considered to be the mildest consequence of WASp deficiency. Herein, we report an additional case of intermittent XLT carrying a novel missense mutation. To further characterize the disease, the fluctuating course of thrombocytopenia, its relationship to infections, and the residual expression of WASp were evaluated.

CASE REPORT

The patient is a 4-year-old male whose medical history began shortly after birth with an abnormal newborn screening test for congenital hypothyroidism. His platelet counts were found to be in the normal range until 19 days of age, when he exhibited a late

rise in thyroid stimulating hormone (30.7 mIU/L) and began replacement therapy with levothyroxine. Thereafter, regular thyroid function testing offered us the opportunity to evaluate the complete blood count longitudinally, and mild thrombocytopenia was noted after one month of age (Figure 1A). He exhibited transient facial eczema during infancy. Ten days after he suffered from an influenza A infection at 8 months of age, the platelet count was elevated to 270 x 10^9/L. At 9 months of age, he was hospitalized for Norovirus gastroenteritis and marked thrombocytopenia (29 x 10^9/L). Platelet-associated immunoglobulin G was not elevated. Absolute neutrophil and lymphocyte counts were 2.73 x 10^9/L and 5.38 x 10^9/L, respectively. Nine days later, his platelet count was elevated to 197 x 10^9/L. At 12 months of age, another episode of gastroenteritis due to Rotavirus occurred. The platelet number initially decreased to 12 x 10^9/L and, 5 days later, recovered to 205 x 10^9/L. Absolute neutrophil and lymphocyte counts at the initial time were 5.69 x 10^9/L and 0.97 x 10^9/L, respectively. A similar fluctuation in platelet counts was also observed after upper respiratory infection; however, infectious episodes did not necessarily result in normalization of platelet counts. No correlation was observed between platelet counts and thyroid hormones levels. The patient was found to have normal thyroid function, and levothyroxine treatment was discontinued at 3 years of age. Consistent with previous observations [5], the mean platelet volume (MPV) was persistently low, regardless of the platelet count (mean, 6.5 fL; Figure 1A).

Immunological studies at 4 years of age revealed that proliferative response to immobilized anti-CD3 and podosome formation were detectable [6], albeit at lower levels than controls (Figure 1 B-C). The patient also exhibited a normal absolute lymphocyte count (4.76 x 10^9/L) and normal immunoglobulin (Ig) levels (IgG 9.23 g/L, IgA 1.74 g/L,

IgM 0.66 g/L). Immunophenotypic analysis of the lymphocytes showed normal or near-normal percentage of $CD3^+$ T (61.3%; normal, 71.4±5.8), $CD4^+$ T (40.9%; normal, 43.2±11.5), $CD8^+$ T (14.1%; normal, 22.3±6.6), $CD16^+$ NK (14.3%; normal, 6.3±3.9), and $CD20^+$ B (19.9%; normal, 12.5±6.7) cells. Serum antibody titers to vaccinations were detected by enzyme immunoassay in the patient: measles-specific IgG 4.9 (normal, < 2.0) and rubella-specific IgG 3.1 (normal, < 2.0). Serum IgG antibody against cytomegalovirus was also positive: 4.9 (normal, < 2.0). Anti-A isohemagglutinin titer (IgM) was 1:64. The patient has shown no clinical evidence of immunodeficiency, autoimmunity or malignancy to date.

As part of the clinical evaluation for thrombocytopenia, mutation analysis of the *WAS* gene was performed at 10 months of age, after informed consent was obtained from the parents. The patient had a G to A substitution at nucleotide 200 in exon 2, resulting in an Ala56Thr substitution (Figure 1D). While this missense mutation is novel, a similar missense mutation Ala56Val has been frequently reported in patients with XLT [7,8]. His mother was a heterozygous carrier of the mutation. We next analyzed WASp expression in peripheral blood mononuclear cells and platelets by flow cytometry and/or Western blot analysis (Figure 1 E-F) [9]. The lymphocytes, monocytes and platelets showed residual expression of the mutated WASp, which was consistent with the missense mutation.

DISCUSSION

In this report, we described a case of intermittent XLT with a missense mutation (Ala56Thr) resulting in residual expression of the mutated WASp. These findings are in line with the previous description of intermittent XLT, in which the missense mutations, Pro58Arg and Ile481Asn, led to substantial protein expression in both lymphocytes and platelets [5]. Studies of platelets and lymphocytes from a series of patients with diverse *WAS* mutations, however, have demonstrated that platelets of most patients were shown to lack WASp, although a complex pattern of WASp expression was found in lymphoid cells [10,11]. Further studies will be necessary to assess whether residual WASp expression in platelets could be directly associated with intermittent thrombocytopenia.

There appears to be an association between normal platelet numbers and a post infectious state in our patient. In healthy children, reactive thrombocytosis may occur following infections, in which significant elevation of thrombopoietin levels during an acute infection precedes the development of thrombocytosis [12]. Although we did not measure any growth factors related to megakaryopoiesis in our patient, the Ala56Thr missense mutation likely permitted the platelet elevation to occur following infections. Further investigations in intermittent XLT may lead to a better understanding of abnormalities in both platelet production and platelet consumption in WASp deficiency.

It may be difficult to conclusively diagnose our patient as being affected with intermittent isolated XLT because of his young age. A scoring system on a scale of 1 to 5 is used to estimate the severity of WAS/XLT-associated symptoms [13]. Progression from a low score representing XLT to the highest score of 5, due to either autoimmunity or malignancy, can occur at any age [8]. Despite the usefulness of the scoring system for assessment of the disease phenotype, it cannot predict the onset of autoimmunity or

malignancy. Indeed, malignancy has been reported in a patient with intermittent XLT carrying the Pro58Arg mutation [8]. Although hematopoietic stem cell transplantation is a treatment of choice for patients with classic WAS, there is a controversy in XLT due to the excellent long-term survival. However, if a human leukocyte antigen-identical donor is available, hematopoietic stem cell transplantation can be considered for patients with XLT because of their high probability of severe disease-related complications [8]. Therefore, careful follow-up is necessary for patients with WASp deficiency, including intermittent XLT. In addition, our findings further support the importance of analysis of WASp in male patients who present with fluctuating courses of thrombocytopenia, similar to idiopathic thrombocytopenic purpura.

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CONFLICT OF INTEREST

Nothing to declare.

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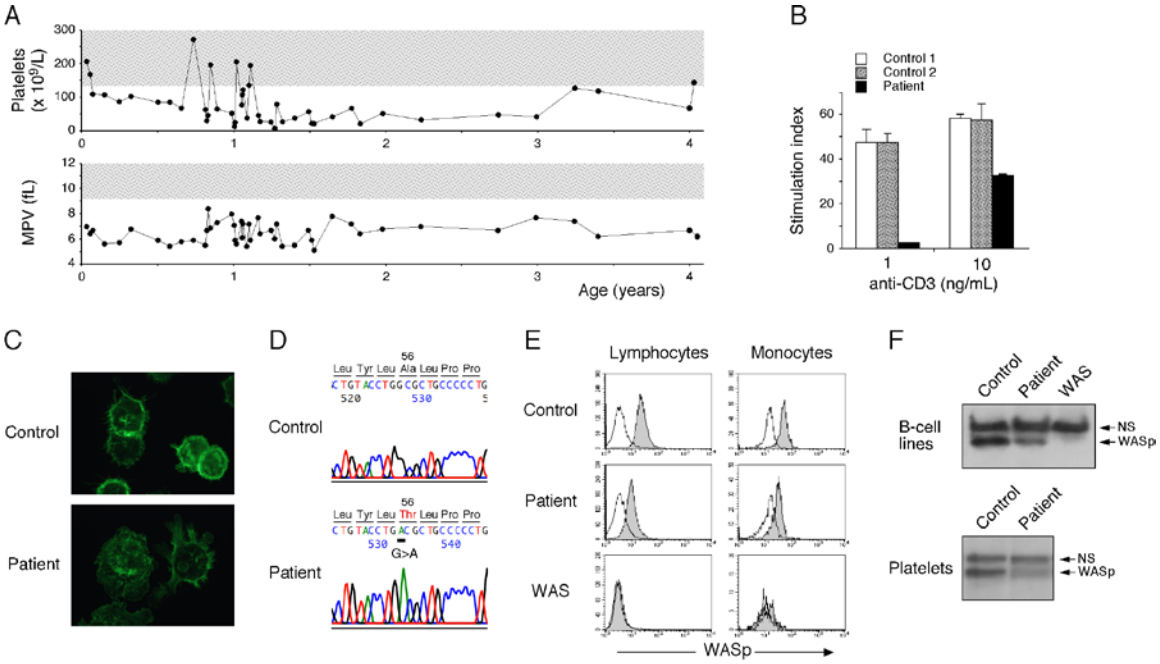
Figure Legends

Fig. 1. Analysis of platelets and WASp.

A: Profiles of the platelet counts and mean platelet volume (MPV). Shaded areas represent the ranges of normal values. **B**: Proliferative responses to anti-CD3. Data are presented of stimulation index (ratio between average incorporated [^{3}H]thymidine in the presence of anti-CD3 to that in the absecence of stimulation). **C**: F-actin distribution in monocyte-derived dendritic cells. Visualization of F-actin was performed with Alexa Fluor 488-conjugated phalloidin. **D**: Mutational analysis of the *WAS* gene. Direct sequencing was performed using an automated sequencer. **E**: Flow cytometric analysis of WASp expression in lymphocytes and monocytes. White histograms indicate control antibody; gray histograms represent anti-WASp monoclonal antibody. **F**: Western blot analysis of WASp expression in B-cell lines and platelets. Arrows indicate the position of the WASp and of a non-specific reactive protein (NS).

Figure 1


A
Platelets (x 10⁹/L)
MPV (fL)
Age (years)
B
Control 1
Control 2
Patient
Stimulation index
anti-CD3 (ng/mL)
C
Control
Patient
D
Control
Patient
G>A
E
Lymphocytes
Monocytes
Control
Patient
WAS
WASp
F
Control
Patient
WAS
B-cell lines
Platelets
NS
WASp