Missense mutations of the WASP gene cause intermittent X-linked thrombocytopenia

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Mutations of the WASP gene have been previously shown to be responsible for classical Wiskott-Aldrich syndrome, isolated X-linked thrombocytopenia, and severe, congenital X-linked neutropenia. We report herewith 2 families in which affected males had a history of intermittent thrombocytopenia with consistently reduced platelet volume, in the absence of other major clinical features, and carried missense mutations of the WASP gene that allowed substantial protein expression. This observation broadens the spectrum of clinical phenotypes associated with WASP gene defects, and it indicates the need for molecular analysis in males with reduced platelet volume, regardless of the platelet number. (Blood. 2002;99:2268-2269) © 2002 by The American Society of Hematology

Introduction

The Wiskott-Aldrich syndrome (WAS, MIM 301 000) is an X-linked disorder characterized by congenital thrombocytopenia with low mean platelet volume (MPV), eczema, increased susceptibility to infections, autoimmune diseases, and malignancies.1 Cloning of the WAS gene, mutated in WAS,2 has allowed the recognition of attenuated forms of the syndrome, with thrombocytopenia and mild eczema or infections, also referred to as X-linked thrombocytopenia (XLT, MIM 313 900).3-5 Most patients with XLT have missense mutations within exons 1 and 2, leading to decreased but detectable protein expression,6,7 whereas a wide spectrum of mutations, most often leading to the absence of protein, have been detected in classical WAS.7,8

We have identified 2 families in which affected males have a history of intermittent thrombocytopenia with persistently reduced MPV. Mutation analysis of the WAS gene disclosed missense mutations in exon 2 (family A) and exon 11 (family B).

Study design

Family A

The index case (patient 1) is a 7-year-old boy in whom petechiae developed at 1 month of age. He had mild and transient antecubital eczema in infancy. The diagnosis of idiopathic thrombocytopenia, based on a platelet count of 38 × 10^9/L, was made when he was 2 years of age. Treatment with high-dose intravenous immunoglobulin (Ig), attempted twice, was ineffective. He continued to have intermittent petechiae and occasional epistaxis associated with variability in the platelet count, but he had consistently low MPV. His idiopathic thrombocytopenia was considered chronic.

His 4-year-old brother (patient 2) and a 39-year-old maternal uncle (patient 3) also had histories of intermittent petechiae, without other symptoms. At the time of first evaluation, his younger brother had 130 × 10^9/L platelets and an MPV of 5.2 fL. His uncle’s most recent platelet count was 64 × 10^9/L, with an MPV of 6.4 fL. Searches for antiplatelet antibodies were performed in patients 1 and 2 and were negative. Immunologic evaluation revealed normal serum IgG, IgA, and IgM levels and normal antibody responses to hepatitis B immunization in the 3 affected males. IgE concentrations, performed in patients 1 and 3, were 346 IU/mL and 403 IU/mL, respectively. In vitro lymphocyte proliferation to mitogens was normal in all 3 patients, but it was markedly decreased (4%-15% of normal controls) to anti-CD3 in the brothers and was normal in the uncle.

Family B

Patient 4, a 7-year-old boy, is the only child of nonconsanguineous parents. At 3 years and 5 months of age, he had petechiae and bruises. Platelet count was 4 × 10^9/L. Treatment with intravenous immunoglobulin (800 mg/kg for 1 day) and prednisone (2 mg/kg for 3 weeks) resulted in a transient and moderate increase in platelet numbers (58 × 10^9/L). He has since maintained a low-normal (106 × 10^9/L) to normal (289 × 10^9/L) platelet count and reduced MPV without treatment. Clinical history was unremarkable for eczema and infections. Serum IgG, IgA, and IgM levels were normal.

Mutation analysis at the WASP locus

Genomic DNA was extracted from peripheral blood. Amplification of each of the 12 exons and flanking splice-sites at the WASP locus was performed as described.9 Mutation analysis was accomplished by single-strand conformation polymorphism and direct sequencing using the ABI Prism 310 sequencer (Applied Biosystem, Foster City, CA).

Analysis of WASP protein expression

The WASP protein was immunoprecipitated from Epstein-Barr virus-transformed lymphoblastoid B-cell lines (LCLs) and from platelets derived from patients and controls, using the 3F3 anti-WASP monoclonal antibody.10 Briefly, 20 × 10^6 LCL cells were lysed in 300 mM NaCl 50 mM Tris, pH 7.5, 2 mM EDTA, pH 8, 0.5% Triton-X plus protease inhibitors (Buffer A). Platelets were prepared from peripheral blood collected in acid-citrate dextrose, after centrifugation at 500g and washing at 700g of platelet-rich plasma with one-third (vol/vol) acid-citrate dextrose. Platelets were resuspended in phosphate-buffered saline–35% bovine serum albumin and were counted; 150 × 10^9 platelets from patients...
Results and discussion

As depicted in Figure 1, patients 1, 2, and 4 showed variability of platelet numbers, ranging from very low to normal, whereas the MPV was consistently reduced, regardless of the platelet count (ranges, 5.6-63 fL for patient 1; 5.2-5.4 fL for patient 2; 4.7-6.4 fL for patient 4; normal range, 7.10 fL). Only limited information is available for patient 3, whose most recent platelet counts were low (37-64 fL) and ranged from very low to normal, whereas the MPV was shown to be 6.2-6.4 fL. Clinically, the platelet counts were normal range for platelet count. Only recent data were available for patient 3 (not shown), who had thrombocytopenia and a small mean platelet volume.

controls and lysates were lysed with Buffer A. For LCL and for platelets, lysates of mutated protein, whereas classical WAS is associated with a variety of genetic defects that usually result in the absence or truncation of WASP.6,8 Our 2 families had missense mutations involving exon 2 (C207G) and 11 (T1476A), respectively. As shown in Figure 2, these mutations allow substantial protein expression. Reduced amounts of WASP protein were detected in LCL and platelets from patient 2 and in platelets from patient 1, whereas normal amounts were detected in patient 4 (for LCL and platelets) and in patient 3 (for LCL).

The 2 families with intermittent thrombocytopenia had in common consistently small platelet size, minimal if any bleeding, and, in most members, no eczema or increased susceptibility to infections. Two of the 3 affected males from family A had low proliferative responses to anti-CD3 in vitro and moderately elevated serum IgE levels. These immunologic abnormalities are typical of WAS/ XLT12,13

The intermittent thrombocytopenia reported herewith represents the mildest consequence of WASP mutations. Because none of the affected males had serious problems, no long-term treatment was indicated. In view of our findings, males with persistently low MPV must be considered for mutation analysis at the WASP locus, regardless of the platelet count.

References

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