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 here two 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)

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and controls were lysed with Buffer A. For LCL and for platelets, lysates were normalized for protein amount using the Bio-Rad Protein Assay (Bio-Rad Laboratories, Hercules, CA). Equal amounts of protein were incubated with protein G-Sepharose coupled with 3F3 monoclonal antibody and were run on 8% sodium dodecyl sulfate–polyacrylamide gel electrophoresis at 80 V for 16 hours. The gel was transferred to a polyvinylidene difluoride membrane (Immobilon-P; Millipore, Bedford, MA) and was blotted with rabbit polyclonal antibody against WASP (H250-SC8353; Santa Cruz Biotechnology, Santa Cruz, CA) using a secondary anti-rabbit IgG POD (Hoffmann-LaRoche, Basel, Switzerland), and then it was revealed with enhanced chemiluminescence (Amersham Pharmacia Biotech, Little Chalfont, United Kingdom).

**Results and discussion**

As depicted in Figure 1, patients 1, 2, and 4 showed variability of platelet count numbers, ranging from very low to normal, whereas the MPV was consistently reduced, regardless of the platelet count (ranges, 5-6.3 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 × 10^3 fL) and who had a reduced MPV (6.2-6.4 fL). Clinically, the platelets counts correlated with the appearance of petechiae. No major episodes of bleeding were recorded, and no transfusions were required. Because of the history and the reduced platelet count and volume, mutation analysis at the WASP locus was performed.

In family A, a C207G nucleotide substitution in exon 2 was identified in all 3 affected males, resulting in a Pro58Arg amino acid change. Heterozygosity for this mutation was detected in the mother of the 2 boys. In family B, a T1476A point mutation in exon 11 was detected in the affected boy, resulting in an Ile481Asn amino acid substitution. The mother was found to be a carrier for this mutation. It is unlikely that these genetic abnormalities are polymorphisms because the nucleotide changes were not detected in more than 300 normal X chromosomes.

Mutations of the WASP gene have been previously shown to be responsible for 3 different clinical phenotypes: classical WAS, XLT, and X-linked congenital neutropenia.1,6,11 The spectrum of phenotypes associated with abnormalities of the WASP gene reflects heterogeneity of the mutations. In particular, XLT usually entails missense mutations in exons 1 and 2 and decreased amounts 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 WAXS/XLT.12,13

The intermittent thrombocytopenia reported hereafter 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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