

Letter to the Editor

Aberrant glycosylation of IgA in Wiskott-Aldrich syndrome and X-linked thrombocytopenia

To the Editor:

Wiskott-Aldrich syndrome (WAS) is a rare X-linked disorder caused by mutations in the *WAS* gene, and it is characterized by the triad of thrombocytopenia, eczema, and susceptibility to infection.¹ WAS exhibits extensive clinical variability, and the genotypes of patients with WAS are also highly variable.¹ Mutations impairing but not abolishing WAS protein expression can cause X-linked thrombocytopenia (XLT), an attenuated form of WAS with minimal or no immunodeficiency.¹ Autoimmune complications are exceedingly common in WAS/XLT and affect 40% to 70% of the patients according to retrospective cohort studies.¹ Glomerulonephritis is a frequent complication of WAS/XLT. It is found in 3.5% to 19% of the cases and can progress to chronic renal failure requiring renal transplantation.² IgA nephropathy (IgAN) has been diagnosed in a majority of patients with WAS/XLT.^{2,3}

Previous reports revealed that aberrantly *O*-glycosylated IgA, which exhibits galactose deficiency in the *O*-linked glycans in the hinge region of the heavy chain, may play a pivotal role in the pathogenesis of these IgANs.⁴ We have previously reported that *Was*-knockout mice develop proliferative glomerulonephritis reminiscent of human IgAN.⁵ We measured serum levels of aberrantly glycosylated IgA in *Was*-knockout mice by using lectin-binding assays with elderberry (*Sambucus nigra*) bark and ricinus communis agglutinin I, which recognize terminal sialic acid and galactose residues.⁶ These results indicated that aberrant IgA production may be critically involved in the pathogenesis of glomerulonephritis in these mice.⁵ Furthermore, aberrant *O*-glycosylation of serum IgA with characteristics similar to those observed in IgAN was detected in a WAS-carrier patient who presented with Henoch-Schönlein purpura.⁶ In this report, we evaluated the role of aberrant IgA production in the development of autoimmunity and glomerulonephritis in patients with WAS/XLT.

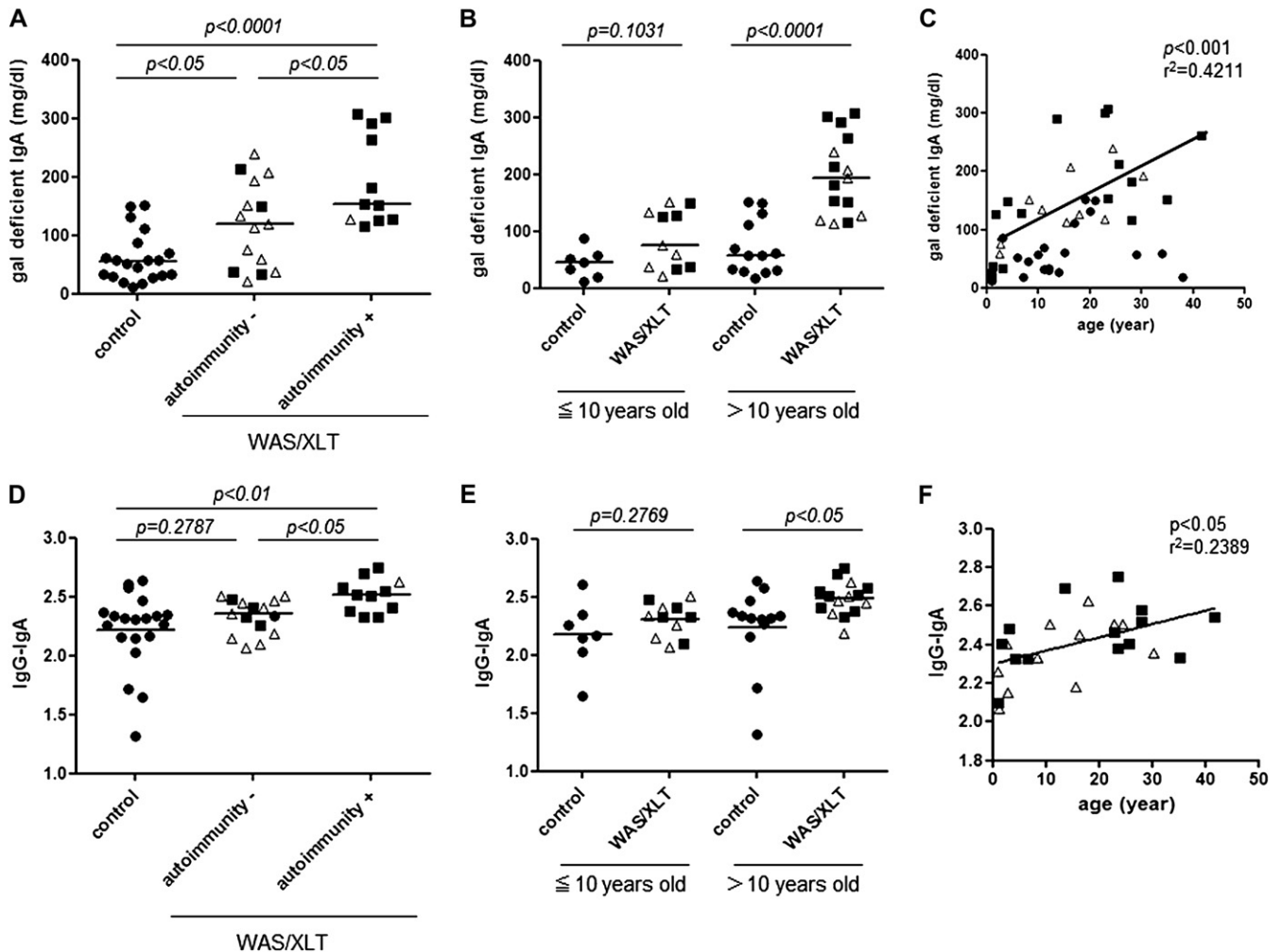


FIG 1. Increased serum galactose-deficient IgA levels and CIC containing IgA and IgG in WAS/XLT. **A**, Serum levels of galactose-deficient IgA. **B** and **C**, Correlation between serum galactose-deficient IgA levels and age. **D**, Serum levels of IgG-IgA CIC. **E** and **F**, The correlation between serum levels of IgG-IgA CIC and age. The bars represent the median values. Open triangles, XLT; filled squares, WAS; filled circles, control.

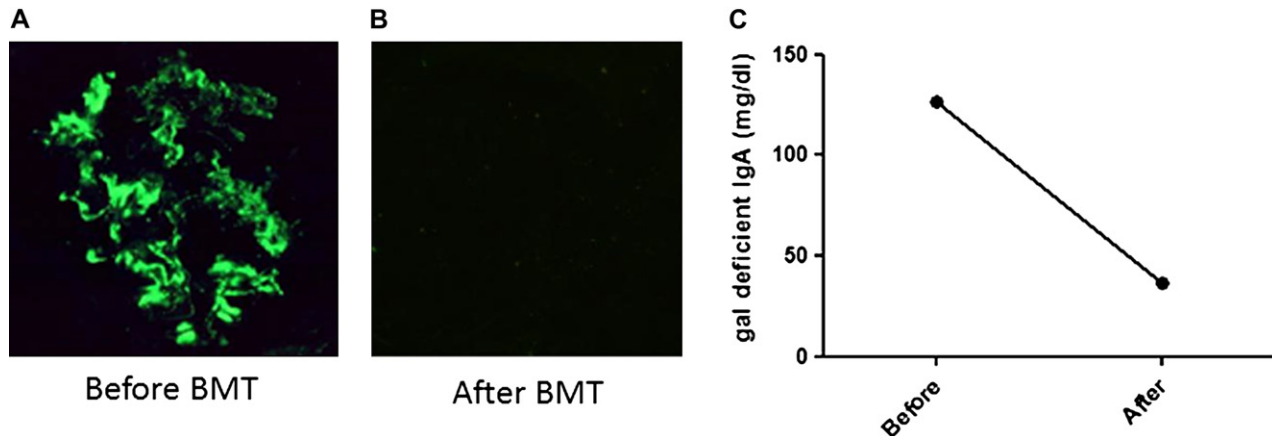


FIG 2. Immunofluorescence study of kidney biopsy specimens and changes in the levels of galactose-deficient IgA in patient 16. **A** and **B**, IgA deposition in mesangial area disappeared after BMT. **C**, The production of galactose-deficient IgA decreased after BMT.

Serum samples were obtained from 26 patients with WAS or XLT and 20 age-matched controls. The clinical characteristics of the patients with WAS/XLT are shown in Table E1 (in this article's Online Repository available at www.jacionline.org). Eleven patients presented with autoimmune complications including IgAN, vasculitis, arthritis, colitis, autoimmune hemolytic anemia, and autoimmune thrombocytopenia.

To examine the terminal galactosylation of IgA molecules, we used lectin-binding assays with *Helix aspersa*, which recognizes terminal *N*-acetylgalactosamine residues⁷ (for more information, see the Methods section in this article's Online Repository at www.jacionline.org).

The serum levels of galactose-deficient IgA in patients with WAS/XLT were significantly higher than those in the control group ($P < .001$) (Fig 1, A). Interestingly, the levels of galactose-deficient IgA were not significantly higher in patients with WAS than in patients with XLT. However, galactose-deficient IgA levels were significantly higher in patients with WAS/XLT with autoimmune complications than in patients without autoimmune complications ($P < .05$) (Fig 1, A). In addition, galactose-deficient IgA levels were significantly higher in patients with WAS/XLT older than 10 years compared with age-matched controls ($P < .0001$) (Fig 1, B). In patients with WAS/XLT, we found that galactose-deficient IgA increased in an age-dependent manner (Fig 1, C).

Next, levels of circulating immune complexes (CIC) containing IgA and IgG (IgG-IgA CIC) in serum were determined by using sandwich ELISA. These levels were found to be significantly higher in patients with WAS/XLT with autoimmune disorders than in the control group ($P < .01$) (Fig 1, D). Notably, IgG-IgA CIC levels were significantly higher in patients with WAS/XLT than in patients without such complications ($P < .05$) (Fig 1, D). In addition, IgG-IgA CIC levels were significantly higher in patients with WAS/XLT older than 10 years of age than in age-matched controls ($P < .05$) (Fig 1, E). Similar to galactose-deficient IgA levels, the elevation in IgG-IgA CIC levels was age dependent (Fig 1, F). Interestingly, IgG-IgA CIC levels were positively correlated with the serum levels of galactose-deficient IgAs (see Fig E1 in this article's Online Repository at www.jacionline.org).

Patient 16 underwent bone marrow transplantation (BMT).³ After BMT, he achieved full donor chimerism and his clinical symptoms were improved. Laboratory findings 24 months after BMT were as follows: platelets increased from $8.7 \times 10^9/L$ to $23.2 \times 10^9/L$; serum IgA levels decreased from 264 mg/dL to 131 mg/dL; urinalysis results changed from 3+ proteinuria and sediment containing more than 100 red blood cells/high-power field with casts to normal findings. The IgA-dominant immune deposits in the renal mesangium and glomerular injury significantly improved after BMT (Fig 2, A), and the serum levels of galactose-deficient IgA also markedly decreased (Fig 2, B).

In this study, we demonstrated that serum galactose-deficient IgA levels and the levels of IgG-IgA CIC increased in patients with WAS/XLT in an age-dependent manner. Interestingly, these levels significantly increased in patients with WAS/XLT with autoimmune manifestations. These findings suggest that increased levels of galactose-deficient IgA with age might be one of the factors contributing to the development of autoimmunity in WAS/XLT. Furthermore, we observed that IgA-dominant immune deposits in the renal mesangium and glomerular injury in a patient with XLT (patient 16) were significantly reduced after BMT. Galactose-deficient IgA levels were also markedly decreased after BMT. These findings indicate that aberrant IgA production in WAS/XLT may result from the immune dysregulation associated with the syndrome and lead to the development of IgAN as one of the several autoimmune complications of WAS that can be corrected with immune reconstitution by BMT.

Some of our patients with WAS/XLT without autoimmune complications showed increased serum levels of galactose-deficient IgA and IgG-IgA CIC. It is possible that increase in IgA and IgG-IgA CIC serum levels precede the clinical presentation of IgAN. Therefore, careful monitoring is considered for these patients.

Altered oligosaccharide biosynthesis in lymphocytes from patients with WAS has been extensively studied⁸; however, the specific mechanisms responsible for the aberrant glycosylation of IgA remain unclear. Previous research suggested the possibility of age-dependent increases in the B-cell populations that produce aberrant IgA.⁵ Interestingly, our results showed that aberrant glycosylation of IgA and production of IgG-IgA CIC in patients

with WAS/XLT were also increased in an age-dependent fashion. Another possibility is that IgA molecules may be influenced by other factors induced by WAS protein deficiency, including TH₂-dominant changes in cytokine balance.^{5,9} Further studies, however, are necessary to validate this hypothesis and clarify the mechanisms responsible for the increased aberrant IgA production in patients with WAS.

Overall, our observations suggest that aberrant IgA production plays a role in the pathogenesis of the renal disease observed in patients with WAS, perhaps through the accumulation of IgA-containing CIC and consequent glomerular injury.

In conclusion, our findings indicate that aberrant IgA production due to mutations in the WAS gene may be critically involved in the development of autoimmune-mediated glomerulonephritis. Further studies are required to clarify the specific mechanisms responsible for the aberrant glycosylation of IgA in WAS.

We thank Ms Chikako Sakai and Mr Hitoshi Moriuchi for their excellent technical assistance. We also acknowledge Dr Hiro Matsukura who performed renal biopsy in patient 16.

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This work was supported by a grant-in-aid for Young Scientists from the Ministry of Education, Culture, Sports, Science and Technology of Japan and by intramural funds of the National Human Genome Research Institute, National Institutes of Health, Bethesda, Md.

Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

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<http://dx.doi.org/10.1016/j.jaci.2012.08.040>

METHODS

Patients

Serum samples were obtained from 26 patients with WAS or XLT and 20 age-matched controls (WAS/XLT, 15.5 ± 12.0 years; controls: 14.6 ± 10.0 years). The clinical characteristics of the patients with WAS/XLT are shown in Table E1. Eleven patients presented with autoimmune complications including IgAN, vasculitis, arthritis, colitis, autoimmune hemolytic anemia, and autoimmune thrombocytopenia. This study was approved by the institutional review boards at Kanazawa University and the National Human Genome Research Institute, National Institutes of Health, Bethesda, Md. All specimens were used after the receipt of informed consent.

Detection of IgA-containing CIC

Levels of CIC containing IgA and IgG in serum were determined by using ELISA. Microtiter plate wells were coated with $15 \mu\text{g/mL}$ F(ab')₂ fragment of goat antihuman IgG (Pierce Biotechnology, Rockford, Ill), washed, and blocked with BSA (Sigma Chemical Company, St Louis, Mo) in PBS (pH 7.4) containing 0.05% Tween-20 (BSA-PBS-Tween). Serum samples diluted 100-fold with the same buffer were then used. After 3 hours of incubation at room temperature, test samples were washed with BSA-PBS-Tween and incubated for 1 hour at room temperature with horseradish peroxidase-labeled goat antihuman IgA (Bethyl Laboratories, Inc, Montgomery, Tex) diluted 1:10,000. Samples were incubated with TMB substrate (Bethyl Laboratories, Inc) after washing with the same buffer, and the optical density was then measured at 450 nm.

Measurement of lectin-binding serum IgA levels

To examine the terminal galactosylation of IgA molecules, lectin-binding assays were designed by using *Helix aspersa* (HAA) (Vector Laboratories, Burlingame, Calif), which recognizes terminal *N*-acetylglucosamine residues (24,25). Microtiter plates coated with $15 \mu\text{g/mL}$ F(ab')₂ fragment of goat antihuman IgA (Jackson ImmunoResearch Labs, West Grove, Pa) were incubated with 100 μL of diluted (100 $\mu\text{g/mL}$) serum samples. In order that the HAA can recognize *N*-acetylglucosamine, the terminal neuraminic acid was removed by incubation with 100 μL of 20 mU/mL neuraminidase (Roche Diagnostic Corp, Indianapolis, Ind) in acetate buffer (pH 5) for 3 hours at 37°C. After washing, 100 μL of biotin-labeled *N*-acetylglucosamine-specific lectin HAA (2 $\mu\text{g/mL}$, Sigma) was added to each well and incubated for 3 hours at 37°C. Peroxidase-conjugated streptavidin (0.1 $\mu\text{g/mL}$; Pierce, Rockford, Ill) was added, and the amount of lectin bound was measured. Total IgA levels were also measured by using horseradish peroxidase-labeled goat antihuman IgA diluted 1:10,000. The ratio of the absorbance at 450 nm between IgA bound to HAA and total IgA (HAA/IgA ratio) was calculated. The amount of galactose-deficient IgA in serum at the time of blood drawing was estimated by using the following formula: serum IgA concentration $\times$ HAA/IgA ratio.

Statistical analysis

The results are expressed as mean $\pm$ SD. Statistical significance was determined by using the Mann-Whitney test. Differences were considered significant if the *P* value was less than .05.

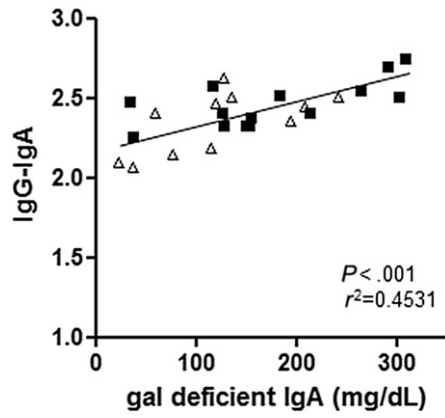


FIG E1. Positive correlation between serum levels of IgG-IgA CIC and galactose-deficient IgAs.

TABLE E1. Clinical characteristics of the patients with WAS/XLT

Patient	Age (y)	Sex	Diagnosis	Autoimmune diseases
1	1	Male	XLT	No
2	2	Male	XLT	No
3	24	Male	XLT	No
4	10	Male	XLT	No
5	22	Male	XLT	No
6	16	Male	XLT	No
7	30	Male	XLT	No
8	2	Male	XLT	No
9	8	Male	XLT	No
10	1	Male	XLT	No
11	15	Male	XLT	No
12	25	Male	WAS	No
13	3	Male	WAS	No
14	4	Male	WAS	No
15	1	Male	WAS	No
16	18	Male	XLT	IgA nephropathy
17	23	Male	WAS	Autoimmune hemolytic anemia
18	6	Male	WAS	Autoimmune thrombocytopenia
19	22	Male	WAS	Systemic vasculitis
20	28	Male	WAS	IgA nephropathy
21	13	Male	WAS	Colitis
22	23	Male	WAS	IgA nephropathy
23	1	Male	WAS	Vasculitis
24	41	Male	WAS	Arthritis
25	28	Male	WAS	IgA nephropathy
26	35	Male	WAS	IgA nephropathy