Aberrant galactosylation of IgA1 is involved in the genetic susceptibility of Chinese patients with IgA nephropathy

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Abstract

Background. Immunoglobulin A nephropathy (IgAN) is associated with genetic and environmental factors, and undergalactosylation of IgA1 in the serum is considered to be a contributor to pathogenesis of IgAN. The present study was conducted to detect the galactose- (Gal) deficient IgA1 level in Chinese IgAN patients and their family members.

Methods. Sixty-three IgAN patients were enrolled, where 32 first-degree relatives of 19 patients and 44 spouses of 44 patients were recruited. Healthy blood donors (n = 39) were used as normal controls. Biotinylated HAA (Helix aspersa) was utilized to detect the Gal-deficient IgA1 in enzyme-linked immunosorbent assay (ELISA). All the results were corrected by serum IgA1 concentration.

Results. Compared with normal controls, the sera IgA1 of patients and their first-degree relatives demonstrated increased Gal-deficient IgA1 level (0.17 ± 0.09 versus 0.10 ± 0.04, P = 0.001; 0.14 ± 0.07 versus 0.10 ± 0.04, P = 0.028); no significant difference between patients and their first-degree relatives was detected (0.17 ± 0.09 versus 0.14 ± 0.07, P = 0.127). In contrast, serum Gal-deficient IgA1 level of IgAN patients was higher than their counterpart spouses and normal controls (0.18 ± 0.13 versus 0.14 ± 0.09, P = 0.009; 0.18 ± 0.13 versus 0.10 ± 0.04, P = 0.001), while that of patients’ spouses was comparable with normal controls (0.14 ± 0.09 versus 0.10 ± 0.04, P = 0.075). There was no correlation between clinicopathological data and serum Gal-deficient IgA1 level.

Conclusion. The patients with IgAN and their first relatives showed significant higher Gal-deficient IgA1 level than healthy controls, whereas patients’ spouses were the same as healthy controls. It can be suggested that the Gal-deficient IgA1 might be inherited in Chinese patients with IgAN.

Keywords: Gal-deficient; genetic susceptibility; glycosylation; HAA; IgA1
Materials and methods

Patients and controls

Sera from 63 patients with biopsy-proven IgAN and 115 controls were recruited. The diagnosis of patients with IgAN was confirmed by the granular deposition of predominant IgA1 mainly in the glomerular mesangium by immunofluorescence detection, as well as by the deposition of mesangial electron density in ultrastructural examination. Systemic lupus erythematosus, Henoch-schonlein purpura and chronic hepatic diseases were excluded by detailed clinical and laboratory examinations. The patients were graded according to the Hass system [11] by two pathologists (J.L. and S.S.F.) blinded to clinical and laboratory data. Twenty-six patients (41.3%) were graded as Haas I–III, and the other 37 (58.7%) patients were graded as Haas IV–V. Nineteen patients and their 32 first-degree relatives (including parents, offspring and siblings) were recruited at first, and then the other 44 patients and their spouses were recruited as well. Sera from 39 additional healthy donors with comparable age and gender were used as normal controls. Urinary analysis and renal function tests were performed in all controls, including first-degree relatives, spouses and healthy donors. The urinary analysis was normal in all controls, and the serum creatinine of first-degree relatives, spouses and healthy donors was 82.5 ± 14.6 (58–111) umol/l, 68.3 ± 16.9 (45–108) umol/l and 57.2 ± 16.2 (45–102) umol/l, respectively, which was in the normal range. Sera of patients were obtained at the time of renal biopsy. The protocol of this study was approved by the ethics committee in our hospital, and informed consent was obtained for sampling sera.

Detection of serum Gal-deficient IgA1

The Gal-deficient IgA1 was detected by specific biotinylated HAA (Sigma-Aldrich, St. Louis, MO, USA) that binds specifically to terminal GalNAc of IgA1 as previously reported [12]. Briefly, the wells in one-half of the plates (Costar, Cambridge, MA, USA) were coated with a bicarbonate buffer alone to act as antigen-free wells. Coated plates were blocked with PBST containing 2% bovine serum albumin at 37°C for 1 h. After three washes, the plates were loaded with the test sera in duplication in a blocking buffer. Every plate contained a blank control and IgA1 that was digested by neuraminidase and galactosidase as a positive control. The plates were treated with neuraminidase (20 mU/ml) to remove terminal sialic acid from O-linked GalNAc of IgA1 as previously reported [12]. Briefly, the wells in one-half of the plates (Costar, Cambridge, MA, USA) were coated with anti-human IgA (Dako, Glostrup, Denmark) in 100 µl of 0.05 mol/l bicarbonate buffer and pH 9.6 at 37°C for 1 h. The wells in the other half were coated with a bicarbonate buffer alone to act as antigen-free wells. The plates were washed with a 0.01 mol/l phosphate-buffered saline (PBS) containing 0.1% Tween-20 (PBST) three times for each step. Coated plates were blocked with PBST containing 2% bovine serum albumin at 37°C for 1 h. After three washes, the plates were loaded with the test sera in duplication in a blocking buffer. Every plate contained a blank control and IgA1 that was digested by neuraminidase and galactosidase as a positive control. The serum were incubated at 37°C for 1 h and followed by washing. Then the plates were treated with neuraminidase (20 mU/ml) to remove terminal sialic acid from O-linked GalNAc on IgA1 and incubated overnight at 37°C. HAA (1 mg/ml, Sigma) diluted to 1:200 in a blocking buffer was added into each plate and incubated for 3 h at 37°C followed by 40 min incubation at 37°C with horseradish peroxidase-conjugated Avidin (Sigma). The second antibody monoclonal antihuman IgA1 (clone No. A1–18, Sigma) in the blocking buffer was added to detect the serum IgA1 concentration. Both Gal-deficient IgA1 and total IgA1 were developed with 0.04% O-phenylenediamine (OPD) in a 0.1 mol/l citrate phosphate buffer (pH 5.0) and 0.3% H2O2 (v/v). The reaction was stopped with 1 mol/l H2SO4. The absorbance (A) was read at 490 nm/630 nm with an ELISA reader (BIORAD550, Japan). Every sample was measured in duplication to obtain the mean value. The relative concentration of serum IgA1 and the level of glycan were calculated as follows: the A value of the blank control was regarded as 0 and the positive control as 100%, and then the A value of each sample was calculated. The relative lectin binding per unit IgA1 (HAA/IgA1) was expressed as the relative concentration of lectin binding level over the relative IgA1 concentration. The CV (coefficient of variance) of the intra- and the inter-test variability of the HAA assay was controlled below 10%.

Statistical analysis

For statistical analysis, the statistical software SPSS 11.0 (SPSS, Chicago, IL, USA) was employed. The Mann-Whitney rank-sum test and the Wilcoxon two-related samples test were used to compare the serum HAA/IgA1 level of patients and control groups. Spearman’s correlation was used to analyse the association between the clinicopathological parameter and the serum HAA/IgA1 level. Statistical significance was considered as a P-value < 0.05.

Results

Clinical features of IgAN patients and their family members

Sixty-three patients with biopsy-proven IgAN were recruited in the present study. The mean age of 63 patients (32 males and 31 females) was 33.7 ± 9.6 years, and the mean duration of disease at the time of renal biopsy was 17 ± 28 months. The mean urine protein excretion of these patients was 1.9 ± 1.9 g/day, and the mean serum creatinine and eGFR were 112.5 ± 79.6 umol/l and 81 ± 28 ml/min/1.73 m², respectively. The mean systolic BP (blood pressure) and mean diastolic BP were 124 ± 18 mmHg and 80 ± 13 mmHg, respectively. First-degree relatives of 19 IgAN patients (n = 32; 16 males and 16 females) were 37 ± 14 years old and spouses of 44 IgAN patients (n = 44; 19 males and 25 females) were 35.8 ± 7.6 years old.

Comparison of Gal-deficient IgA1 level in the serum among IgAN patients, their first-degree relatives and normal controls

Compared with normal controls (n = 39), the sera IgA1 of patients (n = 19) and their first-degree relatives (n = 32) demonstrated increased Gal-deficient IgA1 level (0.17 ± 0.09 versus 0.10 ± 0.04, P = 0.001; 0.14 ± 0.07 versus 0.10 ± 0.04, P = 0.028); no significant difference between patients and their first-degree relatives was detected (0.17 ± 0.09 versus 0.14 ± 0.07, P = 0.127) (Figure 1).
Comparison of Gal-deficient IgA1 level in the serum among IgAN patients, their spouses and normal controls

Gal-deficient IgA1 in the sera of IgAN patients (n = 44) was higher than their counterpart spouses (n = 44) and normal controls (n = 39) (0.18 ± 0.13 versus 0.14 ± 0.09, P = 0.009; 0.18 ± 0.13 versus 0.10 ± 0.04, P = 0.001), while that of patients’ spouses was comparable with normal controls (0.14 ± 0.09 versus 0.10 ± 0.04, P = 0.075) (Figure 2).

Correlation between Gal-deficient IgA1 level in the serum and the clinicopathological parameter

No correlation between the clinical parameter such as the duration from onset to the time of biopsy, the serum creatinine, the amount of urine protein excretion, systolic BP, diastolic BP or eGFR and the Gal-deficient IgA1 level was found in IgAN patients. Also there was no significant difference between the Hass I–III group (n = 26) and the Haas IV–V group (n = 37) (0.17 ± 0.10 versus 0.19 ± 0.13, P = 0.989) in the Gal-deficient IgA1 level.

Discussion

For the past 40 years, familial aggregation of IgAN was gradually reported in different races but no common nephrotoxic factor was found in the families [4,13,14]. Based on linkage analysis, investigators had identified some loci located at identified chromosomes (including 6q22–23, 2q36, 4q26–31 and 17q12–22) [15,16]. However, the underlying pathogenic genes were not yet isolated in these loci and their roles in the development of IgAN were still unknown.

Defection of O-glycosylation in the hinge region of IgA1 has been consistently considered as a critical pathogenic mechanism in the development of IgAN. Therefore, detection of Gal deficiency may be helpful to understand the pathogenesis of IgAN. The ELISA test with HAA lectin was most widely used. Although it may be affected by various influences and cannot present a more precise construction of glycan than matrix-assisted laser desorption ionization time-of-flight MS (MALDI-MS) and liquid chromatograph/electro-spray ionization/MS (LC/EIS/MS), the HAA lectin assay is an available test with high specificity and sensitivity in this patient population. Studies from Gharavi and his colleagues with an HAA-binding test showed that aberrant glycosylation could be inherited not only in familial but also in sporadic IgAN [10]. Their study also revealed that the Gal-deficient IgA1 level of at-risk relatives of Caucasian IgAN patients was higher than normal controls. Our present study demonstrated that the Gal-deficient IgA1 level in the serum of IgAN patients was comparable with their first-degree relatives but significantly higher than normal controls (Figure 1), which was consistent with Gharavi et al’s findings. They revealed that aberrant glycosylated IgA1 could be aggregated in Chinese families of patients with IgAN.

The other possible reason for familial aggregation of aberrantly-glycosylated IgA1 may be the same living environment or food. Some investigations showed that aberrantly-glycosylated IgA1 was an acquired trait, and may be associated with food, viruses or bacteria antigens [17–19], but until now no specific antigen was identified to induce IgAN. In our present study, we recruited additional IgAN patients and their spouses under the same living circumstances to investigate whether environmental factors affected the aberrant glycosylation of IgA1. The results showed that the serum Gal-deficient IgA1 level in IgAN patients was markedly higher than their corresponding spouses, whereas there were no differences between the patients’ spouses and normal controls (Figure 2). The present results suggested that environmental factors might play little role in the production of serum Gal-deficient IgA1.

In the present study, we found that Gal-deficient IgA1 level in some patients was at a low level and in some normal controls Gal-deficient IgA1 was at a high level although with a normal urinary test. This phenomenon points out that high-level Gal-deficient IgA1 alone is not sufficient to induce IgAN and additional ‘bits’ may be needed, including some other genetic defects or environmental factors in the pathway of IgA1 galactosylation to finally disturb the balance of the glycosylation of IgA1. Gal-deficient IgA1 may be easily deposited in the glomerular mesangium, but whether it induces inflammation resulting in clinical presentation is not yet clarified. Other cofactors besides Gal-deficient IgA1 might play an important role in the development of IgAN [10]. Some investigators also reported that 4–16% of healthy people and living donors may have an IgAN1 deposition in the mesangial region [20]. To completely exclude IgA nephropathy from relatives and spouses
Aberrant glycosylated IgA1 in IgAN patients with normal urine test would require them to undergo renal biopsy, which is clearly unethical.

In conclusion, the patients with IgAN and their first relatives showed significant higher Gal-deficient IgA1 level than healthy controls, whereas patients’ spouses were the same as healthy controls. Therefore, it can be suggested that Gal-deficient IgA1 might be inherited in Chinese patients with IgAN.

Acknowledgements. This work was funded by the Projects 30670981 and 30825021 and supported by NSCF, and Project 200802052 was supported by the Foundation of Ministry of Health of China to H.Z.

Conflicts of interest statement. None declared.

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Received for publication: 31.1.09; Accepted in revised form: 26.5.09