Anti-nephrin antibodies in idiopathic nephrotic syndrome in Japanese children

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Abstract

Background

Many patients with childhood idiopathic nephrotic syndrome are steroid-sensitive. Several genome-wide association studies have suggested a polygenic contribution, particularly in the HLA DR/DQ region and a locus including nephrin, but the etiology remains unclear. Anti-nephrin antibodies have recently been reported in both adults and children with biopsy proven minimal change disease (MCD), but the presence of anti-nephrin antibodies in Japanese childhood idiopathic nephrotic syndrome (INS) has not been investigated.

Methods

Anti-nephrin antibodies were measured by ELISA in paired plasma samples obtained from 14 Japanese pediatric patients with INS (male/female: 8/6), at onset (active disease) and following steroid monotherapy.

Results

The median age at the onset was 75.5 months (interquartile range (IQR): 45-113). Steroid sensitivity resulted in complete remission in 13 patients and almost complete remission in one patient after 4 weeks of glucocorticoid monotherapy. Circulating anti-nephrin antibodies were detected in seven of 14 patients during active disease. In all cases, anti-nephrin antibodies were significantly reduced following treatment concordant with clinical response. There were no differences between the positive and negative groups in pre-treatment parameters. Of the 13 patients who achieved complete remission, nine had at least one relapse during a median follow-up of 851 days (IQR: 808-973). There was also no significant difference in the relapse-free period after the onset between the two groups (P=0.658).

Conclusions

We identified circulating anti-nephrin antibodies in half of Japanese pediatric patients with INS at initial presentation, which is higher than has been previously reported in a North American cohort.

Introduction

Idiopathic nephrotic syndrome (INS) in children where complete remission is achieved with corticosteroid monotherapy, is referred to as steroid-sensitive [1], however the underlying etiology and mechanism of steroid response has yet to be fully elucidated [2]. Several genome-wide association studies (GWAS) have been conducted to attempt to understand the disease etiology [3–7], and our group found genome-wide significant associations with pediatric steroid-sensitive nephrotic syndrome (SSNS) in 12 loci, including the HLA-DR/DQ and NPHS1-KIRREL2 regions [7, 8]. Many diseases with significant associations with genetic variation in the HLA-DR/DQ regions have an immunological basis [9] and this is consistent with the efficacy of immune targeted therapies such as rituximab, which depletes B cells, in children with...
SSNS [10, 11]. In membranous nephropathy, significant associations with genetic variants in HLA-DQA1 and PLA2R regions were shown via GWAS [12], and an autoantibody against M-type phospholipase A2 receptor, encoded by the PLA2R gene, is detected in the patients’ serum [13]. Similarly, Watts et al. reported that approximately 30% of patients with nephrotic syndrome and MCD histology have autoantibodies against nephrin encoded by NPHS1 gene [14]. They also observed that a higher proportion of Asians, five out of seven, had circulating anti-nephrin antibodies [14]. Nephrin is an essential structural protein of the slit diaphragm located between the foot processes of podocytes [15]. In this study, we measure anti-nephrin antibodies during active disease (pre-treatment) and following treatment in 14 Japanese patients with first presentation of childhood INS.

**Materials and methods**

**Participants**

In this study, 14 Japanese patients who developed nephrotic syndrome for the first time between 1 and 18 years of age during the one-year period from July 2020 to July 2021 and had preserved plasma in active (pre-treatment) and remission (post-treatment) phases were included. INS was diagnosed according to the International Study of Kidney Disease in Children (ISKDC) criteria [16]. Patients’ characteristics and clinical findings were collected from medical records.

**ELISA for anti-nephrin antibodies**

Paired plasma samples were obtained from each patient at initial disease presentation (pre-treatment) and following steroid monotherapy. Serological testing for nephrin autoantibodies was performed as previously described [13] with the following modifications. A standard curve was generated using a dilution series of a polyclonal sheep anti-human nephrin antibody (R&D, AF4269) from 2000-15.625 ng/ml followed by a biotin-conjugated donkey anti-sheep IgG antibody (Invitrogen, A16045) diluted 1/2000. A concentration of 500 ng/ml of sheep anti-human nephrin was arbitrarily defined as 1000 Relative Units (RU).

**Statistical analysis**

All statistical analyses were conducted using JMP 11 or Prism 6. Data are shown as median and interquartile range (IQR). The Mann–Whitney U test was used for continuous variables and Fisher’s exact test for categorical variables. The occurrence of events (relapse) was analyzed via the Kaplan–Meier method and to calculate p value, we used log-rank test. For interpretation of the ELISA results between states, an unpaired t-test was used. We considered an association to be significant when the p value was < 0.05.

**Results**
Clinical characteristics of participants were shown in Table 1. Eight patients were male (57%) and the median age at disease onset was 75.5 months (IQR: 45–113). All patients were treated with prednisolone (60mg/m²/day; max 60mg/day) based on Japanese Society of Pediatric Nephrology guidelines [16]. Serum albumin levels were low during active disease (median 1.1 g/dL) but normalized following treatment (median 3.6 g/dL, p < 0.001). Similarly, serum IgG levels were low during active disease (median 236 mg/dL) and tended to increase with clinical remission (median 359 mg/dL, p = 0.065). One patient (KU -14) had a Urine protein creatinine ratio (UPCR) of 0.36 g/g and serum Alb of 3.7 g/dL after 28 days of prednisolone monotherapy and was therefore judged to have not entered complete remission. Cyclosporine was then introduced, and he subsequently achieved complete remission.

In the pre-treatment samples, anti-nephrin antibodies were positive in seven out of 14 patients (Fig. 1) and all seven showed a statistically significant reduction in antibody titer concordant with clinical response (Fig. 1). The clinical characteristics of the anti-nephrin antibody positive and negative patients are shown in Table 2. Although the age at onset tended to be higher in the antibody-positive group (p = 0.064), other parameters such as serum Alb, serum IgG and UPCR did not differ between the two groups. Relapse free time was measured in the 13 patients who achieved complete remission based on ISKDC criteria for SSNS. The time from disease onset to the first relapse is shown in Fig. 2A. Nine of thirteen patients had at least one relapse, with a median time to first relapse from disease onset of 488 days (median follow-up of 851 days [IQR: 808–973]). There was no statistically significant difference between the antibody positive and the negative groups (Fig. 2B; p = 0.658).

**Discussions**

This is the first study to search for the anti-nephrin antibodies in an exclusively Japanese pediatric INS cohort. We found that half of the Japanese pediatric patients with INS were positive for anti-nephrin antibodies at initial presentation and all of these patients had a significant reduction or absence of detectable antibodies concordant with treatment response.

We observed no significant differences in the clinical or laboratory characteristics at presentation nor in the relapse-free period between the anti-nephrin antibody positive and negative groups. However, this is a small study and so establishing the prognostic implications of anti-nephrin antibodies in INS warrant further investigation in a larger study.

In the majority of patients, the post treatment sample was obtained within 3–4 weeks of glucocorticoid monotherapy. While the mechanism of steroid sensitivity in these patients remains to be determined, it is intriguing that based on the half-life of IgG (3–4 weeks) this rapid reduction in circulating antibodies may not be fully explained by simply switching off antibody production alone. This raises the possibility that glucocorticoid may act directly on the podocytes in vivo especially since nephrin mRNA expression has been shown to be upregulated in vitro [17].
We identified circulating anti-nephrin antibodies in half of the Japanese pediatric patients with INS, which is a higher proportion than previously reported by Watts et al in a North American cohort of adults and children with biopsy proven MCD [13]. While the association between genotype and presence of anti-nephrin antibodies remains unclear, it is intriguing that we have previously reported that Asians are more likely to have risk variants in the \textit{NPHS1} region [7, 8]. While we have shown that these \textit{NPHS1} risk alleles are associated with allele-specific expression [7], the significance of these risk alleles in autoantibody associated INS remains to be determined. One limitation of this study is that we did not evaluate these patients for the risk variants in \textit{NPHS1} and \textit{HLA DR/DQ} regions.

In those patients who did not have circulating anti-nephrin antibodies, the presence of antibodies targeting other proteins, or another etiological factor, should also be considered. Anti-actin and anti-ATP synthase antibodies or anti-Ubiquitin Carboxyl-Terminal Hydrolase L1 antibody have been reported to be associated with INS [18, 19]. One limitation of our study is that the patients did not have a renal biopsy, as this does not constitute standard clinical practice for new onset INS in children, and so the presence of antibodies targeting nephrin directly in the kidney could not be confirmed.

In conclusion, we have identified circulating anti-nephrin antibodies at initial presentation in half of Japanese pediatric INS, which is a higher proportion than previously reported for a North American cohort of adults and children with biopsy proven MCD. Further studies are needed to establish the prognostic implications of anti-nephrin antibodies in childhood INS and the relationship with the \textit{NPHS1} risk variants in this population.

**Declarations**

**Acknowledgments**

We thank all of the study patients.

Aspects of this work were submitted in abstract form to the American Society of Nephrology Kidney Week 2023 meeting.

**Author contributions**

TH, CN, YI, YT, HK, CU, SN, AK, NS, JF, NK, SI, HK managed the patients and conceived the study; AJBW measured anti-nephrin antibody; TH and AJBW drafted the manuscript; YS, KN, MGS, AW and KI reviewed the manuscript. All the authors have read and approved the final manuscript.

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Availability of data and material

Data from this study can be obtained from the corresponding authors on reasonable request.

Conflicts of interest/Competing interests

The authors declare no conflicts of interest.

Ethics approval

This study involving human participants was conducted in accordance with the Declaration of Helsinki. Ethics approval was obtained from the Ethics Review Committee of Kobe University Graduate School of Medicine (IRB: B210152)

Consent to participate/Consent for publication

Published consent requirements were not necessary because the study was retrospective.

References


Tables
Table 1
Baseline characteristics of participants

<table>
<thead>
<tr>
<th></th>
<th>Pre-treatment</th>
<th>Post-treatment</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender (male/female)</strong></td>
<td>(8/6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td>month</td>
<td>75.5 (45–113)</td>
<td></td>
</tr>
<tr>
<td><strong>Blood test</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC /µL</td>
<td>7800 (7100–9700)</td>
<td>18100 (116000 – 20200)</td>
<td>0.002</td>
</tr>
<tr>
<td>Hb g/dL</td>
<td>14.1 (13.2–15.2)</td>
<td>14.7 (13.8–15.2)</td>
<td>0.408</td>
</tr>
<tr>
<td>Plt 10⁴/µL</td>
<td>35.8 (30.4–41.4)</td>
<td>34.8 (25.8–45.8)</td>
<td>0.696</td>
</tr>
<tr>
<td>TP g/dL</td>
<td>3.9 (3.7–4.2)</td>
<td>6.1 (5.9–6.3)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Alb g/dL</td>
<td>1.1 (1-1.5)</td>
<td>3.6 (3.4–3.9)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>BUN mg/dL</td>
<td>12.8 (9.5–16.0)</td>
<td>14.8 (13.9–18.3)</td>
<td>0.168</td>
</tr>
<tr>
<td>Cr mg/dL</td>
<td>0.33 (0.27–0.44)</td>
<td>0.32 (0.26–0.41)</td>
<td>0.765</td>
</tr>
<tr>
<td>IgG mg/dL</td>
<td>236 (144–329)</td>
<td>359 (287–450)</td>
<td>0.065</td>
</tr>
<tr>
<td><strong>Urine test</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TP/Cr g/gCr</td>
<td>10.7 (7.9–12.8)</td>
<td>0.07 (0.04–0.21)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Anti-nephrin antibody positive</td>
<td>Anti-nephrin antibody negative</td>
<td>P-value</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>-------------------------------</td>
<td>-------------------------------</td>
<td>---------</td>
</tr>
<tr>
<td><strong>Patients</strong> n (%)</td>
<td>7 (50%)</td>
<td>7 (50%)</td>
<td></td>
</tr>
<tr>
<td><strong>Gender (male/female)</strong></td>
<td>(6/1)</td>
<td>(2/5)</td>
<td>0.102</td>
</tr>
<tr>
<td><strong>Age</strong> month</td>
<td>108 (87–116)</td>
<td>46 (26–64)</td>
<td>0.064</td>
</tr>
<tr>
<td><strong>Complete remission after 4 weeks</strong> n (%)</td>
<td>6 (86%)</td>
<td>7 (100%)</td>
<td>1.000</td>
</tr>
<tr>
<td><strong>Patients with at least one relapse</strong> n (%)</td>
<td>4 (67%)</td>
<td>5 (71%)</td>
<td>1.000</td>
</tr>
</tbody>
</table>

**Blood test (pre-treatment)**

<table>
<thead>
<tr>
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<th>Anti-nephrin antibody positive</th>
<th>Anti-nephrin antibody negative</th>
<th>P-value</th>
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</thead>
<tbody>
<tr>
<td>WBC /µL</td>
<td>7500 (6800–9200)</td>
<td>8200 (7100–15000)</td>
<td>0.443</td>
</tr>
<tr>
<td>Hb g/dL</td>
<td>14.0 (13.1–16.2)</td>
<td>14.2 (12.7–14.7)</td>
<td>0.609</td>
</tr>
<tr>
<td>Plt 10^4/µL</td>
<td>33.5 (29.9–38.0)</td>
<td>41.6 (30.2–51.6)</td>
<td>0.085</td>
</tr>
<tr>
<td>TP g/dL</td>
<td>3.8 (3.7–4.2)</td>
<td>4.0 (3.6–5.1)</td>
<td>1.000</td>
</tr>
<tr>
<td>Alb g/dL</td>
<td>1.1 (1.0–1.5)</td>
<td>1.0 (0.9–1.7)</td>
<td>0.748</td>
</tr>
<tr>
<td>BUN mg/dL</td>
<td>16.1 (9.1–18.2)</td>
<td>12.1 (9.2–13.1)</td>
<td>0.142</td>
</tr>
<tr>
<td>Cr mg/dL</td>
<td>0.35 (0.30–0.47)</td>
<td>0.32 (0.20–0.37)</td>
<td>0.371</td>
</tr>
<tr>
<td>IgG mg/dL</td>
<td>306 (194–401)</td>
<td>145 (92–280)</td>
<td>0.168</td>
</tr>
</tbody>
</table>

**Urine test (pre-treatment)**

<table>
<thead>
<tr>
<th></th>
<th>Anti-nephrin antibody positive</th>
<th>Anti-nephrin antibody negative</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP/Cr g/gCr</td>
<td>10.53 (7.91–13.19)</td>
<td>11.51 (5.97–22.1)</td>
<td>0.655</td>
</tr>
</tbody>
</table>

**Figures**
Figure 1

Anti-nephrin antibody titers during active disease and remission in 14 patients measured by indirect ELISA. 8 Healthy control patients were all serologically negative (data not shown). Statistical analysis was performed using unpaired t-test * p < 0.05 ** p ≤ 0.01.
Figure 2

Kaplan-Meier estimates for relapse-free period after primary onset in all participants who achieved complete remission after corticosteroid monotherapy (Figure 2A) and anti-nephrin antibody positive group (solid line) and negative group (dot line; Figure 2B). There was no statistically significant difference in relapse-free period between the antibody-positive and antibody-negative groups (Figure 2B; p=0.658).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- AntinephrinantibodyPedNephGraphicalAbstract.pptx