Type I IgE receptor, interleukin 4 receptor and interleukin 13 polymorphisms in children with nephrotic syndrome

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Abstract

Polymorphisms in the genes encoding the high-affinity IgE receptor, the interleukin 4 (IL4) receptor and IL13 can be associated with the development of asthma and allergy. Although several studies have described an association between atopy and idiopathic childhood nephrotic syndrome (NS), it is not clear whether this association is of a causal nature. Furthermore, it is not known whether these polymorphisms are associated with the clinical course of NS. A total of 84 children (52 male and 32 female; mean age 12.1 years) with NS were included in the present study. Of these, 78 could be classified as either atopic or non-atopic. Atopy was defined by elevated IgE levels (> 100 k-units/l) and/or a positive history of atopy (33 of 78 patients). DNA was extracted from blood collected in EDTA tubes, and polymorphisms at positions 50 and 551 of the IL4 receptor, position 110 of IL13 and position 181 of the high-affinity IgE receptor were investigated by sequence-specific PCR or direct sequencing. Although we noted a strong tendency towards a higher allele frequency of polymorphisms in children with atopy and NS compared with children with NS but without atopy (IL4 50, 30% compared with 18%; IL4 551, 39% compared with 31%; IL13 110, 45% compared with 33%; IgE 181, 12% compared with 13%), these differences did not reach statistical significance. There were no differences in the frequency of polymorphisms between the different clinical courses of NS (frequent relapsers, steroid-dependent or steroid-resistant NS). We conclude that polymorphisms in the IL4 receptor, the high-affinity IgE receptor and IL13 do not seem to predict the clinical course of NS, despite the fact that serum IgE elevations are more frequent in patients with NS than in normal control subjects. The investigated polymorphisms may contribute to the IgE switch in patients with NS.

Introduction

The genetic basis of childhood nephrotic syndrome (NS) remains uncertain. Many authors have suggested that activated T-cells are involved in the pathogenesis of NS [1–4]. This is supported by the detection of increased levels of interleukin 4 (IL4) and IL13 in the serum and urine of children with acute relapse of NS [2–4]. Several studies have described an association between atopic diseases and NS [5–10]. Specifically, it has been

Key words: atopy, IgE receptor, IL4 receptor, IL13, nephrotic syndrome, polymorphism.

Abbreviations: IgE-R, IgE receptor; IL4 (etc.), interleukin 4 (etc.); IL4-R, IL4 receptor; NS, nephrotic syndrome; RAST, radioallergosorbent test.

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observed that children with NS who also suffer from hay fever experience relapses of NS during the pollen season [7,8]. The nature of this association has been debated extensively during the past decades [7–10], and although clinical trials involving desensitization have been carried out [8,9], the results of these trials remain inconclusive.

The pathogenesis of atopic diseases has been elucidated significantly over the past few years. The relationship between polymorphisms of the high-affinity type I IgE receptor (IgE-R) and certain forms of atopic diseases have been widely investigated [11–14]. Changes in certain amino acids of the β-subunit of the high-affinity IgE-R (at position 181 from isoleucine to leucine and at position 183 from valine to leucine) have been found to be associated with atopy [16]. While the pathophysiological consequences of these changes remain uncertain, their presence may alter the phosphorylation capacity of the receptor [15].

In addition to the IgE-R, investigations have also focused on the IL4 receptor (IL4-R) and on IL13 [17–26], because these cytokines promote the isotype switch to IgE in the B cell. Furthermore, they regulate the expression of IgE in the B cells by binding to their receptors [17–20,23,25,26]. The isoleucine variant at position 50 of the IL4-R up-regulates IgE synthesis in cell culture, and is associated with atopic asthma in a Japanese population [20]. The arginine variant at position 551 (equivalent to position 576 in previous studies [22,24–26]) of the IL4-R correlates with asthma severity in an American population [22], and the glutamine variant at position 110 of IL13 has been found to be associated with higher IL13 levels and an increased prevalence of asthma in a British population and a Japanese population [17]. Polymorphisms in the IL4-R and IL13 seem to have effects on the ensuing signal transduction cascade, on the production of IgE [18–20] and on regulation of the expression of low-affinity type II IgE-Rs [27], which are increased in children with NS having high total IgE levels [2]. Both IL4 and IL13 seem to be signal inducers in NS and atopy, and play a role in the regulation of both diseases.

One previous study indicated that the polymorphism at position 551 in the IL4-R gene is frequent in children with NS as in normal controls [28]. However, the levels of total serum IgE were not measured in the subjects of that study, and the clinical course was not reported. Two papers have focused on the IL13 promoter in children with NS [29,30]. One confirmed a polymorphism at position –1055 of the promoter, but showed no difference in allele frequencies between patients with NS and controls [30]. Despite the fact that IL13 has been suspected to play a crucial role in this disease [4,31], no study has addressed the possible association between polymorphisms of the IL13 gene and of the IgE-R gene in children with NS.

The present study was conducted in order to determine the frequencies of known polymorphisms in the genes for the IgE-R, the IL4-R and IL13, and to investigate correlations with clinical features in children with both NS and the presence of atopy.

METHODS

A total of 84 children with a diagnosis of NS (52 male and 32 female) were included in the study. Written informed consent was obtained from parents of all patients, and the study was approved by the Ethics Committees of all participating universities. The mean age of the children was 12.0 ± 4.4 years (± S.D.). According to the guidelines of Arbeitsgemeinschaft Pädiatrische Nephrologie (APN) [32], 29 children were defined as frequent relapers, 35 had steroid-dependent NS, 11 had steroid-resistant NS (five of which had a focal segmental glomerulosclerosis), five children had a primary, steroid-sensitive manifestation and four had an atypical, non-classifiable course of disease. With the exception of the five children with primary, steroid-sensitive disease, all the other children had renal biopsies for exact histological diagnosis. Except for the five children who displayed focal sclerosis in the biopsy specimens, all other patients had minimal glomerular lesions. Patients with childhood NS were divided into two groups (atopic and non-atopic) according to atopic history and/or total serum IgE level. In children with positive history of atopy, either a skin prick test or a radioallergosorbent test (RAST) were available for exact diagnosis. In children without a history of atopy, the cut-off point for an elevated total serum IgE level was 100 k-units/l. For convenience, all children with an IgE level > 100 k-units/l and/or an atopic diagnosis will be classified as atopic in this paper, although we are aware that total serum IgE is not the ideal marker of atopy.

DNA was extracted from EDTA-treated blood (Qiagen blood extraction kit). We analysed position 181 (Leu/Val) of the amino acid sequence of the IgE-R, positions 50 (Ile/Val) and 551 (Gln/Arg) of the IL4-R and position 110 (Arg/Gln) of IL13 (note that in each case the wild-type amino acid is given second). The IgE-R and the IL4-R were analysed by sequence-specific PCR as described by Shirakawa et al. [16] and Hackstein et al. [33] respectively. IL13 was analysed by direct sequencing, using sequencing primers as described by Heinzmann et al. [17].

The genotype frequencies of the polymorphisms were compared between the two groups (atopic and non-atopic), and also between groups defined by the clinical course of NS (frequent relaper (> 4 relapses/year), steroid-dependent and steroid-resistant). Clinical definitions were used according to the APN [32].

In addition, genotype frequencies of all polymorphisms, except for that at position 551 of the IL4-R, were compared with those in a population of 50 asthmatic children (40 atopic, 10 non-atopic) from the Cologne
Table 1  Allele frequencies of investigated polymorphisms in relation to clinical course of NS and atopy: comparison with an asthmatic cohort (Cologne) and with historic study populations

Significant differences compared with particular historic study populations in each column are indicated in bold: * P = 0.02 compared with atopic subjects in [20]; † P = 0.03 compared with control subjects in [22]; ‡ P = 0.02 compared with control subjects in [17].

<table>
<thead>
<tr>
<th>Study population</th>
<th>n</th>
<th>IL4-R Ile50</th>
<th>IL4-R Arg551</th>
<th>IL13 Gln110</th>
<th>IgE-R Leu181</th>
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<tr>
<td>Present study</td>
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<tr>
<td>NS total</td>
<td>84</td>
<td>0.24</td>
<td>0.37</td>
<td>0.36</td>
<td>0.14</td>
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<tr>
<td>Frequent relapers</td>
<td>29</td>
<td>0.23</td>
<td>0.28</td>
<td>0.24</td>
<td>0.1</td>
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<td>Steroid-dependent</td>
<td>35</td>
<td>0.15</td>
<td>0.4</td>
<td>0.37</td>
<td>0.17</td>
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<tr>
<td>Steroid-resistant</td>
<td>11</td>
<td>0.36</td>
<td>0.45</td>
<td>0.54</td>
<td>0</td>
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<td>NS with atopy</td>
<td>33</td>
<td>0.3</td>
<td><strong>0.39†</strong></td>
<td><strong>0.45‡</strong></td>
<td>0.12</td>
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<tr>
<td>NS without atopy</td>
<td>45</td>
<td><strong>0.18</strong>*</td>
<td>0.31</td>
<td>0.33</td>
<td>0.13</td>
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<tr>
<td>Asthma (Cologne)</td>
<td>50</td>
<td>0.26</td>
<td>–</td>
<td>0.4</td>
<td>0.12</td>
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<td>atopics</td>
<td>230</td>
<td><strong>0.48</strong></td>
<td>–</td>
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<tr>
<td>controls</td>
<td>100</td>
<td>0.18</td>
<td>0.22</td>
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<tr>
<td>atopics</td>
<td>150</td>
<td>0.37</td>
<td>0.39</td>
<td>0.38</td>
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<tr>
<td>controls</td>
<td>150</td>
<td>0.31</td>
<td>0.35</td>
<td><strong>0.23</strong></td>
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<tr>
<td>controls</td>
<td>57</td>
<td>–</td>
<td><strong>0.19</strong></td>
<td>–</td>
<td>–</td>
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<tr>
<td>atopics</td>
<td>107</td>
<td>–</td>
<td>0.31</td>
<td>–</td>
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<tr>
<td>NS</td>
<td>100</td>
<td>–</td>
<td>0.28</td>
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<td>Parry et al. [28]:</td>
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<tr>
<td>controls</td>
<td>73</td>
<td>–</td>
<td>0.3</td>
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Statistical analysis was performed with SigmaStat software using the \( \chi^2 \)-test and Fisher’s Z-test.

RESULTS

Exact data on serum IgE levels and atopic history were available for 78 of the children investigated. Of these, 33 (42%) had either a history of atopy, as defined by a positive skin-prick test and/or elevated total serum IgE. Twelve of these children (15%) had clinical signs of an atopic disease (one asthma, seven hay fever and four atopic dermatitis) and 21 (27%) had isolated serum IgE elevation. These data are not statistically different from the frequency of atopy in children living in the western part of Germany (36%), although the frequency of asthma is much lower in our group [34].

Median serum IgE levels were 58 k-units/l for the whole group, 263 k-units/l for children with NS and atopy, and 19.4 k-units/l for children with NS without atopy (\( P < 0.001 \)).

We determined the allele frequencies of polymorphisms in the genes for IL13, the IL4-R and the type I IgE-R (Table 1). We noticed a strong tendency towards higher allele frequencies of the studied polymorphisms in children with atopy than in children without atopy, although these differences did not reach statistical significance. We also analysed the distributions of the studied polymorphisms among patients who experienced different clinical courses of NS, i.e. those who relapsed frequently, those who were steroid-dependent and those who were steroid-resistant. As shown in Table 1, we failed to detect any differences in genotype frequencies between these groups. However, we detected a tendency towards higher IgE levels in children with steroid-
dependent NS compared with children with frequent relapses of NS (78 and 28 k-units/l respectively).

Next we studied the allele frequencies of the same polymorphisms in 50 children with asthma (40 atopic and 10 non-atopic) from the Cologne region. We found no statistically significant differences in the frequencies of the investigated polymorphisms between this group of children and the children with NS (Table 1).

Finally, we compared our group with historical study and control populations studied by other investigators, who had reported on allele frequencies of these polymorphisms in asthmatic and atopic cohorts of patients [17,18,22]. We found no significant differences between our total study group and total study or control groups from other studies. When our study population was divided into atopic and non-atopic groups, we found significant differences between the allele frequencies of polymorphisms in the IL4-R and in IL13 in atopic children with NS when compared with non-atopic control populations of the historical studies. Our results showed that children with NS and atopy had significantly higher allele frequencies of these polymorphisms than control subjects in the previous studies. Furthermore, our non-atopic children with NS showed significantly fewer polymorphisms than atopic patients in the historical studies (Table 1).

DISCUSSION

This present study searched for associations between several polymorphisms in genes relevant to atopic diseases and paediatric NS. The type I IgE-R has pathogenic significance in the development of atopy [11–14], but no study has yet addressed whether polymorphisms of this receptor are associated with the expression of NS in children. Levels of both IL4 and IL13 are elevated in NS and atopic diseases, and polymorphisms in the genes encoding IL13 and the IL4-R have been associated with the development of atopy and more severe forms of asthma [2–4,17–27]. It was therefore reasonable to investigate the frequency of these polymorphisms in children with NS and compare them with clinical manifestations, although a previous study by Parry et al. [28] had failed to find a difference in the frequency of polymorphisms at position 551 of the IL4-R between patients with NS and a control population. Furthermore, an analysis of the promoter region of IL13 by the same group showed no differences between children with NS and normal controls [30], but the patients in both studies were not stratified according to the presence of atopy or to the clinical course of NS.

We did not find any significant difference in the allele frequencies of the investigated polymorphisms between patients with different clinical courses of NS (frequent relapsers, steroid-dependent and steroid-resistant). A sub-analysis comparing the first two groups (combined) with steroid-resistant patients did not show any difference either (results not shown). Therefore these polymorphisms seem to be irrelevant to the clinical course of paediatric NS. However, we cannot exclude the possibility of minor contributions to the course of the disease that could have been missed due to the small patient numbers.

As has been reported for atopic diseases such as asthma and hay fever [17,20–22], we found a higher allele frequency of polymorphisms of the IL4-R and IL13 in atopic children with NS when compared with non-atopic children with NS. However, this difference was not statistically significant, because our study population seemed to be too small. Furthermore, a sub-analysis of those children with signs of atopy other than IgE elevation showed no significantly higher allele frequencies when compared with non-atopic children or with children with IgE elevation alone (results not shown). We therefore compared our group with bigger historical study and control populations studied by other investigators, who had reported on allele frequencies of these polymorphisms in asthmatic and atopic cohorts of patients [17,18,22]. As mentioned in the Results section, we found significant differences between the allele frequencies of polymorphisms in the IL4-R and IL13 in our atopic children with NS when compared with non-atopic control populations in the historical studies. Our children with NS and atopy had significantly higher allele frequencies of these polymorphisms than the control subjects in the previous studies. Furthermore, our non-atopic children with NS had significantly fewer polymorphisms than atopic patients in the historical studies (Table 1).

Many previous studies have focused on the relationship between NS and atopy in different populations [5–10]. Increased IgE levels have been noted in several cohorts with NS, regardless of atopic history [35]. In our cohort, we found a greater tendency towards atopy, defined by total serum IgE and/or a positive history and skin-prick test or RAST, when compared with the normal population in the study of Mutius et al. [34] (42% compared with 36%). Admittedly, the prevalence of an atopic history in our cohort was only 15%, which seems to be lower than in other studies [6,35]. One reason for this finding might be patient selection and the lack of further testing (e.g. skin-prick test), as total serum IgE is not a very sensitive parameter of atopic disease [36]. In the cohort study of Mutius et al. [34], which compared children from former East Germany with children from former West Germany, each child was given a prick test, and a 36% sensitization rate to different allergens was reported in the West German cohort. The overall prevalence of asthma or hay fever was approx. 18% in that population. Another reason for the non-significant difference between our study and that of Mutius et al. [34] might be the increasing frequency of atopy in
children residing in the Western part of Germany during the last few decades.

We defined children with elevated IgE levels but no other signs of atopy to be atopic. This was done for convenience and for data analysis reasons. Elevated IgE levels can be found in several diseases, e.g. intestinal helminthiosis and NS, and are not a perfect marker of atopy [36]. We actually found that 15% of our population had an atopic disease and a further 27% had isolated IgE elevation. It therefore seems to be plausible that, independent of the cause of their elevation (acute relapse of NS or atopic disease), both IL4 and IL13 induce an IgE switch in B cells. The IgE switch is more readily mediated in children with the observed polymorphisms in IL13 and the IL4-R, which seem to increase the functional aspects of these proteins [21]. Therefore we found a higher allele frequency of polymorphisms of the IL4-R and of IL13 in children with elevated IgE levels, and we believe that most of the IgE elevations in NS are not a sign of atopy, but are a result of the IL4 and IL13 elevations in acute relapse of NS. This is consistent with the observation of higher IgE levels in children with steroid-dependent NS compared with frequent relapsers. One explanation for the higher IgE levels in subjects with steroid-dependent NS could be the fact that levels of IL4 and IL13 are more often elevated in these patients than in frequent relapsers. Children with steroid-resistant NS did not show higher IgE levels than those with steroid-dependent NS. Steroid-resistant NS might involve other immunological mechanisms that are independent of elevated levels of IL4 or IL13.

As the IgE-R is of possible relevance to IgE levels in asthma, it was also studied in our investigation of NS, although it is only distributed in mast cells and basophils. We failed to detect differences in the allele frequency of polymorphisms in the IgE-R between atopic and nonatopic children with NS, or between groups characterized by different clinical courses of NS. Furthermore, there was no difference in the frequency of this polymorphism between children with NS and the Cologne asthma group. Therefore a pathological relevance is unlikely, although our study group was too small to draw final conclusions.

In summary, elevated IgE levels are observed in a high proportion of children with NS, regardless of other signs of atopy. Elevated IgE levels were found especially in children with polymorphisms of the IL4-R and of IL13. The increased IgE levels in children with NS are likely to represent a response to the elevated IL4 and IL13 levels, and do not constitute a sign of atopy.

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