No evidence for a role of cosmc-chaperone mutations in European IgA nephropathy patients

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Abstract

Background. Altered IgA1 galactosylation is involved in the pathogenesis of IgA nephropathy (IgAN). The galactosyltransferase core-1 β3-galactosyltransferase-1 (C1GALT1) and its chaperone cosmc are specifically required for O-galactosylation of the IgA1 hinge region. Mutations in the cosmc gene result in a secondary loss of function of C1GALT1 with subsequent undergalactosylation of glycoproteins. Mosaic mutations of cosmc have been shown to result in autoimmune disease. We hypothesized that cosmc mutations might contribute to the altered IgA1 galactosylation in IgAN patients.

Methods. We studied cosmc gene sequences in genomic DNA obtained from male patients with biopsy-proven sporadic (n = 33) and familial IgAN (n = 6 patients from different families). To account for a potential mosaicism we sequenced cosmc in 10 different peripheral blood mononuclear cell DNA clones of every patient. To specifically assess potential mosaic mutations in IgA-producing cells, cosmc mutations were also analysed in DNA isolated from CD20+ B-lymphocytes from three male IgAN patients.

Results. Despite our extensive genomic analysis, the data revealed no functionally relevant cosmc gene variants in sporadic or familial IgAN cases. A cosmc gene polymorphism, rs17261572, was identified in these IgAN patients in a similar frequency as previously reported in healthy adults. A functional consequence of this polymorphism has not yet been determined.

Conclusion. Although decreased C1GALT1 activity has been implicated in the IgAN pathogenesis and cosmc chaperone mutations can cause autoimmune disease, our data provide no evidence for a relevant role of cosmc gene mutations in European patients with sporadic or familial IgAN.

Keywords: chaperone; cosmc; galactosylation; IgA nephropathy; mutation

Introduction

IgA nephropathy (IgAN) is the most common glomerulonephritis in the world [1–3]. The pathogenesis of IgAN is only incompletely understood [1,4]. Considerable evidence exists that altered galactosylation of IgA1 plays a central role in the pathological deposition of glomerular IgA1 in IgAN patients [4,5]. Compared to healthy controls, increased amounts of polymeric IgA1 with reduced O-glycosylation can be detected in serum and mesangium in IgAN patients. The enzyme core-1 β3-galactosyltransferase-1 (C1GALT1) plays a central role in IgA1 hinge region galactosylation. Initial findings in individual IgAN patients indicated a reduced activity of the enzyme C1GALT1 as a probable mechanism underlying the abnormal IgA1 glycosylation in IgAN patients [4,5]. Further studies demonstrated that C1GALT1 polymorphisms are associated with susceptibility to IgAN [6] and that mutations in the C1gal t1 gene in mice resulted in kidney disease [7]. Undergalactosylation of IgA1 results in increased generation of polymeric IgA, possibly by the generation of immune complexes via anti-glycan antibodies and by direct biochemical properties. Consequently, there is increasing evidence that a central mechanism underlying the pathogenesis of IgAN is a glycosylation defect [4,5].

Cummings et al. recently identified a specific molecular chaperone of the enzyme C1GALT1 and named it cosmc [8]. The cosmc gene is located on the X-chromosome (Xq23) and its coding exon consists of 954 base pairs [8]. Loss of function mutations of the cosmc gene can lead to secondary inactivation of C1GALT1 and subsequently to undergalactosylation of glycoproteins. A potential role of cosmc in human pathology is indicated by recent findings of Ju et al., who were able to identify somatic mosaic cosmc gene mutations in two patients with Tn-syndrome, a rare autoimmune disease characterized by undergalactosylation of cell membrane glycoproteins [9]. A mutant cosmc gene can also be responsible for the generation of tumour-specific glycopeptidic neo-epitopes identified in fibrosarcoma as well as neuroblastoma tumours [10]. Functional cosmc mutations have not been reported in IgAN patients so far. Given
Table 1. Clinical data of 29 male, biopsy-proven, sporadic IgAN patients included in the cosmc sequence analysis

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>ESRD</th>
<th>No ESRD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>29</td>
<td>13</td>
<td>16</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>Minimal</td>
<td>Maximal</td>
</tr>
<tr>
<td></td>
<td>49</td>
<td>16</td>
<td>78</td>
</tr>
<tr>
<td>Proteinuria (g/24 h)</td>
<td>1.8</td>
<td>0</td>
<td>6.9</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min)</td>
<td>65</td>
<td>24</td>
<td>122</td>
</tr>
</tbody>
</table>

In four additional IgAN patients clinical data were incomplete.

Table 2. Frequencies and distribution of single basepair substitutions within the cosmc gene detected by sequencing of 10 clones from each of the 39 IgAN patients

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>IgAN sporadic</th>
<th>IgAN familial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>39</td>
<td>33</td>
<td>6</td>
</tr>
<tr>
<td>Basepair substitutions</td>
<td>201</td>
<td>148</td>
<td>53</td>
</tr>
<tr>
<td>Substitutions in only 1/10 clones of a patient</td>
<td>138</td>
<td>104</td>
<td>34</td>
</tr>
<tr>
<td>Substitutions in 2–3/10 clones of the same patient</td>
<td>9</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>5–10 substitutions/patient</td>
<td>13</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>&gt;10 substitutions/patient</td>
<td>4</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

Methods

Patients

Whole blood DNA was obtained from 33 male patients with biopsy-proven, sporadic, primary IgAN that were treated in the Division of Nephrology in Aachen, Germany between 2000 and 2005. Clinical data of these IgAN patients are summarized in Table 1. Additional whole blood DNA was obtained from six male patients with biopsy-proven familial IgAN from six different families, collected within the European IgAN consortium [11]. All samples were collected after signed informed consent of each patient and following approval of the local ethics committees.

Primer, amplification strategy, sequencing

The nucleotide sequence of the cosmc gene was taken from the gene bank of the National Center for Biotechnology Information ncbi (www.ncbi.nlm.nih.gov). Primer sequences for cosmc amplification can be provided on request. Patient DNA was initially amplified with the primers cosmc-F and cosmc-R, and the PCR-product was purified by gel extraction (QIAquick Gel Extraction Kit, Qiagen, Hilden, Germany). The purified PCR product was inserted into a linearized plasmid using a TOPO® TA cloning kit (Invitrogen, Karlsruhe, Germany) and subcloned in competent Top10 cells. Ten different clones per patient were isolated, again PCR-amplified, purified by gel extraction and finally sequenced using the BigDye terminator v.1.1 sequencing kit (Applied Biosystem, Foster City, CA, USA). Sequencing reactions were then run on a ABI3130 sequencing system and analysed by SequencingAnalysis v.5.2 and SeqScape v.2.5 software. This procedure was chosen to increase the sensitivity for the detection of rare mosaic mutations in our patient population.

Isolation of DNA from blood B-lymphocytes of IgAN patients

Following a Ficoll isolation of peripheral blood mononuclear cells (PBMC), B-lymphocytes were isolated by magnetic cell separation (MACS) using the AutoMACS®-Sorting technique (Miltenyi Biotech, Bergisch Gladbach, Germany). Briefly, anti-human CD20 IgG1-MACS®-microbeads (antibodies conjugated with iron oxide containing polysaccharides) were incubated with freshly isolated PBMC. Iron-conjugated, CD20-positive cells were collected using a magnetic field. DNA was isolated from these B-lymphocytes using the QIAamp® DNA mini kit (Qiagen, Hilden, Germany).

Results

We studied the complete cosmc gene sequence (957 bp) of 39 IgAN patients in 10 different clones of each patient resulting in a total number of 373 230 bp analysed. Sequence analysis of the hybridizations revealed 201 basepair substitutions as compared to the wildtype cosmc sequence [NM_001011551, gi:58532583] (Table 2). Indeed, we detected basepair substitutions in at least one clone of nearly every patient, but a common pattern of variations indicating a functional relevance was not obvious. We, therefore, classified these variants as artificial caused by the successive PCR and cloning steps. Furthermore, there was no overall difference in the detection of variations between cases with sporadic and familial IgAN.

In addition to the base variations described above, we detected a known single nucleotide polymorphism (SNP) in the cosmc gene in 36% of the patients (n = 14) at nucleotide position 568 (c.568 T>A, P.D131E; formerly known as c.628T>A) [ncbi: rs17261572]. This SNP has previously been reported in a similar frequency in the course of the HapMap project [www.hapmap.org] in 32% of 120 healthy Europeans. A functional role of this SNP has not yet been established and so far it has not been associated with any pathological phenotype.

To verify the existence and functional relevance of putative cosmc mosaic variations in IgAN patients, we subsequently analysed cosmc in the subpopulation of B-cells. Three patients with non-familial IgAN were selected on the basis of sequence variations with most probable functional relevance. Patient 1 had one identical variation in two different clones and two different stop codon variations in two different clones. Patient 2 had one identical variation in two different clones. Patient 3 had a stop codon variation in one clone. In 10 different clones of each of the B-cell DNA of these three patients, we identified 16 cosmc base
because of the potential dominance of any in our study, we focussed exclusively on male IgAN patients. The reduced controls and patients with other glomerular diseases [12]. patients [13]. Sequencing of the anisms of group extended their study and specifically analysed mech-
a reduced IgA galactosylation in these patients. This same
the disease.

The cosmc gene is located on the X-chromosome [8]. In
our study, we focussed exclusively on male IgAN patients
because of the potential dominance of any cosmc muta-
tions in the absence of a second X-chromosome. IgAN has
a significant male predominance in European countries, a
finding that supports the assumption that a gene on the X-
chromosome such as cosmc is involved in the aetiology of
the disease. Cosmc has also been studied recently in Chi-
inese IgAN patients. Qin et al. have detected a significantly
reduced cosmc mRNA expression in B-lymphocytes iso-
lated from Chinese IgAN patients in comparison to healthy
controls and patients with other glomerular diseases [12].
The reduced cosmc mRNA expression was associated with
a reduced IgA galactosylation in these patients. This same
group extended their study and specifically analysed mech-
isms of cosmc regulation and activity in Chinese IgAN
patients [13]. Sequencing of the cosmc gene in 65 IgAN pa-
patients and 44 controls revealed four cosmc gene SNP’s but
no common mutations in this Chinese population. Again, a
reduced cosmc mRNA expression was detected in IgAN
patients as compared to controls [13]. Further in vitro
studies revealed inhibition of cosmc mRNA expression by
LPS-treatment, a mechanism that might explain infection-
associated aggravation in some IgAN patients [13].

The data of our present study are consistent with and ex-
tend the data from Qin et al. It is our current understanding
that IgAN might not necessarily represent a single dis-
 ease but is rather heterogeneous with different underlying
pathomechanisms resulting in a similar glomerulonephritis
phenotype [3]. Strong support for different IgAN pathome-
chanisms comes from epidemiological data. While in Asian
populations IgAN is typically equally distributed between
both genders, there is a clear male predominance in Cau-
casian IgAN patients. Our finding, showing an absence of
cosmc mutations, both in sporadic and in familial cases of
European IgAN, therefore extends the findings of Qin et al.
in a Chinese IgAN cohort [13].

Another study by Buck et al. reported mRNA expression
levels of cosmc in peripheral blood and bone marrow from
European IgAN patients and controls [14]. This study did
not show a cosmc deficiency or defect. Furthermore, there
was no difference in B-cell O-galactosylation activity of
IgAN patients and controls [14]. However, the authors did
focus on mRNA levels and did not specifically search for
potentially inactivating mutations in the cosmc gene [14].

Was our sample size sufficient enough to detect relevant
cosmc mutations? We decided to include both sporadic and
familial cases of IgAN in our study to deal with potentially
different IgAN subgroups. Furthermore, we substantially
increased our sensitivity for the detection of mosaic mu-
tations by analysing 10 different clones of each patient
(a similar method as used for the detection of mosaic mu-
tations in Tn-syndrome). Indeed, due to the relatively small
number of patients, our data can certainly not exclude the
existence of individual IgAN patients with an underlying
cosmc mutation leading to inactive C1GALT1 and subse-
quent undergalactosylation of IgA1. However, the fact that
relevant cosmc mutations were not detected in 10 indepen-
dently amplified and analysed DNA clones of 39 different
male European IgAN patients does not confirm our ini-
tial hypothesis that cosmc gene mutations are a common
cause of IgA1 undergalactosylation in these patients. Nev-
 ertheless, future mutation and association studies in larger
cohorts of IgAN patients should show whether cosmc vari-
ants contribute to the aetiology of the disease. Furthermore,
genomic variations in the promotor and the non-coding re-
 gion of cosmc might be associated with the disease.

In conclusion, our present data add another piece (al-
though negative) to the still very incomplete puzzle of our
current knowledge of the pathogenesis of IgAN. We were
unable to provide evidence for a role of cosmc gene muta-
tions in IgAN pathogenesis.

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References

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