

Brief Report

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 beta3-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 *C1galt1* 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

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Table 1. Clinical data of 29 male, biopsy-proven, sporadic IgAN patients included in the *cosmc* sequence analysis

	Total	ESRD	No ESRD
Number of patients	29	13	16
	Mean	Minimal	Maximal
Age (years)	49	16	78
Proteinuria (g/24 h) ^a	1.8	0	6.9
Creatinine clearance (ml/min) ^a	65	24	122

In four additional IgAN patients clinical data were incomplete.

^aProteinuria and creatinine only refer to the patients without ESRD.

the known alteration of IgA₁ galactosylation and the potential role of *cosmc* mutations, we hypothesized that such *cosmc* mutations play a role in the pathogenesis of IgAN by altering the IgA₁ galactosylation.

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 v1.1 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 mag-

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

	Total	IgAN sporadic	IgAN familial
Number of patients	39	33	6
Basepair substitutions	201	148	53
Substitutions in only 1/10 clones of a patient	138	104	34
Substitutions in 2–3/10 clones of the same patient	9	4	5
5–10 substitutions/patient	13	10	3
>10 substitutions/patient	4	2	2

netic 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

variations. However, none of these 16 variations in the B-cell DNA had been detected in the DNA isolated from whole blood of the same patients and vice versa none of the variations identified in the whole blood DNA of these three patients were seen in the B-cell DNA clones. The *cosmc* SNP (c.568 T>A) was detected in all clones of two-third of patients in the whole blood DNA as well as in the B-cell DNA.

Discussion

In a comprehensive analysis of the whole genomic sequence of the *cosmc* gene, we were unable to demonstrate functionally relevant mutations in this gene in European patients with sporadic and familial IgAN. The overall rate of variations from the wildtype gene sequence found in our amplified DNA probes can in part be explained by the known error rate of any DNA amplification using DNA polymerases. Each analysed DNA clone was PCR-amplified, subcloned and again PCR-amplified. Furthermore, we were completely unable to reproduce any identical *cosmc* variations in three patients with a significant number of variations in DNA obtained from PBMC as compared to isolated B-lymphocytes. Such a complete lack of reproducibility strongly argues against true biological mutations and rather supports the interpretation of the findings as *in vitro* artefacts. The only true variant detected in *cosmc* in our study was the known variant rs17261572 at nucleotide 568. To answer the question whether such a polymorphism might play a role in IgAN pathogenesis, a far larger sample size would be needed.

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* mutations 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 Chinese IgAN patients. Qin *et al.* have detected a significantly reduced *cosmc* mRNA expression in B-lymphocytes isolated 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 mechanisms of *cosmc* regulation and activity in Chinese IgAN patients [13]. Sequencing of the *cosmc* gene in 65 IgAN patients and 44 controls revealed four *cosmc* gene SNPs 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 extend the data from Qin *et al.* It is our current understanding that IgAN might not necessarily represent a single disease but is rather heterogeneous with different underlying pathomechanisms resulting in a similar glomerulonephritis

phenotype [3]. Strong support for different IgAN pathomechanisms comes from epidemiological data. While in Asian populations IgAN is typically equally distributed between both genders, there is a clear male predominance in Caucasian 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 mutations by analysing 10 different clones of each patient (a similar method as used for the detection of mosaic mutations 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 subsequent undergalactosylation of IgA₁. However, the fact that relevant *cosmc* mutations were not detected in 10 independently amplified and analysed DNA clones of 39 different male European IgAN patients does not confirm our initial hypothesis that *cosmc* gene mutations are a common cause of IgA₁ undergalactosylation in these patients. Nevertheless, future mutation and association studies in larger cohorts of IgAN patients should show whether *cosmc* variants contribute to the aetiology of the disease. Furthermore, genomic variations in the promotor and the non-coding region of *cosmc* might be associated with the disease.

In conclusion, our present data add another piece (although 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 mutations in IgAN pathogenesis.

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Conflict of interest statement. None declared.

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