Crystal-storing histiocytosis with renal Fanconi syndrome: pathological and molecular characteristics compared with classical myeloma-associated Fanconi syndrome

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Abstract

Background. Crystal-storing histiocytosis (CSH) is a poorly described complication of monoclonal gammopathy featuring histiocyte lysosomal storage of κ light chain (κLC) crystals. Although CSH is usually associated with systemic manifestations, renal involvement is uncommon.

Methods. To investigate the molecular mechanisms implicated in κLC crystallization, we performed immunopathological and molecular studies in three patients with CSH and renal Fanconi syndrome (CSH/FS). The Vκ sequences were determined, and resulting molecular models were compared with previously reported myeloma-associated FS κLC sequences.

Results. All patients presented with chronic tubulo-interstitial nephritis and renal FS with accumulation of monoclonal κLC crystals within proximal tubular cells. They showed peri-renal and interstitial infiltration by histiocytes containing eosinophilic crystalline inclusions (pseudo-Gaucher cells). LC sequences were determined and assigned to their germline counterparts, in strong homology with previously reported myeloma-associated FS sequences. Comparison of CSH/FS Vκ domain 3D structures with the germline-encoded structures and those from patients with myeloma-associated FS underlined distinct hydrophobic residues exposed to the solvent in two patients, likely favouring the formation of a variant form of crystals that may further resist degradation after phagocytosis.

Conclusion. Although CSH/FS and myeloma-associated FS are closely related disorders, peculiar mutations in the V domains of CSH/FS monoclonal κLCs, different from those in myeloma-associated FS, may account for crystal morphology, predominant accumulation within histiocytes and multiple organ involvement in CSH.

Keywords: crystal-storing histiocytosis; Fanconi syndrome; molecular modelling; monoclonal κ light chains

Introduction

Monoclonal gammopathy may be associated with accumulation of immunoglobulin (Ig) crystals in various tissues. In cryoglobulinaemia, crystals composed of an entire monoclonal Ig are found in extracellular fluids and within the cytoplasm of various cells [1,2]. Myeloma-associated renal Fanconi syndrome (FS) is a rare disorder characterized by proximal tubule dysfunction related to reabsorption of a monoclonal Ig light chain (LC), almost invariably of the κ type [3]. The hallmark of Fanconi syndrome (FS) is the accumulation of monoclonal κ light chain (κLCs) crystalline inclusions within the endolysosomal compartment of proximal tubular cells. Most κLCs are from the Vκ1 subgroup and derive from two germline genes, O2/O12 or O8/O18 [4,5]. Unlike other monoclonal LCs degraded in tubular cell lysosomes, LCs from FS patients are often partly resistant to proteolysis [4,6]. Peculiarities of the variable (V) κ domain, notably hydrophobic residues at position 30, account for resistance to proteolysis and promote self reactivity and crystallization. Crystal formation is sometimes lacking, as described for monoclonal VκIII LCs encoded by the L2/L16 or L6 genes [4,7].

Accumulation of immunoglobulinic crystals within lysosomes of histiocytes in the bone marrow or other organs defines crystal-storing histiocytosis (CSH). In CSH invariably associated with κLC monoclonal gammopathy, crystals are mostly made up of monoclonal κLCs and more rarely of Ig heavy chains [8]. As histiocytes featuring crys-
tal inclusions in CSH mimic Gaucher cells in genetic lysosomal storage disorders or pseudo-Gaucher cells seen in chronic myeloid leukaemia, they are referred to as ‘pseudo-pseudo-Gaucher cells’ (PPGC) [9]. Renal manifestations have been rarely described in CSH [8]. Inclusion-bearing histiocytes may infiltrate the renal interstitium, while lysosomal inclusions may be present within proximal tubular cells in patients with proximal tubular dysfunction [10]. By contrast, with myeloma-associated FS, morphological and molecular features of CSH featuring proximal tubular dysfunction (CSH/FS) have been poorly described [10–13].

We performed immunopathological and molecular study in three cases of κLC-associated CSH/FS. Vκ sequences and resulting molecular models were compared with previously reported κLCs myeloma-associated FS, in order to analyse the potential effect of mutations in aggregation and crystallization.

### Materials and methods

#### Patients

**Patient 1 (GSC\(^{\text{CSH}}\)).** A 52-year-old man was hospitalized for acute renal failure. Kidney biopsy revealed prominent interstitial plasma cell and lymphocyte infiltration without vascular or glomerular lesions. A diagnosis of drug-induced immuno-allergo-tubulo-interstitial nephritis was suspected. Prednisone therapy resulted in decrease in serum creatinine level from 3.0 to 1.4 mg/dl in 3 months. Over the three following years, the patient presented recurrent episodes of acute renal failure despite steroid therapy. Three yearly kidney biopsies revealed persistent interstitial infiltration by mononuclear cells. Symptoms of FS were detected 4 years after initial admission, along with serum IgM monoclonal gammopathy and full-blown FS with normoglycemic glucosuria and generalized hyperaminoaciduria, discovered 13 years before. At that time, serum creatinine level was 2.8 mg/dl, and a kidney biopsy showed severe tubulointerstitial nephritis with extensive interstitial fibrosis. Upon admission, physical examination was unremarkable. Biological tests revealed: serum creatinine 6.3 mg/dl, slight microscopic haematuria and proteinuria of 2.50 g/day (Table 1). Potassium was 3.8 mmol/l, HCO\(_3\) 23 mmol/l, total protein 8.6 g/dl, albumin 4.8 g/dl, Ca 2.49 mmol/l and phosphate 0.8 mmol/l. Immunoelectrophoresis revealed a serum monoclonal IgG and κ Bence-Jones proteinuria. Serum IgG level was 2.93 g/dl (N 0.69–1.6 g/l) with decreased IgA and IgM levels. No cryoglobulin was detected. Full blood count was normal. Bone marrow smears and biopsy did not show evidence of malignant lymphocytic or plasma cell infiltration but numerous PPGC. Prednisone was reintroduced for 3 years, until the patient died of sepsis after 25 years of follow-up (serum creatinine 4.3 mg/dl).

**Patient 2 (MOR\(^{\text{CSH}}\)).** A 70-year-old man was admitted for chronic renal failure. Clinical examination was unremarkable except for spleen enlargement. Laboratory investigations showed white blood cell count 5500/μl, haemoglobin 11.1 g/l, urea 3.5 mg/dl, serum creatinine 2.4 mg/dl, total protein 70 g/l, K 3.1 mmol/l, Ca 2.59 mmol/l, phosphate 1.09 mmol/l, uric acid 221 μmol/l and proteinuria 0.8 g/day (Table 1). Increased fractional excretion (FE) of uric acid (46%) and phosphate (32%) with orthoglycaemic glucosuria of 0.6 g/day and normal urine amino acid excretion indicated incomplete proximal tubular dysfunction. A monoclonal IgM paraprotein was found in the serum and urine. Serum-free κ LC level was 522 mg/l. Tests for cryoglobulins were negative. Bone marrow smears and skeletal X-rays were normal. A kidney biopsy was performed, and CSH was diagnosed. Six monthly courses of chlorambucil plus prednisone resulted in reduction of serum-free κ LC levels to 388 mg/l and serum IgM level to 1.4 g/l. Two months after treatment withdrawal, the patient developed severe thrombopaenia. Serum creatinine level was 0.9 mg/dl, without glucosuria, but hypophosphataemia (0.63 mmol/l) and hypouricaemia (120 μmol/l) persisted with increased FE of 25% and 59%, respectively. Bone marrow smears revealed diffuse infiltration by CD19+CD20+ slgMr+positive cells suggesting progression to large B-cell lymphoma. Chemotherapy with cyclophosphamide, Adriamycin, vincristin, prednisone and rituximab was initiated. The patient died of sepsis after six courses of treatment (serum creatinine 3.2 mg/dl).

**Patient 3 (MAR\(^{\text{CSH}}\)).** A 65-year-old man was referred for acute worsening of chronic renal failure. He had a past history of IgGk monoclonal gammapathy and full-blown FS with normoglycemic glucosuria and generalized hyperaminoaciduria, discovered 13 years before. At that time, serum creatinine level was 2.8 mg/dl, and a kidney biopsy showed severe tubulointerstitial nephritis with extensive interstitial fibrosis. Upon admission, physical examination was unremarkable. Biological tests revealed: serum creatinine 6.3 mg/dl, slight microscopic haematuria and proteinuria of 2.50 g/day (Table 1). Potassium was 3.8 mmol/l, HCO\(_3\) 23 mmol/l, total protein 8.6 g/dl, albumin 4.8 g/dl, Ca 2.49 mmol/l and phosphate 0.8 mmol/l. Immunoelectrophoresis revealed a serum monoclonal IgGk and κ Bence-Jones proteinuria. Serum IgG level was 2.93 g/dl (N 0.69–1.6 g/l) with decreased IgA and IgM levels. No cryoglobulin was detected. Full blood count was normal. Bone marrow smears, trephine and a second kidney biopsy led to the diagnosis of CSH. No specific treatment was introduced. Chronic renal failure worsened, and haemodialysis was started 19 months after diagnosis and 15 years after the first kidney biopsy.

### Pathological studies

The remaining samples, obtained from routine laboratory investigations, were used for the study according to Helsinki recommendations. All kidney biopsy specimens were examined by light microscopy using standard methods [7]. Direct immunofluorescence (IF) and/or immunohistochemistry (IHC) studies were performed using conjugates specific for human Ig κ, μ, α HCs, κ and λ LCs (Dako). Electron microscopy (EM) and/or immunoEM were performed on urinary sediment (GSC\(^{\text{CSH}}\), bone marrow (GSC\(^{\text{CSH}}\) and κ LCs myeloma-associated FS, in order to analyse the potential effect of mutations in aggregation and crystallization.

### Molecular biology studies

RNA was extracted from bone marrow and prepared in triPure (Roche GmbH). The cDNAs were synthesized using superscript II reverse transcriptase (Invitrogen). To determine each Ig LC subgroup, PCR amplification was performed with cDNA as template, a 3′ primer complementary to the upstream part of the Cκ exon and 5′ primers representing consensus sequences of leader regions for each Vκ subgroup. The 3′ primer was 5′-CGGGAAGATGAGGAGCATGGTGACC-3′, and 5′ primers were: Vκ\(_{\text{iV}}\), Vκ\(_{\text{iV}}\) (5′-ATGGCATGAAGGTTCCGCCTCT-3′), Vκ\(_{\text{iV}}\) (5′-ATGAGGCTCCTGTCTGCTACGTCT-3′), Vκ\(_{\text{iV}}\) (5′-ATGGACCCCAAGCGACCTCTTCT-3′) and Vκ\(_{\text{iV}}\) (5′-ATGGTGTTGCACGACCGTTC-3′).

PCR amplification was performed using Taq DNA polymerase (Qiagen, Valencia, CA). The cDNA was amplified after denaturation for 5 min at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 55°C, 45 s at 72°C and a final elongation step at 72°C for 10 min. PCR products were cloned into PCRII-TOPO vector (Invitrogen, Carlsbad, CA) and sequenced.

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**Table 1. Clinical and laboratory characteristics of GSC\(^{\text{CSH}},\) MOR\(^{\text{CSH}}\) and MAR\(^{\text{CSH}}\) at the time of diagnosis**

<table>
<thead>
<tr>
<th>Patients</th>
<th>Sex/age (year)</th>
<th>Renal features</th>
<th>Bone marrow</th>
<th>Serum creatinine (mg/dl)</th>
<th>Total proteinuria (g/day)</th>
<th>Haemoglobin (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSC(^{\text{CSH}})</td>
<td>M/52</td>
<td>Chronic renal failure/FS</td>
<td>PC &lt; 5% L 9%</td>
<td>2.9</td>
<td>0.29</td>
<td>12.8</td>
</tr>
<tr>
<td>MOR(^{\text{CSH}})</td>
<td>M/70</td>
<td>Chronic renal failure/FS</td>
<td>PC &lt; 5% L 10–15% (μκ +)</td>
<td>2.4</td>
<td>0.8</td>
<td>11.1</td>
</tr>
<tr>
<td>MAR(^{\text{CSH}})</td>
<td>M/65</td>
<td>Chronic renal failure/FS</td>
<td>PC 30% (γκ +) L 15%</td>
<td>6.3</td>
<td>γ globulin: 59%</td>
<td>11</td>
</tr>
</tbody>
</table>

Notes: Bone marrow cellularity was normal. The bone marrow smears and biopsy did not show evidence of malignant lymphocytic or plasma cell infiltration but numerous PPGC. The immunohistochemical (IHC) studies were performed using conjugates specific for human Ig κ, μ, α HCs, κ and λ LCs (Dako). Electron microscopy (EM) and/or immunoEM were performed on urinary sediment.
Sequences repeatedly obtained from independent PCR experiments were assigned to the proliferating clone and compared with the germ line and previously studied κ LCs responsible for myeloma-associated FS. Searches for homologies were made using the Blast server at the National Center for Biotechnology Information and ImMunoGeneTics (IMGT) database.

Theoretical isoelectric points (pI) of CS H and FS κ LCs were calculated using ExPaSy proteomics server (http://www.expasy.ch).

Molecular modelling

Molecular modelling of VsκJκ domains was made by homology to the 1LVE human κ LC LEN crystal structure (Vsκ/Jκ 2 subgroup), determined by X-ray diffraction using a resolution of 1.95 Å [15]. The identity of 1LVE with the three CSH/FS LCs studied here was approximately 80%. The original X-ray structure was optimized using the AMBER force field parameterized for proteins, as implemented in the Insight II software (http://accelrys.com). Water molecules included in the original X-ray structure (1LVE) were kept during the calculation. The convergence criterion was fixed at 0.001 kcal/mol/Å for the maximum gradient. The same structure (1L VE) were kept during the calculation. The convergence criterion was fixed at 0.001 kcal/mol/Å for the maximum gradient. The same structure (1L VE with the three CSH/FS LCs studied here was approximately 80%. The original X-ray structure was optimized using the AMBER force field parameterized for proteins, as implemented in the Insight II software (http://accelrys.com). Water molecules included in the original X-ray structure (1LVE) were kept during the calculation. The convergence criterion was fixed at 0.001 kcal/mol/Å for the maximum gradient. The same structure (1LVE) were kept during the calculation. The convergence criterion was fixed at 0.001 kcal/mol/Å for the maximum gradient. The same structure (1L VE with the three CSH/FS LCs studied here was approximately 80%. The original X-ray structure was optimized using the AMBER force field parameterized for proteins, as implemented in the Insight II software (http://accelrys.com). Water molecules included in the original X-ray structure (1LVE) were kept during the calculation. The convergence criterion was fixed at 0.001 kcal/mol/Å for the maximum gradient. The same structure (1LVE) were kept during the calculation. The convergence criterion was fixed at 0.001 kcal/mol/Å for the maximum gradient. The same structure (1LVE) were kept during the calculation. The convergence criterion was fixed at 0.001 kcal/mol/Å for the maximum gradient. The same structure (1LVE) were kept during the calculation. The convergence criterion was fixed at 0.001 kcal/mol/Å for the maximum gradient. The same structure (1LVE) were kept during the calculation. The convergence criterion was fixed at 0.001 kcal/mol/Å for the maximum gradient. The same structure (1LVE) were kept during the calculation. The convergence criterion was fixed at 0.001 kcal/mol/Å for the maximum gradient. The same structure (1LVE) were kept during the calculation. The convergence criterion was fixed at 0.001 kcal/mol/Å for the maximum gradient. The same structure (1LVE) were kept during the calculation. The convergence criterion was fixed at 0.001 kcal/mol/Å for the maximum gradient. The same structure (1LVE) were kept during the calculation. The convergence criterion was fixed at 0.001 kcal/mol/Å for the maximum gradient. The same structure (1LVE) were kept during the calculation. The convergence criterion was fixed at 0.001 kcal/mol/Å for the maximum gradient. The same structure (1LVE) were kept during the calculation. The convergence criterion was fixed at 0.001 kcal/mol/Å for the maximum gradient. The same structure (1LVE) were kept during the calculation. The convergence criterion was fixed at 0.001 kcal/mol/Å for the maximum gradient. The same structure (1LVE) were kept during the calculation. The convergence criterion was fixed at 0.001 kcal/mol/Å for the maximum gradient. The same structure (1LVE) were kept during the calculation. The convergence criterion was fixed at 0.001 kcal/mol/Å for the maximum gradient.

Results

Pathological findings

Kidney biopsies. Kidney biopsy samples from patients GSCκ (n = 4), MARκ (n = 2) and MORκ (n = 1) were reviewed. In all cases, light microscopy revealed interstitial infiltration by mononuclear cells, with diffuse interstitial fibrosis of variable severity. A moderate population of interstitial histiocytes contained eosinophilic crystalline inclusions with the appearance of Gaucher cells. These PPGCs were numerous within peri-renal adipose tissue in all patients (Figure 1A, G and I).

No specific glomerular lesions were observed, and mesangial cells and podocytes did not contain inclusions. Congo-red staining was negative, and no myeloma casts

Fig 1. GSCκ. (A) Kidney biopsy, adipose pericapsular tissue. Green light Masson trichrome (original magnification >200). Eosinophilic crystalline inclusions in immunoglobulin-producing cells (arrow head). Numerous pseudo-pseudo-Gaucher cells (PPGC) (arrow) infiltrating subcapsular adipose tissue and renal capsule. (B) Kidney biopsy. Masson trichrome (original magnification >200). Atrophy of the proximal tubular epithelium (arrow head) with interstitial fibrosis containing few PPGCs and mononuclear cells. Note the translucent appearance of few proximal tubular cells (arrow) suggestive of crystal storage. (C) Bone marrow biopsy. Haematoxylin and eosin (original magnification >400). Presence of PPGCs (arrow). (D) Bone marrow cytology. Giemsa (original magnification ×1000). Plasma cells with eccentric nucleus and refracting clear crystalline cytoplasmic inclusions (arrow). MORκ. (E) Kidney biopsy. Masson trichrome (original magnification >200). Tubular epithelium showing intra-cytoplasmic crystalline inclusions (arrow). Numerous interstitial cells display the same pattern of PPGCs. (F) Kidney biopsy. Toluidine blue staining (original magnification >1000). One tubular epithelial cell displays intra- cytoplasmic inclusions (arrow), while the neighbouring proximal tubular cells are atrophic (arrowhead). (G) Kidney biopsy. Subcapsular tissue, anti-κ conjugate (original magnification >200). Few plasma cells and numerous histiocytes positively stained with the anti-κ conjugate. Insert: proximal tubular section showing cytoplasmic crystalline inclusions irregularly stained with the anti-kappa conjugate (original magnification ×400). (H) Kidney biopsy. Immunohistochemistry (anti-CD68) monoclonal Ab (original magnification ×200); numerous CD68-positive cells infiltrate the interstitium, the tubular lumen. MARκ. (I) Kidney biopsy. Green light Masson trichrome stain (original magnification >1000). Subcapsular tissue. PPGCs are observed within the subcapsular adipose tissue and the renal capsule (arrow). (J) Bone marrow biopsy. Haematoxylin and eosin (original magnification ×200). Presence of the same PPGC with enlarged clear and multi-lamellar cytoplasm (arrow).
were seen. In all three patients, proximal tubular lesions were prominent, with focal atrophy and brush border disappearance, whereas some cells appeared turgescent and contained intra-cytoplasmic needle-shaped crystals (Figure 1B and E–G[insert]). No staining of proximal tubular cells was initially described with anti-LC conjugates by IF. However, a strong cytoplasmic staining that predominated at the periphery of crystals was observed with anti-kappa LC conjugate by IHC in patients GSC^CSH and MOR^CSH (Figure 1G[insert]). In patient MOR^CSH, interstitial infiltrating cells and cellular casts (Figure 1H) stained for CD68 and kLC, while subcapsular plasmacytoid cells and histiocytes stained with the anti-κ conjugate (Figure 1G and H). IHC studies were not available for patient MAR^CSH.

By EM, needle-shaped crystals were observed in the cytoplasm of altered cells in GSC^CSH urinary sediment (Figure 2D). In patient MOR^CSH, few proximal tubular cells contained numerous electron-dense inclusions organized into 7 to 8 nm diameter amyloid-like fibrils, which were decorated solely by the anti-κ gold conjugate (Figure 2E and F).

Bone marrow smears and biopsies. Initial bone marrow smears revealed the absence of obvious lymphocytic or plasma cell infiltration in patients GSC^CSH and MOR^CSH but the presence of a few PPGC with enlarged and multi-lamellar cytoplasm and a few plasmacytoid cells containing inclusions suggestive of Ig crystals (Figure 1C and D). By EM, bone marrow histiocytes in patient GSC^CSH contained intra-cytoplasmic needle-like crystals (Figure 2C). In MOR^CSH, IF study of cytoplasmic Ig in suspended bone marrow cells showed 10–15% κ-positive lympho-plasma cells with intra-cytoplasmic inclusions that strongly stained for μ and κ chains. Using the same IF study, a small 5–7% population of peripheral blood lymphocytes that displayed strong cytoplasmic staining with anti-μ and anti-kappa conjugates was observed. Bone marrow from patient MAR^CSH showed 30% infiltration by monotypic CD138 and κ-positive plasma cells, some containing eosinophilic inclusions. A diagnosis of multiple myeloma stage I-B was made.

Sequence analysis. Bone marrow cDNA was PCR amplified with Vκ-specific primers, and a single sequence was repeatedly amplified: VκIII for GSC^CSH and MOR^CSH, VκI for MAR^CSH. Highest identities were with germline gene A27 (95.5%)/Jκ1 for GSC^CSH, L2 (99.6%)/Jκ4 for MOR^CSH and O8/O18 (93%)/Jκ3 for MAR^CSH (Figure 3A, Table 2). Deduced protein sequences were aligned with germline and previously published myeloma-associated FS κLCs (Figure 3B). Compared to germline, GSC^CSH revealed 10 unusual residues, with five possibly modifying either hydrophobicity or charge: substitutions of polar with hydrophobic residues occurred in the first complementary determining region (CDR1; position 33), in the CDR2 (position 53) and in the third framework region (FR3) (positions 64 and 73). A charged residue at position 39 replaced a polar residue at position 85, Figure 3B). MAR^CSH sequence exhibited only one substituted residue (Ser replaced Phe at position 85, Figure 3B). MAR^CSH LC displayed eight remarkable substitutions: three apolar residues replaced polar residues were replaced by charged residues at positions 9, 12 (FR1) and 56 (FR3), one charged residue was replaced by a polar residue at position 55 and four polar residues were replaced by charged residues at positions 53, 76, 79 and 93 (Figure 3B).

Isoelectric point. Calculated pl was heterogenous (Table 2). High pl values were found in two CSH/FS LCs (GSC^CSH, MOR^CSH) and three myeloma-associated FS with (CHEB^FS+, TRO^FS+) and without crystals (VAL^FS−). Low pl values were obtained in the remaining cases.
Molecular modelling

The theoretical structures of the pathogenic κ LCs (MAR\(^{\text{CSH}}\), GSC\(^{\text{CSH}}\), MOR\(^{\text{CSH}}\), DEL\(^{\text{FS}}\), MAS\(^{\text{FS}}\), CHEB\(^{\text{FS}}\), TREF\(^{\text{FS}}\), and TRO\(^{\text{FS}}\)) and the germline sequences were compared.

Modelling of 02/O12 encoded FS LCs (Figure 4A and B) showed that: (i) residue 30 in CDR1 was more exposed to the solvent in TRO\(^{\text{FS}}\) compared to TREF\(^{\text{FS}}\) and CHEB\(^{\text{FS}}\), as previously reported by Deret et al. [4]; (ii) the mutated residue 32 in CHEB\(^{\text{FS}}\) (Tyr to Phe) was more exposed to the solvent compared to 02/O12, TREF\(^{\text{FS}}\) and TRO\(^{\text{FS}}\); (iii) mutation in CDR3 also induced a change in the solvent-excluded surface of CHEB\(^{\text{FS}}\), and (iv) an exposed hydrophobic region (60–63) was present in TRO\(^{\text{FS}}\). Carbohydrate (Figure 4D). The GSC\(^{\text{CSH}}\) LC showed exposed hydrophobic region interacting with Ile83 (FR3) and mutated residues Val105 and Phe106 of FR4 – a solvent-excluded surface of CHEB\(^{\text{FS}}\), which was strongly exposed to the solvent in TRO\(^{\text{FS}}\) compared to MAS\(^{\text{FS}}\) and DEL\(^{\text{FS}}\) and was rather hydrophilic, as indicated by the absence of water molecules around this residue after geometry optimization; (ii) in MAR\(^{\text{CSH}}\), two Ala residues (9 and 12) created an exposed hydrophobic region (60–63) was present in TRO\(^{\text{FS}}\), as previously reported by Deret et al. [4]; (ii) the mutated residue 32 in CHEB\(^{\text{FS}}\) (Tyr to Phe) was more exposed to the solvent compared to 02/O12, TREF\(^{\text{FS}}\) and TRO\(^{\text{FS}}\); (iii) mutation in CDR3 also induced a change in the solvent-excluded surface of CHEB\(^{\text{FS}}\), and (iv) an exposed hydrophobic region (60–63) was present in TRO\(^{\text{FS}}\).
drophobic residues Phe33 in CDR1, and Phe53 and Ile73 in CDR2 (Figure 4E and F). MOR\textsuperscript{CSH} LC displayed the same exposed hydrophobic regions as SU\textsuperscript{FS}– and VAL\textsuperscript{FS}– LCs (Figure 4E and F), but after their superimposition, a slight difference in the deviation angle of residue 95 was observed (not shown).

Protease treatment

In patients with CSH/FS, trypsin digestion showed the generation of resistant fragments after 6 h (Figure 5). This fragment was about 12 kDa for MAR\textsuperscript{CSH}, and 8 kDa for MOR\textsuperscript{CSH}. Two fragments were observed for GSC\textsuperscript{CSH} after 3 h (8 and 12 kDa), but only one (8 kDa) was resistant after 6 h. The control BON \kappa LC did not generate any fragment resistant to trypsin.

Discussion

Renal involvement in CSH is poorly documented. Lebeau et al. reported renal disease in six cases out of 60 reviewed [8], often diagnosed post-mortem [17–20]. Few patients with CSH and renal manifestations were further reported. As in the present cases, interstitial lesions predominated with infiltration by mononuclear cells and crystal-containing histiocytes, accompanied by interstitial fibrosis and tubular atrophy [10,21,22]. Because PPGC represent a minor component of infiltrates, renal involvement in CSH may be misdiagnosed as immuno-allergic interstitial nephritis as in GSC\textsuperscript{CSH}. The observation of numerous PPGC in proximal tubules, including hypertrophy of few epithelial cells filled with electron-dense crystalline inclusions, contrasting with atrophy of the adjacent tubular cells [30]. Diagnosis of proximal tubular LC inclusions is challenging, since in the present cases, they were not detected by conventional IF but by immunohistochemistry after antigen activation through oven heating. Other techniques may be required, including IF on pronase-digested tissue [33,34] or immunoEM [22,35]. Pathological findings in CSH/FS are reminiscent of those in classical myeloma-associated FS, where more numerous clarified proximal tubular cells contain \kappa LCs inclusions [36]. However, some characteristics of tubular involvement in CSH appear to be distinct. Free Ig crystals within distal tubule lumens, sometimes described in myeloma-associated FS, were neither observed in the previous nor in the present cases of CSH/FS. In myeloma-associated FS, EM commonly reveals intra-cytoplasmic hexagonal or diamond-shaped crystals, with a lattice-like structure [6,36,37] (Figure 2A and B). By contrast, Ig inclusions in CSH/FS are rarely reported in patients with LC-associated proximal tubulopathy [23,28,29]. A microtubular or fibrillary amyloid-like appearance of crystalline inclusions, similar to that found in patient MOR\textsuperscript{CSH}, has been rarely reported in patients with LC-associated proximal tubulopathy [33,38]. Our results suggest that differences in the ultrastructural patterns of inclusions in CSH/FS and myeloma-associated FS result from distinct structures of monoclonal \kappa LCs.

The mechanisms of LCs crystallization remain unclear. The isoelectric point may influence patterns of tissue depo-
For example, monoclonal κ LCs in non-amyloid LC deposition disease are characterized by high pI [39], whereas amyloidogenic LCs display lower pI [40,41]. In the present CSH/FS cases, crystallization appeared independent of the pI as in myeloma-associated FS κ LCs [5]. Previous studies showed that specific mutations are strongly involved in crystallization in myeloma-associated FS, where substitution of a polar residue by a hydrophobic residue at CDR1 position 30 was found in CHEB<sup>FS+</sup>, TRE<sup>FS+</sup> and TRO<sup>FS+</sup>. In CHEB<sup>FS+</sup>, substitution of Ala 30 by a polar amino acid resulted in the inhibition of crystallization within proximal tubules in a mouse model [42]. LC sequences from our patients were compared with the corresponding germline sequences and to FS κ LCs from patients with or without crystalline inclusions. The most striking feature of GSC<sup>CSH</sup> and MAR<sup>CSH</sup> sequences was the substitution of polar residues.

Fig. 4. Solvent-excluded surfaces of the following κ LCs: (A) and (B) CHEB<sup>FS+</sup>, TRE<sup>FS+</sup>, and TRO<sup>FS+</sup> compared to the corresponding germline sequence (O2/O12) for two different orientations; (C) and (D) MAR<sup>CSH</sup>, MAS<sup>FS−</sup>, and DEL<sup>FS−</sup> compared to the corresponding germline sequence (O8/O18) for two different orientations; (E) and (F) GSC<sup>CSH</sup>, MOR<sup>CSH</sup>, SU<sup>FS−</sup>, and VAL<sup>FS−</sup> for two different orientations. Hydrophobic amino acids are shown in red, and hydrophilic amino acids are blue.
due to hydrophobic amino acids. Molecular modelling showed significant changes in the surface of MAR\textsuperscript{\kappa}H with large hydrophobic solvent-exposed zones which may promote aggregation and crystallization. CSH/FS patients with V\textsubscript{\kappa}\textsuperscript{III} subgroup LCs (GSC\textsuperscript{\kappa}H and MOR\textsuperscript{\kappa}H) presented with crystalline inclusions. In monoclonal LC-associated FS, only two V\textsubscript{\kappa}\textsuperscript{III} LCs (VAL\textsuperscript{\kappa}S and SU\textsuperscript{\kappa}S) without crystals have been extensively studied [4,7]. When compared with SU\textsuperscript{\kappa}S- and VAL\textsuperscript{\kappa}S-LCs, only GSC\textsuperscript{\kappa}H LCs showed mutations with hydrophobic zones exposed to solvent. No hydrophobic mutations were observed for MOR\textsuperscript{\kappa}H but only a slight difference in the deviation angle of the residue 95 compared to SU\textsuperscript{\kappa}S- and VAL\textsuperscript{\kappa}S-LCs. However, beyond the anomalies suggested by molecular modelling, crystallization might also involve parameters such as self-affinity or interactions between protease-resistant fragments, promoting an initial aggregation as a first step towards crystallization [43,44]. All three CSH/FS κLCs were characterized by resistance of the V domain to trypsin digestion that might promote crystallization, as myeloma-associated FS LCs [4,6,7]. Proteolysis-resistant fragments of 8 and 12 kDa were observed for V\textsubscript{\kappa}\textsuperscript{III} and V\textsubscript{\kappa}\textsuperscript{I} CSH/FS κLCs, respectively, but not for control LCs. The 12-kDa fragment was described for myeloma-associated FS κLCs after trypsin or cathepsin B action [4,6,7]. This suggests that complete proteolysis of the V domains cannot occur after endocytosis in proximal tubular cells or macrophages, leading to accumulation of the V fragment within the lysosomal compartment.

In this study, pathological characteristics of CSH/FS and myeloma-associated FS suggest different mechanisms of LC crystallization. In myeloma-associated FS, crystals predominate within monoclonal bone marrow plasma cells. Few crystals are observed within histiocytes, indicating that crystallization occurs after phagocytosis of free crystals or of dying plasma cells. In CSH/FS, plasma cells contain only few cytoplasmic LC inclusions, which are predominantly found within histiocytes and epithelial cells, suggesting that Ig crystallization occurs after endocytosis and proteolysis within the endolysosomal compartment of histiocytes and epithelial cells.

In conclusion, myeloma-associated FS and CSH/FS feature crystal formation by a limited set of monoclonal Ig, most often κLC. These disorders differ by their clinicopathological presentation and by molecular peculiarities of monoclonal κLCs that probably govern location and pattern of crystal formation. Whether aggressive treatment of the underlying B-cell clone, often incident and diagnosed after in-depth bone marrow studies with sensitive techniques, might influence survival and renal prognosis remains to be determined.

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**References**


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