A myeloidosis is a group of diseases characterized by extracellular deposition of insoluble fibrils resulting from abnormal folding of proteins, leading to progressive tissue disruption and organ failure. Amyloid deposits in the kidney may arise from immunoglobulin light chains (AL amyloidosis), amyloid A, fibrinogen $\alpha$-chain, lysozyme, gelsolin, apolipoprotein A-I, apolipoprotein A-II, apolipoprotein A-IV, apolipoprotein C2, apolipoprotein C3, transthyretin and leukocyte chemotactic factor 2. Fibrinogen $\alpha$-chain (AFib) amyloidosis is an autosomal-dominant hereditary systemic amyloidosis caused by the deposition of amyloid fibrils comprising fibrinogen $\alpha$-chain variants induced by mutations in fibrinogen $\alpha$-chain gene ($FGA$). Patients with AFib amyloidosis present with renal disease and typically progress to end-stage renal disease.

Here we report a fibrinogen $\alpha$-chain amyloidosis family with a novel $FGA$ mutation, which was identified by DNA sequencing and mass spectrometry (MS). We also review the literature and discuss the diagnosis and treatments of AFib amyloidosis. To our knowledge, this is the first report of fibrinogen $\alpha$-chain amyloidosis with the p.Lys558Argfs*10 variant in a Chinese family.

**CASE PRESENTATION**

A 33-year-old Chinese women presenting with proteinuria and bilateral lower limb edema for 1-month duration was admitted into hospital in November 2018. She had no complaints of fever, dyspnea, skin rashes, arthralgia, or gastrointestinal symptoms. Past medical history was negative. She denied tobacco use or alcoholism. Physical examination revealed blood pressure 110/70 mm Hg, temperature 37°C, and pulse rate 68 bpm, pitting edema of bilateral lower limb, but no rash, lymphadenopathy, organomegaly, or peripheral neuropathy. Laboratory evaluation showed nephritic range of proteinuria (2230–2870 mg/24 hours) and microscopic hematuria (+). Serum creatinine (0.75 mg/dl) and estimated glomerular filtration rate of (105 ml/min/1.73 m²) were within normal range. Serum albumin was 29g/l. Lipid panel screen showed hypercholesterolemia (total cholesterol 7.95 mmol/l, LDL-C 5.76 mmol/l) with plasma levels of total triglyceride, high-density lipoprotein cholesterol and Apolipoprotein A-I within normal range. Liver function, myocardial enzyme, serum complements were within normal range. Hepatitis, HIV, and syphilis tests were negative. Immunology tests (double-stranded DNA, antinuclear antibody, antineutrophil cytoplasmic antibody, antistreptolysin O, and rheumatoid factor) were normal. Computed tomography scan of the lung, ultrasonic cardiogram, and ultrasound of abdominal organs were normal. There was no evidence of a plasma cell disorder according to sensitive serum free light chain assay, serum protein electrophoresis, and immune fixation electrophoresis.

Renal biopsy was performed and 3 strips of renal cortex containing 39 glomeruli were seen under light microscope. Extensive homogeneous and periodic acid-Schiff–positive stained material was present in glomerular mesangium and subendothelium. These deposits produced apple green birefringence when stained with Congo red and viewed under polarized...
Focal tubular atrophy and mild infiltration of lymphocytes and monocytes without interstitial fibrosis were seen. Arteriolar walls were unaffected. There was no amyloid within the tubules, interstitium, or vessels. Electron microscopy showed massive amorphous deposits with medium to low electron densities in the mesangium, and with a higher power, unbranched fibrils with a diameter of 8 to 12 nm were viewed (Figure 1). Routine immunofluorescence showed nonspecific adhesion of immunoglobulins, complements, and light chains (Supplementary Figure S1).

Since routine kidney biopsy tests showed non–AL amyloidosis, we asked the patient’s family history in detail and found a complicated family history of kidney disease (Figure 2). Her mother (Figure 2 III-2) and cousin (Figure 2 IV-2) had a history of renal amyloidosis and was now receiving maintenance dialysis for uremia. Her grandmother (Figure 2 II-1) and great-grandfather (Figure 2 I-1) died of uremia years earlier. To identify amyloid typing, immunohistochemical analysis of the specimen was carried out and showed strong positive staining of fibrinogen in glomeruli (Figure 1f). Immunohistochemical analysis with antibodies against \( \lambda \)-light chain, \( \kappa \)-light chain, AA amyloid, lysozyme, transthyretin and gelsolin, apolipoprotein A-I, and LECT2 were negative. Genetic analysis of the patient and her parents furthermore revealed a novel frameshift mutation p.Lys558Argfs*10 of FGA gene in our patient and her mother (Figure 3a), resulting from a deletion of an adenine nucleotide (c.1673delA). The new reading frame created by the deletion predicted the premature termination of the protein 10 amino acids downstream from the site of...

Figure 1. Renal biopsy findings of the proband. (a) Massive homogeneous and lightly stained deposits were found in glomeruli (periodic acid-Schiff [PAS] × 200). (b) The amyloid deposits showed PAS positive staining in glomerular mesangium (PAS-Methenamine × 400). (c,d) Positive Congo red staining in glomeruli (Congo red × 200, polarized light). (e) Unbranched fibrils with a diameter of 8 to 12 nm under electron microscopy. (f) Immunohistochemical revealed positive staining for fibrinogen in the glomerular amyloid deposits (×400)
There was no mutation in other genes known to be associated with renal amyloidosis including APOA1, APOA2, and LYZ. MS-based proteomic analysis was run and confirmed the deposits in glomeruli were mutant fibrinogen alpha chain (Figure 3b,c).

Our patient was diagnosed hereditary fibrinogen Aα-chain amyloidosis. She received valsartan 80 mg/day and tripterygium glycosides 10 mg twice a day for treatment. Although the patient’s proteinuria decreased to minimum of 1.11 g/24 hours after 8 months, she developed nephrotic range of proteinuria after we stopped using tripterygium glycosides. Her serum creatinine gradually increased to 3.76 mg/dl at 28-month follow-up (Supplementary Figure S2). She remains on the active waiting list for combined liver and kidney transplantation.

**DISCUSSION**

Fibrinogen is a 340-kDa soluble glycoprotein produced by hepatocyte which plays a crucial role in blood coagulation cascade. Hereditary fibrinogen Aα-chain amyloidosis, first described in 1993 by Benson, is a rare autosomal-dominant inherited disorder resulting from a mutation of FGA gene. The sequences and main clinical manifestations of 16 FGA mutations identified to date are summarized in Table 1. Our case is the first report of p.Lys558Argfs*10 variant in a Chinese family.

Diagnosis of AFib amyloidosis is based on the occurrence of proteinuria, positive family history, and identification of amyloid deposits in affected tissues. Unlike AL amyloidosis and lysozyme amyloidosis, interstitium and vessels are usually free of amyloid accumulation in AFib amyloidosis. Immunofluorescence studies are negative. However, nonspecific smudgy glomerular staining of immunoglobulins can be present because amyloid tends to be made up of sticky proteins, just like our case. In such cases, immunohistochemical staining, MS, and genetic sequencing should be used to confirm amyloid typing. Laser capture microdissection of affected areas followed by tandem MS has become a useful technique for typing of amyloidosis in recent years. However, a major limitation of MS-based proteomics is its dependence on a well-curated database. Previously unreported variants of fibril proteins will not be identified. Some reports described that identification of the mutated fibrinogen alpha chain by MS is more difficult in the case of frameshift mutations, and a score-based algorithm has been designed for diagnosis of AFib from MS data that utilizes knowledge based on large renal amyloidosis data sets. In our case, laser capture microdissection-MS showed predominantly fibrinogen Aα chain in glomeruli, composed of 28 unique peptides including 3 mutant-specific peptides, consistent with the p.Lys558Argfs*10 mutation.
observed in gene analysis. Therefore, sequencing of the $FGA$ gene following the determination of AFib amyloidosis and then confirmation of deposition of the sequence by MS is the best way to determine the theoretical sequence and then confirm expression (Table 2).

Table 1. Gene mutations and clinical features of fibrinogen A alpha-chain amyloidosis

| Number | Protein variant | Sequence variant (mRNA) | Codon change | Clinical features | Ethnic group | Discovery time | Reference |
|--------|-----------------|-------------------------|--------------|-------------------|-------------|----------------|-----------|
| 1      | p.Arg573Leu     | c.1718G>T               | GGT>TTT     | Renal failure     | Peruvian, African American | 1993 | 2      |
| 2      | p.Glu545Val     | c.1634A>T               | GAG>GTG     | Renal failure     | Northern European | 1994 | 3      |
| 3      | p.Ala541Thr*27  | c.1622T>T               | GAA>GTA     | Frame shifting mutation | American | 1996 | 4      |
| 4      | p.Ala269Glu     | c.1626G>G               | GAG>GTA     | Frame shifting mutation | French | 1997 | 5      |
| 5      | p.Met539 >Val   | c.1606_1620delAGTG     | Frame shifting mutation | Renal failure | Korean | 2005 | 6      |
| 6      | p.Pro573His     | c.1712C>A               | CCT>CAT     | Renal failure     | Afro-Caribbean | 2009 | 7      |
| 7      | p.Ala566Val     | c.1676C>A               | GAA>GTA     | Renal failure     | German | 2009 | 7      |
| 8      | p.Ala269Glu     | c.1626T>T               | GAA>GTA     | Renal failure     | French | 1997 | 5      |
| 9      | p.Glu539Glu     | c.1611T>T               | GAG>GTA     | Renal failure     | Russian | 2017 | S1     |
| 10     | p.Glu545Lys     | c.1633C>C               | GAG>GTA     | Renal failure     | Russian | 2017 | S1     |
| 11     | p.Arg573His     | c.1718C>A               | GAA>GTA     | Renal failure     | British | 2017 | S1     |
| 12     | p.Glu574Lys     | c.1720_1721delG        | GGT>TGT     | Renal failure     | Norwegian | 2017 | S1     |

Figure 3. Amyloid typing. (a) Genetic analysis of the proband and her parents showed a deletion of adenine nucleotide resulting a novel $FGA$ mutation in our patient inherited from her mother. (b,c) Mass spectrometry–based proteomic analysis confirmed mutant fibrinogen alpha chain deposits.
Patients with AFib amyloidosis often experience rapid deterioration to end-stage renal disease and begin maintenance dialysis within 5 years. There are currently no effective treatments that can lead to resolution of amyloid deposits. For patients without cardiovascular involvement, combined kidney and liver transplantation has been performed and demonstrated to retard disease progression. In our case, during the waiting time for combined liver and kidney transplantation, supportive therapy including angiotensin receptor blocker and traditional Chinese medicine showed temporary reduction of proteinuria but uncontrolled kidney function loss.

In summary, we report a fibrinogen Aα-chain amyloidosis Chinese family presenting with kidney failure, associated with a novel FGA mutation variant, which was identified by MS combined with DNA sequencing.

**DISCLOSURE**

All the authors declared no competing interests.

**PATIENT CONSENT**

The authors declare that they have obtained consent from the patients discussed in the report.

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**REFERENCES**

1. Straub PW. A study of fibrinogen production by human liver slices in vitro by an immunoprecipitin method. J Clin Invest. 1963;42:130–136.
2. Benson MD, Liepnieks J, Uemichi T, et al. Hereditary renal amyloidosis associated with a mutant fibrinogen alpha-chain. Nat Genet. 1993;3:252–255.
3. Uemichi T, Liepnieks JJ, Benson MD. Hereditary renal amyloidosis with a novel variant fibrinogen. J Clin Invest. 1994;93:731–736.
4. Uemichi T, Liepnieks JJ, Yamada T, et al. A frame shift mutation in the fibrinogen A alpha chain gene in a kindred with renal amyloidosis. Blood. 1996;87:4197–4203.
5. Hamidi L, Liepnieks JJ, Uemichi T, et al. Renal amyloidosis with a frame shift mutation in fibrinogen A alpha-chain gene producing a novel amyloid protein. Blood. 1997;90:4799–4805.
6. Kang HG, Bybee A, Ha IS, et al. Hereditary amyloidosis in early childhood associated with a novel insertion-deletion (indel) in the fibrinogen Aalpha chain gene. Kidney In. 2004;68:1994–1998.
7. Gillmore JD, Lachmann HJ, Rowczenio D, et al. Diagnosis, pathogenesis, treatment, and prognosis of hereditary fibrinogen A alpha-chain amyloidosis. J Am Soc Nephrol. 2009;20:444–451.
8. Yazaki M, Yoshinaga T, Sekijima Y, et al. The first pure form of Osterberg-type amyloidosis in Japan: a sporadic case of hereditary fibrinogen A alpha-chain amyloidosis associated with a novel frameshift variant. Amyloid. 2015;22:142–144.
9. Garnier C, Briki F, Nedelec B, et al. VLITL is a major cross-β-sheet signal for fibrinogen Aα-chain frameshift variants. Blood. 2017;130:2799–2807.