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Fibrinogen A Alpha-Chain Amyloidosis Associated With a Novel Variant in a Chinese Family

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Amyloidosis 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 Aα-chain, lysozyme, gelsolin, apolipoprotein A-I, apolipoprotein A-II, apolipoprotein A-IV, apolipoprotein C2, apolipoprotein C3, transthyretin and leukocyte chemotactic factor 2. Fibrinogen Aα-chain (AFib) amyloidosis is an autosomal-dominant hereditary systemic amyloidosis caused by the deposition of amyloid fibrils comprising fibrinogen Aα-chain variants induced by mutations in fibrinogen Aα-chain gene (*FGA*). Patients with AFib amyloidosis present with renal disease and typically progress to end-stage renal disease.

Here we report a fibrinogen Aα-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 Aα-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) m^2 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

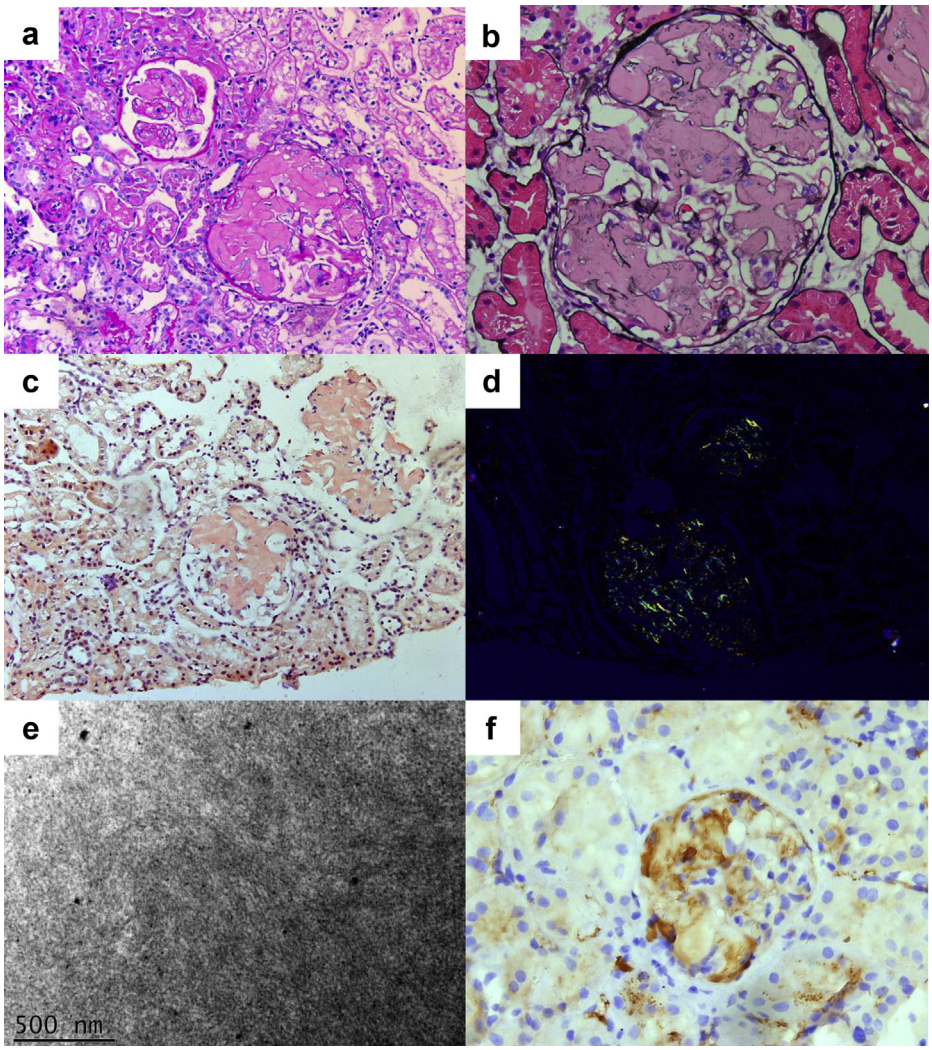

Figure 1. Renal biopsy findings of the proband. (a) Massive homogeneous and lightly stained deposits were found in glomeruli (periodic acid-Schiff [PAS] ×200). (b) The amyloid deposits showed PAS positive staining in glomerular mesangium (PAS-Methenamine ×400). (c,d) Positive Congo red staining in glomeruli (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)

light. 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 λ-light chain, κ-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

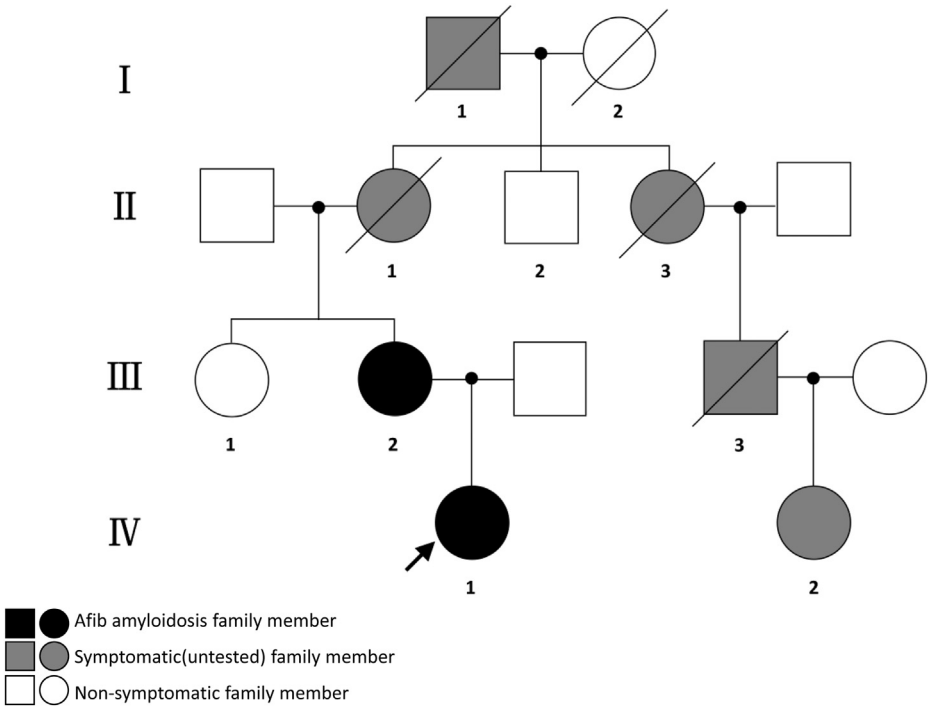

Figure 2. Family tree of the proband's paternal pedigree. Black symbols denote individuals with the *FGA* gene c.1673delA mutation, and gray symbols denote symptomatic but untested family members. The arrow denotes the proband.

mutation. 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.[1] Hereditary fibrinogen Aα-chain amyloidosis, first described in 1993 by Benson,[2] 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,[S2,S3] 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

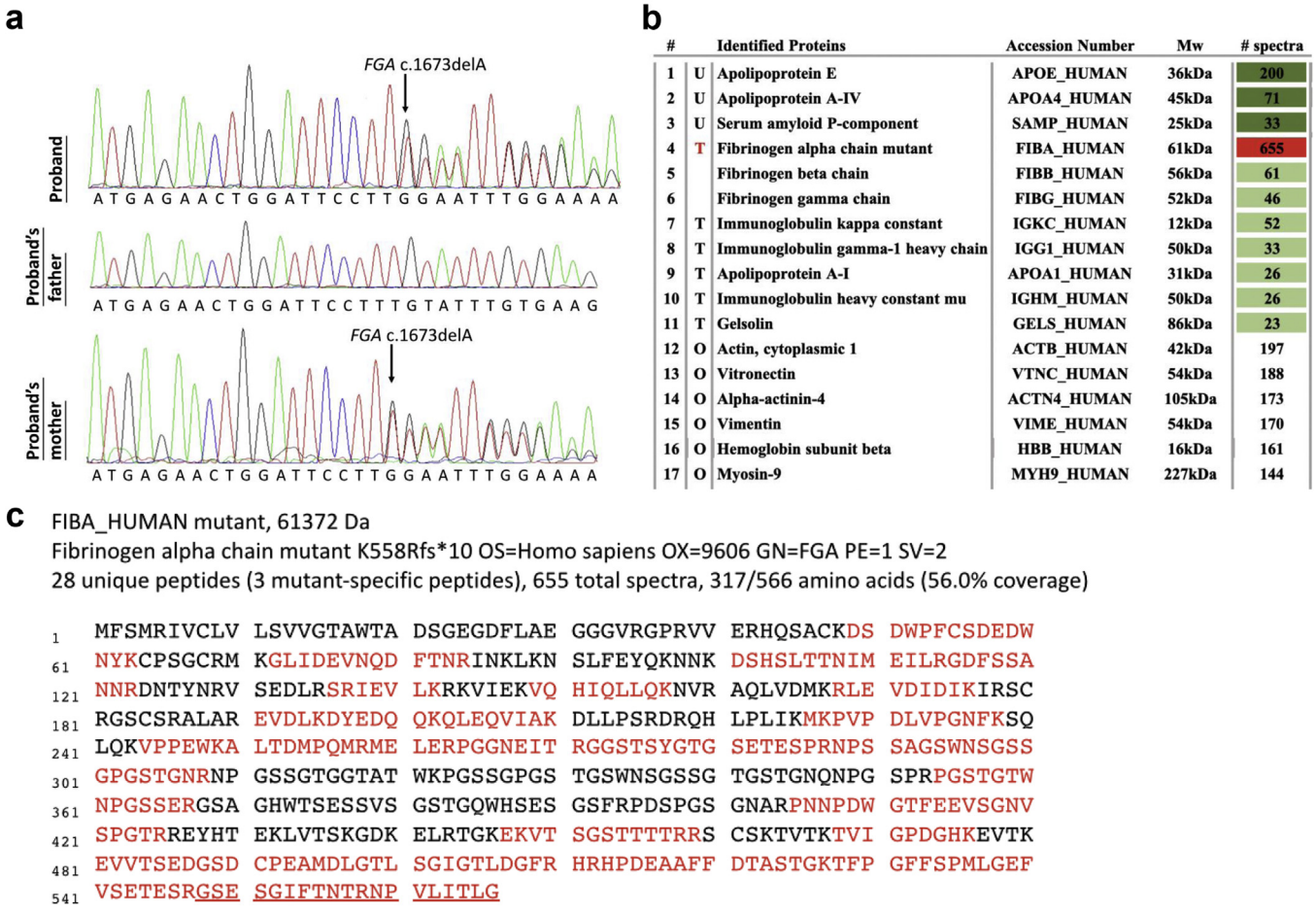

Figure 3. Amyloid typing. (a) Genetic analysis of the proband and her parents showed a deletion of adenine nucleotide resulting a novel *FGA* mutation in our patient inherited from her mother. (b,c) Mass spectrometry–based proteomic analysis confirmed mutant fibrinogen alpha chain deposits.

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 Aα-chain amyloidosis

Number	Protein variant	Sequence variant (mRNA)	Codon change	Clinical features	Ethnic group	Discovery time	Reference
1	p.Arg573Leu	c.1718G>T	CGT>CTT	Renal failure	Peruvian, African American	1993	2
2	p.Glu545Val	c.1634A>T	GAG>GTG	Renal failure	Northern European	1994	3
3	p.Glu543Glufs*25	c.1629delG	Frame shifting mutation	Renal failure	American	1996	4
4	p.Val541Alafs*27	c.1622delT	Frame shifting mutation	Renal failure	French	1997	5
5	p.Met536_Phe540 delinsGlnSerfs*28	c.1606_1620 delATGTTAGGAGAGTTTinsCA	Frame shifting mutation	Renal failure	Korean	2005	6
6	p.Thr544Thrfs*24	c.1632delT	Frame shifting mutation	Renal failure	Chinese	2009	7
7	p.Thr557Lys	c.1670C>A	ACA>AAA	Renal failure	Chinese	2009	7
8	p.Glu559Val	c.1676A>T	GAA>GTA	Renal failure	German	2009	7
9	p.Pro571His	c.1712C>A	CCT>CAT	Renal failure	Afro-Caribbean	2009	7
10	p.Ser523Argfs*25	c.1624_1627delAGTG	Frame shifting mutation	Nephropathy	Japanese	2015	8
11	p.Phe540Leufs*28	c.1620delT	Frame shifting mutation	Nephropathy	French	2017	9
12	p.Gly538Glufs*30	c.1611delA	Frame shifting mutation	Renal failure	French	2017	S1
13	p.Phe540Serfs*27	c.1619_1622delTTGT	Frame shifting mutation	Renal failure	Arab	2017	S1
14	p.Glu543Lys	c.1627G>A	GAG>AAG	Renal failure	Caucasian	2017	S1
15	p.Glu545Lys	c.1633G>A	GAG>AAG	Renal failure	Russian	2017	[S1]
16	p.Arg573His	c.1718G>A	CGT>CAT	non-amyloidogenic	British	2017	[S1]
17	p.Gly574Phe	c.1720_1721delGGinsTT	GGT>TTT	Renal failure	Norwegian	2017	[S1]

Table 2. Teaching points

Fibrinogen A alpha-chain amyloidosis is rare in Asian families. The main pathologic findings include massive amounts of amyloid deposition in the glomeruli without interstitium, vessels or medulla involved, negative routine immunofluorescence and 8- to12-nm unbranched fibrils under electron microscopy.
Clinical awareness and suspicion of hereditary amyloidosis corroborated by laser capture microdissection–mass spectrometry and genetic analysis is valuable to avoid misdiagnosis and imperative for correct management and prognosis.

Patients with AFib amyloidosis often experience rapid deterioration to end-stage renal disease and begin maintenance dialysis within 5 years.[7] There are currently no effective treatments that can lead to resolution of amyloid deposits. For patients without cardiovascular involvement, combined kidney and liver transplant has been performed and demonstrated to retard disease progression.[S4] 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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SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Supplementary material on immunostaining

Supplementary material on follow-up

Supplementary References

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