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Novel gelsolin variant as the cause of nephrotic syndrome and renal amyloidosis in a large kindred

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Abstract

Familial Amyloidosis of Finnish type (FAF) is a rare type of autosomal dominant hereditary amyloidosis associated with genetic variants of gelsolin. Three amyloidogenic mutations have previously been reported characteristically presenting with ophthalmologic abnormalities, progressive cranial neuropathy and cutis laxa. We report a novel gelsolin variant in a 62 year old man with nephrotic range proteinuria of 13.2 grams/day as the only presenting symptom. Renal biopsy followed by laser microdissection and mass spectrometry showed amyloidosis derived from gelsolin. DNA sequencing revealed the novel gelsolin mutation (c.633C>A) encoding p.N211K protein variant. Four of 13 asymptomatic family members were found to be heterozygous for the p.N211K mutation, three of whom had proteinuria of varying degree including one who proceeded to renal biopsy and was confirmed to have renal amyloidosis. Follow up of these cases might give us more insight into pathogenicity and potential treatment strategy of this atypical presentation of gelsolin amyloidosis.

Keywords

Amyloidosis; Gelsolin; Proteinuria

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Authorship contributions: YE saw patients and prepared manuscript; AS and EB took history from family, collected blood samples and prepared pedigree; CH reviewed and edited manuscript and formatted figures; AS, TN and GN read the kidney biopsy and provided biopsy slides; DB reviewed and edited manuscript; JG, PNH and DR did the direct DNA sequencing and edited manuscript.

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Introduction

Familial Amyloidosis of Finnish type (FAF) is a rare type of autosomal dominant hereditary amyloidosis associated with variant gelsolin. Thus far only three causative mutations in the *GSN* gene have been reported, two resulting from change of aspartate at position 214 to either asparagine or tyrosine (p.D214N/Y)[1-6] and one from replacing glycine with arginine at codon 194 (p.G194R)[7] (our nomenclature includes the 27 residues signal peptide omitted in the FAF literature). The distinct clinical features of FAF include cranial neuropathy, corneal lattice dystrophy, distal sensorimotor neuropathy and skin changes[1-3]. Proteinuria is typically observed in the latter stages of the disease, albeit rare cases presenting with nephrotic syndrome and end stage renal failure have been reported [4,5,6].

Here we report a family with nephrotic syndrome due to novel gelsolin variant p.N211K, causing renal amyloidosis in the index patient and his brother. All participants signed informed consent through the genetics department at the Ohio State University to have mutational analysis done.

Methods

Clinical review

The proband was a 62 year old man who presented with hemoptysis from a left upper lobe squamous cell carcinoma. His creatinine was 2.8 mg/dl (0.9-1.3) with nephrotic range proteinuria of 13.2 grams/day. Serum immunoglobulins, electrophoresis, and serum free light chains were normal. He had no cardiac, ophthalmic, neurologic or skin abnormalities on physical exam. While cardiac and thorough skin exam were done, detailed ophthalmic and neurologic exam were not done due to the urgent need to start chemotherapy for the squamous cell lung cancer. He however had no ophthalmic or neurologic complaints and per patient a routine eye exam prior to his diagnosis showed no abnormalities. Of his 6 siblings, 3 had died without any known history of amyloidosis or proteinuria. His maternal parents were from Luxemburg, Germany, and his father's ancestry was unknown.

Histology and Laser microdissection (LDM) and mass spectrometry (MS) of kidney biopsy

Sections 6 μm thick were stained for amyloid with Congo red and viewed under crossed polarized light. LMD followed by liquid chromatography and tandem MS (LC-MS/MS) was performed on the Congo Red–positive glomeruli[4].

Direct DNA sequencing

The proband and subsequently 13 asymptomatic family members were screened for mutations in exon 4 of the *GSN* gene (NCBI RefSeq: NG_012872.1). Peripheral blood was collected and Polymerase-chain-reaction assay (PCR) was carried out with HotStarTaq DNA Polymerase Kit (Qiagen Ltd, Crawley, UK) using the forward 5′-CAAGATAATGGGTATGAAAGT-3′ and reverse 5′-CTGATCAGACCAGGAGCACC-3′ primers. . The PCR products were purified with a QIAquick PCR purification kit (Oiagen, Velno, The Netherlands) according to the manufacturer's protocol and sequenced with the ABI BigDye Terminator v 3.1 Ready Reaction Cycle Sequencing kit (Applied Biosystems,

Foster City, CA). Sequence of the *GSN* gene was analysed on the ABI 3130xl Genetic Analyser, using Sequencing Analysis Software version 5.4 (Figure 1B).

Results

The kidney demonstrated extensive birefringent amyloid deposits primarily in the glomeruli (figure 1A) with immunohistochemical staining negative for immunoglobulin light chain, fibrinogen, lysozyme and transthyretin.

LMD and LC-MS/MS showed spectra matching gelsolin, apolipoprotein E, apolipoprotein-IV and serum amyloid P component. Furthermore, using this technique we were able to demonstrate presence of the N211K variant gelsolin in the amyloid deposits (Figure 1C), which was also confirmed by direct DNA sequencing (Figure 1B).

Four of the 13 family members who underwent genetic screening were heterozygous for the p.N211K (brother, sister, niece, and son). Three of these individuals had proteinuria, his brother with 6700mg/day, sister 2600mg/day and son 454 mg/day (Figure 2). All had normal serum creatinine concentration, and none had abnormal cardiac, ophthalmic or neurologic findings on physical exam. The brother with substantial proteinuria underwent kidney biopsy which in conjunction with LMD and LC-MS/MS demonstrated gelsolin amyloid deposition.

Discussion

Gelsolin amyloidosis is a rare type of autosomal dominant hereditary amyloidosis, the most common mutations resulting from nucleotide substitutions at positions G654A and G654T. Clinical presentation does not usually include renal amyloidosis although this has been reported in homozygous D214N (D187N) patients : a patient from the Middle East reported by Ardalan *et al who presented with nephrotic range proteinuria with normal renal function* and two sisters who presented with severe nephrotic syndrome and end stage renal failure described by Maury *et al*[5,6]. These patients also did have the usual presenting ophthalmic and skin findings. Patients homozygous for this mutation tend to have severe nephrotic range proteinuria in the later stages of the disease, leading to end stage failure, whereas renal failure is uncommon in the heterozygous form[1]. A recently reported case of a 75-year-old woman who presented with progressive kidney failure and was found to have amyloidosis due to novel gelsolin G194R variant [7] was associated with minimal proteinuria (133 mg/day). In contrast, substantial proteinuria was the principal clinical abnormality in the patients with gelsolin N211K we report here, suggesting that the precise microscopic site of amyloid deposition may differ.

This is the first report of a large kindred with a novel gelsolin variant p.N211K associated with a solely renal phenotype, which compellingly emphasizes the application and importance of LC-MS/MS and corroborative direct DNA sequencing utility for making a precise diagnosis.

To date, the treatment for most kinds of hereditary amyloidosis is limited to transplantation of affected organs. Renal transplantation is a potential treatment for members of the current

family should they develop endstage renal failure, although the course and natural history of the disease remains unknown at the present time. Continued follow up and further studies of family members will provide more insight into the pathogenesis and spectrum of clinical disease and its potential treatment.

Acknowledgments

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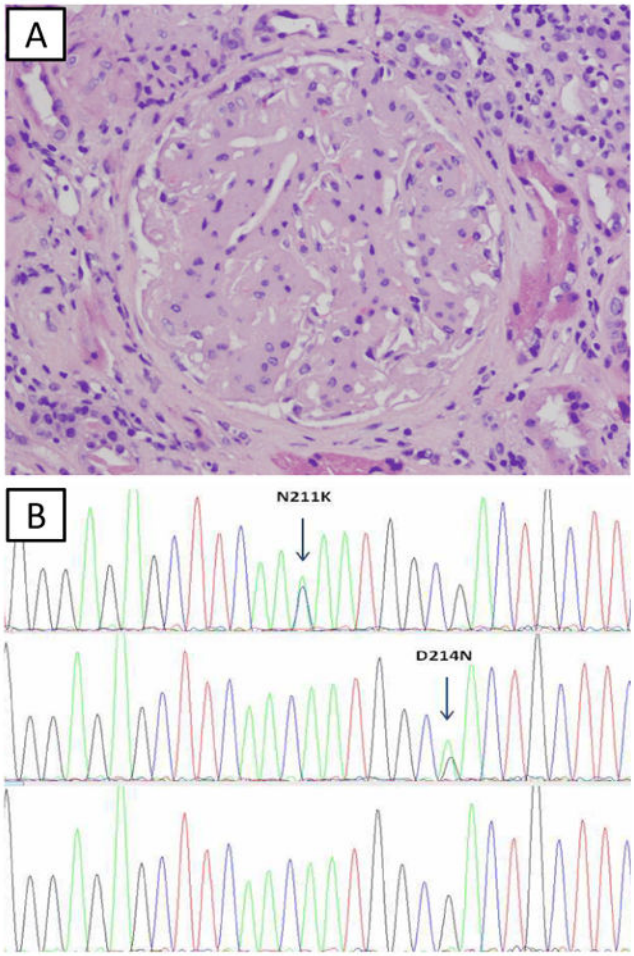
Abbreviations

FAF	Familial Amyloidosis of Finnish type
LDM	Laser microdissection
MS	Mass spectrometry
(LC-MS/MS)	Liquid chromatography and tandem mass spectroscopy



C

Probability Legend:
over 95%
80% to 94%
50% to 79%
20% to 49%
0% to 19%

#	Starred?	Identified Proteins (375)	Accession Number	Molecular Weight	C	1	2	3
1	☆	Apolipoprotein E OS=Homo sapie...	APOE_HUM...	36 kDa		64	61	70
2	☆	Gelsolin OS=Homo sapiens GN=G...	GELS_HUMAN	86 kDa		24	22	32
3	☆	Serum amyloid P-component OS=...	SAMP_HUM...	25 kDa		25	29	32
4		Complement C3 OS=Homo sapien...	CO3_HUMAN	187 kDa		22	17	23
5		Actin, cytoplasmic 1 OS=Homo sa...	ACTB_HUM...	42 kDa		19	20	22
6		Actin, aortic smooth muscle OS=...	ACTA_HUM...	42 kDa		20	24	20
7		Laminin subunit beta-2 OS=Homo...	LAMB2_HU...	196 kDa		8	11	17
8		Serum albumin OS=Homo sapiens...	ALBU_HUM...	69 kDa		18	25	16
9		Collagen alpha-3(VI) chain OS=Ho...	CO6A3_HU...	344 kDa		6	6	16
10		Basement membrane-specific he...	PGBM_HUM...	469 kDa		10	11	15
11		Laminin subunit alpha-5 OS=Hom...	LAMA5_HU...	400 kDa		3	15	15
12		Vimentin OS=Homo sapiens GN=V...	VIME_HUM...	54 kDa		10	9	14
13		Vitronectin OS=Homo sapiens GN...	VTNC_HUM...	54 kDa		13	19	14
14		Complement factor H-related pro...	FHR1_HUM...	38 kDa		10	14	12
15		Hemoglobin subunit alpha OS=Ho...	HBA_HUMAN	15 kDa		9	6	7
16		Collagen alpha-2(I) chain OS=Ho...	CO1A2_HU...	129 kDa		4	8	7
17	☆	Apolipoprotein A-IV OS=Homo sa...	APOA4_HU...	45 kDa		4	6	7
18		Clusterin OS=Homo sapiens GN=C...	CLUS_HUM...	52 kDa		8	9	7
19		Putative trypsin-6 OS=Homo sapi...	TRY6_HUM...	27 kDa		6	6	7
20		Laminin subunit gamma-1 OS=Ho...	LAMC1_HU...	178 kDa		6	6	6
21		Complement component C9 OS=H...	CO9_HUMAN	63 kDa		8	6	6
22		Collagen alpha-2(IV) chain OS=Ho...	CO4A2_HU...	168 kDa		8	10	6
23		Collagen alpha-1(I) chain OS=Ho...	CO1A1_HU...	139 kDa		4	6	6
24	☆	Gelsolin N211K Mutation	GELS_N211...	4 kDa		2	4	6
25		Hemoglobin subunit beta OS=Ho...	HBB_HUMAN	16 kDa		8	4	5
26		Complement factor H-related pro...	FHR5_HUM...	64 kDa		8	2	5

Figure 1.

A: An Hematoxylin & Eosins stains of the kidney biopsy showing diffuse glomerula deposition of eosinophilic material. **B:** Partial DNA sequences of exons 4 of the GSN gene: Top row shows N211K novel mutation indicated by an arrow, the middle shows the D214N variant, and the bottom row shows the corresponding wild-type sequence. **C:** Results of mass spectrometry based proteomic analysis of amyloid deposits. The most abundant proteins identified are listed. The numbers indicate number of total peptide spectra identified for each protein. Results confirm mutant *GSN* as the amyloid fibril protein.

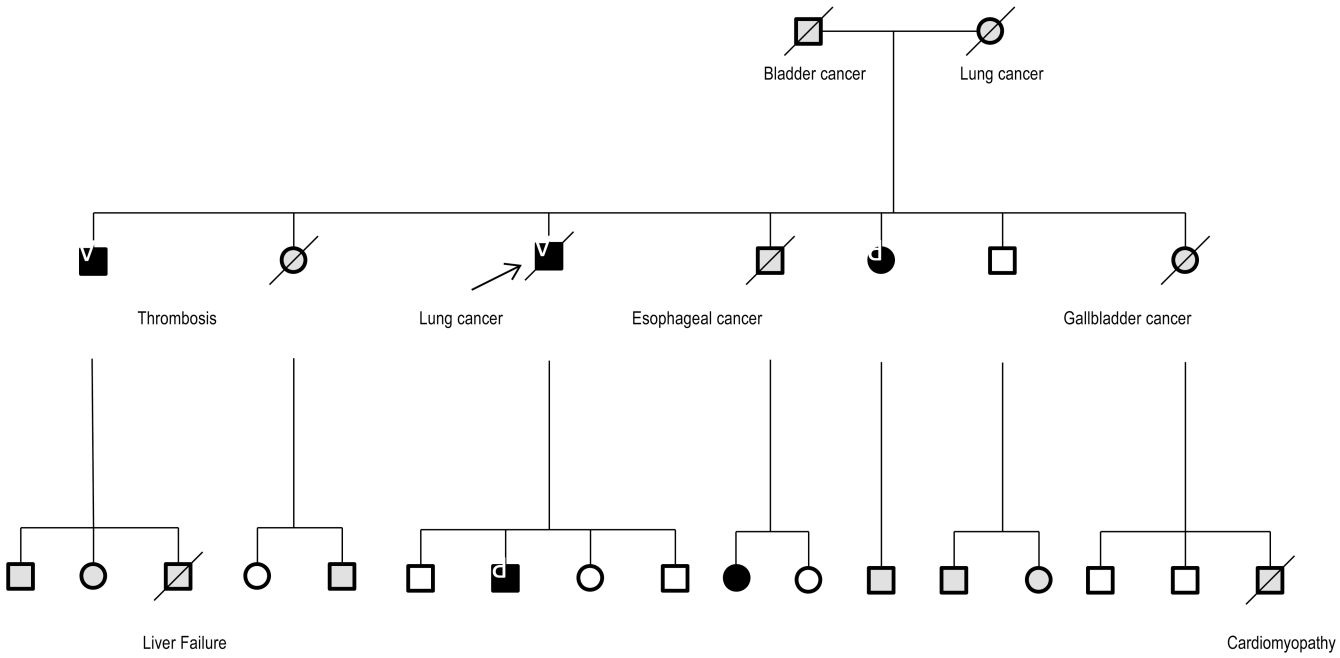


Figure 2.

Family pedigree: The patient is represented by the arrow; male family members are represented by squares, females by circles; deceased members by oblique lines; members tested positive for the mutation by solid symbols, proven amyloidosis by A insertion. asymptomatic affected members with proteinuria by P insertion; unaffected members by white symbols, members not tested by grey symbols. Ca means cancer and CMP means cardiomyopathy.