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The molecular basis of renal amyloidosis in Irish–American and Polish–Canadian kindreds

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Summary

Hereditary amyloidosis of an unusual form has been reported in two separate kindreds; one was Polish–Canadian and the other was Irish–American (*Am J Med* 1975; 59: 121 and *Trans Assoc Am Physicians* 1981; 94: 211). In both kindreds, affected members developed hypertension and nephrotic syndrome due to amyloidosis in their forties or fifties, but the genetic background responsible for the condition has been left undetermined. To identify the genetic defect in these kindreds, a portion of exon 5 of the fibrinogen α-chain gene in members of these kindreds was examined for a mutation by single-strand conformation polymorphism analysis and direct DNA sequencing. DNA analyses revealed an A→T transversion at the second base of codon 526 of the fibrinogen α-chain gene in both of these kindreds. Analysis of DNA polymorphisms in the fibrinogen α-chain gene locus (TCTT repeat in intron 3, *Rsal* site in exon 5, and *Taql* site in the 3′ flanking region of the gene) showed the haplotype B5-*Rsal*(+)-*Taql*(−) for the Val 526 mutant gene in both kindreds studied here, as well as in two kindreds previously described (*J Clin Invest* 1994; 93: 731). The fibrinogen α-chain gene mutation (Val 526) is the genetic defect responsible for hereditary renal amyloidosis in these two kindreds, and the mutant genes in the Val 526 kindreds may have been derived from a single founder.

Introduction

A peculiar form of amyloidosis was reported in two separate kindreds in 1975 and in 1981. A Canadian kindred of Polish origin with renal amyloidosis was reported by Alexander and Atkins.[1] Three siblings developed hypertension and nephrotic syndrome at the ages of 42, 52 and 58, and died at 49, 55 and 60 years of age. Amyloid deposition was found in kidneys, spleen, and adrenals. Mornaghi *et al.* described three Irish–American siblings who developed renal amyloidosis at the ages of 49, 52 and 55.[2,3] These patients also had anaemia and hypertension. Although amyloidosis in these kindreds seemed to be inherited in an autosomal dominant fashion, the clinical manifestation was quite different from familial amyloidosis related to transthyretin, the most common type of hereditary amyloidosis, where heart and/or peripheral nerves are predominantly affected.[4] The disease in these kindreds was similar to that described in a German kindred by Ostertag in 1932 and 1950.[5,6] The genetic defect associated with the disease in these kindreds had been left unknown.

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We examined a part of the fibrinogen α-chain gene in members of these kindreds by single-strand conformation polymorphism and direct DNA sequencing. Using three polymorphic markers in the fibrinogen α-chain gene locus (TCTT repeat in intron 3,[7] *Rsal* site in exon 5,[8] and *Taql* site in the 3′ flanking region of the gene[9]), the genetic linkage between these kindreds was also examined.

Methods

Identification of a mutation in the fibrinogen α-chain gene

DNA isolation

Genomic DNA was isolated from peripheral blood cells or formalin-fixed paraffin-embedded tissue specimens of seven members from the Polish–Canadian kindred[1] (family S) and six members from the Irish–American kindred[2,3](family L) by conventional methods.[10]

Single-strand conformation polymorphism (SSCP) analysis

The fibrinogen α-chain gene was examined for a mutation by SSCP analysis according to the method of Orita *et al.* with some modifications as described previously.[11,12] A part of exon 5 of the fibrinogen α-chain gene (nucleotides 4832–5051[13]) was enzymically amplified by the polymerase chain reaction (PCR) using oligonucleotide primers (Fib3 5′-CTTCGACACTGCCTCAACTG-3′ and Fib2 5′-TCCTCTGTTGTAACTCGTGCT-3′), GeneAmp PCR reagents (Perkin-Elmer Cetus), and α^{32}P dCTP. DNAs from individuals with fibrinogen α-chain gene mutations (Leu 554,[14] Val 526,[12] 4904delG[15]) as well as normal subjects, were used as controls. PCR used 30 cycles of denaturing at 94 °C for 30 s, annealing at 62 °C for 30 s, and extension at 72 °C for 60 s. PCR products were diluted with 100 vol of buffer containing 50% formamide, 0.05% SDS, 0.02% xylene cyanol FF, 0.02% bromophenol blue, and 10 mM EDTA, heated at 95 °C for 5 min, and loaded onto a non-denaturing polyacrylamide gel (5%T, 2%C, 40 × 20 × 0.04 cm). The gel was electrophoresed at 4 °C for 16 h at 3W, dried, and exposed to Kodak X-Omat film.

Direct DNA sequence analysis

DNA was examined by the direct DNA sequence method described previously.[12] PCR was done as described above, only without radiolabelled nucleotides. PCR product (10 μl) was electrophoresed through 3% Nusieve GTG agarose gel (FMC BioProducts). The 220 bp band was cut out and melted in 300 μl Tris-EDTA buffer. Asymmetric PCR was done in 100 μl total volume using 10 ng Fib3 primer, 300 ng Fib2 primer, and 1 μl agarose-gel-purified template DNA. The sample was extracted with chloroform, denatured, and spin-dialysed with a Centricon-30 microconcentrator (Amicon). Of the retentate, 7 μl were used for dideoxynucleotide termination DNA sequencing reaction by Sequenase Version 2.0 (US Biochemicals) according to the manufacturer's protocol, and DNAs were electrophoresed through an 8% denaturing polyacrylamide gel at 1800 V for 3 h.

Restriction-fragment-length polymorphism (RFLP) analysis for the detection of the Val 526 mutation

DNA samples from family members were also examined by the PCR-induced mutation restriction analysis. A Fib4 primer (5′-CAGATTCTGAGCCCCTAGTC-3′), designed to give a *Mae*III recognition site for the PCR product from the mutant gene only, was used for amplification with Fib3 primer using the conditions described above. PCR-amplified DNA fragments were then digested with *Mae*III (Boehringer Mannheim Biochemicals) at 55 °C overnight, electrophoresed through a 3% Nusieve GTG agarose gel, stained with ethidium bromide, and visualized by UV light.

Haplotype analysis of the fibrinogen α-chain gene

DNA microsatellite analysis

The TCTT repeat region in intron 3 of the fibrinogen α-chain gene was amplified by PCR using FMS-1 primer (5′-AATTAGGCATATTTACAAGCTAG-3′) and FMS-2 primer (5′-GATTTGTCTGTAATTGCCAGC-3′) directly incorporating α^{32}P dCTP. The PCR conditions were 30 cycles of 94 °C for 30 s, 62 °C for 30 s, and 72 °C for 60 s. Samples were then electrophoresed through a 5% polyacrylamide denaturing gel. The size of each DNA fragment was determined by direct DNA sequencing.

Rsal polymorphism

Primers FR-1 (5′-GGAAGGCATTAACAGACATG-3′) and FR-2 (5′-TACTTCCAGTTCCAGAGCTC-3′) were used to amplify a part of exon 5 of the gene. PCR was carried out in 20 μl total volume as described above (94 °C for 30 s, 62 °C for 30 s, and 72 °C for 60 s). Five units of *Rsal* (Life Technologies) were added directly to the PCR tubes and incubated at 37 °C overnight. DNA fragments were separated by electrophoresis through a 3% Nusieve GTG agarose gel and visualized by ethidium bromide staining and UV radiation.

TaqI polymorphism

The 3′ flanking region of the fibrinogen α gene was PCR-amplified using FT-1 primer (5′CTTCAGG-TACCCAAAGTGGCA-3′) and FT-2 primer (5′-TGT-CTCAGGTACATTTAGC-3′). The PCR conditions consisted of 40 cycles of a denaturing step of 94 °C for 30 s, an annealing step of 50 °C for 30 s, and an extension step of 72 °C for 120 s. Five units of *TaqI* (Life Technologies) were directly added to 20 μl PCR product and incubated at 65 °C overnight. DNA fragments were examined by 1.5% agarose gel electrophoresis.

To identify the nucleotide sequence variation determining the *TaqI* polymorphism, the region containing the *TaqI* polymorphism was amplified using FT-2 primer and FT-3 primer (5′-CCCTCAGGG-CTGTTCGCATG-3′) from individuals with or without the *TaqI* (+) allele and sequenced as described above.

Results

SSCP analysis revealed an abnormally migrating band for members of family S and family L, which was similar to the band seen in a patient with the fibrinogen α-chain Val 526 mutation (Figure 1).

Direct DNA sequencing of PCR products from these individuals showed both adenine and thymine at position 4909 of the fibrinogen α-chain gene. They were thus heterozygous, with one normal GAG (glutamic acid) and one variant GTG (valine) codon at amino acid position 526 of the fibrinogen α-chain.[16]

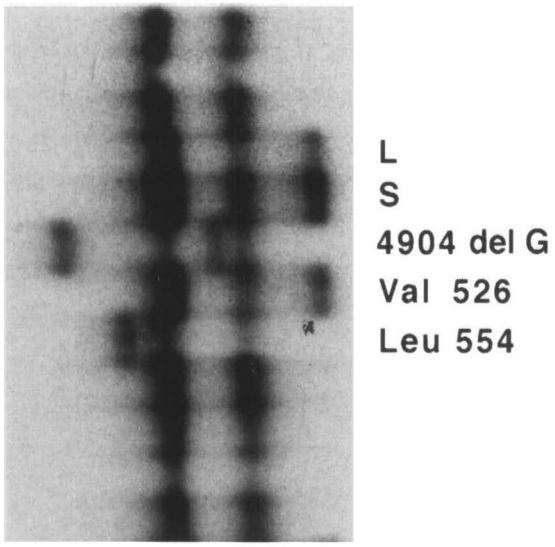

Figure 1. Autoradiography of an SSCP gel. Lanes S and L; a member of family S and a member of family L, respectively. Lanes Leu 554, Val 526, and 4904delG; the fibrinogen α-chain gene mutation controls. Other lanes; normal controls.

PCR-RFLP analysis revealed that five members of family S and two members of family L had the *Mae*III recognition site associated with the Val 526 mutation and therefore had a digestion band of 76 bp, while other individuals showed a normal band of 98 bp.

DNA microsatellite analysis showed that the Val 526 allele segregated with a DNA band of an identical size in all four kindreds (Figure 2). Sequence analysis revealed the DNA fragment to be 144 bp long, which corresponded to the B5 allele reported by Mills *et al.*[7]

RFLP analyses showed the Val 526 mutant gene to have an *RsaI* but not a *TaqI* site. The DNA sequence of the *TaqI*(−) allele was identical to that published previously.[17] Sequence analysis of the *TaqI*(+) allele showed that an extra 28 bp of the normal sequence (GAAGTGGGAATGGGAGCACT-CTGTCTTC, nucleotide position 3312–3339[17]) was inserted as a tandem repeat in the 3′flanking region of the gene, which created the *TaqI* site (TCGA) at the junction of the repetitive sequence. This insertion was not present in the Val 526 allele.

Discussion

In this study, we demonstrated a mutant fibrinogen α-chain gene (Val 526) in two kindreds with renal amyloidosis. This mutation was previously shown to be associated with the condition in two other kindreds.[12] Since DNAs available in this study were mostly from asymptomatic individuals, we could not confirm the segregation of the mutation with the disease. However the fact that the mutation causes the disease is supported by (i) identification of this portion of the fibrinogen α-chain with an amino acid substitution as the amyloid subunit in a patient with renal amyloidosis,[14] (ii) the segregation of the mutation with the disease in two kindreds previously described,[12] and (iii) a mutation in this part of the gene is not a common polymorphism, according to screening of the American population by SSCP.[15]

Identification of the variant fibrinogen as the amyloid precursor protein in these kindreds is important for therapy for the condition as well as for diagnosis of the disease. Since fibrinogen is mainly produced in the liver and circulates in plasma, patients with this type of amyloidosis should be considered as candidates for not only kidney but also liver transplantation, the latter of which could be a curative therapy.

Three fibrinogen α-chain mutations are known to be related to hereditary amyloidosis with autosomal dominant inheritance. A mutation (Leu 554) was found in a Peruvian–Mexican kindred with renal amyloidosis.[14] Two members of the kindred died of renal failure at the ages of 24 and 28. One patient

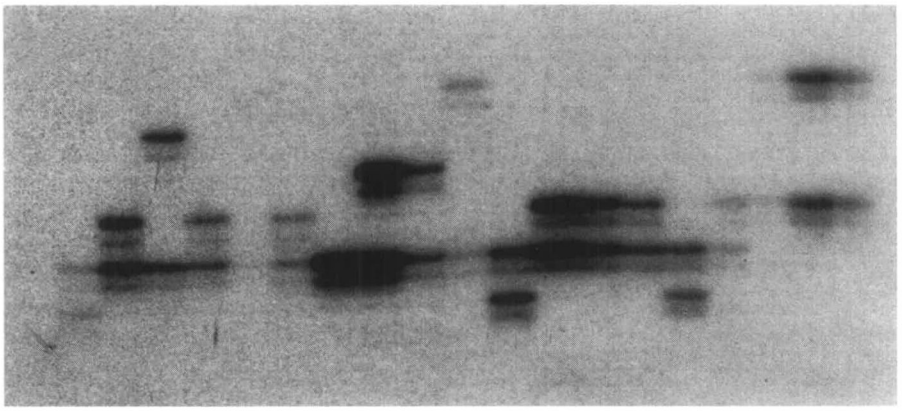

Figure 2. Autoradiography of DNA microsatellite analysis of the fibrinogen α chain gene. Lanes 1–5, mutant gene carriers from family D; lanes 6–10, mutant gene carriers from family R; lanes 11–15, mutant gene carriers from family S. Lane 16, a mutant gene carrier from family L; lanes 17–19, children of lane 16 who are non-mutant gene carriers. Families S and L are from the present study and families D and R are from reference 12. The Val 526 mutation segregated with the B5 allele (indicated by arrow) in all these kindreds.

developed nephrotic syndrome at age 36. He received a cadaver renal transplant at age 40, but had a recurrence of renal amyloidosis and died at the age of 50 with septicaemia after receiving a second renal allograft. Another mutation (4904delG) was associated with renal amyloidosis in an American kindred.[15] The propositus was found to have proteinuria at the age of 41, subsequently developed renal insufficiency and cardiac failure, and died at age 46. Her mother and uncle died of renal failure at ages 38 and 41. Clinical features of the disease in four kindreds with the Val 526 mutation are summarized in Table 1. While mainly offspring of affected individuals are tested for the mutation, most of the mutant gene carriers in these kindreds are younger than 50 years old and thus are asymptomatic. It is notable that all four kindreds have the same clinical manifestations and similar onset ages. The disease related to the Val 526 mutation may be less aggressive when compared to that of the other fibrinogen mutations. Also, although the biological nature of the amyloid protein in the German kindred described by Ostertag remains unknown, this mutant fibrinogen α-chain gene (Val 526) might not be the cause of the disease in that kindred, because their death occurred earlier at ages of 18, 35, 36, 39, and 52.[5,6]

DNA polymorphisms including restriction fragment length polymorphism (RFLP) and short tandem repeat polymorphism (STRP, DNA microsatellite) are

Table 1 Four kindreds with fibrinogen α-chain Val 526

Family	S	L	D	R
Residence	Alberta, Canada	New Jersey, USA	Illinois, USA	California, USA
Ethnic background	Polish	Irish	Irish	Irish
Number affected in the kindred	6	4	6	2
Range of onset ages	42–58	49–55	43–61	60, 66
Clinical features	Nephrotic syndrome, hypertension, anaemia	Nephrotic syndrome, hypertension, anaemia	Nephrotic syndrome, hypertension, anaemia	Nephrotic syndrome, hypertension, anaemia
Organs where amyloid deposition was proven	Kidney, Spleen, Adrenal	Kidney, Lung	Kidney, Spleen, Liver	Kidney
Asymptomatic mutant gene carriers (*n*)	5	1	6	9
Reference	1 (Present study)	2, 3 (Present study)	12	12

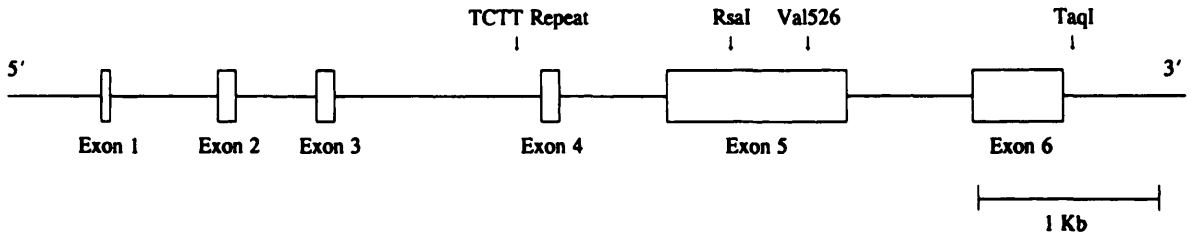

Figure 3. Schematic illustration of the fibrinogen α-chain gene showing the Val 526 mutation and the three polymorphic sites.

often used to determine whether mutant genes in separate kindreds are derived from a common origin. In the field of amyloidosis, the transthyretin and gelsolin genes are among those examined by DNA haplotype analysis. The transthyretin gene coding for Met 30 variant is associated with familial amyloidotic polyneuropathy in a number of kindreds all over the world. Yoshioka *et al.* demonstrated that there were three different haplotypes of the transthyretin Met30 gene, and therefore hypothesized that these mutant genes were probably derived from two or more founders.[18] The mutant gelsolin gene ($G_{654}A$) is also found in numerous kindreds with familial amyloidosis in Finland, Japan, and the USA. Microsatellite marker analyses suggested that, while at least one kindred in the USA shared the Finish haplotype,[19] the Japanese and Finnish gelsolin mutations had most probably evolved independently.[20]

A DNA microsatellite (TCTT repeat) and two RFLPs (*RsaI* and *TaqI*) are known in the fibrinogen α-chain gene locus (Figure 3). This study revealed that the fibrinogen α-chain gene coding the Val 526 variant in all four kindreds shared the same haplotype, B5-*RsaI*(+)-*TaqI*(−). We do not know the linkage between these polymorphic sites, but the allelic frequency at each site has been reported. *RsaI*(+) and *TaqI*(−) are the more common allelic types, with frequencies of 0.759 and 0.73, respectively,[8,9] and our preliminary study indicates a tight linkage between these two polymorphisms. On the other hand, the microsatellite in the intron 3 shows high heterogeneity and the frequency of the B5 allele has been reported to be 0.15.[7] Although family S is reported to be of Polish origin and the other three are Irish, this study suggests that the mutant genes in these four kindreds may be derived from a single ancestor. Extensive genealogical studies of these kindreds in North America revealed no relation between them. It would be of interest to see if there are patients with this mutation in Europe who might provide the missing link between these kindreds.

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