## Acquired dysfibrinogenemia caused by monoclonal production of immunoglobulin λ light chain

**Disorders of fibrinogen are usually caused by genetic mutations that result in low protein levels (hypofibrinogenemia) or an abnormal molecule (dysfibrinogenemia). However, environmental and plasma factors can have an *acquired* effect on its expression or function. For example, antibodies can bind fibrinogen and/or fibrin to interfere with polymerization and inhibit coagulation. The objective here was to determine the cause of dysfibrinogenemia in a 63-year-old man. Despite a low functional fibrinogen concentration and prolonged thrombin time, no inherited fibrinogen abnormality could be detected after extensive protein analysis and gene sequencing. Thus, electrophoresis methods and fibrinogen binding studies were used to establish the cause of the acquired dysfibrinogenemia. An immunoglobulin λ light chain was found to bind fibrinogen as a monomer. It had no significant effect on fibrinopeptide release, but caused substantial defects in all other stages of thrombin-catalyzed fibrin polymerization. Binding to fibrinogen also seemed to prevent the light chain from being filtered through the kidneys, causing only low levels of it in the urine. Once in the urine, the λ chain lost its anti-fibrinogen activity, apparently due to dimerization. The 63-year-old patient acquired dysfibrinogenemia from a monoclonal production of λ light chain that bound and inhibited the function of fibrinogen. At age 64.5 he was diagnosed with monoclonal gammopathy of undetermined significance, explaining the abnormal immunoglobulin chain production. This case was particularly unusual in that the inhibition of fibrin polymerization was caused by a single immunoglobulin light chain, rather than by a whole antibody molecule.**

**Haematologica 2007;** 92:e111-e117 DOI: 10.3324/haematol.11837

Fibrinogen is synthesized in hepatocytes as a 340 kDa disulfide-bonded dimer of three pairs of polypeptide chains; $(A\alpha, B\beta, \gamma)_2$. Once secreted into the circulation, it is intimately involved in health and disease through its pivotal roles in blood coagulation, platelet aggregation and wound healing, and is maintained at a plasma concentration of 1.5-4.0 mg/mL. In the final stages of the coagulation cascade, thrombin activates fibrinogen by cleaving fibrinopeptides from the Aα and Bβ chains. Exposure of the *A* polymerization site is sufficient to initiate fibrin polymerization, whereby the new a chain N-terminus docks with a constitutively exposed cavity in the C-terminal domain of the γ chain. The resulting protofibrils aggregate to form the matrix of a blood clot and become stabilized by factor XIIIa-mediated covalent cross-linking. The fibrin matrix preserves the integrity of the hemovascular system by restricting blood loss and promotes wound healing by providing a scaffold for the migration and adhesion of other proteins and cells.[1]

Most disorders of fibrinogen are caused by genetic mutations that result in low levels of normal protein (hypofibrinogenemia) or normal levels of an abnormal molecule (dysfibrinogenemia). However, acquired disorders highlight a number of environmental and plasma factors that can have an effect on fibrinogen expression and function. Acquired abnormalities of fibrinogen occur in patients with a number of underlying diseases. For example, acquired dysfibrinogenemia associated with liver disease is caused by increased sialylation of the Bβ and γ chains, with the increased negative charge causing a decrease in polymerization rate.[2] Diseases that cause increased immunoglobulin production (myeloma) or autoantibody activation (systemic lupus erythematosus (SLE)) have also been associated with abnormal fibrin polymerization, but this is not usually caused by specific activity against fibrinogen.[3,4] That said, two patients with multiple myeloma[5,6] and three patients with SLE[7,8] have been reported to have prolonged thrombin times caused by anti-fibrinogen autoantibodies. Other disease states associated with autoantibodies toward fibrinogen include renal insufficiency,[9] migratory thrombophlebitis,[10] sarcoidosis with isoniazid therapy,[11] chronic liver disease[12] and Down's syndrome.[13] There are also examples of anti-fibrinogen antibodies developing spontaneously for no determined reason.[14-16] Anti-fibrinogen alloantibodies are equally rare, but there have been reports of their appearance in afibrinogenemic patients who have received fibrinogen replacement therapy.[17-19] Another patient developed alloantibodies against fibrinogen, thrombin and factor V after receiving bovine topical thrombin as a hemostatic agent.[20] Non-clinically relevant antibodies also develop toward fibrinogen degradation products in most normal individuals[21,22] and during pregnancy.[23] These may provide a bridge between the hemostatic and immune systems to control the pathogenic actions of fibrinogen fragments.

Acquired antibodies to fibrinogen and/or fibrin can interfere with fibrinopeptide release, fibrin polymerization or factor XIIIa-mediated cross-linking and usually create an abnormal coagulation profile, although there has been no consistent correlation with bleeding. They are usually whole immunoglobulin molecules and are rare enough to merit case reports. The case presented here was particularly unusual in that the inhibition of fibrin polymerization was caused by a single immunoglobulin λ light chain, rather than by a whole antibody molecule. There has only been one previous report of a light chain binding and inhibiting the function of fibrinogen.[24]

### Design and Methods

*Hematological assays.* Routine hematological tests were performed with fresh citrate plasma and included fibrin clot weight, thrombin time, prothrombin time, international normalized ratio (INR), activated partial thromboplastin time (aPTT) and assays for factor VIII, von Willebrand factor (vWF) and fibrin degradation products. Functional fibrinogen concentration was measured

using a modification of the Clauss method.[25] Gravimetric fibrinogen concentration was measured by quantification of fibrinopeptide A release as previously described.[26]

*Fibrinogen purification and fibrin polymerization.* Fibrinogen was purified by precipitation with 22% saturated ammonium sulfate.[26] The pellet was washed twice with 25% saturated ammonium sulfate before being dissolved in 20 mmol/L Hepes, pH 7.4, 150 mmol/L NaCl and dialyzed extensively against the same buffer. Thrombin-catalyzed fibrin polymerization was initiated by addition of 20 µL of l NIH U/mL human α-thrombin (Enzyme Research Laboratories Inc., South Bend, IN) to purified fibrinogen (40 µg in 180 µL 20 mmol/L Hepes, pH 7.4, 150 mmol/L NaCl) and absorbance at 350 nm was monitored for 45 minutes at room temperature in triplicate.[27]

*SDS-PAGE and Western blot.* Purified protein was analyzed by reducing and non-reducing sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Western blots utilized rabbit polyclonal antibody to human fibrinogen or human λ chains (immunoglobulin fraction, 1:1000 dilution in casein blocking solution; DakoCytomation, Glostrup, Denmark), an enhanced chemiluminescence Western blotting analysis system (Amersham Biosciences, Buckinghamshire, England) and BioMax XAR film (Kodak, Rochester, NY, USA).

*Reverse phase high-performance liquid chromatography.* Fibrinogen (5 mg/mL) was reduced by incubation in 8 mol/L urea, 0.1 mol/L Tris-HCl, pH 8.0, 15 mmol/L dithiothreitol (DTT) for 16 hours at room temperature, and the individual chains separated by reverse phase high-performance liquid chromatography (HPLC) on a 25x0.46 cm C4 column (Phenomenex, Torrance, CA, USA). After injecting 0.5 mg of protein, the column was monitored at 215 nm and developed with a 0.05% trifluoroacetic acid/acetonitrile gradient system at a flow rate of 0.75 mL/min.[28]

*Electrospray ionization mass spectrometry and tryptic peptide mapping.* Purified fibrinogen chains or immunoglobulin λ chains were analyzed by electrospray ionization mass spectrometry (ESI MS) on a VG Platform II quadrupole analyzer (Micromass, Manchester, England) as previously described.[28] A 20 µL sample was injected directly into the ion source at a flow rate of 10 µL/min. The probe was charged at 3500 V and the source maintained at 60°C. Raw data was acquired and processed using Mass-Lynx software and transformed onto a true molecular mass scale using maximum entropy software. For tryptic peptide mapping, purified fibrinogen chains were dried at 55°C under $N_2$, redissolved in 50 µL 50 mmol/L $NH_4HCO_3$ and digested with 2 µg trypsin for 16 hours at 37°C. After being dried under vacuum with $P_2O_5$ and redissolved in 50 µL 50% acetonitrile, 0.1% formic acid, a 20 µL sample of each tryptic digest was analyzed by ESI MS.

*DNA analysis.* Genomic DNA was isolated from whole blood as previously described.[29] All exons and intron-exon boundaries of the three fibrinogen genes were amplified using standard PCR protocols and primers designed from the published FGA (Genbank M64982), FGB (M64983) and FGG (M10014) sequences. PCR products were purified in MultiScreen$_{96}$ PCR plates (Millipore, Billerica, MA, USA) and sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit and a 3100-Avant capillary DNA sequencer (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions.

*Immunoglobulin analysis.* For native protein electrophoresis of plasma, urine (concentrated 100x), serum and purified protein, 1% agarose gels were electrophoresed at 250 V in 38 mmol/L Tris, 46 mmol/L sodium barbitone, 16 mmol/L diethyl barbituric acid, pH 8.6. Plasma, serum and purified protein gels were stained with Coomassie Brilliant Blue R (Sigma-Aldrich Co., St. Louis, MO, USA) and urine gels were stained with silver nitrate.

Immunofixation electrophoresis (IFE) of plasma, urine, serum and purified protein was carried out using rabbit polyclonal antibodies for human fibrinogen and each of the immunoglobulin heavy chains (γ, α, µ, δ and ε) and light chains (κ and λ) (DakoCytomation). Following native protein electrophoresis of diluted samples, strips of filter paper that had been soaked in the appropriate antibodies were laid over each sample lane. They were left to form insoluble immune complexes, which after extensive washing were detected by staining with Coomassie Brilliant Blue R (Sigma-Aldrich Co.).

Plasma levels of IgG, IgA, IgM, free κ and free λ, as well as urine levels of free κ and free λ, were determined using rate nephelometry immunoassay. Total urine protein was measured using the biuret method.[30]

*Purification of λ light chain from urine*

Immunoglobulin λ light chain was purified from a 24-hour urine (~1.5 L), which was concentrated 25x and dialyzed. It was then subjected to cation exchange chromatography at 1 mL/min on a sulphopropyl (SP)-Sephadex column (1.6x15 cm) equilibrated with 25 mmol/L sodium acetate, pH 5.0. After elution of unbound proteins (albumin and transferrin), bound ¥ chain was eluted as a single peak with a linear gradient to 25 mmol/L sodium acetate, pH 6.5, 300 mmol/L NaCl and then dialyzed and freeze-dried.

## Results

*Case history.* A 63-year-old Caucasian man was investigated for a possible dysfibrinogenemia when an abnor-

mal coagulation profile was identified during tests before a radical prostatectomy. Despite a negative history of bleeding or easy bruising, the decision was made to administer cryoprecipitate before the operation, which was subsequently performed uneventfully. Results of coagulation studies are presented in Table 1. The low functional fibrinogen concentration (0.4 mg/mL), normal fibrin clot weight (3.9 mg/mL) and prolonged thrombin time (56 s) suggested a dysfibrinogenemia when he first presented. Fibrinopeptide release assays subsequently yielded normal amounts of the A and B peptides after 15 min and provided a gravimetric fibrinogen concentration of 3.9 mg/mL, indicating normal rates of release for both peptides. Consistent with a qualitative fibrinogen disorder, prothrombin time, aPTT and INR were slightly raised. A mild reduction of factor VIII could also be explained by the dysfibrinogenemia, as the factor VIII assay relies on normally functioning fibrinogen. Fibrin degradation products, vWF levels and liver function tests were all normal.

*Protein analysis.* Significant ($p$-value <0.01) defects were identified in all stages of thrombin-catalyzed fibrin polymerization. Purified fibrinogen polymerized with a lag time of 370 s, a $V_{max}$ of $0.7x10^{-4}$ U/s and a final turbidity of 0.07 U, compared with normal control values of 120 s, $3.0x10^{-4}$ U/s and 0.13 U, respectively. This confirmed that there was abnormally functioning fibrinogen present in his plasma.

Detailed protein analyses gave no clues as to the location of a possible mutation. In particular, 7.5% reducing and 4% non-reducing SDS-PAGE of purified fibrinogen showed normal patterns of bands and the three fibrinogen chains reacted normally with rabbit polyclonal antibody to human fibrinogen on Western blot. ESI MS analysis of purified fibrinogen chains demonstrated normal masses for all three and a normal sialylation pattern for the Bβ and γ chains. Further *m/z* mapping of all three chains failed to detect any abnormal tryptic peptides (data not shown).

*DNA analysis.* Surprisingly, DNA sequencing of all exons and intron-exon boundaries of all three fibrinogen genes revealed no potential causative mutation. These sequencing results were confirmed in a repeat sample and at this time, at age 64, the patient continued to exhibit low functional fibrinogen and a prolonged thrombin time consistent with a dysfibrinogenemia (Table 1).

*Characterization of a polymerization inhibitor.* The possibility of an acquired dysfibrinogenemia was considered at this point. A functional defect due to liver disease or abnormal fibrinogen degradation due to circulating proteases were excluded on the basis of the normal sialylation pattern on ESI MS and the intact nature of chains on SDS-PAGE. Native protein electrophoresis, however, revealed that the patient's fibrinogen migrated slightly faster than normal in both plasma and purified fibrinogen samples (Figure 1). In order to determine whether this was due to antibody binding, IFE was performed for the five immunoglobulin heavy chains (γ, α, μ, δ and ε) and two light chains (κ and λ). This demonstrated a pair of clonal l light chain bands, one migrating slightly cathodic to the origin and the other co-migrating with fibrinogen, but only normal polyclonal smears for the other six chains (Figure 2A). The same clonal λ chain bands were also demonstrated in the patient's purified fibrinogen (Figure 2B). Quantitative plasma levels of IgG, IgA, IgM and free κ light chains were all below normal, whereas free λ light chain was greatly elevated, resulting in a significantly lower κ:λ ratio than normal (Table 2).

**Table 1. Coagulation profile.**

| *Assay* | *Normal range* | *Patient* |
|---|---|---|
| *Age 63 years* | | |
| Functional fibrinogen (mg/mL) | 1.5-4.0 | 0.4 |
| Gravimetric fibrinogen (mg/mL) | 1.5-4.0 | 3.9 |
| Fibrin clot weight (mg/mL) | 1.8-4.0 | 3.9 |
| Thrombin time (s) | 18-25 | 56 |
| Prothrombin time (s) | 7.6-10.6 | 12.2 |
| INR | 0.8-1.2 | 1.3 |
| aPTT (s) | 26-38 | 40 |
| Factor VIII (%) | 50-150 | 42 |
| vWF ristocetin cofactor (%) | 50-150 | 96 |
| vWF antigen (%) | 50-150 | 103 |
| Fibrin degradation products | negative | negative |
| *Age 64 years* | | |
| Functional fibrinogen (mg/mL) | 1.5-4.0 | 0.7 |
| Thrombin time (s) | 18-26 | 50 |
| *Age 64.5 years* | | |
| Functional fibrinogen (mg/mL) | 1.5-4.0 | 0.4 |
| Thrombin time (s) | 18-26 | 51 |

**Table 2. Blood and urine analyses.**

| *Assay* | *Normal* | *Patient (years)* | | |
|---|---|---|---|---|
| | | *Age 64* | *Age 64.5* | *Age 66.5* |
| *Immunoglobulins* | | | | |
| IgG (g/L) | 7.0-14.0 | 5.1 | 8.1 | 5.7 |
| IgA (g/L) | 0.8-3.5 | 0.7 | 1.3 | 1.9 |
| IgM (g/L) | 0.5-2.0 | 0.2 | 0.3 | <0.5 |
| Free κ (mg/L) | 3.30-19.40 | 3.26 | 4.70 | 0.83 |
| Free λ (mg/L) | 5.71-26.30 | 1410 | 1050 | 1130 |
| κ:λ | 0.26-1.65 | 0.0023 | 0.0045 | 0.0007 |
| *Urine* | | | | |
| Free κ (mg/L) | 1.35-26.19 | n.d. | 42.0 | n.d. |
| Free λ (mg/L) | 0.24-6.66 | n.d. | 28.5 | n.d. |
| κ:λ | 2.04-10.37 | n.d. | 1.47 | n.d. |
| Total protein | <0.25 | n.d. | 0.08 | n.d. |
| *Blood screen* | | | | |
| Hemoglobin (g/L) | 130-180 | n.d. | 142 | 137 |
| Platelets ($x10^9$/L) | 150-400 | n.d. | 142 | 124 |
| Neutrophils ($x10^9$/L) | 2.0-7.5 | n.d. | 3.1 | 1.65 |
| β2-microglobulin (mg/L) | 1.0-3.5 | n.d. | 1.76 | 1.79 |
| Corrected calcium (mmol/L) | 2.05-2.22 | n.d. | 2.20 | 2.14 |

*n.d., not determined.*

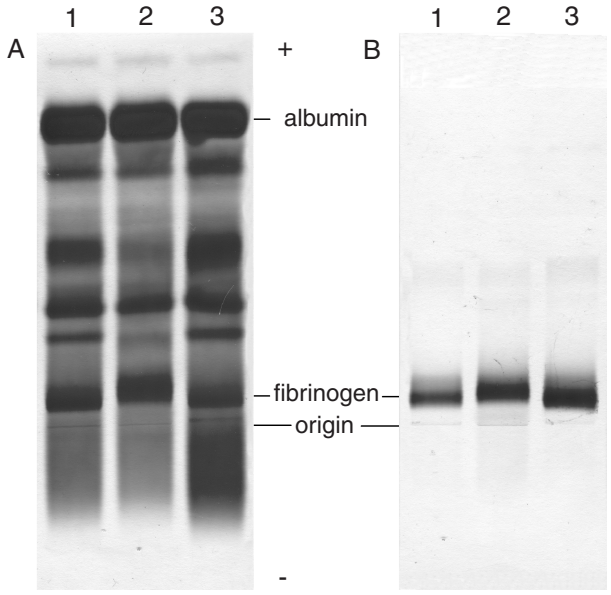


Figure 1. Native protein electrophoresis of samples from normal controls (lanes 1 and 3) and the patient (lane 3). A. Plasma protein electrophoresis. B. Purified fibrinogen electrophoresis. The patient's fibrinogen consistently migrated slightly faster than normal.

Collectively, these results suggested a monoclonal production of λ light chain that binds to fibrinogen and acts as a polymerization inhibitor. Since there is a molar excess of λ chain over fibrinogen, this would explain the altered fibrinogen mobility on electrophoresis and the pair of λ chain bands seen on plasma IFE (unbound and bound to fibrinogen; Figure 2A). It was suspected that the two λ bands detected on IFE of purified fibrinogen (Figure 2B) were due to some λ chain dissociating from fibrinogen during electrophoresis or to some unbound λ chain co-purifying during ammonium sulfate precipitation.

Fibrinogen-bound λ light chain was subsequently purified from 22% ammonium sulfate-saturated plasma by subjecting the precipitate to reverse-phase HPLC under reducing conditions. A small abnormal peak consistently eluted after the breakthrough and before the Aa peak (data not shown). This peak electrophoresed at ~20 kDa on 10% reducing SDS-PAGE and ESI MS analysis indicated a mass of 22,818 Da, consistent with an immunoglobulin λ light chain without an accompanying heavy chain.

By the time the patient was aged 64.5 years he had noticed a bruising and bleeding tendency that had increased during the preceding 12 months. His functional fibrinogen concentration and thrombin time were consistent with previous measurements (Table 1), as were his immunoglobulin levels (Table 2). Also as before, his purified fibrinogen displayed impaired thrombin-catalyzed fibrin polymerization. A 24-hour urine had an elevated free λ chain concentration of 28.5 mg/L, but normal total protein levels (Table 2), and native protein electrophoresis and IFE showed a single clonal λ light chain band running slightly cathodic to the origin (Figure 2C). The level of Bence-Jones Protein (BJP) in our patient's urine was somewhat lower than expected, suggesting that binding to fibrinogen might impair free λ chain from passing through the kidneys.

Because only small amounts of plasma were available, the 24 h urine was used as a source of λ chain. Elution from an SP-Sephadex column yielded 13 mg of protein that was confirmed as λ chain by SDS-PAGE (Figure 3A, Lane 3), native protein electrophoresis and IFE. Surprisingly, incubation of this purified λ chain with normal plasma had no effect on the functional fibrinogen concentration or thrombin time. Native protein electrophoresis and IFE further verified that the λ chain purified from urine did not bind to exogenous fibrinogen. Specifically, a single λ band remained near the origin when 280 μg urine-derived λ chain was incubated with 200 μL of normal plasma containing 480 μg fibrinogen (data not shown).

Non-reducing SDS-PAGE (10%) revealed a possible explanation for the difference in anti-fibrinogen activity of plasma- and urine-derived λ chain (Figure 3A). That is, the λ chain is mostly monomeric when bound to fibrinogen in plasma, but dimeric in urine. From this it can be hypothesized that binding to fibrinogen prevents λ chain dimerization. Figure 3A also shows that the fibrinogen-λ chain interaction is non-covalent and that there were not significant levels of any other protein co-precipitating with fibrinogen-λ. Further ESI MS analysis confirmed that urine-derived λ chain was dimeric at 45,646 Da and reverted to a mass of 22,822 Da when reduced with 15 mmol/L DTT at 37∞C for 30 min (Figure 3B). This measurement was consistent with the molecular mass of plasma-derived λ chain (22,818 Da), indicating that no further modification other than dimerization had occurred in the kidneys or urine.

In order to confirm that monomeric λ light chain was capable of binding exogenous fibrinogen, purified fibrinogen was added to patient serum in both the presence and absence of DTT. IFE clearly showed that the initial patient serum had a single λ chain band running slightly cathodic to the origin (unbound λ dimer). When exogenous fibrinogen was added (up to 5 mg/mL final concentration) there was a dose dependant decrease in intensity of the unbound lower band and concomitant increase in fibrinogen-bound monomeric λ chain (Figure 4). However, fibrinogen binding only occurred to the extent seen in plasma when the λ dimers were first reduced by incubation with 15 mmol/L DTT. These results confirm that the circulating λ chain was able to bind normal exogenous fibrinogen as a monomeric unit.

*Diagnosis and Follow-up.* Because high levels of monoclonal light chain are indicative of a plasma cell dyscrasia, the patient had a bone marrow biopsy soon after diagnosis of his B cell proliferation. He was classified as a case of monoclonal gammopathy of undetermined sig-

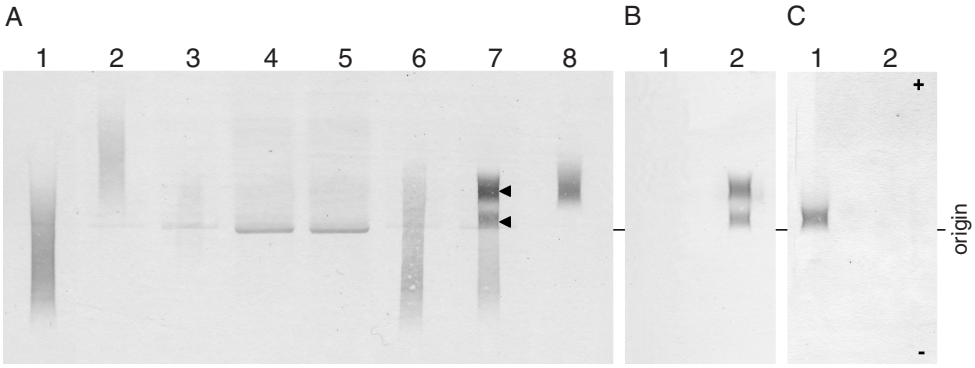


**Figure 2. Immunofixation electrophoresis. A. IFE of plasma for the immunoglobulin heavy chains γ, α, μ, δ and ε (lanes 1-5, respectively), light chains κ and λ (lanes 6 and 7, respectively) and fibrinogen (lane 8). The arrowheads indicate the location of a pair of clonal l light chain bands. B. λ light chain IFE of purified fibrinogen from a normal control (lane 1) and the patient (lane 2). C. IFE of the patient's urine for λ light chains (lane 1) and κ light chains (lane 2).**

nificance (MGUS), with a marrow plasma cell count of 6% at age 64.5 (the normal limit for small plasma cells is 5%). A second marrow obtained almost a year later at age 65.5 showed essentially the same appearances (5% plasma cells).

Since then, the patient has been monitored six-monthly and his overall lab status has not significantly changed, apart from falling κ chain levels and κ:λ ratio. A slowly developing thrombocytopenia and mild neutropenia are suggestive of slow marrow progression. No further marrow has been obtained and these tests will only be repeated if he shows a significant fall in immunoglobulins or cell counts, or a rise in λ light chain. Elevated levels of β2-microglobulin and calcium in the blood are further indications of progression to a hematological malignancy such as multiple myeloma, but as yet these remain normal (Table 2).

The bone marrow also provided a sample for mRNA purification and subsequent cDNA synthesis, from which the λ chain cDNA sequence was determined. It was completely unique, not only throughout the variable region, but also in the joiner and constant regions. Comparisons with germline sequences suggested an *IGLV2-23/J3/C3* configuration, with most variations occurring in complementarity-determining regions and with further diversity created at the $V_\lambda$-$J_\lambda$ junction by DNA exonuclease activity. The theoretical molecular mass of the mature protein is 22,818 Da, which is identical to the mass of the plasma-derived λ chain measured by ESI MS. Furthermore, tryptic peptide mass mapping was able to identify all the predicted λ chain peptides except for those containing cysteine residues. It also confirmed the presence of a unique arginine residue in the constant region, but because our mass spectrophotometer is a single quadrupole instrument we were unable to obtain more specific amino acid sequence information.

## Discussion

A 63-year-old patient acquired dysfibrinogenemia from a monoclonal production of immunoglobulin λ light chain. This λ chain was initially overlooked because it ran off the bottom of the 7.5% reducing SDS-polyacrylamide gels that are routinely used for fibrinogen analysis. The λ chain non-covalently bound to fibrinogen, causing significant polymerization defects, but only a mild bruising and bleeding tendency. The most substantial aberrations to thrombin-catalyzed fibrin polymerization were a more than three-fold increase in lag time and a four-fold decrease in Vmax. Final turbidity was approximately halved and the amount of fibrinopeptide release was normal. These results point to impaired protofibril formation and growth into fibers. The effect on fibrin polymerization may be a useful tool for monitoring progression of the patient's disease or therapy. Over the course of this study, his thrombin time remained relatively constant (50-56 s), but this may extend further if levels of λ chain increase.

The monoclonal antibody 7E9 has provided insights into the effects of an IgG molecule on fibrin polymerization and fibrin clot structure.[32] Some of these same mechanisms may contribute to the effect that the λ chain has on fibrin polymerization. For example, binding to fibrinogen D regions may prevent the A:a interaction, causing significant defects at all stages of fibrin polymerization. This could also produce a cap on the end of a protofibril, preventing its growth and lateral aggregation. However, λ light chain is unlikely to produce the short fibers and large pores of 7E9 clots because it lacks the bulk that is required to produce severe aberrations in clot architecture. It is also unlikely that the monomeric λ chain has the ability to bind two fibrinogen molecules to bring fibrinogen or fibrin monomers together during fibrin polymerization, and this is consistent with the prolonged lag time. At this stage it is unclear whether the λ chain binds the D region, E region, coiled-coil or AαC domain of fibrinogen, or how many λ chains bind to fibrinogen. However, assuming that Coomassie staining is proportional to peptide mass, densitometry of 10% reducing SDS-PAGE of λ chain co-purified with the patient's fibrinogen suggested that two monomers were bound to each fibrinogen molecule. This is consistent with the dimeric nature of fibrinogen, but confirming this proposition will certainly be a main aim of future work. With a lack of material for purifying the λ chain from plasma for further investigations, urine was

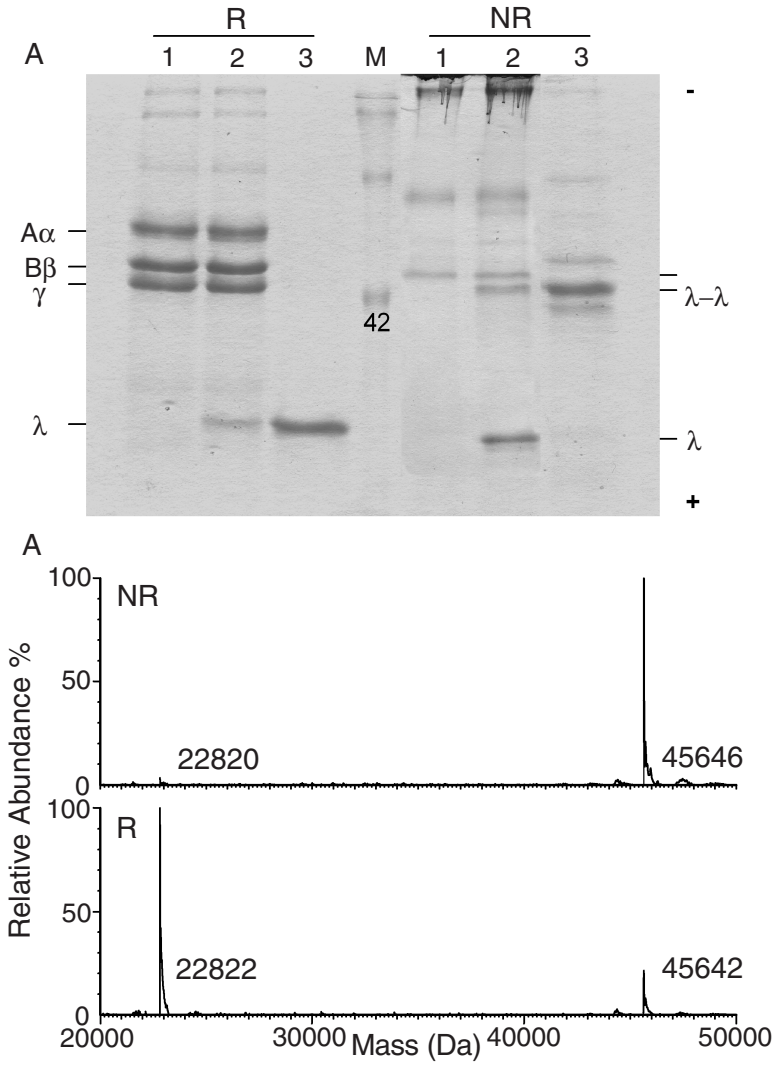


Figure 3. Comparison of plasma- and urine-derived λ chain. A. 10% reducing (R) and non-reducing (NR) SDS-PAGE of normal control fibrinogen (lane 1), patient fibrinogen (lane 2) and λ light chain purified from urine (lane 3). The 42 kDa marker of the Invitrogen E-PAGE SeeBlue pre-stained standard (M) is labeled. B. Transformed ESI MS of λ light chain from urine. Spectra of non-reduced (NR) dimeric λ chain and reduced (R) monomeric λ chain (reduced with 15 mmol/L DTT at 37∞C for 30 min).

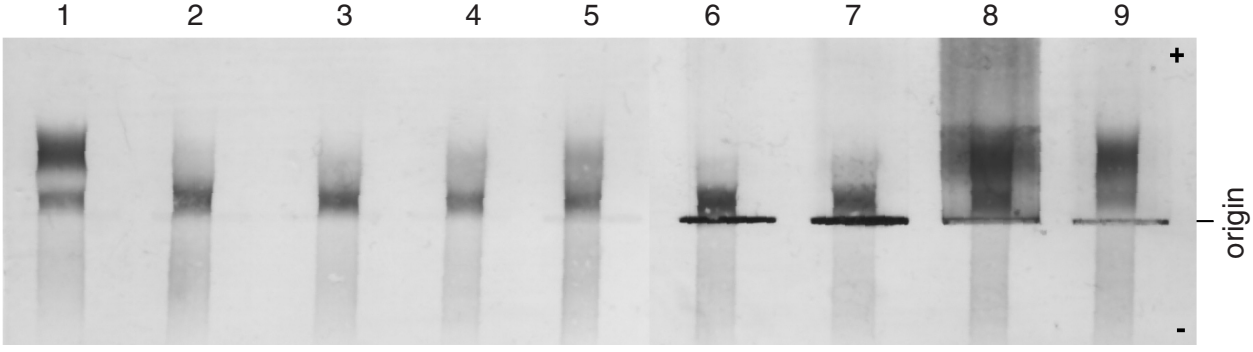


Figure 4. λ light chain IFE of patient serum in the presence of increasing final concentrations of added fibrinogen. Lane 1, patient plasma control; lanes 2 and 6, serum controls, 0 mg/mL added fibrinogen; lanes 3 and 7, 1.25 mg/mL added fibrinogen; lanes 4 and 8, 2.5 mg/mL added fibrinogen; lanes 5 and 9, 5 mg/mL added fibrinogen. All serum samples were preincubated with 10 mmol/L EDTA. In addition, serum in lanes 6-9 was preincubated with 15 mmol/L DTT. There is dose dependant binding of reduced λ chain to fibrinogen.

explored as a possible source. However, dimerization appeared to make the urine-derived protein inactive, perhaps by occluding the fibrinogen binding site. Thus, being active as a monomeric unit, it is clear that the λ chain does not mimic the Fab region of an immunoglobulin molecule or bind fibrinogen in the usual antigen binding mechanism between two variable regions.

The only other reported case of an immunoglobulin light chain causing acquired dysfibrinogenemia occurred in a Japanese woman who remained asymptomatic until the age of 70 when she suffered a pretibial edema.[24] She was diagnosed with nephrotic syndrome, for which treat-

ment was ineffective. Instead, she received maintenance dialysis until her death from heart failure at age 74. Post-mortem examination revealed amyloid deposits in her kidneys, pancreas, thyroid gland, gastrointestinal tract, adrenal glands and vascular walls of systemic vessels. These were probably derived from the light chain, which had been detected as a monoclonal band in her serum.

In both cases there was a low tendency toward bleeding and the light chains had similar effects on fibrinogen function. The Japanese woman had a functional fibrinogen concentration of 1.05 mg/mL (NR: 1.30-2.70) and abnormal fibrin polymerization. Both light chains were identified as Kabat subgroup VλII and were similar in size. Their migration on IFE and SDS-PAGE was comparable and they were both inhibitory as monomeric units in plasma. These two cases highlight the fact that, even without heavy chains, λ chains may have sufficient binding affinity through their variable or even constant regions to exert an effect on fibrinogen function.

The clinical presentations of the two cases were quite different; the New Zealand patient was pre-symptomatic and diagnosed following routine pre-operative tests. Upon further investigation he was found to have MGUS, with as yet no evidence for multiple myeloma, amyloidosis or other hematological malignancy. Also, the Japanese woman's gravimetric fibrinogen concentration was significantly high (5.81 mg/mL; NR: 1.50-2.64) and urine BJPs were not detectable. This may be a reflection of her condition, as increased levels of light chain in plasma may prolong the half-life of fibrinogen, and formation of amyloid fibrils may decrease excretion of BJP into the urine. A moderately high fibrinogen concentration and relatively low urine BJPs were seen in the New Zealand patient, probably also due to fibrinogen-λ binding. These concentrations may become exaggerated as the patient's condition worsens and his plasma concentration of λ chain increases. Clearly, it will be vital to continue monitoring progression of the patient's disease and frequency of bleeding episodes.

Light chain production is a random process involving variable/joiner recombination and somatic mutation that occurs prior to B cell maturation and proliferation. It is unclear why a light chain developed with affinity for fibrinogen in both these cases, but their similarities are likely to be coincidental. It may be that production of antibody chains with the ability to bind human proteins is more common than suspected, following the monoclonal production of immunoglobulins or BJPs in plasma cell dyscrasias.

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*Acknowledgements: we wish to thank the patient for his participation in this study.*

*Key words: fibrinogen, acquired dysfibrinogenemia, immunoglobulin light chain, monoclonal gammopathy of undetermined significance (MGUS).*

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