Vol. 191, No. 1, 1993 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 26, 1993 Pages 41-44

DEMONSTRATION OF A CIRCULATING 65K GELSOLIN VARIANT SPECIFIC FOR FAMILIAL AMYLOIDOSIS, FINNISH TYPE

C.P.J.Maury and Hannele Rossi

Fourth Department of Medicine, University of Helsinki,
SF-00170 Helsinki, Finland

Received December 18, 1992

Summary: Familial amyloidosis , Finnish type (FAF), is a dominantly inherited form of systemic amyloidosis caused by a point mutation G654 to A654 in the gelsolin gene. The mutation leads to the expression of mutant Asn-187 gelsolin and the accumulation of amyloid in tissues. Here we demonstrate that patients with FAF have an abnormal 65K gelsolin species in the circulation that cosegregates with the disease. The 65K variant is detected by immunoblotting using a monoclonal antigelsolin antibody or polyclonal antipeptide (P-gel 231-242) antibodies. The 65K gelsolin variant is lacking in normal subjects and unaffected family members. It is the putative circulating precursor of tissue amyloid in FAF.

Familial amyloidosis, Finnish type (FAF, Finnish hereditary amyloidosis, McKusick No. 105120) is an autosomal dominant form of systemic amyloidosis characterized by corneal lattice dystrophy, cranial neuropathy, skin changes, as well as renal and cardiac manifestations(1,2) and the deposition in tissues of a novel amyloid protein that is derived from mutant Asn-187 gelsolin by limited proteolysis(3-6). The complete primary structure of the accumulating subunit protein has been elucidated(6) showing that it is derived from an actin modulating domain of gelsolin homologous to residues 173-243 of mutant Asn-187 gelsolin. The disease is caused by a point mutation G to A at the first position of codon 187 in the gelsolin gene (7-9) located on chromosome 9 at q32-q34 (10). Here we show that patients with FAF have an abnormal circulating 65K gelsolin species that cosegregates with the disease. Normal subjects and unaffected family members lack the 65K gelsolin variant. The abnormal gelsolin can be detected by immunoblotting providing a rapid test for FAF.

MATERIALS AND METHODS

Samples. Plasma samples were collected from 13 patients with FAF, 10 unaffected family members, and 10 other control subjects. Based on allele-specific oligonucleotide hybridization tests, 12 of the FAF

0006-291X/93 $4.00

patients were heterozygous and one homozygous for the Asn-187 mutation (7,9).

Affinity chromatography.Antipeptide antibodies raised against a synthetic dodecapeptide homologous to residues 231-242 of human gelsolin were purified on a peptide 231-242- agarose column (ProtOn Kit 1, Multiple Peptide Systems,CA).Affinity chromatography on immobilized cibacron blue F3GA was carried out as described(11).

Western blotting.Proteins were separated on 10% SDS-PAGE, transferred to nitrocellulose membranes (Bio-Rad,CA) and incubated with either murine monoclonal antigelsolin antibody raised using a chymotryptic cleavage product of gelsolin containing the COOH-terminal actin-binding site (Sigma, MO) or rabbit polyclonal antipeptide 231-242 antibody (6).Peroxidase-conjugated swine immunoglobulins to murine or rabbit, respectively,immunoglobulins were used as second antibodies. Development was performed with 3,3´-diaminobenzidine tetrahydrochloride and hydrogen peroxide.

RESULTS

Figure 1 shows immunoblotting with antigelsolin P 231-242 antibodies of isolated gelsolin from homozygous FAF plasma and control plasma.The abnormal gelsolin species in FAF plasma has a relative molecular mass of about 65000 (65K) in contrast to the normal 93000 (93K) band. Immunoblotting with the monoclonal antigelsolin antibody is shown in Figure 2. The abnormal 65K band is found in FAF plasma,but not in normal plasma. Gelsolin of normal 93K size is the major band in heterozygous FAF plasma, but is present in homozygous FAF plasma in markedly decreased amounts only. In addition, a smaller gelsolin band of about 55K was detected in homozygous and heterozygous FAF plasma,as well as, although as a very

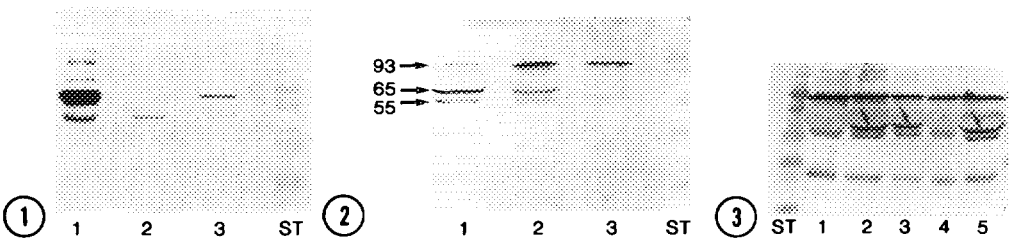

Figure 1.Immunoblotting with polyclonal antigelsolin(P 231-242)antibodies of gelsolin isolated by ammonium sulfate fractionation, DEAE-Sepharose CL-6B chromatography and cibacron blue affinity chromatography (11) from homozygous FAF plasma(lane 2, relative molecular mass of about 65000)and normal plasma (lane 3, relative molecular mass of about 93000).Lane 1 shows a gelsolin preparation from heterozygous FAF plasma.ST, prestained molecular mass standards(from top to bottom): 106000,80000,49500,32500,27500,and 18500.

Figure 2.Immunoblotting with monoclonal antigelsolin antibodies of gelsolin from homozygous FAF(lane 1),heterozygous FAF(lane 2) and normal plasma(lane3). $M_r \times 10^{-3}$.The markers are the same as in Fig.1.

Figure 3.Screening for 65K gelsolin variant from 0.15ml plasma samples purified on cibacron blue columns (0.5x1.5cm) eluted 1mM ATP containing buffer(15).Monoclonal antigelsolin antibodies were used in the immunoblotting procedure.Lanes 2,3,and 5 represent plasma from heterozygous FAF patients,and lanes 1 and 4 plasma from control(unaffected)subjects. Arrows show the abnormal gelsolin component in FAF plasma.

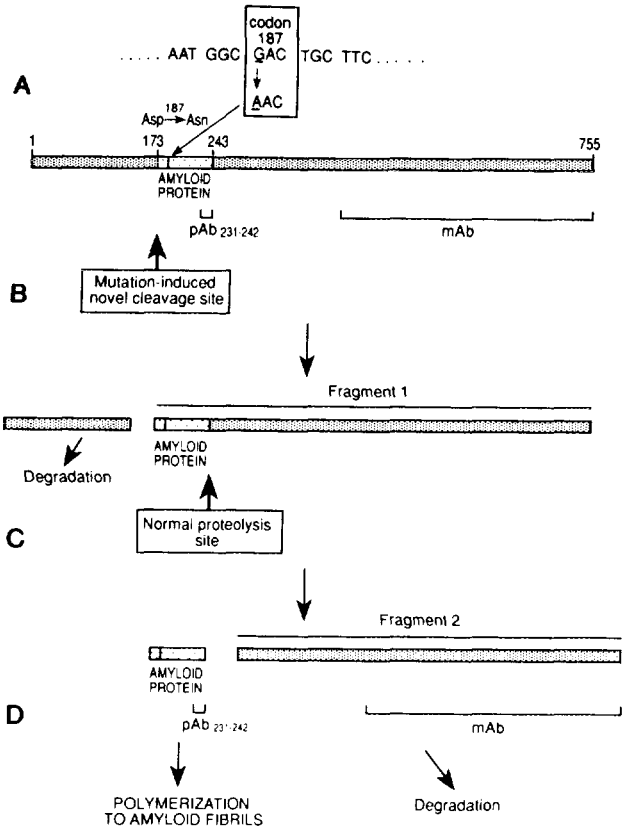

Figure 4. Proposed mechanism of amyloid formation in FAF(Asn-187). A) The G to A mutation at the first position of codon 187 in the gelsolin gene (7) leads to the expression of Asn-187 gelsolin. The amyloid subunit protein is homologous to residues 173-243 of Asn-187 gelsolin(6). pAb, polyclonal antibodies; mAb, monoclonal antibodies (lines indicate parts with which antibodies were raised). B) The Asn-187 mutation induces a novel proteolysis site; fragment 1 contains the amyloid sububit protein and represents the FAF-specific 65K gelsolin variant. Fragment 2 represents the 55K gelsolin species generated through cleavage at a proteolysis site present on both normal and mutant Asn-187 gelsolin(C). Fragment 173-243, the amyloid subunit protein, rapidly polymerizes to amyloid fibrils due to the highly amyloidogenic Asp→Asn mutation-induced intrinsic amino acid sequence(D).

weak band, in normal plasma. Figure 3 shows Western blots of cibacron blue purified plasma samples from three heterozygous FAF patients and two control subjects. The 65K gelsolin band is present in FAF plasma only. In the family studies the abnormal 65K gelsolin band was present in all 13 FAF subjects, including one asymptomatic gene carrier, but in none of the 20 control subjects, including 10 unaffected family members.

DISCUSSION

The results show that patients with FAF have a disease-specific 65K gelsolin variant in the circulation. In addition to the abnormal gelsolin,

a normal-sized gelsolin band of 93K was also present in homozygous FAF plasma, suggesting that full-length mutant gelsolin is produced and secreted. The most likely explanation for the presence of the FAF-specific 65K gelsolin is that it is derived from mutant Asn-187 gelsolin by cleavage at a novel proteolysis site generated by the substitution of the charged aspartic acid with the uncharged asparagine at residue 187 (Figure 4).The 65K fragment represents the novel cleavage product and is the putative circulating precursor of tissue amyloid in FAF.It is generated from mutant gelsolin only and is therefore disease-specific.The smaller fragment of 55K is derived from both mutant and normal gelsolin and is not specific for FAF.As a consequence of the highly amyloidogenic intrinsic amino acid sequence induced by the Asn-187 mutation (12), the release of the 8K fragment is associated with rapid polymerization of this fragment to amyloid fibrils(Figure 4).

Recently, we have described another mutation at the first position of codon 187 of the gelsolin gene, a G to T transition predicting an Asp to Tyr 187 substitution (13).This mutation was found in a Danish and a Czech family with a clinical syndrome similar to FAF.In vitro studies with synthetic peptides homologous to normal Asp and mutant Tyr-187 gelsolin have demonstrated highly accelerated amyloid fibril formation from Tyr-187 peptides(14).The mechanism of amyloidogenesis in FAF(Tyr-187) may thus be similar to that in FAF(Asn-187).

REFERENCES

1. Meretoja,J.(1969) Ann.Clin.Res 1,314-324.
2. Maury, C.P.J.,Kere,J.,Tolvanen,R.,and de la Chapelle,A.(1992) Genomics 13,902-903.
3. Maury,C.P.J.,Alli,K., and Baumann,M.(1990) FEBS Lett. 260,85-87.
4. Maury,C.P.J. and Baumann,M. (1990) Biochem.Biophys.Acta 1096,84-86.
5. Ghiso,J.,Haltia,M.,Prelli,F.,Novello,J., and Frangione,B. (1990) Biochem.J. 272,827-830.
6. Maury,C.P.J. (1991) J.Clin.Invest. 87,1195-1199.
7. Maury,C.P.J.,Kere,J.,Tolvanen,R., and de la Chapelle,A. (1990) FEBS Lett. 276,75-77.
8. Levy,E.,Haltia,M.,Fernandez-Madrid,I.,Koivunen,O.,Ghiso,J., Prelli,F,and Frangione,B(1990) J.Exp.Med 172,1865-1867.
9. de la Chapelle,A.,kere,J.,Sack,G.H.Jr.,Tolvanen,R., and Maury,C.P.J. (1992) Genomics 13,898-901.
10. Kwiatkowski,D.J.,Westbrook,C.A.,Bruns,G.A.P.,and Morton,C.(1988) Am.J.Hum.Genet. 42,565-572.
11. Yamamoto,H.,Terabayashi,M.,Egawa,T.,Hayashi,E.,Nakamura,H.,and Kishimoto,S. (1989) J.Biochem.105,799-802.
12. Maury C.P.J. and Nurmiaho-Lassila,E.-L. (1992)Biochem.Biophys.Res. Commun. 183,227-231.
13. de la Chapelle,A.,Tolvanen,R.,Boysen,G.,Santavy,J.,Bleeker-Wagemakers,L., Maury,C.P.J., and Kere,J. (1992) Nature Genetics 2, 157-160.
14. Maury,C.P.J.,Nurmiaho-Lassila,E-L., Rossi,H. Submitted for publication.
15. Yamamoto,H.,Ito,H.,Nakamura,H.,Hayashi,E.,Kishimoto,S.,Hashimoto,T., and Tagawa,K(1990)J.Biochem. 108,505-506.