Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 7389–7393, August 1992
Medical Sciences

Apolipoprotein AI mutation Arg-60 causes autosomal dominant amyloidosis

(mass spectrometry/protein sequencing)

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Communicated by David Weatherall, May 5, 1992 (received for review March 2, 1992)

ABSTRACT **A mutation in the gene for apolipoprotein AI (apoAI) was identified in an English family with autosomal dominant non-neuropathic systemic amyloidosis. The plasma of all affected individuals contained a variant apoAI with one additional charge, as well as normal apoAI. The propositus was heterozygous; the coding region of his apoAI gene contained both the normal sequence and a single-base substitution changing the codon for residue 60 of the mature protein from CTG (leucine) to CGG (arginine). Allele-specific oligonucleotide hybridization showed that the other affected individuals were also heterozygotes and that there was concordance of the mutant allele with the presence of variant plasma apoAI. Amyloid fibrils isolated from the spleen of the propositus consisted of proteins that ran as a doublet with an apparent mass of ≈10 kDa in SDS/PAGE and a trace band at 28 kDa. Electrospray mass spectrometry of the purified 10-kDa material revealed components with mass corresponding to the N-terminal 88, 92, 93, and 94 residues of apoAI each with substitution of arginine for leucine. These observations were confirmed by direct protein sequencing and laser desorption time-of-flight mass analysis. No material with the normal apoAI sequence was detected. The trace band at 28 kDa yielded the N-terminal sequence of mature apoAI, indicating that intact or minimally degraded apoAI was also present in the fibril preparation. Discovery of this mutation and the detailed characterization of the apoAI fragments that form the amyloid fibrils open additional avenues for investigation of amyloidogenesis.**

Amyloidosis is a disorder of protein metabolism in which autologous proteins or their fragments are deposited as abnormal fibers in the tissues (1). Most forms of amyloidosis are fatal, but the molecular mechanisms by which different proteins aggregate as amyloid fibrils and then persist *in vivo* remain obscure. One potentially powerful investigative approach is the identification of the rare amyloidogenic variants of already well-characterized proteins, which should enable the structural and functional determinants of amyloidogenicity to be elucidated.

In neuropathic hereditary systemic amyloidosis, commonly known as familial amyloid polyneuropathy (FAP), the fibril protein is usually derived from variants of plasma transthyretin (2). In the rare Finnish type of FAP, a variant of gelsolin (3) has been identified; and in FAP of the Iowa type, a variant of apolipoprotein AI (apoAI) (4, 5), the major apolipoprotein of high density lipoprotein (HDL) (6), has been reported. The apoAI Iowa variant protein, with arginine substituted for glycine at position 26, has also been found postmortem in a single patient with non-neuropathic hereditary systemic amyloidosis (7). This latter condition, which is much rarer than hereditary neuropathic amyloidosis, was first described by Ostertag (8) in 1932 and is generally fatal by middle age, usually due to renal involvement. We have recently investigated a previously unreported English family with Ostertag-type hereditary amyloidosis and detected a different mutation in the gene for apoAI that results in substitution of arginine for leucine at position 60 and causes deposition of a fragment of the mutant protein as amyloid fibrils.

MATERIALS AND METHODS

Clinical Subjects. Individual III-3 presented with renal amyloidosis and was found to have a family history of amyloid, which had been or was subsequently confirmed histologically in each clinically affected case. All other consenting family members were screened by *in vivo* scintigraphy with ^{123}I-labeled serum amyloid P component (SAP) (9).

Characterization of Plasma apoAI. To characterize apoAI protein itself, plasma was delipidated (10) and then subjected to isoelectric focusing for 2000 V·h at 4 W constant power in an agarose gel (11) using Pharmalyte, pH 4.0–6.5 (Pharmacia). The separated proteins were then transferred to a pure nitrocellulose membrane (Schleicher & Schull, Anderman, Kingston-upon-Thames, Surrey, U.K.) by pressure blotting (12) and were immunostained with sheep anti-human apoAI antiserum (Immuno Ltd., Dunton Green, Kent, U.K.), horseradish peroxidase-labeled rabbit anti-sheep IgG antibody (Zymed Laboratories; Cambridge Biosciences, Cambridge, U.K.), and diaminobenzidine (Sigma) according to the Bio-Rad immunoblot protocol. The association of apoAI with HDL was compared in affected individuals and controls by fast protein liquid chromatography gel filtration of whole plasma on a Superose 12 column (Pharmacia) and immunochemical detection of apoAI in the fractions.

DNA Analysis. Genomic DNA was isolated from frozen whole blood (200 μl) by a rapid method (13) and solubilized in 400 μl of 10 mM Tris, pH 7.5/1 mM EDTA. Three fragments making up the entire coding region of the apoAI gene were amplified by the polymerase chain reaction (PCR) (14) with the following primers: 5′-CCACCCTCAGGGAGCCAGGCTCGG (primer 44, 5′ end) and 5′-TAGGTGAGGACTCGGCCAGTCTGG (primer 45, 3′ end) (255-base-pair fragment of exon 3); 5′-CAGCCCTCAACCCTTCTGTCTCACC (primer 46, 5′ end) and 5′-CAGATGCGTGCGCAGCGCG-

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Abbreviations: apoAI, apolipoprotein AI; FAP, familial amyloid polyneuropathy; HDL, high density lipoprotein; SAP, serum amyloid P component.

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TCCACA (primer 48, 3′ end) (391-base-pair fragment of exon 4); and 5′-AGCTGCAAGAGAAGCTGAGCCCACT (primer 49, 5′ end) and 5′-AACGTTTATTCTGAGCACCGGGAAG (primer 47, 3′ end) (371-base-pair fragment of exon 4). The PCR reaction mixtures contained 100 ng of each oligonucleotide, 10 nmol of each dNTP, 5.0 μl of DNA solution (≈100 ng), and 1.25 units of Amplitaq (Perkin–Elmer/Cetus) in a total volume of 50 μl of 10 mM Tris (pH 8.3) containing 50 mM KCl, 1.5 mM $MgCl_2$, and 1% (vol/vol) dimethylformamide. The amplified fragments were purified by electrophoresis in low melting point agarose [1% (wt/vol) Nusiere agarose; FMC] and extracted from the gel slice with phenol (15), and their sequences were determined directly (16). The mutation that altered the codon for residue 60 was demonstrated by allele-specific hybridization. The PCR products (5–10 μl of the reactive mixture) containing the amplified fragment of the 5′ end of exon 4 (primers 46 and 48) were fractionated by electrophoresis on 1.5% agarose and transferred to a positively-charged nylon membrane (Boehringer Mannheim) by alkaline transfer (15), and duplicate membranes were hybridized with one of a pair of digoxigenin-labeled allele-specific oligonucleotides (5′-GCAAGCTGCGCGA for Leu-60 or 5′-GCAAGCGGCGCGA for Arg-60). Oligonucleotides were labeled with a commercially available kit (digoxigenin oligonucleotide 3′-end labeling kit; Boehringer Mannheim), and hybridization conditions were as recommended by the supplier. Hybridization was for 2 h at 37°C, and the final stringent wash was for 10 min at 39°C in 0.1× standard saline citrate (15)/0.1% (wt/vol) SDS. Bound oligonucleotides were detected by chemiluminescence (digoxigenin luminescence detection kit; Boehringer Mannheim). Exposure of the film was for 15 min at ambient temperature after preincubation of the blot for 30 min at 37°C.

Isolation and Characterization of Amyloid Fibrils. Frozen sections of the amyloid-laden spleen of the propositus were tested by standard immunohistochemical staining procedures, with the same antiserum to apoAI used for immunoblotting. Specificity was demonstrated by complete abolition of staining when the antiserum was absorbed before use with isolated HDL as a source of apoAI. Fibrils were isolated from the spleen tissue by water extraction (17) after repeated homogenization in 10 mM EDTA/140 mM NaCl/10 ml Tris/0.1% (wt/vol) NaN_3, pH 8.0 and were analyzed by SDS/8–18% gradient PAGE (ExcelGel; Pharmacia) after solubilization by boiling in 10 mM Tris at pH 8.0 containing 1 mM EDTA, 2.5% SDS, 5% (vol/vol) 2-mercaptoethanol, and 10% (vol/vol) glycerol. Isolated fibrils were also solubilized in 6 mM guanidine hydrochloride/0.5 M Tris, pH 8.5 and fractionated on Sephacryl S-200 HR (Pharmacia) equilibrated and eluted with 4 M guanidine hydrochloride/0.05 M Tris, pH 8.2. The major included peak containing the 10-kDa doublet, identified by SDS/PAGE, was dialyzed into distilled water and lyophilized.

Electrospray Mass Spectrometry. The lyophilized amyloid fibril subunit peptide was redissolved in 2% acetic acid/acetonitrile, 50:50 (vol/vol), at a concentration of ≈20 pmol/μl and was analyzed on a VG Bio-Q mass spectrometer (VG Biotech, Altrincham, Cheshire, U.K.) by using electrospray ionization at atmospheric pressure (18). The sample (20 μl) was introduced with a solvent flow of 3 μl/min, and data were acquired in a multiple-channel analysis mode at 10 sec per scan over the m/z range of 800–1600. The mass scale was calibrated with bovine ubiquitin (mass = 8564.9 Da).

Polypeptide Sequence Analysis. Purified amyloid fibril subunits were digested with lysyl endopeptidase, and the products were separated by HPLC on an Applied Biosystems OD300 (C_{18}) column. Automated amino-terminal sequencing of the purified peptides and of material transferred by electroblotting from SDS/PAGE analysis was performed with Applied Biosystems instruments 473A and 477A (19). The masses of the HPLC-purified products of fibril subunit digestion were determined by time-of-flight in a Finnigan MAT Lasermat (Finnigan-MAT, Hemel Hempstead, Herts, U.K.),

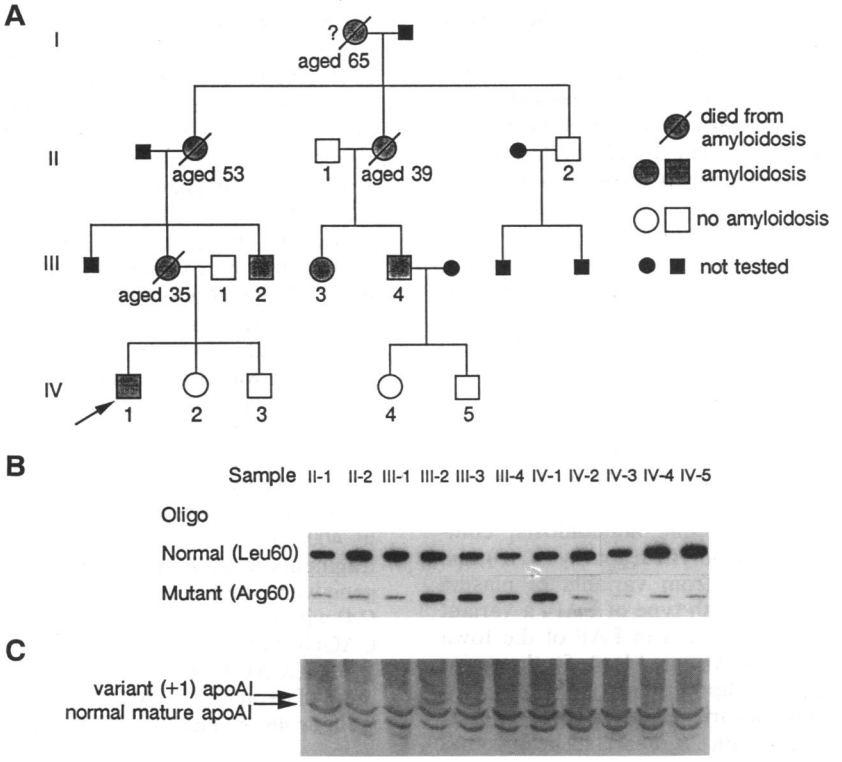

FIG. 1. (*A*) Family tree of the propositus (arrow) with autosomal dominant hereditary nonneuropathic systemic amyloidosis. (*B*) Hybridization with allele-specific oligonucleotides showing the presence of the apoAI Arg-60 mutation in genomic DNA. (*C*) Isoelectric focusing and immunoblotting of plasma apoAI showing the variant apoAI.

which was calibrated by inclusion of substance P or renin on a sinapinic acid matrix. The average masses of the sequenced peptides were calculated by using the Finnigan-MAT GPMAW program, version 1.02.

RESULTS

Hereditary Amyloidosis in an English Family. The family shown in Fig. 1 is of English origin as far as is known. Major visceral amyloidosis has been confirmed in three generations. The propositus (IV-1) was completely asymptomatic at the age of 24 years, when his extensive splenic and hepatic amyloidosis were discovered by ^{123}I-labeled SAP scintigraphy (20). He subsequently developed progressive hypertension, thrombocytopenia, and easy bruising. He also complained of left upper quadrant pain, and, in view of his active life style and the grave risk of splenic rupture, splenectomy was undertaken. An amyloid-laden spleen (1.5 kg) was removed and the platelet count then returned to normal; the bruising ceased and his hypertension became easier to control. None of the remaining family members at risk have any clinical symptoms; IV-2 and IV-3 have had normal ^{123}I-labeled SAP scintigraphs, whereas IV-4 and IV-5 (young children) and II-2 have not been scanned.

Characterization of apoAI and its Gene. When plasma was analyzed by isoelectric focusing and immunoblotting, the propositus and other family members with amyloidosis all showed an abnormal additional apoAI band with a pI corresponding to an increase of one positive charge, compared to normal mature apoAI in unaffected family members and controls (Fig. 1*C*). The bands cathodal to mature apoAI are deamidation products (6); those anodal to the variant apoAI are proapoAI (normal and variant) (21). Plasma from the propositus, from nonaffected family members, and from other controls was fractionated by fast protein liquid chromatography gel filtration. All detectable apoAI eluted in the same, expected position, indicating that both the variant and the normal apoAI were associated with HDL. Amplification and sequencing of the coding region of the apoAI gene (22) revealed that the propositus was heterozygous for a single-base substitution in exon 4, changing the codon for residue 60 of the mature protein from CTG (leucine) to CGG (arginine) (Fig. 2). The remainder of the sequence, including the codon for residue 26, was normal. Hybridization of the amplified fragment of exon 4 from the propositus and other family members with allele-specific oligonucleotides showed concordance of the mutant allele with the presence in plasma of the variant apoAI (Fig. 1 *B* and *C*).

Characterization of Amyloid Fibril Protein. The splenic amyloid deposits of the propositus stained intensely with antibodies to apoAI, identifying apoAI as the amyloid fibril protein. Fibrils were purified from the spleen and analyzed in reduced SDS/PAGE (Fig. 3). All the bands stained on immunoblotting with anti-apoAI antibodies. Preliminary protein sequencing of the predominant 10-kDa doublet, after electroblotting from the polyacrylamide gel, revealed the N-terminal six amino acid residues of mature apoAI. Fibrils solubilized in guanidine were fractionated by gel filtration, and the low molecular mass peak containing the 10-kDa doublet was subjected to protein sequencing (Table 1). The complete N-terminal sequence of mature apoAI up to residue 88 was identified, with only arginine at position 60, indicating that the fibrils contained exclusively the variant apoAI. Electrospray mass spectrometry of the isolated fibril subunits (Fig. 3*B*) revealed three pairs of species of approximately equal abundance (23) corresponding precisely to the expected masses of the N-terminal 88, 92, and 93 residues of mature apoAI with a single substitution of arginine for leucine. The higher mass material in each pair was compatible with oxidation of the single methionine residue (Table 2). A trace peak corresponding to the 94-residue fragment was also present (Fig. 3 and Table 2). The bulk of the fibrils thus consisted of these N-terminal fragments of the Arg-60 mutant apoAI. In addition, the unfractionated fibril preparation contained a very faint 28-kDa band (Fig. 3) in which the N-terminal sequence both of apoAI and of SAP (a universal nonfibrillar constituent of all amyloid deposits) (1) were detected. This suggests that traces of intact or minimally degraded apoAI were present in the fibril preparation, together with traces of SAP that had not been removed from the tissue before fibril extraction.

DISCUSSION

The concordance of the apoAI Arg-60 mutation with the phenotypic expression of amyloid disease and the presence in amyloid fibrils of N-terminal fragments derived exclusively from the mutant protein provide compelling evidence that the mutation is causative. Furthermore this apoAI variant has

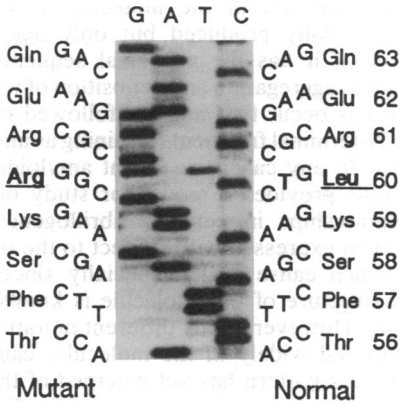

FIG. 2. DNA sequence of part of the apoAI gene corresponding to residues 56–63 of mature apoAI protein, showing that the propositus is heterozygous for a single base change of T to G in codon 60.

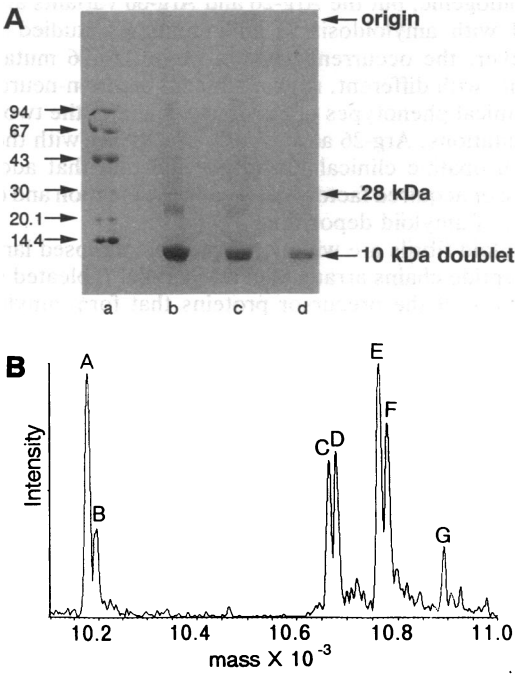

FIG. 3. Characterization of amyloid fibril protein of the propositus. (*A*) SDS/PAGE of solubilized amyloid fibrils. Lanes: a, marker proteins; b, c, and d, three serial doubling dilutions of amyloid fibrils isolated from spleen. (*B*) Electrospray mass spectrometry of the purified 10-kDa component shown in *A*. The molecular masses of peaks A–G are shown in Table 2.

Table 1. Polypeptide analysis of the purified amyloid fibril subunit proteins (10-kDa doublet)

Peptide	Retention time, min	Sequence	TOF mass, Da	Average mass,* Da
1	10.1	QLNLK	617	614.7
2′, 2	8.5, 12.4	ETEGLRQEMSK†	1309, 1324	1307.4
3	17.9	DEPPQSPWDR (VK)‡	1456	1453.6
4	21.6	DSGRDYVSQFEGSAL (GK)‡	1816	1815.9
5	26.7	LLDNWDSVTSTFSK	1611	1612.8
6	28.4	DLATVYVDVLK	1237	1235.4
7	28.8	RREQLGPVTQEFWDNLEK	2245	2245.5

Peptides released by lysyl endopeptidase digestion of the purified fibril subunits were separated by HPLC and subjected to automated N-terminal sequencing as described in *Materials and Methods*; their order in the intact apoAI fragment, by alignment with the known sequence (22), is shown below.

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1         10        20        30        40
DEPPQSPWDR[VK]|DLATVYVDVLK|DSGRVYVSQFEGSAL[GK]|QLNLK|
  peptide 3   peptide 6     peptide 4          peptide 1

      50        60        70        80      88
LLDNWDSVTSTFSK|RREQLGPVTQEFWDNLEK|ETEGLRQEMSK
  peptide 5     peptide 7           peptide 2
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TOF, Time-of-flight mass spectrometry. The boldface R in peptide 7 is the arginine substitution at position 60 of the mature protein.

*Average masses were deduced from peptide sequences.

†The presence of two species of the only methionine-containing peptide, which differ in mass by 15, confirmed the electrospray mass spectrometry results on the intact proteins suggesting the presence of methionine sulfoxide.

‡C-terminal residues of these peptides could not be identified by automated sequence analysis, and the residues expected from the known full-length sequence and confirmed by mass analysis are shown in parentheses.

not been reported, even in a study of 32,000 subjects screened by isoelectric focusing (24). Variants of apoAI are extremely rare (25) and only one, Arg-26, has previously been related to amyloid formation (4, 7). It is intriguing that both the Arg-26 and the Arg-60 mutations replace neutral residues and both produce Arg-Arg doublets in the apoAI sequence, but we have no direct evidence about the possible role of this structural alteration in amyloidogenesis. Normal apoAI is not amyloidogenic, but the Arg-26 and Arg-60 variants are associated with amyloidosis in all individuals studied so far. However, the occurrence of the same Arg-26 mutation in patients with different, neuropathic (4) and non-neuropathic (7), clinical phenotypes of amyloidosis and of the two different mutations, Arg-26 and Arg-60, in patients with the same nonneuropathic clinical phenotype indicate that additional genetic or acquired factors determine the location and clinical effects of amyloid deposition.

Amyloid fibrils are widely held to be composed largely of polypeptide chains arranged in antiparallel β-pleated sheets, and many of the precursor proteins that form amyloid are richly endowed with β structure [for example, immunoglobulin light chains in acquired monoclonal type (AL) amyloid and transthyretin in FAP (26)]. However, the fibrils in reactive systemic amyloidosis are composed of amyloid A protein, derived from serum amyloid A protein, which is not notable for its content of β structure (27). Interestingly, serum amyloid A protein is an apoprotein of HDL as is apoAII, a variant form of which causes senile amyloidosis in a number of inbred mouse strains (28). That three different HDL apoproteins can produce amyloid fibrils suggests that their association with lipid or with this particular lipoprotein may be involved in amyloidogenesis, but there is no direct evidence for this. The lipid-binding amphipathic helices of apoAI are located in the major C-terminal portion of the molecule (29). Less is known about the secondary structure of the N-terminal region, the fragment found in our patient's amyloid fibrils, but it is probably not involved in lipid–protein interactions, and both the mutant and the normal apoAI in the plasma or our propositus were completely associated with HDL.

Table 2. Molecular masses of the peaks observed by mass spectrometry of purified 10-kDa subunits of isolated amyloid fibrils and the calculated masses of the predicted apoAI fragments

Peak	Measured mass*	Predicted apoAI fragment	Calculated mass†, Da
A	10,177.3	Residues 1–88	10,177.3
B	10,195.0	Residues 1–88 including methionine sulfoxide	10,193.3
C	10,665.3	Residues 1–92	10,663.7
D	10,680.2	Residues 1–92 including methionine sulfoxide	10,679.7
E	10,763.5	Residues 1–93	10,762.9
F	10,779.8	Residues 1–93 including methionine sulfoxide	10,778.9
G	10,890.9	Residues 1–94	10,891.1

*Mean of duplicate determinations.

†With arginine at position 60.

It is not clear whether the N-terminal portion of the arginine-substituted mutant protein is unduly susceptible to cleavage from the rest of the molecule or whether this fragment is normally produced but only aggregates into amyloid fibrils if it has the abnormal sequence. Another possibility is that aggregation and deposition of whole variant apoAI molecules occur first and are followed by cleavage, with just the N-terminal fragment remaining as amyloid fibrils in the tissues. In any case, the potent amyloidogenicity of these mutations provides a model for study of structure–function relationships in amyloid fibrillogenesis. Similar hopes have been expressed with respect to the transthyretin mutations, which cause FAP, especially since the three-dimensional structure of this molecule is known to atomic resolution (30). However, many different mutations in transthyretin, scattered widely in the molecule, cause amyloid (31), and no clear pattern has yet emerged of the perturbations these may cause in its conformation. Furthermore, normal transthyretin is itself inherently amyloidogenic, causing senile systemic amyloidosis in about 25% of the elderly population (32). On the other hand, the commonest trans-

thyretin mutation associated with FAP, methionine for valine at residue 30, is not completely penetrant in some populations, and even an individual homozygous for the trait has been reported without amyloid in the sixth decade (33). The facts that normal apoAI is not inherently amyloidogenic, that mutations in its gene are extremely rare, and that there is evidence for complete penetrance, at least in the present family, all suggest that it may provide a more useful model.

Finally, regardless of its fundamental significance, the identification of the present mutation permits informed genetic counseling and definitive pre- and postnatal diagnosis in this rare but devastating hereditary disease.

We thank Ruth Gallimore and Alistair Sterling for technical assistance and Beth Sontrop for expert preparation of the manuscript. This work was supported in part by the Medical Research Council, U.K., and the Wellcome Trust.

1. Pepys, M. B. (1988) in *Immunological Diseases*, eds. Samter, M., Talmage, D. W., Frank, M. M., Ansten, K. F. & Claman, H. N. (Little, Brown, Boston), 4th Ed., Vol. 1, pp. 631–674.
2. Benson, M. D. & Wallace, M. R. (1989) in *Metabolic Basis of Inherited Disease*, eds. Beaudet, A., Scriver, C., Sly, W. & Valle, D. (MacGraw-Hill, New York), 6th Ed., pp. 2439–2460.
3. Maury, C. P. J. (1991) *J. Clin. Invest.* **87**, 1195–1199.
4. Nichols, W. C., Dwulet, F. E., Liepnieks, J. & Benson, M. D. (1988) *Biochem. Biophys. Res. Commun.* **156**, 762–768.
5. Nichols, W. C., Gregg, R. E., Brewer, B., Jr., & Benson, M. D. (1990) *Genomics* **8**, 318–323.
6. Jackson, R. L., Morrisett, J. D. & Gotto, A. M., Jr. (1976) *Physiol. Rev.* **56**, 259–316.
7. Jones, L. A., Harding, J. A., Cohen, A. S. & Skinner, M. (1991) in *Amyloid and Amyloidosis 1990*, eds. Natvig, J. B., Forre, O., Husby, G., Husebekk, A., Skogen, B., Sletten, K. & Westermark, P. (Kluwer, Dordrecht, The Netherlands), pp. 385–388.
8. Ostertag, B. (1932) *Zentralbl. Aug. Pathol.* **56**, 253–254.
9. Hawkins, P. N., Lavender, P. J. & Pepys, M. B. (1990) *N. Engl. J. Med.* **323**, 508–513.
10. Menzel, H.-J. & Uterman, G. (1986) *Electrophoresis* **7**, 492–495.
11. McDowell, I. F. W., Wisdom, G. B. & Trimble, E. R. (1989) *Clin. Chem.* **35**, 2070–2073.
12. Keir, G., Walker, R. W. H., Johnson, M. H. & Thompson, E. J. (1982) *Clin. Chim. Acta* **121**, 231–236.
13. Talmud, P., Tybjaerg-Hansen, A., Bhatnagar, D., Mbewu, A., Miller, J. P., Durrington, P. & Humphries, S. (1991) *Atherosclerosis* **89**, 137–141.
14. Saiki, R. K., Gelfond, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B. & Erlich, H. A. (1988) *Science* **239**, 487–491.
15. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY), 2nd Ed.
16. Casanova, J.-L., Pannetier, C., Jaulin, C. & Kourilsky, P. (1990) *Nucleic Acids Res.* **18**, 4028.
17. Pras, M., Schubert, M., Zucker-Franklin, D., Rimon, A. & Franklin, E. C. (1968) *J. Clin. Invest.* **47**, 924–933.
18. Poulter, L., Green, B. N., Kaur, S. & Burlingame, A. L. (1990) in *Biological Mass Spectrometry*, eds. Burlingame, A. L. & McCloskey, J. A. (Elsevier, Amsterdam), pp. 119–128.
19. Totty, N. F., Waterfield, M. D. & Hsuan, J. J. (1992) *Protein Science* **1**, in press.
20. Hawkins, P. N., Feest, T. G. & Pepys, M. B. (1991) in *Amyloid and Amyloidosis 1990*, eds. Natvig, J. B., Forre, O., Husby, G., Husebekk, A., Skogen, B., Sletten, K. & Westermark, P. (Kluwer, Dordrecht, The Netherlands), pp. 789–792.
21. Zannis, V. I., Karathanasis, S. K., Keutmann, H. T., Goldberger, G. & Breslow, J. L. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 2574–2578.
22. Shoulders, C. C., Kornblihtt, A. R., Munro, B. S. & Baralle, F. E. (1983) *Nucleic Acids Res.* **11**, 2827.
23. van Dorsselaer, A., Bitsch, F., Green, B., Jarvis, S., Lepage, P., Bischoff, R., Kolbe, H. V. J. & Roitsch, C. (1990) *Biomed. Environ. Mass Spectrom.* **19**, 692–704.
24. von Eckardstein, A., Funke, H., Walter, M., Altland, K., Benninghoven, A. & Assmann, G. (1990) *J. Biol. Chem.* **265**, 8610–8617.
25. Assman, G., Schmitz, G., Funke, H. & von Eckardstein, A. (1990) *Curr. Opin. Lipidol.* **1**, 110–115.
26. Glenner, G. G. (1980) *N. Engl. J. Med.* **302**, 1283–1292, 1333–1343.
27. Turnell, W., Sarra, R., Glover, I. D., Baum, J. O., Caspi, D., Baltz, M. L. & Pepys, M. B. (1986) *Mol. Biol. Med.* **3**, 387–407.
28. Higuchi, K., Kitagawa, K., Naiki, H., Hanada, K., Hosokawa, M. & Takeda, T. (1991) *Biochem. J.* **279**, 427–433.
29. Segrest, J. P., Jackson, R. L., Morrisett, J. D. & Gotto, A. M. (1974) *FEBS Lett.* **38**, 247–250.
30. Blake, C. C. F., Geisow, M. J., Oatley, S. J., Rerat, B. & Rerat, C. (1978) *J. Mol. Biol.* **121**, 339–356.
31. Saraiva, M. J. M. (1991) *Neuromuscular Dis.* **1**, 3–6.
32. Pitkanen, P., Westermark, P. & Cornwell, G. G. (1984) *Am. J. Pathol.* **117**, 391–399.
33. Holmgren, G., Drugge, U., Haettner, E., Lundgren, E., Sandgren, O., Steen, L. & Wahlqvist, J. (1990) in *Familial Amyloidotic Polyneuropathy and Other Transthyretin Related Disorders*, eds. Costa, P. P., de Freitas, A. F. & Saraiva, M. J. M. (Arquivos de Medicina, Porto, Portugal), pp. 193–197.