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A Homozygous Transthyretin Variant Associated with Senile Systemic Amyloidosis: Evidence for a Late-Onset Disease of Genetic Etiology

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Summary

Senile systemic amyloidosis (SSA) is a late-onset disease characterized by deposition of amyloid fibrils containing transthyretin (TTR). Amino acid sequencing of protein isolated from the amyloid fibrils of a patient with SSA identified TTR containing a position−122 isoleucine-for-valine substitution. This change led to the prediction of a genomic G-to-A transition, destroying an *Mae*III restriction site. We confirmed the presence of the variant DNA fragment both by Southern blotting and by visualization of *Mae*III digests of DNA amplified around codon 122, by using the polymerase chain reaction. The patient's DNA was entirely resistant to *Mae*III cleavage; therefore, only the mutant sequence was present. DNA from none of either 24 controls or six other SSA patients contained the variant. Quantitative Southern blotting demonstrated that the patient's DNA contained two copies of the TTR gene per genome; the mutation was therefore homozygous rather than hemizygous. In the present case, the homozygous mutation TTR (122 Val→Ile) is associated with SSA, a finding which is consistent with autosomal recessive inheritance of this condition.

Introduction

In recent years it has become evident that a number of diseases with characteristic onset at age 40 years or older have a genetic etiology. In some cases, such as Huntington disease and Alzheimer disease, the chromosomal loci associated with disease and linked genetic markers have been identified, although the specific genes involved have not yet been determined (Gusella 1989). In other cases, such as adult-onset G_{M2} gangliosidosis (Tay-Sachs disease), specific point mutations in well-characterized genes are known to cause disease (Paw et al. 1989). We present data suggesting that senile systemic amyloidosis (SSA) is another disease in the latter category.

SSA is a disease of late onset (usually after age 60 years), characterized by deposits of amyloid material consisting of the serum protein transthyretin (TTR, formerly called prealbumin). In SSA, the clinically dominant site of amyloid deposition is the heart, a characteristic causing congestive heart failure and conduction-system disturbances (hence its previous name, "senile cardiac amyloidosis") (Smith et al. 1979; Westermark et al. 1979; Pitkanen et al. 1984; Olson et al. 1987).

TTR is a tetrameric serum protein of four identical subunits, containing substantial β pleated sheet structure (Blake et al. 1978), which transports thyroxine and retinol-binding protein (Van Jaarsveld et al. 1973; Ferguson et al. 1975). Its gene, consisting of four exons and three introns, has been sequenced (Sasaki et al. 1985; Tsuzuki et al. 1985). TTR constitutes the major fibril protein not only in SSA but also in the related syndromes of familial amyloidotic polyneuropathy (FAP) and vitreous amyloid. In these diseases it appears that a TTR point mutation is always present, leading, via unknown mechanisms, to fibril formation. FAP behaves as an autosomal dominant disease. In the most

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common type of FAP, seen in kindreds of Portuguese, Japanese, and Swedish descent, a position-30 valine-for-methionine substitution is present (Dwulet and Benson 1983; Tawara et al. 1983; Saraiva et al. 1984). Other kindreds contain TTR with mutations at positions 33 (Jacobson et al. 1988), 60 (Wallace et al. 1986), 77 (Wallace et al. 1988), and 84 (Dwulet and Benson 1986). The position-30 mutation is present also in some cases of isolated vitreous amyloid (Gorevic et al. 1987; Sandgren et al. 1988). Another TTR variant, at position 111, has been described in a kindred with "familial cardiomyopathy" (Nordlie et al. 1988). In contrast, until recently, the reason for TTR deposition in SSA was unknown; it has been hypothesized that the amino acid sequence of the deposited TTR might be normal (Pitkanen et al. 1984; Cornwell et al. 1988). The first complete amino acid sequence of TTR isolated from the fibrils of a patient (HAR) with SSA was recently determined: a position-122 substitution of isoleucine for valine was present, and the sequence was otherwise normal (Gorevic et al. 1989).

The position-122 substitution found by protein sequencing led to the prediction of a G-to-A point mutation at the first position of codon 122 in the DNA. This mutation should destroy a recognition site for the restriction endonuclease *Mae*III (GTNAC), occurring in codon 122 and the first two bases of codon 123 (fig. 1). In order to confirm the protein studies, we analyzed the TTR gene in this patient.

Material and Methods

Source and Preparation of Genomic DNA

HAR was a 68-year-old black male from West Virginia who died of congestive cardiomyopathy. Amyloid protein isolated from his heart consisted of TTR with a position-122 substitution (Gorevic et al. 1989). Genomic DNA was extracted (Blin and Stafford 1976) from cardiac tissue frozen during autopsy. DNA was also prepared from six other patients with SSA and from 24 controls: nine patients with colonic malignancy, four patients with lymphoma, three normal controls, four patients with FAP (one patient with a position-33 mutation [Jacobson et al. 1988] and three patients with incompletely defined mutations [Jacobson et al. 1987]), and four unaffected family members of one of the patients with FAP. Considering duplication of alleles resulting from the testing of DNA samples from related individuals, a minimum of 43 control alleles were tested. The control samples used for the gene dosage experiment were both from patients with FAP who were known to be heterozygous for TTR variants as detected by a unique *Fnu*4HI restriction pattern (Jacobson et al. 1987); thus, each had two copies of the TTR gene. One was male, and the other was female.

	121	122	123
(normal)	VAL	VAL	THR
(normal)	GTC	GTC	ACC
(variant)	GTC	ATC	ACC
(variant)	VAL	ILE	THR

Mae III cuts at ^GTNAC

Figure 1 Sequence of normal and variant TTR gene around codon 122.

Southern Blotting and Hybridization

For *Mae*III digests, 30 μg of genomic DNA were digested overnight in threefold excess enzyme and then for an additional 4 h after addition of further, twofold excess enzyme. Samples were electrophoresed on a 2% agarose gel and vacuum blotted to a positively charged nylon membrane (Nytran; Schleicher and Schuell, Keene, NH). For the gene dosage experiment, 50 μg of DNA from patient HAR and from two controls were digested simultaneously with fivefold excess of *Eco*RI, *Bam*HI, and *Pvu*II. After 12 h, an additional threefold excess of the enzymes was added, and the incubations continued for 4 h. The samples were extracted with phenol and then with chloroform, were ethanol precipitated, and were resuspended in Tris 10 mM, EDTA 1 mM, pH 8.3 (TE). The concentration of digested DNA was measured by absorbance at 260 nm. Fifteen micrograms of each sample were electrophoresed through a 0.8% agarose gel and vacuum blotted to a nitrocellulose filter. Filters were fixed by baking for 2 h at 80°C under vacuum; were prehybridized at 42°C for 18 h in 5 × Denhardt's solution (1 × Denhardt's = 0.2 g/liter Ficoll, 0.2 g/liter polyvinylpyrrolidone, 0.2 g/liter BSA), 5 × SSC (1 × SSC = 0.15 M NaCl, 0.015 M sodium citrate), 50% formamide, 50 mM sodium phosphate pH 6.8, 1% glycine, 0.1% SDS, 0.25–0.5 mg sheared, denatured salmon sperm DNA/ml; and were hybridized for 18 h in 1 × Denhardt's 5 × SSC, 50% formamide, 20 mM sodium phosphate pH 6.8, 0.1% SDS, 0.25–0.5 mg sheared, denatured salmon sperm DNA/ml, and radiolabeled probes. Filters were washed at a final stringency of 0.2 × SSC, 0.5% SDS, at 59°C

(for the nylon filter) or at 65°C (for the nitrocellulose filter) and were exposed to Kodak XAR-5 film in cassettes with intensifying screens.

Oligonucleotides and Gene Amplification

Oligonucleotide primers, designed to be complementary to the expected genomic TTR sequences at either end of exons 3 and 4 and including artificial restriction sites at the 5′ ends, were synthesized by Dr. Bernard Goldschmidt (table 1). For the initial *Mae*III mapping experiments, on patient HAR and the controls, amplification of TTR exon 4 was performed by the polymerase chain reaction (PCR) (Kogan et al. 1987) in a final volume of 50 µl containing 1 µg genomic DNA and 200 ng (14 pmol) of each of the fourth-exon oligonucleotide primers, 10 mM Tris HCl pH 8.3, 0.2 mM each deoxynucleotide triphosphate, 5 mM KCl, 2 mM MgCl2, 0.01% (w/v) gelatin, 1 mM β-mercaptoethanol. Samples were incubated at 93°C for 5 min to denature the DNA, after which 1.5 units of *Taq* polymerase (New England Biolabs, Beverly, MA) were added to each sample. Samples were transferred among heating blocks at 37°C for 30 s (primer annealing), at 63°C for 90 s (DNA synthesis), and at 93°C for 60 s (denaturation), for 30 cycles. After the last cycle, samples were incubated for an additional 5 min at 63°C and were allowed to cool to room temperature. In later experiments, on samples from other patients with SSA and for synthesis of exon-specific probes (see below), annealing was performed at 55°C–60°C × 2 min, synthesis was performed at 72°C × 1 min, and denaturation was performed at 94°C × 1 min in an automated temperature cycler (Perkin Elmer–Cetus, Norwalk, CT). An aliquot of 5 µl from each sample was electrophoresed on an agarose gel to check for successful amplification, as shown by a single band of 123 bp after ethidium bromide staining. A few samples showed a weak or absent band after 30 cycles; further cycles were performed until successful amplification could be demonstrated.

Probes

A TTR cDNA probe was cloned according to a method described elsewhere (Jacobson et al. 1988). Probes corresponding to exons 3 and 4 of the TTR gene were made by PCR, in a final volume of 200 µl containing 1 µg of normal genomic DNA, 80 pmol of each primer, and 2 units of *Taq* polymerase in standard PCR buffer (see above), and were amplified for 30 cycles. For use as a control probe in the gene dosage experiment, DNA from p114.2, a 647-bp genomic clone from coagulation factor VIII in plasmid pUC12 (Gitschier et al. 1985), was obtained from the American Type Culture Collection. The insert was released from the plasmid by digestion with *Sac*I and *Bam*HI. The probes corresponding to TTR exons 3 and 4 and to the factor VIII insert were applied to 2% agarose gels and were electrophoresed, and the bands were visualized by ethidium bromide staining. Bands were removed from the gels by electrophoresis into filter paper backed by dialysis membranes and were removed from the filter papers by centrifugation (Girvitz et al. 1980). Eluted samples were extracted once with phenol and once with chloroform, were purified on Elutip columns (Schleicher and Schuell), were ethanol precipitated, and were resuspended in TE. The concentration of each DNA sample was measured by optical density. *Mae*III digests were probed with 250 ng of the TTR cDNA probe nick-translated with ^{32}P-dCTP to a specific activity of at least 5×10^8 cpm/µg. For the quantitative Southern blotting experiment, 10 ng of each of the three probes (TTR exon 3, TTR exon 4, and factor VIII) were individually ^{32}P-labeled to a specific activity of at least 5×10^9 cpm/µg by random priming, and the probes were combined for hybridization.

Table 1

Sequences of Synthetic Oligonucleotides Used for DNA Amplification

Oligonucleotide Sequence[a]	Location of Sequence
1. 5′ ****** AGAATTCCAGACTTTCACACCTTATAG 3′	Exon 3, 5′ end
2. 5′ ****** ATCTAGACCTCTGCATGCTCATGGAATG 3′	Exon 3, 3′ end
3. 5′ ****** ACTGCAGGTGGTATTCACAGCCAACGA 3′	Exon 4, 5′ end
4. 5′ ****** AGAATTCCCTCATTCCTTGGGATTGGTG 3′	Exon 4, 3′ end

[a] Underlined regions are complementary to the genomic sequence. Bases under asterisks constitute a synthetic endonuclease recognition site (*Eco*RI for 1 and 4, *Xba*I for 2, and *Pst*I for 3).

Digestion of Amplified DNA Sequences

An aliquot of 20 μL of each amplified sample was digested with 10 units of *Mae*III, was electrophoresed on a 4% agarose gel, and was visualized by UV fluorescence after ethidium bromide staining. *Msp*I-digested PBR 322 DNA was used as a size standard; bands are seen at 622, 527, 404, 309, 242, 238, 217, 201, 190, 180, 160, 147, 123, 110, 90, 76, and 67 bp.

Mixing Experiment

DNA from the patient with SSA and DNA from a normal control were combined prior to amplification and *Mae*III digestion. In all cases, a total of 1 μg of genomic DNA was amplified; the patient DNA consisted of 0%, 25%, 50%, 75%, or 100% of the total. These samples were then amplified and cut with *Mae*III as described above.

Calculation of Gene Dosages

The filter of the sample from HAR and from two FAP controls was exposed to Kodak XAR film for several periods of time, ranging from 14 to 36 h. Intensity of the bands on autoradiograms developed after 15, 18, and 36 h were measured by automated densitometry, and numerical values for the relative optical density of the six bands were obtained. Gene dosages were calculated from ratios of optical densities measured from a film in the linear range.

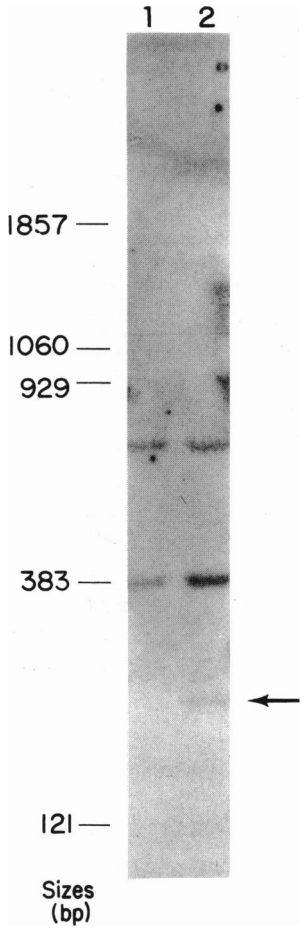

Figure 2 Southern blot of *Mae*III digests of control DNA (lane 1) and DNA of the patient with SSA (lane 2). The band of 207 bp seen in the SSA sample (arrow) results from the loss of an *Mae*III site; in the presence of the normal TTR sequence, this fragment should be cut into pieces of 139 and 68 bp. The other bands result from binding of the probe to fragments from other exons, and they agree with the pattern predicted from the genomic sequence.

Results

A Southern blot of *Mae*III-digested DNA from the patient with SSA revealed a band of 207 bp not present in control DNA (fig. 2). Analysis of the published sequence indicated that in the presence of the normal sequence *Mae*III further cleaves this piece into fragments of 139 and 68 bp (fig. 3). These fragments were smaller than the lower size limit at which we were able to detect any bands; no fragment smaller than 200 bp was seen in either the SSA sample or control sample.

DNA amplified around codon 122 yielded a segment of 123 bp for all samples, as expected (fig. 4). Digestion of these samples by *Mae*III revealed that DNA from the patient with SSA was entirely resistant to *Mae*III cleavage, as demonstrated by persistence of the single band at 123 bp. All other samples, from other patients with SSA and from controls, showed both complete disappearance of the 123-bp fragment and the appearance of new bands at 93 and 30 bp (fig. 5).

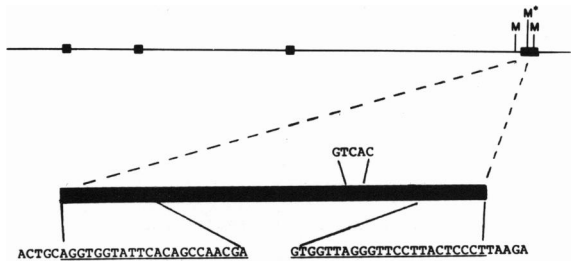

Figure 3 Schematic diagram of TTR gene. *Top,* Exons are (indicated by blackened boxes) and introns and flanking regions (both indicated by lines). The *Mae*III restriction site normally present at codon 122 but absent in HAR is marked (M*), as are the two flanking *Mae*III sites (M). The 25 other *Mae*III sites in the gene are unmarked. *Bottom,* Enlargement of exon 4, showing the positions of the *Mae*III site at codon 122 and the amplification primers.

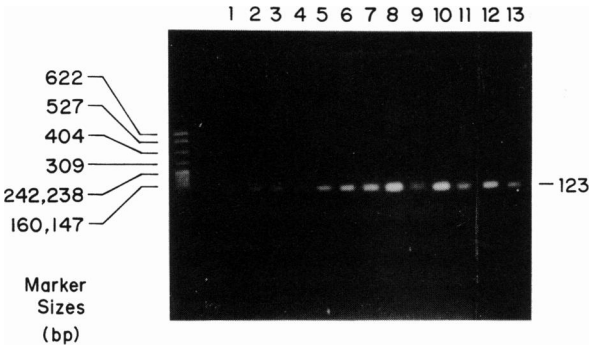

Figure 4 DNA from the SSA patient (lane 1) and from 12 controls (lanes 2–13) after PCR amplification. DNA from 12 additional controls and from six other patients with SSA showed an amplified segment of identical size.

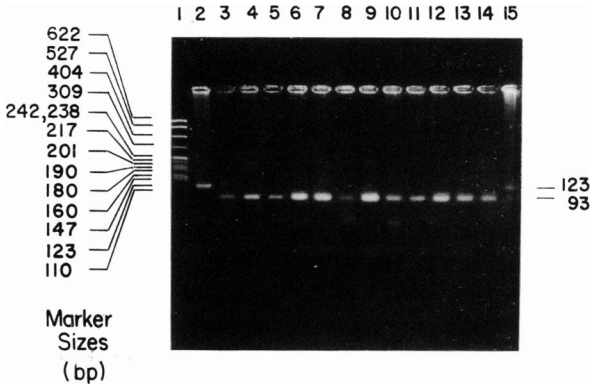

Figure 5 *Mae*III digests of amplified DNA segments from the SSA patient (lane 2) and from 12 controls (lanes 3–14). Lane 15, 50:50 mixture of the samples in lanes 1 and 2, showing the pattern that would be expected in a heterozygote. An additional band, at 30 bp, can be seen faintly in lanes 3–15 on an overexposed photograph of this gel (not shown). DNA from 12 additional controls and from six other unrelated patients with SSA showed the same pattern as seen in lanes 2–13. Lane 1, DNA from PBR 322, cut with *Msp*I.

To eliminate the possibility that the DNA sample from the SSA patient inhibited the activity of *Mae*III, SSA and control DNA were mixed in varying proportions prior to amplification and digestion. No such inhibition occurred; the relative intensity of the bands at 93 bp (normal) and 123 bp (mutant) varied with the control:SSA DNA ratio in the mixture prior to amplification (fig. 6).

Quantitative Southern blotting using DNA from HAR and from two TTR heterozygote controls, hybridized with TTR and factor VIII probes, revealed one TTR band of 4.4 kb and one factor VIII band of 3.5 kb for each sample, as expected both from previous mapping data and from the known TTR genomic sequence (fig. 7). Visual inspection revealed that the TTR bands were of roughly equal intensity in all samples and that the factor VIII band in the female control sample was more intense than the corresponding band in the other (male) samples, a finding consistent with the expected 2:1:1 ratio. As a quantitative test of the protocol's ability to distinguish one gene copy from two gene copies, the ratio (B1/B3):(A1/A3) was calculated, where B1, B3, A1, and A3 represent the relative optical densities of the four bands in the control lanes (table 2). This ratio equaled 1.96, in agreement with the expected value of 2.0. The TTR gene dosage was then calculated as the average of the dosage calculated by comparing the test sample with each of the two controls, i.e., $(A2/A3)/(B2/B3) + [2 \times (A2/A1)/(B3/B1)] = 2.02$. Repeated measurements from the same and other films yielded ratios between 1.90 and 2.15; thus, HAR DNA contained two copies of the TTR gene.

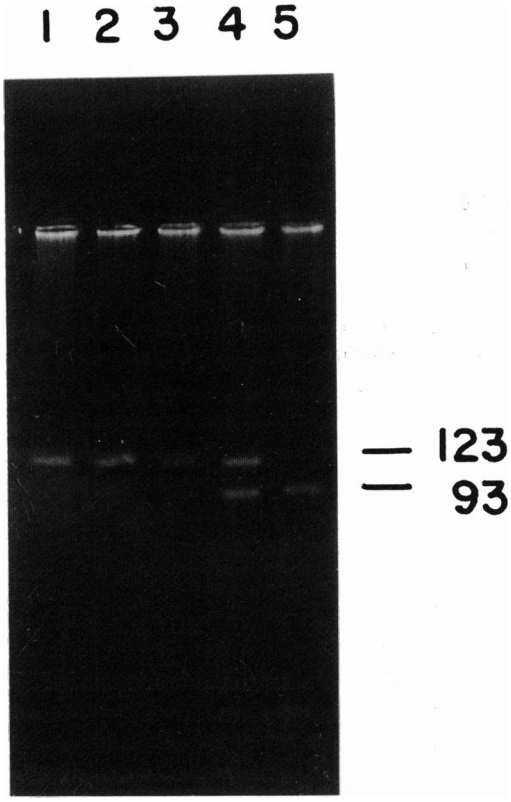

Figure 6 Mixing experiment. SSA and control DNA were mixed in 3:1, 1:1, and 1:3 ratios prior to amplification and *Mae*III digestion. Lane 1, 1.0 μg SSA DNA. Lane 2, 0.75 μg SSA and 0.25 μg control DNA. Lane 3, 0.50 μg SSA and 0.50 μg control DNA. Lane 4, 0.25 μg SSA and 0.75 μg control DNA. Lane 5, 1.0 μg control DNA.

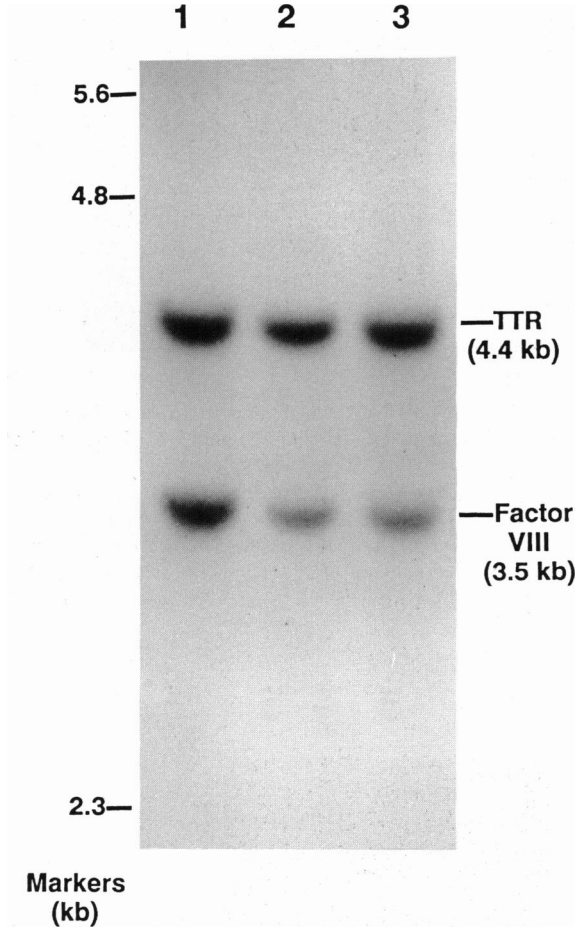

Figure 7 Southern blot of DNA from SSA patient HAR and from two controls, hybridized simultaneously to probes for TTR and factor VIII. Lane 1, Female control. Lane 2, HAR. Lane 3, male control. Each sample hybridized to probes for both TTR and factor VIII.

Discussion

The first report of a TTR amino acid substitution associated with SSA was the position-122 substitution of isoleucine for valine (Gorevic et al. 1989). No valine was found at position 122; this could have resulted either because normal TTR did not codeposit with the variant form or because no normal TTR was present; these two possibilities could not be distinguished on the basis of the protein data. This point mutation should result from an alteration in a single base in codon 122, causing a change in the restriction pattern seen after *Mae*III digestion. Such a change was, indeed, seen on Southern blotting; however, because of the small sizes of the fragments involved (139 and 68 bp in the normal DNA sample and 207 bp in the variant DNA sample), only a weak autoradiographic signal corresponding to the 207-bp fragment could be seen even after a 4-wk exposure time. No information regarding the presence of normal bands was obtained from Southern blotting. The normal *Mae*III fragments are below the size limit for detection in our hands; no fragment smaller than 150 bp could be demonstrated in either the SSA sample or the control sample, even when each lane contained 30 μg of genomic DNA and when the autoradiogram exposure time was 4 wk. In general, such small fragments are difficult to detect in Southern blots of genomic DNA.

The use of PCR enabled us to overcome the technical limits imposed by Southern blotting and to demonstrate that in the DNA from HAR no normal TTR allele was present; thus, the mutation was either hemizygous, accompanied by deletion of the other allele, or homozygous. The possibility that the SSA DNA sample inhibited *Mae*III, thereby preventing detection of a normal allele, was ruled out by a mixing experiment. In this experiment, the normal:variant fragment ratio approximated the control:SSA DNA ratio in the original amplification mixture. Combining patient and control DNA prior to amplification yielded the pattern that would be expected in a heterozygote.

Ideally one would like to study both parents of a patient demonstrating a potentially homozygous mutation and show a heterozygous pattern in each; however, such a study is difficult for a disease with onset in the seventh decade. If the parents are unavailable, information from other relatives may clarify the pattern of inheritance. In the present case, however, no

Table 2

Relative Optical Density of Autoradiographic Bands in Gene Dosage Experiment

DNA Sample	1 Female Control	2 SSA Patient	3 Male Control
TTR ("A")	791	647	756
Factor VIII ("B")	682	279	332

NOTE.—Values are a relative measure of intensity of the six bands seen on the autoradiogram in fig. 7 and were obtained by integrating the area under the curve, on a plot of optical density vs. distance. Each band was assigned an identifying letter (A = TTR; B = factor VIII) and number (1 = female control; 2 = SSA patient; 3 = male control). If the protocol is able to distinguish one gene copy from two gene copies the ratio (B1/B3):(A1/A3) should equal 2.0. The calculated ratio equaled 1.96. The TTR gene dosage was calculated by the formula (A2/A3)/(B2/B3) + [2 × (A2/A1)/(B2/B1)] and equaled 2.02.

family members of patient HAR have been available for study. Therefore, to determine whether HAR was homozygous for the TTR variant or hemizygous with deletion of the opposite allele, quantitative Southern blotting was performed. DNA from HAR and two controls (one male and one female, both with FAP and known to contain two copies of the TTR gene) was hybridized to probes for TTR and for factor VIII. TTR probes binding to both exons 3 and 4 were used in order to increase the signal intensity over that obtained using an exon 4 probe alone. A control probe on the X chromosome, rather than an autosomal probe, was chosen in order to provide an internal test of the protocol's power to distinguish between gene dosages of one and two per genome. This strategy ensured that if negative results were obtained, i.e., if HAR DNA contained the same gene dosage as the controls (as turned out to be the case), we could rule out the possibility that the results simply reflected an assay insufficiently sensitive for detecting the difference in the signal generated by one versus two copies per genome. The ratios of the optical density of the TTR and factor VIII bands in the control samples demonstrated that the method used did distinguish between gene dosages of one and two. DNA from HAR gave a measured TTR gene dosage of 2.02; therefore, HAR was homozygous for the position-122 variant.

The term "senile systemic amyloidosis" was originally used by Pitkänen et al. (1984) to refer to the small amount of TTR-amyloid material found incidentally at autopsy in the hearts and elsewhere in upward of 25% of elderly (over age 80 years old) individuals (Wright et al. 1969; Hodkinson and Pomerance 1977; Cornwell et al. 1983; Pitkänen et al. 1984; Smith et al. 1984; Lie and Hammond 1988). They noted that such deposition usually has minimal or no adverse effects on cardiac function, although a small proportion of patients have "massive cardiac involvement" (Cornwell et al. 1983). They predicted that the generally benign deposits commonly found in elderly individuals were likely to consist of TTR of normal amino acid sequence, in contrast to the situation seen in FAP, where an abnormal molecule is always found (Pitkänen et al. 1984). This prediction is supported by a preliminary report of a normal TTR sequence found in amyloid material isolated from one such individual (Westermark et al. 1990). Three other partial amino acid sequences of TTR from patients with SSA have also been reported; the regions sequenced were normal in all cases, one of which included position 122 (Sletten et al. 1980; Cornwell et al. 1988); however, mutations may have been present elsewhere in the molecule.

In contrast, Olson et al. (1987) used the same label, "senile systemic amyloidosis," in reporting five men, age 57–72 years, who had severe congestive heart failure secondary to TTR amyloid deposition (Olson et al. 1987). These patients and the small proportion of patients with "massive cardiac involvement" mentioned by Cornwell et al. (1983) appear to constitute a distinct small group in which the stimulus for TTR deposition is accelerated, causing increased amyloid formation and true "disease" (i.e., symptoms). Neither our patient nor any of the patients described by Olson et al. (1987) were known to have a family history of amyloidosis or cardiac disease.

Our patient and those of Olson et al. apparently have disease resembling that of a Danish kindred with "familial amyloidotic cardiomyopathy," in which several family members developed symptomatic cardiac amyloidosis in their forties and fifties, with the amyloid fibrils containing TTR with a position-111 mutation (Frederiksen et al. 1962; Nordlie et al. 1988). These patients appear similar also to many patients in an Appalachian kindred with "familial amyloidotic polyneuropathy" due to a TTR position-60 variant, many of whom have cardiomyopathy but little or no neurologic involvement (Wallace et al. 1986; Benson et al. 1987).

We believe that the term "senile systemic amyloidosis" has been used to describe two related but distinct entities. As used originally, the term refers to a usually incidental finding of high prevalence in the elderly population. The stimulus for this amyloid deposition is unknown, and it may relate to normal processes of aging. In most or all such cases the TTR deposits may be of normal sequence, as there is presently no evidence for the existence of TTR polymorphisms of high prevalence in the general population. In a smaller number of individuals, the process of TTR amyloid deposition in the heart is accelerated, causing true disease. Our prediction is that in this subgroup, with heavy TTR-amyloid deposits causing congestive heart failure and arrhythmias in the sixth and seventh decades, the stimulus for amyloid deposition will always be found to be a TTR point mutation, similar to the case in FAP.

In addition, some patients may fall between these two extremes. It would appear likely that patients older than age 80 years will be described with moderate TTR-amyloid cardiac deposition and cardiac symptoms; such patients may have TTR containing mutations which

have only a minor effect on the amyloidogenic properties of TTR, or they may be heterozygous for TTR variants associated with accelerated amyloid deposition when present as homozygous variants, such as the position-122 variant.

We have looked for the position-122 variant in six additional DNA specimens from unrelated individuals with SSA and in specimens from controls with no clinical evidence of SSA. The control group included three kindreds with FAP associated with three different TTR mutations, patients with diseases unrelated to TTR-associated amyloidosis (lymphoma and colon carcinoma), and normal controls. The position-122 variant was found in no specimen other than HAR. This variant could represent a normal population polymorphism, associated with disease only in the homozygous state. The fact that none of 43 control alleles and none of 12 alleles in other SSA patients demonstrated the variant suggests that, if it is a normal population polymorphism, it may be a rare allele, or one found only in certain ethnic populations. The patient we studied was a black American from West Virginia. Only two of our control samples and one of the other SSA samples were from blacks. Supporting the hypothesis that the position-122 variant may cause SSA in the black population is the recent identification of the same TTR variant in two other patients with SSA; in at least one case the variant may be homozygous, and in both cases the patient was black (M. J. M. Saraiva and W. C. Nichols, personal communication). Whether this variant appears in other racial groups, whether it is always associated with disease in the homozygous state, whether heterozygous carriers of the variant will also develop SSA, and what the gene frequency is in the black population remain unanswered at this time. Of note is that FAP has not been reported in the black population. The primary site of deposition (the peripheral nerves or cardiac system) may be related to other genetic or environmental factors, separate from the primary TTR amino acid sequence; at present, this too is unknown.

This is the first instance of any form of amyloidosis in which the index case had a homozygous mutation. In FAP, the TTR variant typically causes disease in an autosomal dominant manner—affected patients usually have one normal and one abnormal TTR allele (although Swedish homozygotes for the position-30 TTR variant have also been reported; Holmgren et al. 1988). Why the position-122 variant may behave differently is not clear. Indeed, the process by which a soluble precursor becomes an insoluble fibril is not understood for any of the amyloidoses. In the present case, perhaps the location of the mutation—i.e., five amino acids from the carboxy-terminus of the molecule—or the nature of the amino acid substitution—i.e., one hydrophobic amino acid for another—caused only a slight change either in the molecule's conformation or in the interaction between protein subunits, thereby leading to deposition only in the homozygote. The absence of the position-122 variant in the other SSA samples tested suggests that SSA may be a heterogeneous TTR disorder; perhaps, as in the case of FAP, a variety of TTR variants will be found in SSA patients from different ethnic and racial backgrounds.

Our data suggest that SSA may fall into the group of diseases in which a genomically encoded protein abnormality, present from birth, fails to produce disease until late in life. This is consistent with either the accumulation of the mutant gene product over time, an age-related reduction in the ability to dispose of a structurally abnormal protein, or both. The identification of TTR variants may help clarify the mechanisms by which proteins in all forms of amyloidosis, initially synthesized as soluble molecules, become deposited in tissues under physiologic conditions, thereby causing disease.

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