Tissue distribution and levels of gelsolin mRNA in normal individuals and patients with gelsolin-related amyloidosis

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Abstract We measured quantitatively the mRNA levels of intracellular and secretory forms of gelsolin, an actin-modulating protein, in human tissues from subjects of different ages. The intracellular gelsolin mRNA constituted the major type of gelsolin steady-state mRNA in all tissues analyzed. Both forms of gelsolin were expressed in most adult tissues, with particularly high mRNA levels in all types of muscle and interestingly in skin. Between the adult and infantile tissues the most striking difference in expression levels was found in liver, as the adult liver contained only a subtle amount of gelsolin mRNA. Skin and muscle samples from patients with gelsolin-related amyloidosis (FAF), with significantly increased concentrations of serum gelsolin, did not reveal an increased expression of the gene, and both mutant and wild-type alleles were expressed in equal amounts. The high level of expression of the gelsolin gene in the skin in general could locally contribute to the characteristic skin amyloidosis in FAF patients.

Key words: Quantitative polymerase chain reaction; mRNA quantification; Gelsolin; Amyloidosis

1. Introduction

Gelsolin is a protein of higher eukaryotes that was first identified as a factor mediating the change of a gel-like cytoplasmic extract to a soluble phase in the presence of calcium [1]. The *gel-sol* transition is the consequence of the severing of actin filaments, but gelsolin is also capable of capping the barbed ends of actin filaments and, under appropriate conditions, of promoting actin polymerization (reviewed in [2]). Gelsolin occurs in two forms, intracellular (cytoplasmic) and secreted (plasma), which are encoded by a single gene located on chromosome 9 [3–5]. The intracellular gelsolin participates in the regulation of the cellular actin network, and is required in rapid stress responses involving locomotion of cells, such as responses to hemostasis or inflammation [6,7]. Recent studies have suggested an additional role of the intracellular gelsolin in signal transduction through its binding to phosphatidylinositol 4,5-bisphosphate [8,9]. The physiological significance of the secretory form of gelsolin is not well known, but it has been suggested to depolymerize and sequester actin that has been released during cellular damage [10].

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Abbreviations: GSN, gelsolin; S-GSN, secretory gelsolin; Tot-GSN, total gelsolin; PCR, polymerase chain reaction; RT, reverse transcription; FAF, familial amyloidosis of the Finnish type

After their initial discovery, the two forms of gelsolin have been shown to be synthesized in most types of cells and tissues and, interestingly, variations in the level of expression of the gelsolin gene have been observed both during cell differentiation and in carcinogenesis [11–15]. However, despite numerous studies on the synthesis of gelsolin in cells, the expression pattern of the gelsolin gene in different human tissues has not been systematically studied, most previous reports concentrating mainly on animal tissues or only on a few types of human tissues or cells [16–19].

A mutation in the gelsolin gene results in an autosomal dominantly inherited amyloid disease (familial amyloidosis, Finnish type or FAF) histologically characterized by accumulation of gelsolin fragments as amyloid deposits in most tissues [20–22]. We have previously demonstrated that FAF patients have significantly elevated levels of gelsolin in their serum [23]. The underlying mechanism of this feature, which might have a role in the disease pathogenesis by enhancing the amyloid formation, could involve increased compensatory synthesis of the possibly functionally impaired mutant gelsolin in patients [24].

To study the expression levels of gelsolin mRNA in tissues of healthy individuals of varying age as well as in those of FAF patients, we developed a method for quantification of the gelsolin mRNA. The method is based on reverse transcription (RT) of the sample mRNAs followed by the polymerase chain reaction (PCR) together with an internal quantification standard that differs only by a single nucleotide from the analyzed mRNA region. Furthermore, by separately determining the levels of the unique 5′ sequence of the secretory gelsolin (S-GSN) mRNA and of the 3′ region shared by the two forms of gelsolin (Tot-GSN) mRNA, we were able to calculate the ratios of the mRNA species encoding the secretory and intracellular forms of gelsolin.

2. Materials and methods

2.1. Tissue samples

We studied tissues from six healthy adults (A1–A6), a newborn (NB1), a fetus of 26 weeks (F1) and five FAF patients (P1–P5) (Table 1). All the FAF patients had been previously shown to carry the $G_{654}A$ mutation of the gelsolin gene [25], and they all showed typical signs of corneal lattice dystrophy as well as cranial, especially facial, neuropathy [26]. Tissue specimens from all of them had been histopathologically demonstrated to contain amyloid [26].

The tissue specimens from the individuals A1–A5 and P1–P5 were frozen in liquid nitrogen and stored at −70°C. The autopsy samples from subjects A6, NB1 and NB2 were taken within 24 h of death, frozen and stored at −70°C. Total cellular RNA was extracted using the RNA-zol procedure (Cinna/Biotech Laboratories International, Texas, USA). The concentration of the extracted RNA was immedi-

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ately determined by UV absorption at 260 nm, after which the RNA samples were stored at −70°C in 70% ethanol. Possible DNA contamination in the RNA samples was excluded by the absence of product when the samples were subjected to PCR without a reverse transcription step. The quality of the RNA specimen was assessed by performing successful RT-PCR amplifications of two control genes, aspartylglucosaminidase and fibrillin, that are routinely analyzed in our laboratory [27,28].

2.2. Quantification standards for PCR

Gelsolin cDNA cloned into M13mp18 served as a standard with the normal sequence [29]. A standard with a mutant sequence was prepared from this cDNA clone by site-specific mutagenesis of the nucleotides at positions 128 (G→A) and 654 (G→T) of the gelsolin cDNA. The replicative form of the M13 clones was purified by the $CsCl_2$ method with two subsequent gradient centrifugations. The quality of the DNA was assessed by agarose gel electrophoresis and the DNA concentration was determined by UV absorption at 260 nm. In calculating the number of molecules of standard, the fact that both strands of the standard DNA will serve as a template in the PCR amplification, while the target sequence is single stranded, was taken into account. Before use, the gelsolin insert was excised from the vector using *Eco*RI and the digested sample was stored in aliquots at −70°C.

Standard curves for the quantitative analysis were established by mixing the normal and mutant standard sequences in known ratios, followed by PCR and detection of the nucleotide at positions 128 and 654 by solid-phase minisequencing (Figs. 1 and 2) [27,30]. For determining the relative levels of normal and mutant GSN-mRNA in FAF patients, mixtures of genomic DNA from a FAF patient and normal individual were used for preparation of a standard curve [25,28].

2.3. Reverse transcription and PCR

Table 2 gives the sequences of the primers used in the RT and PCR reactions. 200–500 ng of total cellular RNA was reverse transcribed using 25 pmol of the primer GSN-764 or GSN-223, 20 nmol of each dNTP, 20 U of RNAsin (Promega Biotech, Helsinki, Finland) and 25 U of AMV reverse transcriptase (Promega) in 20 μl of buffer containing 50 mM Tris-HCl, pH 8.4, 40 mM KCl_2, 5 mM $MgCl_2$ and 2 mM DTT. Four or 9 μl of the RT mixture was subjected to PCR amplification together with 10^4–10^6 molecules of the mutant standard sequence. The amount of the mutant standard sequence had previously been titrated to give a ratio of 0.1–10 in the minisequencing assay. The PCR reaction mixtures contained 20 pmol of the biotinylated primer GSN-B543 and 100 pmol of the primer GSN-707, or 20 pmol of the biotinylated primer GSN-B16 and 100 pmol of the primer GSN-162, 20 nmol of each dNTP and 2.5 U of DNA polymerase (Dynazyme II, Finnzymes, Helsinki, Finland) in 100 μl buffer containing 40 mM Tris-HCl, pH 8.8, 15 mM $(NH_4)_2SO_4$, 0.01% gelatin, 0.1% Triton X-100 and 1.5 mM $MgCl_2$. For amplification with the primers GSN-B16 and GSN-162, 5% (v/v) of DMSO was included in the buffer and the DNA polymerase was added to the PCR reactions employing a 'hot start' procedure. The amplification was performed at 95°C for 1 min, at 59–60°C for 1 min and at 72°C for 1 min for 30–40 cycles.

2.4. Solid-phase minisequencing

To allow for duplicate assays of both sequences in the sample mixture, four 10 μl aliquots of each PCR product were captured in streptavidin-coated microtitration wells (Combiplate 8, Labsystems, Helsinki, Finland), the wells were washed and the DNA was denatured as described in detail previously [25,31]. The minisequencing reaction mixture contained 10 pmol of the primer GSN-674 (Table 2) or primer GSN-148 (Table 2), 3 pmol of [^{3}H]dNTP ([^{3}H]dATP, sp.ac 75 Ci/mmol; [^{3}H]dCTP, sp.ac. 64 Ci/mmol; [^{3}H]dTTP, sp.ac. 121 Ci/mmol, Amersham, UK) and 0.05 U of DNA polymerase in 50 μl of its buffer. The minisequencing reactions were carried out for 10 min at 55°C. After washing and denaturation, the incorporated radioactivity was measured in a liquid scintillation counter. The final quantitative result was obtained by comparing the ratio between the incorporated [^{3}H]dNTPs and a standard curve (Figs. 1 and 2).

2.5. Immunochemical analyses of gelsolin

The gelsolin of the serum samples of individuals P1–5 was quantified using a radioimmunoassay described in [23]. For Western blot analysis of the tissue specimens, 20 μg of tissue (skin, heart, intestine, kidney and brain) kept on dry ice were crushed and dissolved in 800 μl of 0.5% Triton X-100 in phosphate buffered saline containing EDTA (1.2 mg/ml) and PMSF (35 mg/ml) as protease inhibitors. The samples were freeze-thawed twice, and the supernatant was used in the further assays. The protein concentration of the tissue samples was determined using a protein detection assay (Bio-Rad, Hercules, CA, USA) [32]. 20 μg of protein solubilized from the tissue samples was subjected to 10% SDS-PAGE, electroblotting and immunodetection using monoclonal antibody recognizing carboxy-terminal epitope of gelsolin, as described previously [23].

3. Results

We applied a quantitative method based on RT-PCR and solid-phase minisequencing to determine the levels of gelsolin mRNA in tissues derived from normal adults, a fetus, a newborn and patients with FAF. Because a single gene encodes

Table 1
Subjects and analyzed tissues

Subjects	Age	Tissue	Health status
Normal adults			
A1 (female)	30 years	Placenta	Healthy
A2 (male)	30 years	Skin	Healthy
A3 (female)	37 years	Muscle	Post polio syndrome
A4 (male)	54 years	Muscle, skin	Healthy
A5 (male)	56 years	Skin (healthy area)	Carcinoma basocellulare
A6 (female)	83 years	Muscle, heart, liver, kidney, spleen, brain	Accidental death
Fetus/newborn			
F1	26 weeks[a]	Muscle, heart, large intestine, liver, kidney, brain, peripheral nerve	Idiopathic respiratory distress syndrome (2nd of twins)
NB1	41 weeks[a]	Muscle, heart, small intestine, liver, kidney, brain	Hypoglycemia, metabolic acidosis
FAF patients			
P1	43 years	Muscle, skin	Familial amyloidosis, Finnish
P2	47 years	Muscle, skin	Familial amyloidosis, Finnish
P3	54 years	Skin	Familial amyloidosis, Finnish
P4	55 years	Muscle, skin	Familial amyloidosis, Finnish
P5	75 years	Skin	Familial amyloidosis, Finnish

[a]For F1 and NB1, the gestational age is given.

both the secretory and the intracellular forms of gelsolin [4], we quantified separately the unique 5′ end of the secretory form of gelsolin mRNA and the RNA region shared by the two gelsolin forms. A known amount of an internal standard sequence differing from the normal sequence at nucleotide positions 128 and 654 of the gelsolin gene is added to the cDNA sample prior to the PCR amplification in both assays (Fig. 1). As the sequences originating from the mRNA sample and from the quantification standard differ only by a single nucleotide, they are amplified with equal efficiency and their relative amounts can be accurately determined in the minisequencing reaction [27].

3.1. Tissue distribution of total gelsolin mRNA

Gelsolin mRNA was present in all analyzed adult tissues (Fig. 3A and Table 3). Particularly high transcript levels were measured in skin, skeletal and cardiac muscle as well as in placenta (range of mean values $1.1–2.5\times10^7$ of Tot-GSN mRNA molecules per μg of total RNA). The interindividual differences in the skin samples were high, more than 10-fold, while only 2-fold differences were observed in the muscle samples. Intermediate mRNA levels were observed in brain, kidney and spleen ($2.5–4.9\times10^6$ molecules per μg of total RNA), while only a subtle amount of gelsolin mRNA (2.4×10^5 molecules per μg total RNA) was detectable in liver.

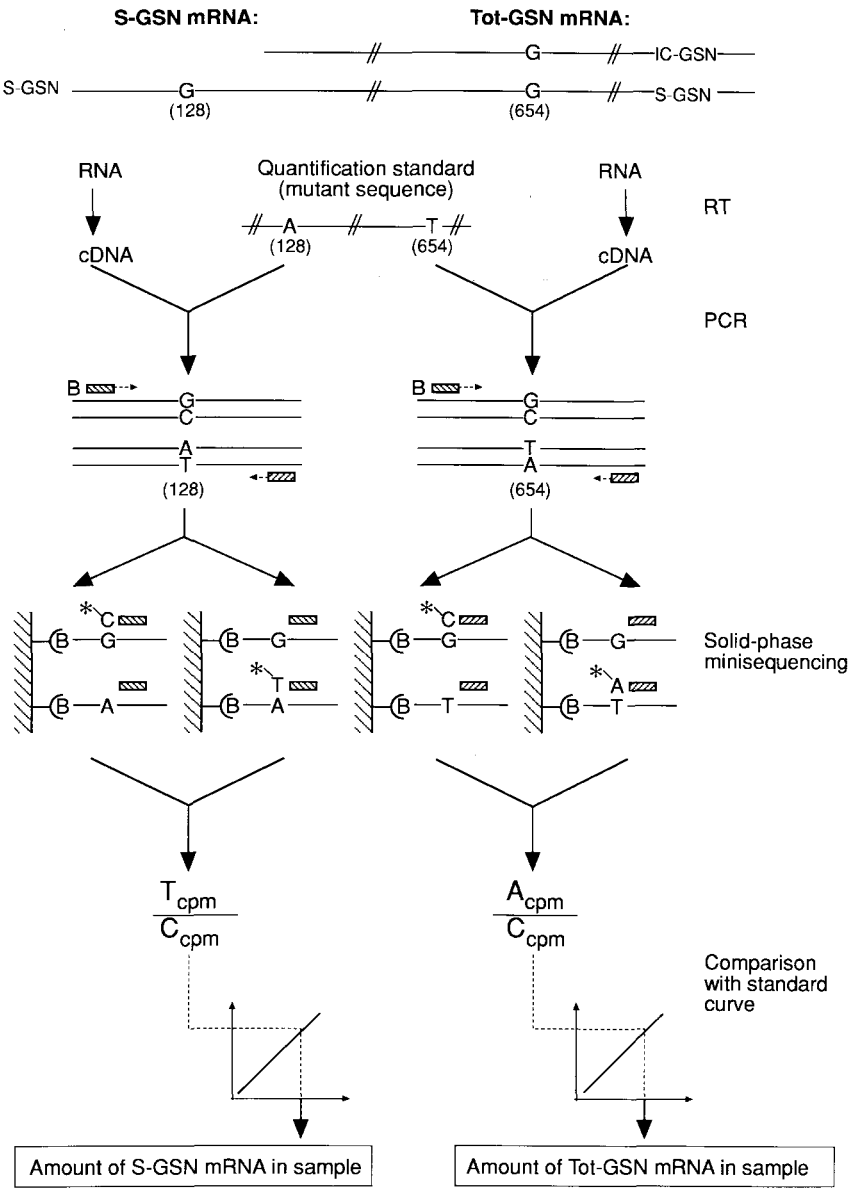

Fig. 1. Strategy for quantitative analysis of S-GSN and Tot-GSN mRNAs by RT-PCR and solid-phase minisequencing. The figure illustrates the positions of the PCR primers and the nucleotides with which the S-GSN (left) and Tot-GSN (IC-GSN and S-GSN) (right) mRNAs were quantified separately. The sequences are shown schematically, and the nt numbering is not in scale. In IC-GSN, the transcribed region common with S-GSN begins at nt 159. After the RT reaction a known amount of quantification standard is added to the sample and the mixed sample is amplified by PCR using primers flanking nt positions 128 and 654, respectively. The PCR product is captured on a solid support by the biotin-streptavidin interaction and denatured. In the minisequencing reaction a specific primer annealing adjacent to the analyzed nucleotide position (128 or 654) is extended by a DNA polymerase with a single labeled nucleoside triphosphate. The incorporated label is measured and the result is obtained by comparing the ratio between the incorporated labels and the quantification standard curve. S-GSN, secretory gelsolin; IC-GSN, intracellular gelsolin; Tot-GSN, total gelsolin.

The steady-state mRNA level of the gelsolin gene differed in the tissues of the 26-week fetus (F1) and the newborn (NB1) when compared to adult tissues (Fig. 3B). The amount of gelsolin mRNA in the liver and heart from F1 and NB1 was 32 and 3 times higher than in the adults, respectively, while a low level of gelsolin mRNA compared to that of adults was observed in the brain tissue of F1 and NB1 and in the peripheral nerve from F1 (range 9.4–9.5$\times 10^5$ molecules per μg of total RNA). F1 and NB1 gelsolin mRNA levels differed most from each other in the cardiac muscle (4-fold) and intestine (4-fold), the mRNA level of F1 being higher in the intestine and that of NB1 being higher in the heart tissue. Thus, no striking differences were found between these two subjects representing different developmental stages.

3.2. Levels of mRNA encoding secretory gelsolin

After determining the level of total gelsolin mRNA in various tissues, we determined separately the level of S-GSN mRNA in skeletal and cardiac muscle, spleen, kidney and brain of the adult A6 and in the skin of one of the patients, P3. The amount of mRNA encoding S-GSN was between 13 and 23% of the Tot-GSN mRNA. The highest levels of S-GSN mRNA were measured in heart and skin (2.7×10^6 and 2.2×10^6 molecules per μg of total RNA), and the largest proportion of S-GSN mRNA was determined in the heart (23%). The mRNA level in the brain was below 5.0×10^5 molecules per μg of total RNA. We confirmed these unexpectedly low levels of mRNA encoding S-GSN [16] by conventional dot blot hybridization analysis of RNA from the muscle sample using PCR products obtained from regions defining the S-GSN or Tot-GSN DNA as probes. In this analysis, the hybridization signals obtained from S-GSN mRNA were less than one third of those obtained for the Tot-GSN mRNA, thereby being in accordance with the result obtained using the minisequencing method.

3.3. Gelsolin mRNA and serum gelsolin levels in FAF

In order to study the possible relationship between the previously shown elevated gelsolin levels in the serum of FAF patients and the expression of the gelsolin gene [23], we determined the mRNA levels in skin and/or muscle samples, and the serum gelsolin concentration of patients P1–P5. The mean values of Tot-GSN mRNA in skin and muscle from the patients (1.1×10^7 and 1.5×10^7 molecules per μg of total RNA) were 61% and 136% of those of the controls (Table 4 and Fig. 3). The levels of serum gelsolin ranged from 160 to 270 μg/ml, and did not correlate with the mRNA levels in these tissues with highest steady-state mRNA amounts. Consequently, the elevated serum gelsolin levels of FAF patients [23] are not caused by an increased steady-state level of gelsolin mRNA.

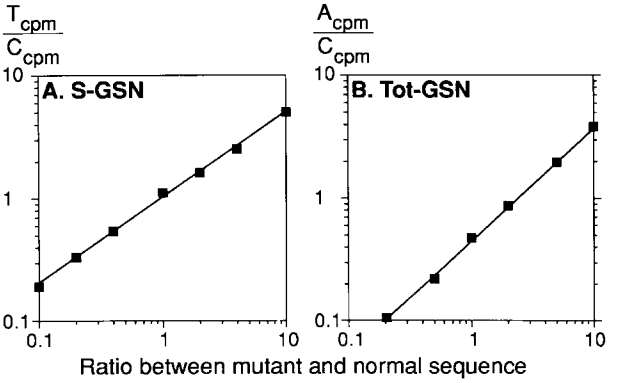

Fig. 2. Solid-phase minisequencing standard curves for quantification of S-GSN mRNA (A) and of Tot-GSN mRNA (B). The ratio between the cpm values obtained from the incorporated nucleotides at position 654 or 128 is plotted as a function of the ratio between the sequences in the original mixture. The nucleotides of the non-coding strand of the GSN gene were analyzed. The obtained ratios depend on the specific activities of the [^{3}H]dNTPs used, and on the fact that two [^{3}H]dCTPs will be incorporated in the allele with G_{128}.

The relative levels of mRNA encoded by the normal allele (with nucleotide G at position 654) and the mutant allele (A_{654}) were obtained by quantitative minisequencing analysis of patient skin and muscle samples without the addition of the standard sequence. The relative amounts of the two mRNAs ranged from 0.93 to 1.1 (mean value 1.0±0.058). Thus, although the total gelsolin mRNA levels showed large inter-individual differences, especially in skin, the normal and the FAF-associated alleles of the gelsolin gene were present in equal ratios (Table 4).

3.4. Gelsolin in normal and FAF tissues

Finally, we analyzed gelsolin extracted from the tissue specimens of individuals A5, NB1, F1 and P3 that had been subjected to GSN mRNA quantification, by Western blot analysis using gelsolin-specific antibodies. We found a relatively good concordance between the gelsolin mRNA levels and the amount of the gelsolin polypeptides seen in the im-

Table 2
RT and PCR primers

Primer	Use	Sequence and position as nt number of the gelsolin cDNA
Tot-GSN mRNA[a]		
GSN-764	RT	5′-GTTGTCCCGGATGCCCTT (764–747)
GSN-B543[b]	PCR	5′-AAGCACGTGGTACCCAAC (543–560)
GSN-707	PCR	5′-GTTGGAACCACACCACTG (707–690)
GSN-674	minisequencing	5′-CAGGTCCAGGATGAAGCAGT (674–655)
S-GSN mRNA[a]		
GSN-223	RT	5′-ATCTGCAGGCCAGGCTCCTT (223–204)
GSN-B16[b]	PCR	5′-TGGCTCCGCACCGCCCCGCGCC (16–37)
GSN-162	PCR	5′-TGGGCCGCGCCTCGGGCACCCG (162–141)
GSN-148	minisequencing	5′-GGCACCCGCCCCTGGGGCGC (148–129)

[a]The primers were synthesized on an Applied Biosystems 392 DNA synthesizer and they were designed according to the published sequence of the GSN gene [4].
[b]The primers GSN-B543 and GSN-B16 were biotinylated at their 5′ end.

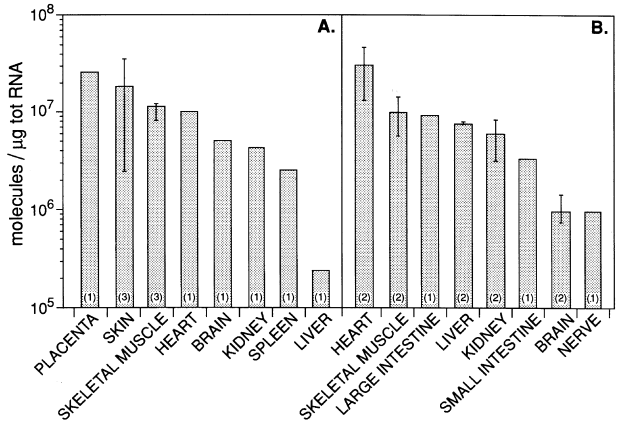

Fig. 3. Levels of Tot-GSN mRNA determined in tissue samples from normal adults (A) and from a fetus and a newborn (B). The number of samples analyzed is shown in parentheses. When tissues from more than one individual were analyzed, the mean values and variation ranges are given.

munoblots of most tissue samples (Fig. 4). Two main gelsolin species of 80 and 83 kDa in size, corresponding to the intracellular and secretory forms of the protein respectively, were detected using an anti-gelsolin antibody that recognizes the carboxy-terminal part of the protein (Fig. 4).

4. Discussion

We developed a method for quantifying gelsolin mRNA and applied it to analyze the steady-state mRNA levels in various tissue samples from healthy adults, from a fetus and a newborn, as well as from patients with gelsolin-related amyloidosis (FAF). Our PCR-based technique enabled us to detect gelsolin mRNA more accurately and sensitively than would be possible by more conventional methods, such as blot hybridizations or nuclease protection assays. The problems associated with quantitative PCR analysis were avoided by addition of an internal quantification standard that differs from the GSN sequence by a single nucleotide to the samples before PCR [27,33]. Ideally a quantification standard for RT-PCR should be added to the sample before the RNA extraction step to control for possible degradation of the GSN mRNA during the processing of the tissue samples and for the reverse transcription step. The efficiency of the RT reaction has, however, been shown to be close to 100%, and independent of the RNA concentration in the sample [34], and careful precautions to avoid RNA degradation were taken in our study. However, the measured GSN mRNA amounts reflect their levels at the moment of sampling, and conclusions about the implication of the mRNA levels on the actual GSN synthesis are indirect since, even within a given organism, the half-lives of different mRNA species may vary considerably [35]. The half-life of neither the secretory nor the intracellular type of gelsolin is known, but we were able to demonstrate a relatively good correlation between the mRNA levels and the amount of polypeptide in immunoblots of the same tissue samples.

This is to our knowledge the first report in which several human tissues have been systematically compared for their content of gelsolin mRNA. Interestingly, we found that the epidermal and dermal layers of the skin contain high levels of the steady-state mRNA of both the intracellular and secretory forms of gelsolin, although there were strikingly great differences between the study samples. These results suggest that gelsolin has an important physiological role in skin, possibly related to some adaptive mechanisms, such as wound healing, where locomotion of the fibroblasts and epithelial cells as well as destruction of the extracellular debris containing e.g. actin is needed. The requirement for gelsolin in wound healing has also been suggested in a recent study, in which gelsolin knockout fibroblasts were shown to migrate more slowly than normal fibroblasts in tissue culture [36]. Our finding of high levels of intracellular gelsolin mRNA in different types of muscle, in which the physiological target of gelsolin (actin) is abundant, was not surprising, and this finding agrees with previous reports (see for example [16–18]). Although actin is considered to have an important role in the cytoskeletal plasticity of the neurons [37], we found only intermediate levels of the total gelsolin mRNA in adult brain, and, moreover, in the newborn and fetal brains, gelsolin mRNA was scarce as compared to the other tissues. Therefore the present results are slightly discordant with those obtained by analyzing murine cerebrum

Table 3
Example of quantification of Tot-GSN mRNA in tissue samples from individual A6

Tissue	Amount of standard added (No. of molecules per sample[a])	Acpm[b]	Ccpm[b]	Ratio Acpm / Ccpm	Ratio Mutant sequence / Normal sequence[c]	Amount of Tot-GSN mRNA (No. of molecules per µg of Tot-RNA)[d]
Heart	1.4×10^6	1370	3410	0.4	0.92	1.5×10^7
	2.7×10^6	2414	2591	0.93	2.2	1.2×10^7
Kidney	6.8×10^5	2460	3320	0.74	1.7	4.0×10^6
	6.8×10^5	2620	3270	0.80	1.9	3.6×10^6
Brain	2.7×10^5	523	2620	0.20	0.44	6.1×10^6
	2.7×10^6	3090	1420	2.2	5.6	4.8×10^6
Spleen	8.2×10^5	4320	2560	1.7	4.4	1.9×10^6
	8.2×10^5	3060	2330	1.3	3.3	2.5×10^6
Liver	6.8×10^4	1450	1260	1.2	2.8	2.4×10^5
	2.7×10^5	3270	940	3.5	9.2	2.9×10^5
Skeletal muscle	1.4×10^6	1502	4340	0.35	0.80	1.8×10^7

[a]500 ng of Tot-RNA was applied to the RT reaction and one fifth of the RT reaction mixture was subjected to the PCR amplification together with the standard sequence.
[b]Mean values of duplicates.
[c]The ratio between amounts of the standard sequence and the GSN sequence of the sample was obtained from the standard curve in Fig. 1B.
[d]The amount of Tot-GSN mRNA in the sample is calculated from the mutant/normal ratio based on the known amount of added standard.

Table 4
Expression levels of the normal and mutant gelsolin alleles in FAF patients

Tissue	Muscle		Skin	
	Tot-GSN mRNA[a]	mRNA ratio Normal / Mutant	Tot-GSN mRNA[a]	mRNA ratio Normal / Mutant
Patient				
P1	1.6×10^7	1.1	1.8×10^7	0.93
P2	2.1×10^7	0.97	2.6×10^6	1.0
P3	ND[b]		3.2×10^7	1.0
P4	7.2×10^6	1.1	1.7×10^6	0.94
P5	ND		1.5×10^6	1.1

[a]In quantification of the amount of Tot-GSN mRNA in patients' tissues, the presence of transcripts from two gelsolin alleles with G_{654} and A_{654}, respectively, was taken into account.
The results are given as molecules per μg of total RNA.
[b]ND=not determined.

or cerebellum, where maximal expression of gelsolin was found during active myelinogenesis in the oligodendrocytes (20–30 day old mice), after which the protein levels decreased [19,38].

We observed that the intracellular gelsolin constitutes the major form of gelsolin synthesized in all the analyzed tissues. Since detectable amounts of S-GSN mRNA could, however, be found in the majority of the tissues, the relatively high abundance of gelsolin in serum (100–400 μg/ml) [23,39] seems to result from the combined production of the protein by cells in various organs. In this regard, the net influence of the skin and skeletal muscle, comprising 5 and 43% of the body weight, respectively, and relatively high S-GSN mRNA levels, is of the great importance. Our results disagree with an earlier report, in which up to 100% of tissue gelsolin mRNA was found to be of the secretory form, as measured by the nuclease protection assay [16]. These discrepant results could be explained by methodological factors of that study, in which a probe was used that hybridizes to S-GSN mRNA by its whole length, but only partially to the mRNA encoding intracellular gelsolin. Moreover, experiments with rabbit tissues showed that less than half (0–50%) of the newly synthesized gelsolin was secreted into the culture medium, e.g. representing the secretory form of gelsolin [16].

FAF is the only inherited disease that has so far been linked to gelsolin. The FAF mutation at nt position 654 of the gene, resulting in the change of an amino acid at position 187, leads to alternative cleavage of the mutant gelsolin in the secretory pathway of the cells [29]. This results in the secretion of an amino-terminally truncated fragment of gelsolin, and eventually in the accumulation of gelsolin peptides as amyloid in patients' tissues, in particular in the cornea, skin and nerves as well as skeletal and cardiac muscles [20,26,40–44]. Our present results suggest that, at least in the skin and muscle, where mRNA encoding for both the intracellular and secretory forms of gelsolin was abundant, and, moreover, both the wild-type and mutant alleles were expressed in equal amounts, amyloid accumulation could be due to local production of mutant gelsolin. Although we did not specifically analyze adult peripheral nerves or mRNA from peripheral nerve cells, it is likely that the amyloid accumulation in the nerves of the FAF patients cannot be explained by a particularly high level of synthesis of mutant gelsolin in the nerves.

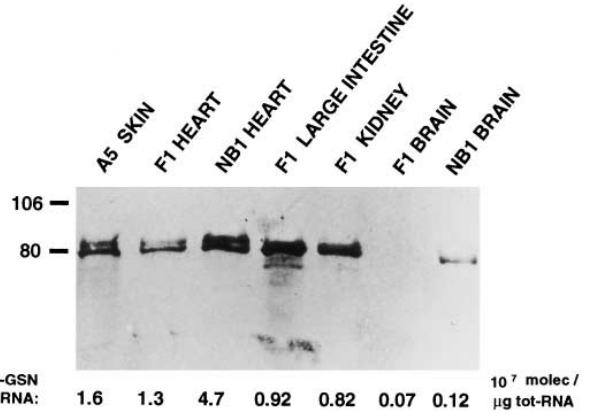

Fig. 4. Western blot analysis of gelsolin in normal and FAF tissues. The protein samples from individuals A5 (skin), F1 (heart, large intestine, kidney) and NB1 (heart and brain) were analyzed. The corresponding values for Tot-GSN mRNA for the studied samples are given ($\times10^7$ molecules/μg of Tot-RNA).

We have reported previously that FAF patients have increased levels of gelsolin in their serum and the levels both in serum and in liquor increase with age [23]. One plausible explanation for this feature could be that the mutant gelsolin would be functionally defective [24] and that gelsolin synthesis would therefore become up-regulated, especially upon aging when some unknown compensatory mechanisms would fail to function. We were, however, able to show here that the gelsolin mRNA levels are not increased in the patients' tissues and, furthermore, do not correlate with the serum gelsolin levels. The present results therefore strongly suggest that the elevated serum gelsolin levels are not caused by increased expression of the gelsolin gene nor by increased stability of the transcript of the mutant GSN allele, but rather by translational or some catabolic events.

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