Induction of Protein Conformational Change in Mouse Senile Amyloidosis*

Received for publication, December 5, 2001, and in revised form, June 11, 2002
Published, JBC Papers in Press, June 20, 2002, DOI 10.1074/jbc.M111570200

Yanming Xing‡, Akihiro Nakamura‡, Tatsumi Korenaga‡, Zhanjun Guo‡, Junjie Yao‡, Xiaoying Fu‡, Takatoshi Matsushita§, Kumiko Kogishi§, Masanori Hosokawa§, Fuyuki Kametani¶, Masayuki Mori¶, and Keiichi Higuchi¶

From the ‡Department of Aging Angiology, Research Center on Aging and Adaptation, Shinshu University School of Medicine, Matsumoto 390-8621, §Field of Regeneration Control, Institute for Frontier Medical Science, Kyoto University, Kyoto 606-8507, and ¶Department of Molecular Biology, Tokyo Institute of Psychiatry, Tokyo 156-8585, Japan

Aggregated amyloid fibrils can induce further polymerization of precursor proteins in vitro, thus providing a possible basis for propagation or transmission in the pathogenesis of amyloidoses. Previously, we postulated that the transmission of amyloid fibrils induces conformational changes of endogenous amyloid protein in mouse senile amyloidosis (Xing, Y., Nakamura, A., Chiba, T., Kogishi, K., Matsushita, T., Fu, L., Guo, Z., Hosokawa, M., Mori, M., and Higuchi, K. (2001) Lab. Invest. 81, 493–499). To further characterize this transmissibility, we injected amyloid fibrils (AApIII(C)) of amyloidogenic C type apolipoprotein A-II (APOAIIC) intravenously into 2-month-old SAMR1 mice, which have B type apolipoprotein A-II (APOAIIB) and develop few if any amyloid deposits spontaneously. 10 months after amyloid injection, deposits were detected in the tongue, stomach, intestine, lungs, heart, liver, and kidneys. The intensity of deposition increased thereafter, whereas no amyloid was detected in distilled water-injected SAMR1 mice, even after 20 months. The deposited amyloid was composed of endogenous APOAIIB with a different amyloid fibril conformation. The injection of these amyloid fibrils of APOAIIB (AAapoAII(B)) induced earlier and more severe amyloidosis in SAMR1 mice than the injection of AApIII(C) amyloid fibrils. Thus, AApIII(C) from amyloidogenic APOAIIB could induce a conformational change of less amyloidogenic APOAIIB to a different amyloid fibril structure, which could also induce amyloidosis in the less amyloidogenic strain. These results provide important insights into the pathogenesis of amyloid diseases.

Prion, an abnormal form of the host cellular prion protein (PrP*)1, is responsible for the transmissible spongiform encephalopathy, including scrapie of sheep, bovine spongiform encephalopathy, and human Creutzfeldt-Jakob disease (2, 3). In transmissible spongiform encephalopathy, prion induces the conformational change of PrPC to the prion form PrPSc and causes a detectable phenotype or disease in the affected individual. Recent studies with yeast have broadened the definition of prion from proteinaceous infectious agent of transmissible spongiform encephalopathy to infectious proteins or protein-based genetic elements. Like PrPSc, prions URE3 and PSI in Saccharomyces cerevisiae can induce the conversion of the cytoplasmic proteins ure2p and sup35p to the prion form. This infectious agent propagates in the cytoplasm and is also transmissible to other yeast (4–6).

The term amyloidosis refers to a group of diverse conditions characterized by the extracellular accumulation of fine amyloid fibrils to which normally innocuous soluble proteins polymerize (7). The nucleation-dependent polymerization model is postulated to explain well the kinetics of amyloid fibrilization and conversion of PrPC to PrPSc (8, 9). This model consists of nucleation and extension phases. Preformed amyloid fibrils accelerate conformational changes of many amyloid precursor proteins and result in rapid extension of amyloid fibrils in vitro (10–12). Although Alzheimer’s disease is not known to be infectious like prion disease (13, 14), intracerebral injection of brain homogenate from an Alzheimer’s patient into marmoset monkeys induced the formation of amyloid plaques in these primates (15). Injection of synthetic amyloid-like fibrils or modified silk accelerated amyloid protein A (AA) amyloidosis and showed amyloid-enhancing activity, possibly by serving as a seed on which new AA amyloid fibrils could form (16, 17). These results suggested that amyloid β and AA amyloidoses may be transmissible under experimental conditions.

We established the Senescence-Accelerated Mouse (SAM) strains as a good model for senile amyloidosis (18). In mouse senile amyloidosis, apolipoprotein A-II (apoA-II), the second most abundant apolipoprotein of serum high density lipoprotein, polymerizes to form amyloid fibrils and deposits systemically but not in the brain and bone (19, 20). Three variants of apoA-II (types A, B, and C) with different amino acid substitutions at four positions (5, 20, 26, and 38 from the N terminus) are present in inbred strains of mice (21). Senescence-Accelerated Mouse-resistant 1 (SAMP1) mice with wild-type B apoA-II (APOAIIB, Pro5, and Val26) show few, if any, signs of senile amyloidosis. The R1.P1-Apoa2c mice are a congenic strain of mice that have the amyloidogenic allele of the apoA-II gene from the Senescence-Accelerated Mouse prone 1 (SAMP1) strain on the genetic background of the SAMR1 strain. This strain with the variant C type apoA-II (APOAIIC, Gln5, and Ala26) spontaneously exhibits a high incidence of amyloidosis.

* This work was supported in part by Grants-in-Aid 09278209 for Priority Areas and B114700596 for Scientific Research (B) from the Ministry of Education of Japan and by a grant from the Ministry of Health and Welfare of Japan. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 To whom correspondence should be addressed. Dept. of Aging Angiology, Research Center on Aging and Adaptation, Shinshu University School of Medicine, Asahi 3-1-1, Matsumoto 390-8621, Japan. Tel.: 81-263-37-2691; Fax: 81-263-36-3662; E-mail: kiguchi@sch.md.shinshu-u.ac.jp.

2 The abbreviations used are: PrP, prion protein; SAM, Senescence-Accelerated Mouse; AA, amyloid protein A; SAMRI, Senescence-Accelerated Mouse-resistant; DW, distilled water; AIF, amyloid index; Tricine, N-(2-hydroxy-1,1-bis(hydroxymethyl)ethyl)glycine.

This paper is available online at http://www.jbc.org
and severe amyloid deposition with aging (22).

In previous studies, we found that intravenous injection of AApAII(C) fibrils isolated from livers of old R1.P1-ApoA2C mice markedly accelerated amyloid deposition in young R1.P1-ApoA2C mice (23). The *in vitro* experiment also demonstrated that the extension of AApAII(C) proceeds by the association of APOAIC to the ends of existing fibrils (24). The nucleation-dependent polymerization model addressed these phenomena. We fed young R1.P1-ApoA2C mice with AApAII(C) fibrils or reared the young mice with old severe amyloid-deposited R1.P1-ApoA2C mice in the same cage. All of the treated mice developed amyloid deposits (1). These results suggested that the oral transmission of amyloid fibrils results in the fibril formation of amyloid proteins in AApAII amyloidosis and presented a possible pathogenesis of amyloidosis. In this study, we investigated whether exogenous AApAII fibrils can induce amyloid deposition in less amyloidogenic SAMR1 mice. Our results revealed that exogenous amyloid fibrils can change the less amyloidogenic wild-type apoA-II monomer to amyloid fibrils; these new amyloid fibrils can more easily induce de novo amyloid deposition in SAMR1 mice. The results of this experiment provide further evidence for the hypothesis of transmission in mouse senile amyloidosis.

**EXPERIMENTAL PROCEDURES**

**Animals**—R1.P1-ApoA2C and SAMR1 mice were raised in the Institute for Frontier Medical Science of Kyoto University under conventional conditions at 24 ± 2 °C with a light-controlled regimen (12-h light/dark cycle). A commercial diet (CE-2, Nihon Clea, Tokyo, Japan) and tap water were available ad libitum. Mice were killed by cardiac puncture under diethyl ether anesthesia. All of the experiments were performed with the consent of the Animal Care and Use Committee of both Kyoto University Graduate School of Medicine and Shinshu University School of Medicine.

**Isolation and Injection of Amyloid Fibrils**—AApAII(C) amyloid fibril fractions were isolated from the livers of 18–21-month-old R1.P1-ApoA2C mice using Pras’ method with some modification (25, 26). Purified AApAII(C) fibrils were suspended in distilled water (DW) at a concentration of 1.0 mg/ml and sonicated five times on ice for 30 s at 30-s intervals using a microtip-equipped Astrason ultrasonic processor W-350 (Heat System-Ultrasonics Inc., Farmingdale, NY) (25). A single dose of 0.1 ml of sonicated AApAII(C) fraction (0.1 mg) was immediately injected into the tail veins of 2-month-old SAMR1 mice. An equal volume of DW was injected to SAMR1 and R1.P1-ApoA2C mice of the same age. After 1–20 months, the treated mice were sacrificed and their organs were subjected to pathologic or biochemical analysis as described below. The amyloid fractions from the tongue and lungs of each animal were used for biochemical and electron microscopy studies. The amyloid fraction from the severely deposited tongue of one SAMR1 mouse was injected into an additional group of 2-month-old SAMR1 mice. After 1–12 months, these mice were killed and amyloid deposition was determined. We also injected 0.1 mg of sonicated AApAII(C) into six 18-month-old SAMR1 mice. These mice were killed for pathologic examination 3 months after injection.

**Detection of Amyloid Deposition**—Half of each organ was fixed in 10% neutral buffered formalin, embedded in paraffin, and cut into serial 4-μm sections. The other half was stored frozen for biochemical analysis. The amyloid was identified by green birefringence in Congo Red-stained sections under polarizing microscopy. Amyloid deposits were also immunohistochemically stained by the horseradish peroxidase-labeled streptavidin-biotin method (DAKO, Glostrup, Denmark) using anti-apoA-II antisera as the primary antibody (23) and 3,3 diaminobenzidine as the chromogen. The intensity of the AApAII amyloid deposition was determined semi-quantitatively using the amyloid index (AI) as a parameter. The AI is the average of the degree of AApAII deposition graded from 0 to 4 in the seven organs examined (liver, spleen, skin, heart, stomach, small intestine, and tongue) in sections stained with Congo Red after immunohistochemical confirmation of the AApAII deposition (18). The amount of AApAII deposited in each organ was categorized into the following five grades: grade 0 no AApAII found; grade 1 with a minute amount of AApAII deposits; grade 2 with small amounts of AApAII deposits only in the perivascular areas of the liver, in the perifollicular regions of the spleen, in the intestinal tissues covering <10% of the areas of both ventricles, atria, ventricular septum, and both atria of the heart, only in the glandular portion and squamous glandular junction of the stomach and in <30% papillary layer of the dermis of the skin; grade 3 with a moderate amount of AApAII deposits in <30% of the area of the lobules of the liver, in <30% of the area of the red pulp of the spleen, in the interstitial tissues covering 10–30% of the area of the lamina propria and submucosa of the squamous epithelium of the stomach, and in 30–80% of the area of the papillary layer of the dermis of the skin; and grade 4 with extensive AApAII deposits in 30–80% of the area of the lobules of the liver, in 30–80% of the area of the red pulp of the spleen, in the interstitial tissues covering >30% of the area of the heart muscles, in >50% lamina propria and submucosa of the squamous epithelium of the stomach, and in almost all parts of the papillary layer of the dermis and around the hair follicles and sebaceous glands of the skin. The grading in the small intestine and tongue was described previously (23). Two observers who had no information regarding the examined tissue graded and averaged the AI independently for each mouse.

**Electron Microscopy**—10 μl of purified amyloid fractions (0.4 mg/ml) and 10 μl of 2% phosphotungstic acid (pH 7.0) were mixed. Half of the carbon-coated plastic grid (Oukens, Tokyo, Japan) was immersed in this mixture for 1 s. The negatively stained samples were observed with a JEOL 1200 EX electron microscope (JEOL, Tokyo, Japan) operated at 80 kV. We measured amyloid fibrils if both ends were visible on the photographs. Absolute breadth and length were calculated using the calibration bar on the photographs.

**Biochemical Analysis**—Amyloid fractions were purified from the tongue and lungs of a 17-month-old AApAII(C)-treated SAMR1 mouse with severe amyloid deposition. The isolated amyloid fraction was applied to Tris-Tricine SDS-PAGE gels (16.5%/8%) (27) and stained with Coomassie Brilliant Blue R-250 (ICN Biomedicals, Aurora, Ohio). Proteins were botted onto immunobilized polyvinilidene difluoride membranes (Bio-Rad). AApAII on the blots was detected with anti-apoA-II antiserum (1:4000), horseradish peroxidase-linked anti-rabbit IgG antibody (1:3000, New England Biolabs, Beverly, Massachusetts), and the ECL Western blotting analysis system (Amersham Biosciences). To examine the type of apoA-II in the amyloid deposits, a part of each purified amyloid fraction was subjected to preparative two-dimensional PAGE with the Multiphore II system (Amersham Biosciences) essentially as described in the manufacturer’s manual. The fraction was soaked into Immobiline Drystip (pH 3.0, 10 NL, 13 cm, Amersham Biosciences) overnight, and then the strips were processed by isoelectric focusing electrophoresis in the presence of 8% urea, 0.5% Triton X-100, and 9.7 m dithiothreitol. After equilibration, the strips were processed by 16.5%/Tris-Tricine SDS-PAGE. Separated proteins were botted onto a polyvinilidene difluoride membrane and immuno-detected as described above. The apoA-II spot located by immunoblotting of a separate gel was excised and digested with 200 μl of 25 μg/ml pyroglutamin aminopeptidase deblocking buffer (Roche Diagnostics) for 24 h in a 30 °C-water bath. The membrane then was air-dried and processed by an automatic peptide sequencer (Applied Biosystems 477 A and 120 A, Foster City, CA).

**Statistic Analysis**—We used the StatView software package (Abacus Concepts, Berkeley, CA) to analyze the data. Because the AI is a non-linear index, the AI of different groups of mice was compared using the nonparametric Mann-Whitney-U test. The average lengths of amyloid fibrils were compared with the Student’s t test.

**RESULTS**

**AApAII(C) Injection-induced Amyloid Deposition in SAMR1 Mice**—At 1, 2, and 3 months after injection of AApAII(C) or DW to 2-month-old SAMR1 mice, no amyloid deposition was identified in any tissue. At 10 months after AApAII(C) injection, we observed mild or moderate amounts of amyloid deposition in the lamina propria of the tongue, the lamina propria and submucosa of the small intestine, the glandular portion and squamous glandular junction of the stomach, and the ves sel wall and alveolar septa of the lungs. A slight deposition was also observed in the collagen nucleus in the papillae of the kidney, around the central veins in the liver, and in the subendo-
treatment, we detected severe amyloid deposition in the tongue (Fig. 2, A and C), stomach (Fig. 2, B and D), small intestine, and lungs. We detected mild deposition in the heart and kidneys and slight deposition in the liver, skin, and spleen. In contrast, we did not observe any amyloid deposition in DW-injected SAMR1 mice, even 20 months after treatment. To study the effect of age at the time of injection on amyloid induction, we also injected AApoAII(C) into 18-month-old SAMR1 mice, but no amyloid deposition was apparent in the tissues 3 months after injection.

R1.P1-Apoa2C mice were injected with 0.1 ml of DW at 2 months of age. Three months after treatment, mild amyloid deposition was seen in only one mouse. At five months after injection, the AI increased obviously (Fig. 1), and at ten months after treatment, severe amyloid deposition was seen systematically but not in the bone and brain parenchyma.

Characterization of AApoAII from AApoAII(C)-treated SAMR1 Mice—We analyzed amyloid fractions from the tongue and lungs of a 17-month-old AApoAII(C)-treated SAMR1 mouse. The biochemical results indicated no differences between these two organs. We applied the amyloid fractions to 16.5% Tris-Tricine SDS-PAGE gels. One major band with a molecular mass of 6.5 kDa and several faint bands with high molecular masses were detected in the Coomassie Brilliant Blue R-250-stained gel (Fig. 3A). Subsequent immunoblot analysis showed three immunoreactive signals with molecular masses of 6.5, 16.2, and 26.4 kDa (Fig. 3A). Judging by their molecular masses and immunoreactivities, the prominent 6.5-kDa polypeptide and the other larger bands were determined most likely to be the monomeric and oligomeric forms of apoA-II, respectively. Immunoblot analysis after two-dimensional PAGE revealed that the oligomeric forms were almost completely depolymerized to the monomeric form. AApoAII monomers formed four isoforms, one major and three minor forms (data not shown). The major apoA-II membrane spot was cut and subjected to sequencing. The result revealed that the fifth amino acid at the N terminus was proline, which specified the B type apoA-II monomer.

Characterization of AApoAII injection-induced amyloid deposition in SAMR1 mice. Two-month-old SAMR1 mice were injected with AApoAII(C) (+) and killed at 1 (n = 5), 2 (n = 4), 3 (n = 5), 10 (n = 6), 12 (n = 3), and 15 (n = 4) months after injection. Control mice were injected with DW (−) and killed at 1 (n = 5), 2 (n = 4), 3 (n = 5), 10 (n = 5), 12 (n = 3), 15 (n = 3), and 20 (n = 3) months after injection. Two-month-old SAMR1 mice were injected with AApoAII(B) fibrils (+) and killed at 1 (n = 5), 3 (n = 5), 7 (n = 3), 10 (n = 3), and 12 (n = 3) months after injection. Two-month-old R1.P1-Apoa2C were injected with DW (−) and killed at 1 (n = 3), 2 (n = 3), 3 (n = 5), 5 (n = 3), 7 (n = 3), and 10 (n = 4) months after injection. The intensity of amyloid deposition was determined using AI as a parameter. The AI of each mouse was the average of grades in seven organs.

AApoAII injection-induced amyloid deposition in SAMR1 mice. Amyloid deposition in the tongue and stomach of a 17-month-old AApoAII(C)-treated SAMR1 mouse was detected by immunohistochemical staining (A and B) and green birefringence in Congo Red-stained sections and examination by polarized microscopy (C and D). AApoAII deposits (grade 4) in the papillary layer of the tongue of a SAMR1 mouse 15 months after AApoAII injection (A and C) are shown. AApoAII deposits (grade 4) in the lamina propria and submucosa of the squamous glandular junction of the stomach (B and D) are shown. The scale bar indicates 15 μm.

AApoAII(C)-induced amyloid fibrils in SAMR1 mice are composed of APOAIIB. A, Coomassie Brilliant Blue R-250 staining (lane 1) and immunoblot (lane 2) analysis of an amyloid fraction purified from the lungs of an AApoAII(C)-treated SAMR1 mouse. One major 6.5-kDa band was detected in the gel. Three anti-apoA-II-reactive bands were detected in the blot with molecular masses corresponding to the apoA-II monomer (6.5 kDa) and its oligomeric forms (16.2 and 26.4 kDa). B, N-terminal amino acid sequencing of AApoAII from AApoAII(C)-treated SAMR1 mice. Because the enzyme removes the first amino acid, only numbers 2–6 N-terminal amino acids are shown here. The fifth amino acid at the N terminus is proline, the same as in the B type apoA-II monomer.
sequencing. These results show that the major component of the deposited amyloid in AApoAII(C)-treated SAMR1 mice was endogenous APOAIIB and not exogenous AApoAII(C).

**Electron Microscopy of Amyloid Aggregation**—To analyze the ultrastructure of the AApoAII(B) fibrils, we observed the negatively stained lung and tongue amyloid samples from two AApoAII(C)-treated SAMR1 mice with transmission electron microscopy. Both of the samples exhibited amyloid-characteristic straight and unbranched fibril images with 9-nm mean breadths, lacking any very obvious surface features (Fig. 4A).

AApoAII(C) fibrils were also examined for comparison and were ~9 nm in diameter (Fig. 4B). The average length of AApoAII(B) measured was 87.58 ± 10.39 nm (50 fibrils), whereas that of the amyloid fibril AApoAII(C) was 439.28 ± 43.38 nm (80 fibrils). The former fibrils are shorter than the latter fibrils ($p < 0.0001$). Compared with AApoAII(B), we could see more obvious helically wound structures in AApoAII(C) fibrils.

**AApoAII(B) Injection Induced Amyloid Deposition in SAMR1 Mice**—We injected 0.1 mg of AApoAII(B) in solution into 2-month-old SAMR1 mice. Similar to results in AApoAII(C)-treated mice, no amyloid deposition was detected at 1 or 3 months after injection. However, at seven months after treatment, we detected severe amyloid deposition in the lamina propria of the tongue and interstitium of the myocardium. We detected moderate amyloid deposition in the glandular portion and squamous glandular junction of the stomach and in the lamina propria and submucosa of the small intestine. Mild amyloid deposits were observed in the extracellular sites around the central veins in the liver. The intensity of the deposition increased after seven months (Fig. 1). The average AI of the AApoAII(B)-treated group at 10 and 12 months after induction was 2.21, whereas that of AApoAII(C)-treated mice at 10 and 12 months after injection was 1.36 ($p = 0.0018$). We compared the average level of amyloid in five major organs with deposits in tongue, heart, stomach, small intestine, and liver at 10 and 12 months after AApoAII injection (Fig. 5A). In AApoAII(B)-treated 12- and 14-month-old SAMR1 mice, intense amyloid plaques were detected in the heart such that the plaques occupied and destroyed the histologic structure (Fig. 5B). The average AI of the heart was 3.67, which is significantly higher than that of hearts from AApoAII(C)-injected mice of the same age ($AI = 1.22, p = 0.0022$). Although, in general, the liver did not contain extensive deposits of amyloid, more amyloid deposited in the livers of AApoAII(B)-treated SAMR1 mice ($AI = 1.17$) than in those of AApoAII(C)-injected mice ($AI = 0.11, p = 0.0157$).

**FIG. 5.** AApoAII deposits in AApoAII(B)-treated SAMR1 mice. A, comparison of amyloid deposition in major organs of AApoAII(B)-treated (solid bar) or AApoAII(C)-treated (hatched bar) 12- and 14-month-old SAMR1 mice. The AI of the heart and liver are significantly different between the two groups. B, AApoAII deposition in the interstitium of the heart of a SAMR1 mouse 12 months after injection detected by immunohistochemical staining (a) and green birefringence in Congo Red-stained sections examined by polarized microscopy (b). The scale bar indicates 15 μm.
Amyloid Fibrils and Mouse Senile Amyloidosis

REFERENCES

1. Xing, Y., Nakamura, A., Chiba, T., Kogishi, K., Matsuzaki, T., Fuku, L., Gwh, Z., Hosokawa, M., Mori, M., and Takakushi, K. (1997) Lab. Invest. 81, 493–499
2. Prusiner, S. B. (1991) Science 252, 1515–1522
3. Pan, K. M., Baldwin, M., Nguyen, J., Gasse, M., Serban, A., Groth, D., Mielhorn, I., Huang, Z., Fletterick, R. J., Cohen, P. F., and Prusiner, S. B. (1993) Proc. Natl. Acad. Sci. U.S.A. 90, 10962–10966
4. Wickner, R. B., Edeske, H. K., Maddelein, M. L., Taylor, K. L., and Miyari, H. (1999) J. Biol. Chem. 274, 555–558
5. Taylor, K. L., Cheng, N., Williams, R. W., Steven, A. C., and Wickner, R. B. (1999) Science 283, 1339–1343
6. Glover, J. R., Kowal, A. S., Schirmer, E. C., Patino, M. M., Liu, J. J., and Lindquist, S. (1997) Proc. Natl. Acad. Sci. U.S.A. 94, 811–819
7. Sipe, J. D. (1992) Annu. Rev. Biochem. 61, 947–975
8. Kelly, J. W., and Lansbury, P. T. (1994) Amyloid 3, 186–225
9. Harper, J. D., and Lansbury, P. T. (1995) Annu. Rev. Biochem. 66, 385–407
10. Jarrett, J. D., and Lansbury, P. T. Jr. (1995) Cell 73, 1055–1058
11. Nakai, H., and Nakakuki, K. (1996) Lab. Invest. 74, 374–383
12. Clark, A., Chargem S. B., Badman, M. K., MacArthur, D. A., and de Koning, H. (1997) Proc. Natl. Acad. Sci. U.S.A. 94, 10557–10562
13. Csekebse, B. (1998) Science 278, 42–43
14. Caughey, B. (2000) Nat. Med. 6, 751–754
15. Baker, H. F., Ridley, R. M., Duchen, L. W., Crow, T. J., and Bruton, C. J. (1994) Mol. Neurobiol. 8, 25–39
16. Johan, K., Westermark, G., Engstrom, U., Gustavsson, A., Hultman, P., and Westermark, P. (1998) Proc. Natl. Acad. Sci. U.S.A. 95, 2558–2563
17. Kiselevsky, R., Remieux, L., Boudreau, L., Yang, D. S., and Fraser, P. (1996) Amyloid 3, 88–106
18. Higuchi, K., Matusumura A., Honna, A., Takeshita, S., Hashimoto, K., Hosokawa, M., Yashina K., and Takeda, T. (1983) Lab. Invest. 48, 231–239
19. Yonezu, T., Higuchi, K., Tsunawasawa, S., Takagi, S., Sakiyama, F., and Takeda, T. (1986) FEBS Lett. 203, 149–152
20. Higuchi, K., Yonezu, T., Kogishi, K., Matsuzuma, A., Takeshita, S., Higuchi, K., and Kogishi, K., and Matsuzuma, A., Takeshita, S., Higuchi, K.
Kohno, A., Matsushita, M., Hosokawa, M., and Takeda, T. (1986) J. Biol. Chem. 261, 12834–12840
21. Higuchi, K., Kitagawa, K., Naiki, H., Hanada, K., Hosokawa, M., and Takeda, T. (1991) Biochem. J. 270, 427–433
22. Higuchi, K., Naiki, H., Kitagawa, K., Kogishi, K., Matsushita, T., and Takeda, T. (1995) Lab. Invest. 72, 75–82
23. Higuchi, K., Kogishi, K., Wang, J., Chen, X., Chiba, T., Matsushita, T., Hoshii, Y., Kawano, H., Ishihara, Y., and Yokota, T. (1998) Lab. Invest. 78, 1535–1542
24. Naiki, H., Higuchi, K., Nakakuki, K., and Takeda, T. (1991) Lab. Invest. 65, 104–110
25. Pras, M., Schubert, M., Zucker-Franklin, D., Rimon, A., and Franklin, E. C. (1968) J. Clin. Invest. 47, 924–933
26. Naiki, H., Higuchi, K., Hosokawa, M., and Takeda, T. (1989) Anal. Biochem. 177, 244–249
27. Schagger, H. J., and Von Jagow, G. (1987) Anal. Biochem. 166, 368–379
28. Prasner, S. B. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 13363–13383
29. Sparrer, H. E., Santoso, A., Szoka, F. C., Jr., and Weissman, J. S. (2000) Science 289, 595–599
30. Raymond, G. J., Hope, J., Kocisko, D. A., Priola, S. A., Raymond, L. D., Bossers, A., Ironside, J., Will, R. G., Chen, S. G., Petersen, R. B., Gambetti, P., Rubenstein, R., Smits, M. A., Lansbury, P. T. Jr., and Caughey, B. (1997) Nature 388, 285–288
31. Santoso, A., Chien, P., Osherovich, L. Z., and Weissman, J. S. (2000) Cell 100, 277–288
32. Derkatch, I. L., Bradley, M. E., Hong, J. Y., and Liebman, S. W. (2001) Cell 106, 171–182
33. Stangou, A. J., Hawkins, P. N., Heaton, N. D., Rela, M., Monaghan, M., Nihoyannopolus, P., O’Gardy, J., Pepys, M. B., and Williams, R. (1998) Transplantation 66, 229–233
34. Yazaki, M., Tokuda, T., Nakamura A., Higashikata, T., Koyama, J., Higuchi, K., Harihara, Y., Baba, S., Kametani, F., and Ikeda, S. (2000) Biochem. Biophys. Res. Commun. 274, 702–706
35. Hammarstrom P., Schneider, F., and Kelly, J. F. (2001) Science 293, 2459–2462
36. Nichols, W. C., Gregg, R. E., Brewer, H. B., Jr., and Benson, M. D. (1990) Genomics 8, 318–323
37. Booth, D. R. Tan, S., Booth, S. E., Tennent, G. A., Hutchinson, W. L., Hsuan, J. J., Totty, N. F., Truong, O., Soutar, A. K., Hawkins, P. N., Bruguera, M., Caballeria, J., Sole, M., Campistol, J. M., and Pupps, M. B. (1996) J. Clin. Invest. 97, 2714–2721
38. Hamidi Asl, L., Liepnieks, J. L., Hamidi Asl, K., Uemichi, T., Moulin, G., Desjoyaux, E., Loire, R., Delpech, M., Grateau, G., and Benson, M. D. (1999) Am. J. Pathol. 154, 221–227
39. Kindy, M. S., King, A. R., Perry, G., de Beer, M. C., and de Beer, F. C. (1995) Lab. Invest. 73, 469–475
40. Kisilevsky, R., Gruys, E., and Shirahama, T. (1995) Amyloid 2, 128–133
41. Hoshii, Y., Kawano, H., Cui, D., Takeda, T., Gondo, T., Takahashi, M., Kagishi, K., Higuchi, K., and Ishihara, T. (1997) Am. J. Pathol. 151, 911–917
42. Benson, M. D., Liepnieks, J. J., Yazaki, M., Yamashita, T., Hamidi Asl, K., Guenther, B., and Klueve-beckerman, B. (2001) Genomics 15, 272–277