The Role of Cystatin C in Cerebral Amyloid Angiopathy and Stroke: Cell Biology and Animal Models

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A variant of the cysteine protease inhibitor, cystatin C, forms amyloid deposited in the cerebral vasculature of patients with hereditary cerebral hemorrhage with amyloidosis, Icelandic type (HCHWA-I), leading to cerebral hemorrhages early in life. However, cystatin C is also implicated in neuronal degenerative diseases in which it does not form the amyloid protein, such as Alzheimer disease (AD). Accumulating data suggest involvement of cystatin C in the pathogenic processes leading to amyloid deposition in cerebral vasculature and most significantly to cerebral hemorrhage in patients with cerebral amyloid angiopathy (CAA). This review focuses on cell culture and animal models used to study the role of cystatin C in these processes.

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The cysteine protease inhibitor, cystatin C (23), also known as γ trace (67), is found in all mammalian body fluids and tissues (23). In vitro experiments have indicated that it can inhibit the cysteine proteases cathepsins B, H, K, L and S (for review, see 18). In addition to being a protease inhibitor, cystatin C itself is a target of proteolysis (138, 143) and is inactivated by proteolytic degradation by cathepsin D and elastase (1, 92). Cathepsins are lysosomal proteases required for housekeeping function during protein turnover and they differ in structure, substrate-specificities and biochemical characteristics (159). Uncontrolled proteolysis as a result of imbalance between active proteases and their endogenous inhibitors has been associated with different diseases such as Alzheimer disease (AD) (116), ischemia (70, 122, 129, 158, 180), rheumatoid arthritis (157), renal failure (80), multiple sclerosis (19), osteoporosis (41), muscular dystrophy (148), inflammatory periodontal disease (87), inflammatory lung disease (29), inflammation and trauma (12), and various types of cancer (30, 44, 154). For review of the involvement of proteases and their inhibitors in the processes of neuronal degeneration and repair of the nervous system, see (156). This review will describe the data implicating cystatin C in pathological processes of the central nervous system, mainly in CAA and cerebral hemorrhage.

MOLECULAR MECHANISM OF VARIANT CYSTATIN C FIBRIL FORMATION AND AMYLOID DEPOSITION IN HEREDITARY CEREBRAL HEMORRHAGE WITH AMYLOIDOSIS, ICELANDIC TYPE

Hereditary cerebral hemorrhage with amyloidosis, Icelandic type (HCHWA-I) (9, 61), also called hereditary cystatin C amyloid angiopathy (HCCAA) (126), is an autosomal dominant form of CAA. Amyloid deposition in cerebral and spinal arteries and arterioles leads to recurrent hemorrhagic strokes causing serious brain damage and eventually fatal stroke (61). The amyloid deposited is composed mainly of a Leu68Gln variant of cystatin C (5, 36, 51, 95, 130). A heterozygous point mutation, identical to that found in the cystatin C gene of these patients, was also identified in a Croatian man with CAA and intracerebral hemorrhage (56). Thus, sporadic CAA in some patients may be associated with mutations in the cystatin C gene (56, 110).

The cystatin C isolated from the leptomeninges of HCHWA-I patients was found to be amino-terminally truncated, starting at position 11 of normal cystatin C (36, 51). A serine protease, elastase, cleaves cystatin C in vitro, generating a very stable product that lacks the amino-terminal 10 residues of full-length cystatin C (4). There is evidence to indicate that this modification may occur extracellularly in vivo as well because a truncated form of cystatin C was isolated from human urine (64). However, cystatin C isolated from cerebrospinal fluid (CSF) of HCHWA-I patients and from unaffected individuals is amino-terminally intact (11, 99, 125, 172), although a minor component starting at residue Leu9 was also found (11). Moreover, cystatin C synthesized by monocytes of carriers of the mutation also has full-length sequence (11, 155), and cells transfected with either the wild-type or variant genes secrete only full-length cystatin C (172). The cleavage of the amino-terminal decapeptide of the amyloid may occur in vivo during long-term exposure of the protein to proteases before, during and/or after amyloid fibril formation. Structural studies revealed that the amino-terminal truncation likely is an event secondary to amyloid formation, and of no relevance for the development of the disease (48). Mass spectrometric characterization showed that after three months of storage of human CSF at -20°C, cystatin C was cleaved in the peptide bond between Arg8 and Leu9 and lost its eight amino-terminal residues, whereas this cleavage did not occur when the CSF was stored at -80°C, indicating a storage-related artifact rather than a physiological or pathological processing of the protein (31).

The biochemical properties of the variant cystatin C have been extensively studied. It was demonstrated that the substitution does not affect the inhibitory activity, because wild-type and Leu68Gln variant cystatin C produced in *Escherichia coli* expression sys-

tems and by tissue culture cells effectively inhibited the cysteine protease cathepsin B (2, 172). However, the Leu68Gln is within the hydrophobic core of cystatin C (8), thus reducing the stability of the protein. It was hypothesized that this amino acid substitution may induce conformational changes, affecting the proteolytic susceptibility of the variant protein, as well as its predisposition to dimerization and amyloid fibril formation.

Effect of the Leu68Gln substitution on cystatin C dimerization. It was shown that recombinant wild-type cystatin C dimerizes when exposed to denaturing agents, low pH, or high temperature (45). These conditions lead to partial unfolding of cystatin C molecules with subsequent refolding into a dimeric state (45), in which the protein loses its capacity to inhibit papain-like cysteine proteases. Analysis of wild-type and variant cystatin C dimerization using recombinant proteins expressed in *Escherichia coli* (46, 49) has shown that the proteins differed in their tendency to dimerize and form aggregates. The wild-type protein was monomeric even after prolonged storage at elevated temperatures, while the variant proceeded to dimerize and lose biological activity immediately after refolding (2). The dimers and aggregates of the Leu68Gln variant recombinant protein were formed at normal body temperature, nearly 25°C lower than that needed for wild-type cystatin C dimerization (2). The most likely site for physiological dimerization would be in acidic cellular compartments. The pH in lysosomes is in the range of 4.6 to 5.0 which corresponds to the pH at which recombinant cystatin C dimerized to some extent in vitro. Consistent with this, a possible role of lysosomes in amyloid fibril formation has been proposed (52, 147). The lysosome could be the site of partial protein denaturation yielding an intermediate, which can associate into amyloid fibrils faster than it can be degraded. Similarly, it was shown that partial denaturation of transthyretin under conditions which mimic the acidic environment of a lysosome is sufficient to effect amyloid fibril formation by self-assembly of a conformational intermediate (37).

Cystatin C also forms dimers in mammalian cells overexpressing either the wild-type or variant forms of the gene (22, 112, 172).

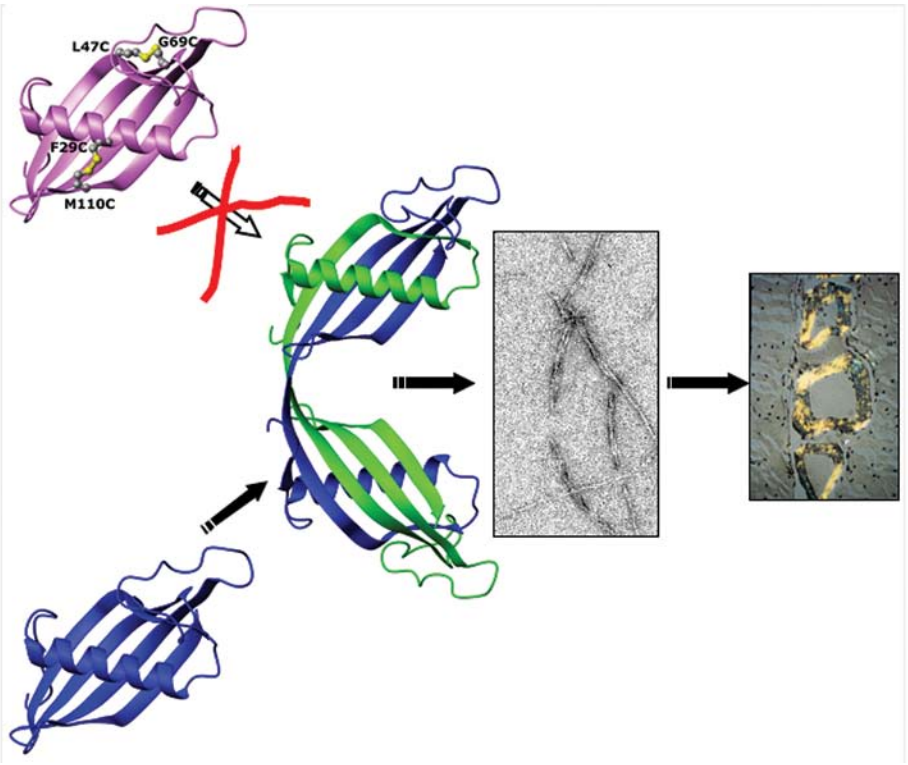

Figure 1. Domain swapping (blue and green) seems to be involved in the formation of amyloid fibrils from wild-type and Leu68Gln variant cystatin C. Proteins stabilized against domain swapping by disulfide bridges (purple) do not produce amyloid fibrils. Amyloid fibrils were formed following incubation of non-stabilized cystatin C (blue) at a concentration of 3 mg/ml in 10 mM glycine buffer, pH 2.0., containing 0.2% sodium azide, at 48°C.

A study of stably transfected NIH/3T3 cells suggested that cells expressing the gene encoding Leu68Gln cystatin C had intracellular accumulation of insoluble variant cystatin C mainly in the endoplasmic reticulum (22). In Chinese hamster ovary transfected cells, variant cystatin C formed stable intracellular dimers that are partially retained in the endoplasmic reticulum and degraded. The bulk of mutant cystatin C that is secreted does not dissociate and is exported as an inactive dimer (16). Blocking exit from the endoplasmic reticulum with brefeldin A resulted in increased dimerization of cystatin C and removal of brefeldin A caused an increase in the intracellular monomeric form. It was suggested that cystatin C dimerization occurs in the endoplasmic reticulum and dimer dissociation occurs later in the secretory pathway (112). Some degree of intracellular dimerization was identified by chromatography on AcA-44 column of lysates of Chinese hamster ovary cells transfected with the wild-type cystatin C cDNA (79). Mass spectrometry and Western blot analyses of several human astrocytic glioma U251 and human kidney 293 stably transfected cell lines, showed the existence of cystatin C dimers in cell homogenates with high levels of overexpression of cystatin C (172). Dimerization directly correlated with cystatin C concentration in the sample, although the variant cystatin C formed dimers at concentrations lower than those necessary for dimerization of the wild-type protein. The mature, active form of cystatin C was secreted as a monomer into the culture medium (112, 172). The temporal profile of cystatin C production and secretion by pulse-chase studies revealed that both wild-type and variant cystatin C were expressed and cleared from the cells at the same rate and to a similar extent (172). The variant was found along with wild-type cystatin C in plasma and CSF of patients. Approximately equal amounts of cystatin C dimers and monomers were demonstrated in plasma from HCHWA-I patients, whereas only monomers could be found in normal plasma. Leu68Gln-wild-type cystatin C heterodimers seem to be present in the dimeric cystatin C population. CSF from patients also contained cystatin C dimers and monomers, but the dimeric fraction was minute. CSF from control patients did not

contain dimeric cystatin C (21). These data suggest that dimerization of wild-type and variant cystatin C occurs in compartments with high concentrations of the protein and that the Leu68Gln variant dimerizes at lower concentrations than the wild-type protein.

The crystal structure of human cystatin C revealed that the protein refolds to produce very tight 2-fold symmetric dimers while retaining the secondary structure of the monomeric form (75). The dimerization occurs through 3-dimensional (3D) domain swapping, a process involving exchange of sub-domains of the monomeric proteins. The exchange of domains is symmetric, or closed-ended, leading to intertwined and closed dimeric species. It has been suggested that in an open-ended, run-away mode, the mechanism of domain swapping could lead to infinite linear polymerization (76), such as is characteristic of amyloid fibrils. Since domain swapping of cystatin C involves an extended β-sheet, the final aggregate could be consistent with cross-β structure, which is believed to be at the heart of the molecular architecture of amyloid fibrils (76). Association of 3D domain-swapped cystatin C dimers into larger structures with involvement of intermolecular β-sheet interactions has been confirmed in another crystallographic study (74). In addition, it has been demonstrated that cystatin C with amino-terminal truncation forms essentially identical 3D domain-swapped dimers (73). However, the absence of the N-terminal decapeptide may facilitate further association of the protein via β-sheet interactions. Prevention of domain swapping inhibits dimerization and amyloid fibril formation of cystatin C (119) (Figure 1). The process of 3D domain swapping destroys one of the key elements of the cysteine protease-binding epitope. This explains the complete loss of the protease inhibitory activity reported for dimeric cystatin C (45, 46). The dimerization process influences the equilibrium between the proteolytic and inhibitory activities, and may be, therefore, relevant for physiological regulation of cysteine protease activity, for amyloid formation, and for the occurrence of hemorrhages.

Effect of the Leu68Gln substitution on cystatin C structure and proteolytic susceptibility. Most native proteins are relatively resistant to cleavage, whereas unfolded proteins are usually good substrates for proteolysis. Thus, the rate at which a protein is degraded by a protease is indicative of its stability, enabling comparison of a mutant protein to its wild-type counterpart. The fraction of molecules that are unfolded varies as a function of temperature for both proteins, but the transition for the mutant may occur over a lower temperature range than that for the wild-type protein. Some proteins aggregate because they are unfolded or incompletely folded, and thus escape proteolysis (128). Thus, a common mechanism of fibril formation emerges (101), and it was suggested that the effect of the Leu68Gln substitution on cystatin C is similar to that caused by amino acid replacements in other amyloid forming proteins: Glu22Gln variant of Aβ deposited in cerebral vasculature of patients with HCHWA, Dutch type (HCHWA-D) (94, 176), immunoglobulin light-chain associated with light-chain amyloidosis (69), transthyretin variants found in familial amyloid polyneuropathy (111), and amyloid formed in lysozyme amyloidosis (24). The first step of this mechanism is a change in conformation followed by the opening of new hydrophobic surfaces, finally leading to the fibrillogenic step.

Circular dichroism, NMR spectroscopy, and 1-anilinonaphthalene-8-sulfonic acid binding analyses of the recombinant proteins isolated from *Escherichia coli* inclusion bodies showed that the Leu68Gln variant is structurally very similar to human wild-type cystatin C. However, the variant protein was stable only in a restricted range of conditions. Outside of this range, the variant differed from the wild-type protein by an increased amount of hydrophobic components on the protein surface (49). Experiments that used cystatin C proteins expressed in a mammalian tissue culture system indicate that the variant cystatin C has a more unfolded structure than the wild-type protein, with stretches of hydrophobic residues exposed to the solvent (172). The exposure of hydrophobic fragments to a polar environment is an unfavorable thermodynamic state that often results in protein oligomerization and aggregation. This could be the driving force of amyloid formation. Moreover, the unfolded structure of variant cystatin C may also expose critical residues of the protein to proteases, resulting in increased degradability. Thus, the unfolding of the protein leads to either aggregation or degradation depending on the proteins' concentration. When variant cystatin C is present in a low concentration in body fluids, such as the CSF, it is highly susceptible to extracellular proteolysis by a serine protease. High concentration of the variant protein within neuronal cells, as was shown within the cytoplasm and cell processes of pyramidal neurons, mainly in layers three and four of the cortex of aged individuals (96), may result in its aggregation and fibrillogenesis.

The concentration of cystatin C in the CSF of HCHWA-I patients is lower compared to normal controls (60). Mass spectrometry of tryptic peptides of cystatin C isolated from the CSF of patients or from media of cultured monocytes isolated from patients revealed that only wild-type cystatin C was detectable, raising questions about the expression and fate of the mutant protein (11, 155). While accumulation of this protein in vessel walls may explain its depletion from the CSF, reduced secretion or enhanced proteolysis may be additional explanations. It was shown that the wild-type and variant cystatin C are similarly expressed and constitutively secreted by a variety of tissue culture cell lines transfected with either gene (28, 35, 39, 172). Both had a relatively short intracellular half-life (about 73 minutes) in human glial and kidney culture cells stably transfected with the cystatin C genes. The mature, active form of cystatin C was secreted as a monomer into the culture medium (112, 172). However, media of clones expressing the gene encoding Leu68Gln cystatin C had lower amounts of the protein than clones expressing wild-type human cystatin C (22, 172). It was shown that the secreted wild-type protein is resistant to extracellular proteolysis, whereas the secreted variant cystatin C is quickly degraded. Wild-type cystatin C secreted into the culture media was stable for at least 5 days. Conversely, the level of the Leu68Gln variant cystatin C was significantly reduced in the media in one day and only traces were observed after 2 days of culture (172). Protease inhibitory profile showed that the serine protease inhibitor, DFP, blocked the protease activity. Thus, a serine protease is involved in the observed reduction of the variant protein. This activity is cell line specific, since different levels of proteolytic activity are re-

leased by many cell types. Furthermore, a significant amount of the protease activity is also present in CSF of normal individuals (172). This suggests that the low level of cystatin C in CSF of affected patients is due to enhanced proteolysis of the variant protein.

INVOLVEMENT OF CYSTATIN C IN ALZHEIMER DISEASE

Aβ, a processing product of the larger β amyloid precursor protein (APP) (82), is the major constituent of the amyloid fibrils deposited in the brain of patients with AD, Down syndrome, sporadic CAA, and HCHWA-D (27, 53, 107, 178). Several APP missense mutations within or flanking the Aβ region were found in the APP gene in a few early onset familial AD pedigrees (151). APP amino acid substitutions located in the middle of the Aβ peptide, at positions 21, 22, and 23 (26, 65, 81, 94) are associated with various degrees of cerebrovascular pathology, some of which are extensive and result in cerebral hemorrhage (25, 26, 38, 55, 58, 63, 65, 81, 94, 102, 124, 162, 171). However, amyloid fibrils are usually formed from processing products of normal precursor proteins (152). Thus, mechanisms other than mutations expressed as protein variants might play a crucial role in the process of amyloid formation (88). Some proteins associated with AD lesions may have a role in the pathological processes leading to amyloidogenesis and neuronal degeneration and others may bind secondarily to amyloid deposits.

Immunohistochemical studies revealed that cystatin C co-localizes with Aβ predominantly in amyloid-laden vascular walls, and in senile plaque cores of amyloid in brains of patients with AD, Down syndrome, HCHWA-D, and cerebral infarction (62, 71, 96, 104, 167) and of non-demented aged individuals (96). Cystatin C also co-localizes with Aβ amyloid deposits in the brain of aged rhesus and squirrel monkeys (173), dogs (160, 161), and transgenic mice overexpressing human APP (96, 149) (Figure 2).

The neuronal staining of cystatin C in AD brains was primarily limited to pyramidal neurons in cortical layers duplicating the pattern of neuronal susceptibility in AD brains: the strongest staining was found in the entorhinal cortex, in the hippocampus, and in the temporal cortex; fewer pyramidal neurons were stained in the frontal, parietal, and occipital lobes (42). Immunostaining of cystatin C within neurons showed similar distribution to that of the endosomal/lysosomal proteases cathepsin B (42) and cathepsin D (33). Upregulation of cathepsin synthesis in AD neurons and accumulation of hydrolase-laden lysosomes indicate an early activation of the endosomal/lysosomal system in vulnerable neuronal populations, possibly reflecting early regenerative or repair processes (32, 33). These neuropathological observations support an association between cystatin C and cathepsin B and cathepsin D in AD, and suggest a model of cystatin C involvement in the process of neuronal death in AD. Using an antibody specific to the carboxyl-terminus of Aβ42, intracellular immunoreactivity was observed in the same neuronal subpopulation strongly stained for cystatin C (96). This suggests that Aβ accumulates in a specific population of pyramidal neurons in the brain, the same cell type in which cystatin C is highly expressed. Co-localization of cystatin C with APP has been demonstrated in transfected human embryonic kidney HEK293 cells, mouse neuroblastoma N2a cells (145), in APP-overexpressing cultured human muscle fibers, and in muscle cells of patients with sporadic inclusion-body myositis (s-IBM) (163).

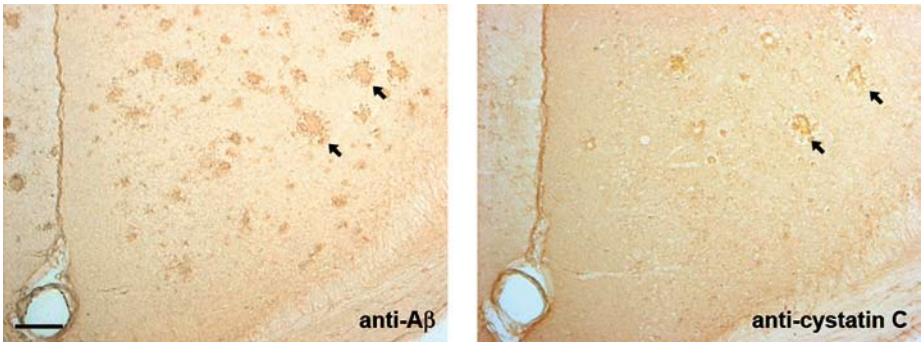

Figure 2. Immunohistochemical staining of amyloid deposits in the brain of an APP Tg2576 transgenic mouse at 18 months of age with anti-Aβ antibody (6E10) and with anti-cystatin C antibody. Arrows indicate same plaques stained with both antibodies and scale bar represents 50 μm.

Co-localization of cystatin C with Aβ results from binding of cystatin C to Aβ, as well as to its precursor protein. Western blot analysis of immunoprecipitated cell lysate or medium proteins revealed binding of cystatin C to full-length APP and to secreted APPα (145). Deletion mutants of APP localized the cystatin C binding site within APP to the extracellular region of Aβ. This binding location seems to protect APP from β-secretase processing, resulting in an increase in the non-amyloidogenic α-secretase cleavage, with no effect on the γ-secretase cleavage site. Accordingly, co-expression of cystatin C and APP in neuroblastoma cells resulted in increased secretion of APPα, while production of both Aβ40 and Aβ42 remained unchanged (145). The same observation was made in vivo, in transgenic mice expressing the human cystatin C gene (134). Enzyme-linked immunosorbent assay (ELISA) analysis of Aβ40 and Aβ42 concentrations in the brain of these mice showed no difference between cystatin C transgenic mice and their non-transgenic littermates (134). Thus, in vitro and in vivo overexpression of human cystatin C does not affect the levels of the potentially toxic Aβ.

Cystatin C binds not only to Aβ sequences within APP, but also to the peptide itself. Analysis of the association of cystatin C and Aβ by ELISA demonstrated that cystatin C interacts with both Aβ40 and Aβ42 in a concentration dependent manner at physiological pH and temperature. A specific, saturable and high affinity binding between cystatin C and Aβ was observed (145). Electron microscopic analysis of fibril formation revealed that incubation of cystatin C with either Aβ40 or Aβ42 inhibits Aβ fibril formation in a concentration-dependent manner (145). Thus, binding of cystatin C to soluble Aβ inhibits amyloid fibril formation, and the occurrence of the inhibitor in amyloid deposits may represent a residual effect of this association.

Genetic studies support a role for cystatin C in AD. The cystatin C gene (CST3), localized on chromosome 20 (3, 144) has three genetically linked base substitutions in the 3′ region (14, 15). A G/A transition in exon 1 results in Ala/Thr variation in the coding region of CST3, within the signal

peptide. While the allele containing Ala at that position was called the A allele, the one containing Thr in the same position was called B allele. Several studies have linked CST3 gene polymorphisms with an increased risk of developing AD (20, 34, 40, 47, 54, 97, 127) and a possible interaction with apolipoprotein E (ApoE) genotype was noted. However, some studies have failed to show an association between the cystatin C gene and AD in a German cohort (43), a Dutch sample with early-onset AD (140), Japanese AD patients (103), and in early onset AD families (132).

Crawford et al (40) divided a multicentric AD population by age at onset and found an age related increased influence of the A allele of CST3. A significant interaction between the homozygous A genotype of CST3 and age of onset of AD was found, such that in the over 80 years age group this genotype was responsible for a 2-fold increased risk for the disease. This interaction was independent of the ApoE genotype (40). Another study of large European and American populations, with mean age at onset of 73.1 and 75.0 for AD and controls, respectively, showed linkage between the B allele and late onset AD with no synergistic association with ApoE allele (47). A synergistic association between the CST3 and ApoE ε4 alleles was found in a Spanish sample. The CST3 B allele caused a 3-fold elevated risk of AD before age 70 and there was an 8-fold increase in risk for ApoE ε4 carriers with this allele (20). In one study the combination of one or two CST3 B alleles and ApoE ε4 carried a 14-fold increased risk for men and 16-fold for women. These risks apply to a shift in risk from ages 65 and older to younger ages (34). When it was attempted to determine the association between CST3 polymorphism and AD or vascular dementia, associations between CST3 B genotype and AD patients older than 75 years, or vascular dementia patients younger than 75 years were evident. A synergistic association of CST3 and ApoE ε4 alleles was observed in predicting vascular dementia patients (97).

The amino acid exchange from Ala to Thr at the -2 position for signal peptide cleavage alters the hydrophobicity profile of the signal sequence (47), resulting in a less efficient cleavage of the signal peptide and thus a reduced secretion of cystatin C (17). The CST3 gene G73A polymorphism functionally affects cystatin C plasma levels (123). Furthermore, a study of the targeting of the Thr haplotype in cultured retinal pigment epithelial and HeLa cells have shown that a proportion of the Thr protein undergoes incorrect trafficking. In contrast to the Ala haplotype that is targeted to the Golgi apparatus, the Thr variant was associated primarily with mitochondria, resulting in a substantial reduction in the efficiency of targeting cystatin C for secretion (131). A multicenter electroencephalographic (EEG) study analyzed the effects of CST3 haplotypes on resting cortical rhythmicity in subjects with AD and mild cognitive impairment. A relationship between the CST3 Thr haplotype and global neurophysiological phenotype (ie, cortical delta and alpha rhythmicity) was found. While ApoE ε4 affects EEG rhythms in AD (77, 90, 91), the effects of CST3 polymorphism were independent of ApoE ε4 co-presence (13).

Thus, multiple lines of research support a role for cystatin C in AD. It remains to be determined in what pathological, or alternatively, protective process(es) of the disease it is involved, and what the mechanism of its action is. Similar to a variety of activities that have been associated with other protease inhibitors in the brain (7, 68, 89, 139, 150), cystatin C has been implicated in the processes of neuronal degeneration and repair of the nervous system. Enhanced cystatin C expression was observed in response to injury, including facial nerve axotomy (113), perforant path transections (181), and hypophysectomy (83), in morphologically degenerative CA1 pyramidal neurons and reactive astrocytes of hippocampus following transient forebrain ischemia (70, 122, 129, 180) and after induction of epilepsy (10, 66, 100). It was proposed that cystatin C might facilitate the establishment of an axonal growth-promoting environment by protecting neurite growth-associated molecules from degradation by cysteine proteases in the deafferented hippocampus (6, 135). Oxidative stress also stimulates an increase in cystatin C expression in cultured neurons (120, 121) and in cerebral microvascular smooth muscle cells (170), suggesting a role in regulation of apoptosis. Moreover, a glycosylated form of rat cystatin C was found to be an autocrine/paracrine factor, required for the mitogenic activity of basic fibroblast growth factor on neural stem cells (153). These data suggest that cystatin C may have a protective role in neurodegenerative diseases, including AD, in addition to its role in inhibition of Aβ fibrilogenesis.

INVOLVEMENT OF CYSTATIN C IN CEREBRAL AMYLOID ANGIOPATHY

While Aβ usually accumulates both in the cerebral blood vessels and in brain parenchyma as amyloid plaques, in some cases there is predominantly vascular Aβ deposition (165). The factors leading to vascular rather than parenchymal amyloid deposition are unknown and it is still unclear when CAA leads to hemorrhage. In vessels affected by CAA, local smooth muscle and elastic elements are lost and replaced by amyloid fibrils, thereby weakening the overall structure of the vessel (84, 104, 142, 164). Consequently, it was suggested that CAA predisposes towards cerebral infarction and cerebral hemorrhage. However, CAA is usually asymptomatic and only a subpopulation is at high risk of hemorrhage (57, 71, 78, 106, 109, 141, 164, 166, 168). Thus, CAA appears to be a prerequisite but not sufficient for vessel rupture, with additional factors playing important roles. Multiple genetic factors can be associated with the risk of CAA in the elderly. The role of ApoE in the genetics and pathogenesis of AD and of CAA both in AD and in non-demented elderly individuals has been well established. Whereas the ApoE ε4 allele promotes deposition of Aβ in the cerebral vasculature (59, 108, 109, 117, 146, 177, 179), possession of ApoE ε2 promotes the development of hemorrhage in vessels already laden with amyloid (57, 59, 109, 117, 118, 177).

A role for cystatin C in CAA-related hemorrhage is implicated from immunohistochemical studies of patients with AD, Down syndrome, HCHWA-D, intracranial hemorrhage, cerebral infarction, and of elderly patients without any neurological disorder, that revealed co-localization of cystatin C and Aβ in amyloid-laden vascular walls (62, 71, 104, 167). Maruyama et al (104) reported that only patients showing co-localization of cystatin C and Aβ immunoreactivity in their diseased cerebral vessels suffered fatal subcortical hemorrhages. The degree of cerebrovascular amyloid deposition in these patients was also greater than in patients without cerebral hemorrhages. Studies were conducted to find out

whether cystatin C exists as amyloid fibrils or as unpolymerized cystatin C absorbed onto or trapped within the bundles of Aβ amyloid fibrils. ELISA analysis of crude amyloid fibrils isolated from cerebral blood vessels of one patient revealed that cystatin C and Aβ have been included at the ratio of about 1:100 (115). In another case of sporadic CAA, isolation and chemical analysis of amyloid fibril proteins from leptomeningeal vessels revealed that while Aβ was fibrillar, cystatin C was soluble (105). It has been suggested that cystatin C deposition occurs secondarily to Aβ deposition and may increase the predisposition to cerebral hemorrhages (71).

Genetic studies show a role for cystatin C in CAA and stroke. In support of the interaction of CST3 with ApoE ε4 in conferring vascular pathologies, a logistic regression analysis revealed that neither CST3 polymorphism nor its interaction with ApoE ε4 were significant predictors of AD. However, a synergistic association of CST3 and ApoE ε4 alleles was observed in predicting vascular dementia patients (97). Izumihara et al (72) suggested that while severe Aβ deposition and ApoE ε2 and ε4 alleles are risk factors for the occurrence of the hemorrhage, severe cystatin C accumulation is a risk factor both for the occurrence and enlargement of the hemorrhage, as well as for the tendency to induce recurrent hemorrhages. In addition, loss of vascular smooth muscle was observed in the intensely amyloid-laden vascular walls that showed cystatin C-immunoreactivity.

Cystatin C co-localization with amyloid, other than Aβ, was observed in a variety of disorders, such as hereditary gelsolin amyloidosis (familial amyloidosis, Finnish type) (85, 86) and familial cerebral amyloid angiopathy, British type, (50). Thus, cystatin C may have a role in CAA and hemorrhage in a variety of diseases that involve deposition of heterogeneous types of amyloid proteins.

CYSTATIN C IN ANIMAL MODELS OF CAA

A striking increase in CAA with aging was found in the APP transgenic mouse line APP23 (174). However, no significant correlation was found between CAA frequency or severity and amyloid load within age groups. 19-month-old APP23 mice had several focal cerebral hemorrhages that dramatically increased in frequency and size in 27-month-old mice. The anatomical distribution of the hemorrhages appeared very similar to the distribution of CAA. Antibodies to cystatin C showed appreciable staining of cerebrovascular amyloid in these mice, suggesting that mouse cystatin C is part of the amyloid. Similar to cystatin C staining of Aβ deposits in human brains, the cystatin C immunoreactivity was restricted to a subpopulation of amyloid-laden vessels and was clearly less intense than Aβ staining (96, 149, 174).

Non-human primates are good models to study cerebral changes that occur through aging. Neuropathologies characteristic of AD and normal aging in humans were found in senescent non-human primates (137, 169, 175). In aged rhesus monkeys (*Macaca mulatta*), amyloid deposition predominates in senile plaques with relatively minor vascular involvement. However, cerebrovascular deposits in aged squirrel monkeys (*Saimiri sciureus*), usually are more conspicuous than senile plaques (169). Because CAA in squirrel monkey resembles that in HCHWA-D it was hypothesized that a species-specific amino acid difference in Aβ between rhesus and squirrel may contribute to CAA in aged squirrel monkeys. However, it was shown that the predicted amino acid sequence of Aβ in squirrel monkeys is identical to that of rhesus monkeys and normal humans (93). It was also demonstrated that species differences in loci of amyloid deposition are not related to ApoE allotype because both rhesus and squirrel monkeys are homozygous for the same ApoE allele (114, 136, 182). However, sequence analysis of rhesus and squirrel monkey cystatin C cDNA revealed that squirrel monkey has Met at position 68, which is Leu in the rhesus and wild-type human cystatin C and Gln in HCHWA-I patients (173). An additional difference between squirrel and rhesus monkeys in cystatin C sequence was found at position 10, a residue that was shown to affect the specificity of the inhibitor for different cysteine proteases (98). The species-specific cystatin C sequences in humans, rhesus, and squirrel monkeys may be responsible for the variability of the amyloid deposits observed.

In humans co-localization of cystatin C with Aβ elevates the risk of hemorrhagic stroke. While co-localization of Aβ and cystatin C was demonstrated in vascular and parenchymal deposits in the brains of rhesus monkeys and in vascular amyloid in brains of aged squirrel monkeys, there was no evidence of intracerebral hemorrhage (173). Abundant amyloid deposition and mild hemorrhage was found in the brain of one female squirrel monkey, known as "Baker," who died at the estimated age of 27 years from renal failure (169, 173). However, no systematic, large-scale study of hemorrhage in aged monkeys has been conducted. Thus, the specific cystatin C sequences in squirrel monkeys may contribute to the predominant deposition of amyloid in cerebral vessel walls, but not to an increased risk of hemorrhage.

OVEREXPRESSION OF CYSTATIN C AS A CAUSE OF CEREBRAL HEMORRHAGE

Cystatin C transgenic mice were generated in order to elucidate the role of increased expression of this protease inhibitor in vivo (134). These mice express either human wild-type or the Leu68Gln variant cystatin C genes under the transcriptional control of its own promoter (95), overexpressing the transgene along with its endogenous counterpart in the appropriate tissues (134). Lines of mice expressing various levels of cystatin C in the brain were selected. All selected lines had very high concentrations of the transgene in the blood. None of the mice had amyloid deposits either in the vessel walls or in the neuropil. Neuropathological examination of dead or ailing aged transgenic mice revealed mice with cerebral or subarachnoid hemorrhages (133). Conversely, no hemorrhages were observed in their non-transgenic siblings. These data demonstrate that elevated brain and/or blood levels of cystatin C can cause hemorrhagic strokes in the absence of vascular amyloid deposits. In the brain of aged individuals and AD patients, cystatin C co-deposits with Aβ. It has been shown that the risk of cerebral hemorrhage increases when high levels of cystatin C are present in cerebrovascular Aβ deposits. We suggest that cystatin C binding to Aβ may cause local accumulation of the protease inhibitor, contributing to hemorrhages.

SUMMARY

Accumulating data implicate cystatin C in Alzheimer disease, in CAA, and in cerebral hemorrhage. A variant cystatin C with a single amino acid substitution can form

amyloid fibrils and directly cause CAA and the resulting hemorrhages. Wild-type cystatin C binds to proteins that form amyloid fibrils, such as Aβ, and the resulting local accumulation of the inhibitor can result in hemorrhages as well. Furthermore, animal models suggest that high systemic levels of cystatin C can cause cerebral hemorrhages. Future studies will determine the mechanism by which cystatin C causes damage to vessel walls and leads to hemorrhage.

Two proteins, the cysteine protease inhibitor cystatin C and ApoE, are linked to late onset AD and to CAA, and both proteins have been implicated in CAA-related hemorrhage. While the ApoE ε4 and ApoE ε2 isoforms are risk factors for the occurrence of hemorrhages, severe cerebrovascular cystatin C accumulation is a risk factor not only for the incidence but also for enlargement and recurrence of hemorrhages. While both ApoE and cystatin C are factors contributing to cerebral hemorrhage, neither factor is sufficient to always produce hemorrhages. The relative significance of each of the factors involved, and the possible synergy between them remain to be determined.

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REFERENCES

1. Abrahamson M, Buttle DJ, Mason RW, Hansson H, Grubb AO, Lilja H, Ohlsson K (1991) Regulation of cystatin C activity by serine proteinases. *Biomed Biochim Acta* 50:587-593.

2. Abrahamson M, Grubb AO (1994) Increased body temperature accelerates aggregation of the Leu-68→Gln mutant cystatin C, the amyloid-forming protein in hereditary cystatin C amyloid angiopathy. *Proc Natl Acad Sci U S A* 91:1416-1420.

3. Abrahamson M, Islam MQ, Szpirer J, Szpirer C, Levan G (1989) The human cystatin C gene (CST3), mutated in hereditary cystatin C amyloid angiopathy, is located on chromosome 20. *Hum Genet* 82:223-226.

4. Abrahamson M, Mason RW, Hansson H, Buttle DJ, Grubb AO, Ohlsson K (1991) Human cystatin C. Role of the N-terminal segment in the inhibition of human cysteine proteinases and its inactivation by leukocyte elastase. *Biochem J* 273:621-626.

5. Abrahamson M, Olafsson I, Palsdottir A, Ulvsback M, Lundwall A, Jensson O, Grubb AO (1990) Structure and expression of the human cystatin C gene. *Biochem J* 268:287-294.

6. Aghajanyan HG, Arzumanyan AM, Arutunyan AA, Akopyan TN (1988) Cystatins from bovine brain: purification, some properties, and action on substance P degrading activity. *Neurochem Res* 13:721-727.

7. Akopyan T (1991) Protein inhibitors of proteinases from brain. *Neurochem Res* 16:513-517.

8. Alber T (1989) Mutational effects on protein stability. *Annu Rev Biochem* 58:765-798.

9. Arnason A (1935) Apoplexie und ihre Vererbung. *Acta Psychiatr Neurol Scand* (Suppl) VII:1-180.

10. Aronica E, van Vliet EA, Hendriksen E, Troost D, Lopes da Silva FH, Gorter JA (2001) Cystatin C, a cysteine protease inhibitor, is persistently up-regulated in neurons and glia in a rat model for mesial temporal lobe epilepsy. *Eur J Neurosci* 14:1485-1491.

11. Asgeirsson B, Haebel S, Thorsteinsson L, Helgason E, Gudmundsson KO, Gudmundsson G, Roepstorff P (1998) Hereditary cystatin C amyloid angiopathy: monitoring the presence of the Leu68Gln cystatin C variant in cerebrospinal fluids and monocyte cultures by MS. *Biochem J* 329:497-503.

12. Assfalg-Machleidt I, Jochum M, Nast-Kolb D, Siebeck M, Billing A, Joka T, Rothe G, Valet G, Zauner R, Scheuber HP, et al (1990) Cathepsin B-indicator for the release of lysosomal cysteine proteinases in severe trauma and inflammation. *Biol Chem Hoppe Seyler* 371 Suppl:211-222.

13. Babiloni C, Benussi L, Binetti G, Bosco P, Busonero G, Cesaretti S, Dal Forno G, Del Percio C, Ferri R, Frisoni G, et al (2006) Genotype (cystatin C) and EEG phenotype in Alzheimer disease and mild cognitive impairment: A multicentric study. *Neuroimage* 29:948-964.

14. Balbin M, Abrahamson M (1991) SstII polymorphic sites in the promoter region of the human cystatin C gene. *Hum Genet* 87:751-752.

15. Balbin M, Grubb AO, Abrahamson M (1993) An Ala/Thr variation in the coding region of the human cystatin C gene (CST3) detected as a SstII polymorphism. *Hum Genet* 92:206-207.

16. Benedikz E, Merz GS, Schwenk V, Johansen TE, Wisniewski HM, Rushbrook JI (1999) Cellular processing of the amyloidogenic cystatin C variant of hereditary cerebral hemorrhage with amyloidosis, Icelandic type. *Amyloid* 6:172-182.

17. Benussi L, Ghidoni R, Steinhoff T, Alberici A, Villa A, Mazzoli F, Nicosia F, Barbiero L, Broglio L, Feudatari E, et al (2003) Alzheimer disease-associated cystatin C variant undergoes impaired secretion. *Neurobiol Dis* 13:15-21.

18. Bernstein HG, Kirschke H, Wiederanders B, Pollak KH, Zipress A, Rinne A (1996) The possible place of cathepsins and cystatins in the puzzle of Alzheimer disease: a review. *Mol Chem Neuropathol* 27:225-247.

19. Bever CT, Jr., Garver DW (1995) Increased cathepsin B activity in multiple sclerosis brain. *J Neurol Sci* 131:71-73.

20. Beyer K, Lao JI, Gomez M, Riutort N, Latorre P, Mate JL, Ariza A (2001) Alzheimer's disease and the cystatin C gene polymorphism: an association study. *Neurosci Lett* 315:17-20.

21. Bjarnadottir M, Nilsson C, Lindstrom V, Westman A, Davidsson P, Thormodsson F, Blondal H, Gudmundsson G, Grubb A (2001) The cerebral hemorrhage-producing cystatin C variant (L68Q) in extracellular fluids. *Amyloid* 8:1-10.

22. Bjarnadottir M, Wulff BS, Sameni M, Sloane BF, Keppler D, Grubb A, Abrahamson M (1998) Intracellular accumulation of the amyloidogenic L68Q variant of human cystatin C in NIH/3T3 cells. *Mol Pathol* 51:317-326.

23. Bobek LA, Levine MJ (1992) Cystatins-inhibitors of cysteine proteinases. *Crit Rev Oral Biol Med* 3:307-332.

24. Booth DR, Sunde M, Bellotti V, Robinson CV, Hutchinson WL, Fraser PE, Hawkins PN, Dobson CM, Radford SE, Blake CCF, et al (1997) Instability, unfolding and aggregation of human lysozyme variants underlying amyloid fibrillogenesis. *Nature* 385:787-793.

25. Brooks WS, Kwok JB, Halliday GM, Godbolt AK, Rossor MN, Creasey H, Jones AO, Schofield PR (2004) Hemorrhage is uncommon in new Alzheimer family with Flemish amyloid precursor protein mutation. *Neurology* 63:1613-1617.

26. Bugiani O, Padovani A, Magoni M, Andora G, Sgarzi M, Savoiardo M, Bizzi A, Giaccone G, Rossi G, Tagliavini F (1998) An Italian type of HCHWA. *Neurobiol Aging* 19:S238.

27. Burgermeister P, Calhoun ME, Winkler DT, Jucker M (2000) Mechanisms of cerebrovascular amyloid deposition. Lessons from mouse models. *Ann N Y Acad Sci* 903:307-316.

28. Burnett D, Abrahamson M, Devalia JL, Sapsford RJ, Davies RJ, Buttle DJ (1995) Synthesis and secretion of procathepsin B and cystatin C by human bronchial epithelial cells in vitro: modulation of cathepsin B activity by neutrophil elastase. *Arch Biochem Biophys* 317:305-310.

29. Buttle DJ, Abrahamson M, Burnett D, Mort JS, Barrett AJ, Dando PM, Hill SL (1991) Human sputum cathepsin B degrades proteoglycan, is inhibited by α 2-macroglobulin and is modulated by neutrophil elastase cleavage of cathepsin B precursor and cystatin C. *Biochem J* 276:325-331.

30. Calkins CC, Sloane BF (1995) Mammalian cysteine protease inhibitors: biochemical properties and possible roles in tumor progression. *Biol Chem Hoppe Seyler* 376:71-80.

31. Carrette O, Burkhard PR, Hughes S, Hochstrasser DF, Sanchez JC (2005) Truncated cystatin C in cerebrospiral fluid: Technical artefact or biological process? *Proteomics* 5:3060-3065.

32. Cataldo AM, Barnett JL, Berman SA, Li J, Quarless S, Bursztajn S, Lippa C, Nixon RA (1995) Gene expression and cellular content of cathepsin D in Alzheimer's disease brain: evidence for early up-regulation of the endosomal- lysosomal system. *Neuron* 14:671-680.

33. Cataldo AM, Hamilton DJ, Barnett JL, Paskevich PA, Nixon RA (1996) Properties of the endosomal-lysosomal system in the human central

nervous system: disturbances mark most neurons in populations at risk to degenerate in Alzheimer's disease. *J Neurosci* 16:186-199.

34. Cathcart HM, Huang R, Lanham IS, Corder EH, Poduslo SE (2005) Cystatin C as a risk factor for Alzheimer disease. *Neurology* 64:755-757.

35. Chapman HA, Jr., Reilly JJ, Jr., Yee R, Grubb A (1990) Identification of cystatin C, a cysteine proteinase inhibitor, as a major secretory product of human alveolar macrophages in vitro. *Am Rev Respir Dis* 141:698-705.

36. Cohen DH, Feiner H, Jensson O, Frangione B (1983) Amyloid fibril in hereditary cerebral hemorrhage with amyloidosis (HCHWA) is related to the gastroentero-pancreatic neuroendocrine protein, γ trace. J Exp Med 158:623-628.

37. Colon W, Kelly JW (1992) Partial denaturation of transthyretin is sufficient for amyloid fibril formation in vitro. Biochemistry 31:8654-8660.

38. Coria F, Castano EM, Frangione B (1987) Brain amyloid in normal aging and cerebral amyloid angiopathy is antigenically related to Alzheimer's Disease β-protein. *Am J Pathol* 129:422-428.

39. Corticchiato O, Cajot J-F, Abrahamson M, Chan SJ, Keppler D, Sordat B (1992) Cystatin C and cathepsin B in human colon carcinoma: expression by cell lines and matrix degradation. *Int J Cancer* 52:645-652.

40. Crawford FC, Freeman MJ, Schinka JA, Abdullah LI, Gold M, Hartman R, Krivian K, Morris MD, Richards D, Duara R, et al (2000) A polymorphism in the cystatin C gene is a novel risk factor for late-onset Alzheimer's disease. *Neurology* 55:763-768.

41. Delaisse JM, Ledent P, Vaes G (1991) Collagenolytic cysteine proteinases of bone tissue. Cathepsin B, (pro)cathepsin L and a cathepsin L-like 70 kDa proteinase. *Biochem J* 279 (Pt 1):167-174.

42. Deng A, Irizarry MC, Nitsch RM, Growdon JH, Rebeck GW (2001) Elevation of cystatin C in susceptible neurons in alzheimer's disease. *Am J Pathol* 159:1061-1068.

43. Dodel RC, Du Y, Depboylu C, Kurz A, Eastwood B, Farlow M, Oertel WH, Muller U, Riemenschneider M (2002) A polymorphism in the cystatin C promoter region is not associated with an increased risk of AD. *Neurology* 58:664.

44. Duffy MJ (1996) Proteases as prognostic markers in cancer. *Clin Cancer Res* 2:613-618.

45. Ekiel I, Abrahamson M (1996) Folding-related dimerization of human cystatin C. *J Biol Chem* 271:1314-1321.

46. Ekiel I, Abrahamson M, Fulton DB, Lindahl P, Storer AC, Levadoux W, Lafrance M, Labelle S, Pomerleau Y, Groleau D, et al (1997) NMR structural studies of human cystatin C dimers and monomers. *J Mol Biol* 271:266-277.

47. Finckh U, von Der Kammer H, Velden J, Michel T, Andresen B, Deng A, Zhang J, Muller-Thomsen T, Zuchowski K, Menzer G, et al (2000) Genetic association of a cystatin C gene polymorphism with late-onset Alzheimer disease. *Arch Neurol* 57:1579-1583.

48. Gerhartz B, Abrahamson M (2002) Physicochemical properties of the N-terminally truncated L68Q cystatin C found in amyloid deposits of brain haemorrhage patients. *Biol Chem* 383:301-305.

49. Gerhartz B, Ekiel I, Abrahamson M (1998) Two stable unfolding intermediates of the disease-causing L68Q variant of human cystatin C. *Biochemistry* 37:17309-17317.

50. Ghiso J, Plant GT, Revesz T, Wisniewski T, Frangione B (1995) Familial cerebral amyloid angiopathy (British type) with nonneuritic amyloid plaque formation may be due to a novel amyloid protein. *J Neurol Sci* 129:74-75.

51. Ghiso J, Pons-Estel B, Frangione B (1986) Hereditary cerebral amyloid angiopathy: the amyloid fibrils contain a protein which is a variant of cystatin C, an inhibitor of lysosomal cysteine proteases. *Biochem Biophys Res Commun* 136:548-554.

52. Glenner GG, Ein D, Eanes ED, Bladen HA, Terry W, Page DL (1971) Creation of "amyloid" fibrils from Bence Jones proteins in vitro. *Science* 174:712-714.

53. Glenner GG, Wong CW (1984) Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. *Biochem Biophys Res Commun* 120:885-890.

54. Goddard KA, Olson JM, Payami H, Van Der Voet M, Kuivaniemi H, Tromp G (2004) Evidence of linkage and association on chromosome 20 for late-onset Alzheimer disease. *Neurogenetics* 5:121-128.

55. Grabowski TJ, Cho HS, Vonsattel JP, Rebeck GW, Greenberg SM (2001) Novel amyloid precursor protein mutation in an Iowa family with dementia and severe cerebral amyloid angiopathy. *Ann Neurol* 49:697-705.

56. Graffagnino C, Herbstreith MH, Schmechel DE, Levy E, Roses AD, Alberts MJ (1995) Cystatin C mutation in an elderly man with sporadic amyloid angiopathy and intracerebral hemorrhage. *Stroke* 26:2190-2193.

57. Greenberg SM (1998) Cerebral amyloid angiopathy: prospects for clinical diagnosis and treatment. *Neurology* 51:690-694.

58. Greenberg SM, Shin Y, Grabowski TJ, Cooper GE, Rebeck GW, Iglesias S, Chapon F, Tournier-Lasserve E, Baron JC (2003) Hemorrhagic stroke associated with the Iowa amyloid precursor protein mutation. *Neurology* 60:1020-1022.

59. Greenberg SM, Vonsattel JP, Segal AZ, Chiu RI, Clatworthy AE, Liao A, Hyman BT, Rebeck GW (1998) Association of apolipoprotein E (2 and vasculopathy in cerebral amyloid angiopathy. *Neurology* 50:961-965.

60. Grubb AO, Jensson O, Gudmundsson G, Arnason A, Lofberg H, Malm J (1984) Abnormal metabolism of γ-trace alkaline microprotein. The basic defect in hereditary cerebral hemorrhage with amyloidosis. *N Engl J Med* 311:1547-1549.

61. Gudmundsson G, Hallgrimsson J, Jonasson TA, Bjarnason O (1972) Hereditary cerebral haemorrhage with amyloidosis. *Brain* 95:387-404.

62. Haan J, Maat-Schieman MLC, van Duinen SG, Jensson O, Thorsteinsson L, Roos RAC (1994) Co-localization of β/A4 and cystatin C in cortical blood vessels in Dutch, but not in Icelandic hereditary cerebral hemorrhage with amyloidosis. *Acta Neurol Scand* 89:367-371.

63. Haan J, Roos RA, Algra PR, Lanser JB, Bots GT, Vegter-van der Vlis M (1990) Hereditary cerebral haemorrhage with amyloidosis--Dutch type. Magnetic resonance imaging findings in 7 cases. *Brain* 113:1251-1267.

64. Hall A, Dalboge H, Grubb AO, Abrahamson M (1993) Importance of the evolutionarily conserved glycine residue in the N-terminal region of human cystatin C (Gly-11) for cysteine endopeptidase inhibition. *Biochem J* 291:123-129.

65. Hendriks L, van Duijn CM, Cras P, Cruts M, Van Hul W, van Harskamp F, Warren A, McInnis MG, Antonarakis SE, Martin JJ, et al (1992) Presenile dementia and cerebral haemorrhage linked to a mutation at codon 692 of the β-amyloid precursor protein gene. *Nature Genet* 1:218-221.

66. Hendriksen H, Datson NA, Ghijsen WE, van Vliet EA, da Silva FH, Gorter JA, Vreugdenhil E (2001) Altered hippocampal gene expression prior to the onset of spontaneous seizures in the rat post-status epilepticus model. *Eur J Neurosci* 14:1475-1484.

67. Hochwald GM, Pepe AJ, Thorbecke GJ (1967) Trace proteins in biological fluids. IV. Physicochemical properties and sites of formation of γ trace and β trace proteins. *Proc Soc Exp Med* 124:961-966.

68. Hoffmann MC, Nitsch C, Scotti AL, Reinhard E, Monard D (1992) The prolonged presence of glia-derived nexin, an endogenous protease inhibitor, in the hippocampus after ischemia-induced delayed neuronal death. *Neuroscience* 49:397-408.

69. Hurle MR, Helms LR, Li L, Chan W, Wetzel R (1994) A role for destabilizing amino acid replacements in light-chain amyloidosis. *Proc Natl Acad Sci U S A* 91:5446-5450.

70. Ishimaru H, Ishikawa K, Ohe Y, Takahashi A, Maruyama Y (1996) Cystatin C and apolipoprotein E immunoreactivities in CA1 neurons in ischemic gerbil hippocampus. *Brain Res* 709:155-162.

71. Itoh Y, Yamada M, Hayakawa M, Otomo E, Miyatake T (1993) Cerebral amyloid angiopathy: a significant cause of cerebellar as well as lobar cerebral hemorrhage in the elderly. *J Neurol Sci* 116:135-141.

72. Izumihara A, Ishihara T, Hoshii Y, Ito H (2001) Cerebral amyloid angiopathy associated with hemorrhage: immunohistochemical study of 41 biopsy cases. *Neurol Med Chir (Tokyo)* 41:471-477; discussion 477-478.

73. Janowski R, Abrahamson M, Grubb A, Jaskolski M (2004) Domain swapping in N-truncated human cystatin C. *J Mol Biol* 341:151-160.

74. Janowski R, Kozak M, Abrahamson M, Grubb A, Jaskolski M (2005) 3D Domain-swapped human cystatin C with amyloidlike intermolecular β-sheets. *Structure Function, and Bioinformatics* 61:570-578.

75. Janowski R, Kozak M, Jankowska E, Grzonka Z, Grubb A, Abrahamson M, Jaskolski M (2001) Human cystatin C, an amyloidogenic protein, dimerizes through three-dimensional domain swapping. *Nat Struct Biol* 8:316-320.

76. Jaskolski M (2001) 3D domain swapping, protein oligomerization, and amyloid formation. *Acta Biochim Pol* 48:807-827.

77. Jelic V, Julin P, Shigeta M, Nordberg A, Lannfelt L, Winblad B, Wahlund LO (1997) Apolipoprotein E ε4 allele decreases functional connectivity in Alzheimer's disease as measured by EEG coherence. *J Neurol Neurosurg Psychiatry* 63:59-65.

78. Jellinger K (1977) Cerebrovascular amyloidosis with cerebral hemorrhage. *J Neurol* 214:195-206.

79. Johansen TE, Vogel CK, Schwartz TW (1990) C-terminal KDEL-modified cystatin C is retained in transfected CHO cells. *Biochem Biophys Res Commun* 172:1384-1391.

80. Kabanda A, Goffin E, Bernard A, Lauwerys R, van Ypersele de Strihou C (1995) Factors influencing serum levels and peritoneal clearances of low molecular weight proteins in continuous ambulatory peritoneal dialysis. *Kidney Int* 48:1946-1952.

81. Kamino K, Orr HT, Payami H, Wijsman EM, Alonso ME, Pulst SM, Anderson L, O'Dahl S, Nemens E, White JA, et al (1992) Linkage and mutational analysis of familial Alzheimer disease kindreds for the APP gene region. *Am J Hum Genet* 51:998-1014.

82. Kang J, Lemaire HG, Unterbeck A, Salbaum JM, Masters CL, Grzeschik KH, Multhaup G, Beyreuther K, Mueller-Hill B (1987) The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. *Nature* 325:733-736.

83. Katakai K, Shinoda M, Kabeya K, Watanabe M, Ohe Y, Mori M, Ishikawa K (1997) Changes in distribution of cystatin C, apolipoprotein E and ferritin in rat hypothalamus after hypophysectomy. *J Neuroendocrinol* 9:247-253.

84. Kawai M, Kalaria RN, Cras P, Siedlak SL, Velasco ME, Shelton ER, Chan HW, Greenberg BD, Perry G (1993) Degeneration of vascular muscle cells in cerebral amyloid angiopathy of Alzheimer disease. *Brain Res* 623:142-146.

85. Kiuru S, Salonen O, Haltia M (1999) Gelsolin-related spinal and cerebral amyloid angiopathy. *Ann Neurol* 45:305-311.

86. Kiuru-Enari S, Somer H, Seppalainen AM, Notkola IL, Haltia M (2002) Neuromuscular pathology in hereditary gelsolin amyloidosis. *J Neuropathol Exp Neurol* 61:565-571.

87. Lah TT, Babnik J, Schiffmann E, Turk V, Skaleric U (1993) Cysteine proteinases and inhibitors in inflammation: their role in periodontal disease. *J Periodon* 64:485-491.

88. LeBlanc A (1995) Increased production of 4 kDa amyloid β peptide in serum deprived human primary neuron cultures: possible involvement of apoptosis. *J Neurosci* 15:7837-7846.

89. Lee KS, Frank S, Vanderklish P, Arai A, Lynch G (1991) Inhibition of proteolysis protects hippocampal neurons from ischemia. *Proc Natl Acad Sci U S A* 88:7233-7237.

90. Lehtovirta M, Partanen J, Kononen M, Hiltunen J, Helisalmi S, Hartikainen P, Riekkinen P, Sr., Soininen H (2000) A longitudinal quantitative EEG study of Alzheimer's disease: relation to apolipoprotein E polymorphism. *Dement Geriatr Cogn Disord* 11:29-35.

91. Lehtovirta M, Partanen J, Kononen M, Soininen H, Helisalmi S, Mannermaa A, Ryynanen M, Hartikainen P, Riekkinen P, Sr. (1996) Spectral analysis of EEG in Alzheimer's disease: relation to apolipoprotein E polymorphism. *Neurobiol Aging* 17:523-526.

92. Lenarcic B, Krasovec M, Ritonja A, Olafsson I, Turk V (1991) Inactivation of human cystatin C and kininogen by human cathepsin D. *FEBS Letters* 280:211-215.

93. Levy E, Amorim A, Frangione B, Walker LC (1995) β-amyloid precursor protein gene in squirrel monkeys with cerebral amyloid angiopathy. *Neurobiol Aging* 16:805-808.

94. Levy E, Carman MD, Fernandez-Madrid IJ, Power MD, Lieberburg I, van Duinen SG, Bots GTAM, Luyendijk W, Frangione B (1990) Mutation of the Alzheimer's disease amyloid gene in hereditary cerebral hemorrhage, Dutch type. *Science* 248:1124-1126.

95. Levy E, Lopez-Otin C, Ghiso J, Geltner D, Frangione B (1989) Stroke in Icelandic patients with hereditary amyloid angiopathy is related to a mutation in the cystatin C gene, an inhibitor of cysteine proteases. *J Exp Med* 169:1771-1778.

96. Levy E, Sastre M, Kumar A, Gallo G, Piccardo P, Ghetti B, Tagliavini F (2001) Codeposition of cystatin C with amyloid-β protein in the brain of Alzheimer's disease patients. *J Neuropathol Exp Neurol* 60:94-104.

97. Lin C, Wang ST, Wu CW, Chuo LJ, Kuo YM (2003) The association of a cystatin C gene polymorphism with late-onset Alzheimer's disease and vascular dementia. *Chin J Physiol* 46:111-115.

98. Lindahl P, Ripoll D, Abrahamson M, Mort JS, Storer AC (1994) Evidence for the interaction of valine-10 in cystatin C with the S2 subsite of cathepsin B. *Biochemistry* 33:4384-4392.

99. Lofberg H, Grubb AO, Nilsson EK, Jensson O, Gudmundsson G, Blondal H, Arnason A, Thorsteinsson L (1987) Immunohistochemical characterization of the amyloid deposits and quantitation of pertinent cerebrospinal fluid proteins in hereditary cerebral hemorrhage with amyloidosis. *Stroke* 18:431-440.

100. Lukasiuk K, Pirttila TJ, Pitkanen A (2002) Upregulation of cystatin C expression in the rat hippocampus during epileptogenesis in the amygdala stimulation model of temporal lobe epilepsy. *Epilepsia* 43 Suppl 5:137-145.

101. Lundgren E, Persson H, Andersson K, Olofsson A, Dacklin I, Goldsteins G (1999) Mapping protein conformations in fibril structures using monoclonal antibodies. *Methods Enzymol* 309:591-605.

102. Luyendijk W, Bots GT, Vegter-van der Vlis M, Went LN, Frangione B (1988) Hereditary cerebral haemorrhage caused by cortical amyloid angiopathy. *J Neurol Sci* 85:267-280.

103. Maruyama H, Izumi Y, Oda M, Torii T, Morino H, Toji H, Sasaki K, Terasawa H, Nakamura S, Kawakami H (2001) Lack of an association between cystatin C gene polymorphisms in Japanese patients with Alzheimer's disease. *Neurology* 57:337-339.

104. Maruyama K, Ikeda S, Ishihara T, Allsop D, Yanagisawa N (1990) Immunohistochemical characterization of cerebrovascular amyloid in 46 autopsied cases using antibodies to β protein and cystatin C. *Stroke* 21:397-403.

105. Maruyama K, Kametani F, Ikeda S, Ishihara T, Yanagisawa N (1992) Characterization of amyloid fibril protein from a case of CAA showing immunohistochemical reactivity for both β protein and cystatin C. *Neurosci Lett* 144:38-42.

106. Mastaglia FL, Byrnes ML, Johnsen RD, Kakulas BA (2003) Prevalence of cerebral vascular amyloid-β deposition and stroke in an aging Australian population: a postmortem study. *J Clin Neurosci* 10:186-189.

107. Masters CL, Simms G, Weinman NA, Multhaup G, McDonald BL, Beyreuther K (1985) Amyloid plaque core protein in Alzheimer disease and Down syndrome. *Proc Natl Acad Sci U S A* 82:4245-4249.

108. McCarron MO, Nicoll JA, Stewart J, Cole GM, Yang F, Ironside JW, Mann DM, Love S, Graham DI (2000) Amyloid β-protein length and cerebral amyloid angiopathy-related haemorrhage. *Neuroreport* 11:937-940.

109. McCarron MO, Nicoll JA, Stewart J, Ironside JW, Mann DM, Love S, Graham DI, Dewar D (1999) The apolipoprotein E ε2 allele and the pathological features in cerebral amyloid angiopathy-related hemorrhage. *J Neuropathol Exp Neurol* 58:711-718.

110. McCarron MO, Nicoll JA, Stewart J, Ironside JW, Mann DM, Love S, Graham DI, Grubb A (2000) Absence of cystatin C mutation in sporadic cerebral amyloid angiopathy-related hemorrhage. *Neurology* 54:242-244.

111. McCutchen SL, Lai Z, Miroy GJ, Kelly JW, Colon W (1995) Comparison of lethal and nonlethal transthyretin variants and their relationship to amyloid disease. *Biochemistry* 34:13527-13536.

112. Merz GS, Benedikz E, Schwenk V, Johansen TE, Vogel LK, Rushbrook JI, Wisniewski HM (1997) Human cystatin C forms an inactive dimer during intracellular trafficking in transfected CHO cells. *J Cell Physiol* 173:423-432.

113. Miyake T, Gahara Y, Nakayama M, Yamada H, Uwabe K, Kitamura T (1996) Up-regulation of cystatin C by microglia in the rat facial nucleus following axotomy. *Mol Brain Res* 37:273-282.

114. Morelli L, Wei L, Amorim A, McDermid J, Abee CR, Frangione B, Walker LC, Levy E (1996) Cerebrovascular amyloidosis in squirrel monkeys and rhesus monkeys: apolipoprtein E genotype. *FEBS Letters* 379:132-134.

115. Nagai A, Kobayashi S, Shimode K, Imaoka K, Umegae N, Fujihara S, Nakamura M (1998) No mutations in cystatin C gene in cerebral amyloid angiopathy with cystatin C deposition. *Mol Chem Neuropathol* 33:63-78.

116. Nakamura Y, Takeda M, Suzuki H, Hattori H, Tada K, Hariguchi S, Hashimoto S, Nishimura T (1991) Abnormal distribution of cathepsins in the brain of patients with Alzheimer's disease. *Neurosci Lett* 130:195-198.

117. Nicoll JA, Burnett C, Love S, Graham DI, Dewar D, Ironside JW, Stewart J, Vinters HV (1997) High frequency of apolipoprotein E ε2 allele in hemorrhage due to cerebral amyloid angiopathy. *Ann Neurol* 41:716-721.

118. Nicoll JA, Burnett C, Love S, Graham DI, Ironside JW, Vinters HV (1996) High frequency of apolipoprotein E ε2 in patients with cerebral hemorrhage due to cerebral amyloid angiopathy. *Ann Neurol* 39:682-683.

119. Nilsson M, Wang X, Rodziewicz-Motowidlo S, Janowski R, Lindstrom V, Onnerfjord P, Westermark G, Grzonka Z, Jaskolski M, Grubb A (2004) Prevention of domain swapping inhibits dimerization and amyloid fibril formation of cystatin C: use of engineered disulfide bridges, antibodies, and carboxymethylpapain to stabilize the monomeric form of cystatin C. *J Biol Chem* 279:24236-24245.

120. Nishio C, Yoshida K, Nishiyama K, Hatanaka H, Yamada M (2000) Involvement of cystatin C in oxidative stress-induced apoptosis of cultured rat CNS neurons. *Brain Res* 873:252-262.

121. Nishiyama K, Konishi A, Nishio C, Araki-Yoshida K, Hatanaka H, Kojima M, Ohmiya Y, Yamada M, Koshimizu H (2005) Expression of cystatin C prevents oxidative stress-induced death in PC12 cells. *Brain Res Bull* 67:94-99.

122. Nitatori T, Sato N, Waguri S, Karasawa Y, Araki H, Shibanai K, Kominami E, Uchiyama Y (1995) Delayed neuronal death in the CA1 pyramidal cell layer of the gerbil hippocampus following transient ischemia is apoptosis. *J Neurosci* 15:1001-1011.

123. Noto D, Cefalu AB, Barbagallo CM, Pace A, Rizzo M, Marino G, Caldarella R, Castello A, Pernice V, Notarbartolo A, et al (2005) Cystatin C levels are decreased in acute myocardial infarction: effect of cystatin C G73A gene polymorphism on plasma levels. *Int J Cardiol* 101:213-217.

124. Obici L, Demarchi A, de Rosa G, Bellotti V, Marciano S, Donadei S, Arbustini E, Palladini G, Diegoli M, Genovese E, et al (2005) A novel AβPP mutation exclusively associated with cerebral amyloid angiopathy. *Ann Neurol* 58:639-644.

125. Olafsson I, Gudmundsson G, Abrahamson M, Jensson O, Grubb AO (1990) The amino terminal portion of cerebrospinal fluid cystatin C in hereditary cystatin C amyloid angiopathy is not truncated: direct sequence analysis from agarose gel electropherograms. *Scan J Clin Lab Invest* 50:85-93.

126. Olafsson I, Thorsteinsson L, Jensson O (1996) The molecular pathology of hereditary cystatin C amyloid angiopathy causing brain hemorrhage. *Brain Pathol* 6:121-126.

127. Olson JM, Goddard KA, Dudek DM (2002) A second locus for very-late-onset Alzheimer disease: a genome scan reveals linkage to 20p and epistasis between 20p and the amyloid precursor protein region. *Am J Hum Genet* 71:154-161.

128. Pakula AA, Sauer RT (1989) Genetic analysis of protein stability and function. *Ann Rev Genet* 23:289-310.

129. Palm DE, Knuckey NW, Primiano MJ, Spangenberger AG, Johanson CE (1995) Cystatin C, a protease inhibitor, in degenerating rat hippocampal neurons following transient forebrain ischemia. *Brain Res* 691:1-8.

130. Palsdottir A, Abrahamson M, Thorsteinsson L, Arnason A, Olafsson I, Grubb AO, Jensson O (1988) Mutation in cystatin C gene causes hereditary brain haemorrhage. *Lancet* 2:603-604.

131. Paraoan L, Ratnayaka A, Spiller DG, Hiscott P, White MR, Grierson I (2004) Unexpected intracellular localization of the AMD-associated cystatin C variant. *Traffic* 5:884-895.

132. Parfitt M, Crook R, Roques P, Rossor M, Chartier-Harlin MC (1993) The cystatin-C gene is not linked to early onset familial Alzheimer's disease. *Neurosci Lett* 154:81-83.

133. Pawlik M, Danilov V, Mancevska K, Morbin M, Tagliavini F, Levy E. (2002) Hemorrhages caused by overexpression of cystatin C in transgenic mice. *Neurobiol Aging* 23:S242.

134. Pawlik M, Sastre M, Calero M, Mathews PM, Schmidt SD, Nixon RA, Levy E (2004) Overexpression of human cystatin C in transgenic mice does not affect levels of endogenous brain amyloid β peptide. *J Mol Neurosci* 22:13-18.

135. Peppard JV, Knap AK (1999) Effect of the selective and non-selective cysteine protease inhibitors on the intracellular processing of interleukin 6 by HEPG2 cells. *In Vitro Cell Dev Biol Anim* 35:459-464.

136. Poduri A, Gearing M, Rebeck GW, Mirra SS, Tigges J, Hyman BT (1994) Apolipoprotein E4 and β amyloid in senile plaques and cerebral blood vessels of aged Rhesus monkeys. *Am J Pathol* 144:1183-1187.

137. Price DL, Martin LJ, Sisodia SS, Walker LC, Voytko ML, Wagster MV, Cork LC, Koliatsos VE (1994) The aged nonhuman primate. A model for the behavioral and brain abnormalities occurring in aged humans. In: *Alzheimer's disease*, RD Terry, R Katzman, KL Blick (eds). Raven Press: New York. pp. 231-245.

138. Rider BJ, Fraga E, Yu Q, Singh B (1996) Immune responses to self peptides naturally presented by murine class II major histocompatibility complex molecules. *Mol Immunol* 33:625-633.

139. Roberts-Lewis JM, Marcy VR, Zhao Y, Vaught JL, Siman R, Lewis ME (1993) Aurintricarboxylic acid protects hippocampal neurons from NMDA- and ischemia-induced toxicity in vivo. *J Neurochem* 61:378-381.

140. Roks G, Cruts M, Slooter AJ, Dermaut B, Hofman A, Van Broeckhoven C, Van Duijn CM (2001) The cystatin C polymorphism is not associated with early onset Alzheimer's disease. *Neurology* 57:366-367.

141. Rosand J, Hylek EM, O'Donnell HC, Greenberg SM (2000) Warfarin-associated hemorrhage and cerebral amyloid angiopathy: a genetic and pathologic study. *Neurology* 55:947-951.

142. Rozemuller AJ, Roos RA, Bots GT, Kamphorst W, Eikelenboom P, Van Nostrand WE (1993) Distribution of β/A4 protein and amyloid precursor protein in hereditary cerebral hemorrhage with amyloidosis-Dutch type and Alzheimer's disease. *Am J Pathol* 142:1449-1457.

143. Rudensky A, Preston-Hurlburt P, Hong SC, Barlow A, Janeway CA, Jr. (1991) Sequence analysis of peptides bound to MHC class II molecules. *Nature* 353:622-627.

144. Saitoh E, Sabatini LM, Eddy RL, Shows TB, Azen EA, Isemura S, Sanada K (1989) The human cystatin C gene (CST3) is a member of the cystatin gene family which is localized on chromosome 20. *Biochem Biophys Res Commun* 162:1324-1331.

145. Sastre M, Calero M, Pawlik M, Mathews PM, Kumar A, Danilov V, Schmidt SD, Nixon RA, Frangione B, Levy E (2004) Binding of cystatin C to Alzheimer's amyloid β inhibits amyloid fibril formation. *Neurobiol Aging* 25:1033-1043.

146. Schmechel DE, Saunders AM, Strittmatter WJ, Crain BJ, Hulette CM, Joo SH, Pericak-Vance MA, Goldgaber D, Roses AD (1993) Increased amyloid β-peptide deposition in cerebral cortex as a concequence of apolipoprotein E genotype in late-onset Alzheimer's disease. *Proc Natl Acad Sci U S A* 90:9649-9653.

147. Shirahama T, Cohen AS (1975) Intralysosomal formation of amyloid fibrils. *Am J Pathol* 81:101-116.

148. Sohar I, Laszlo A, Gaal K, Mechler F (1988) Cysteine and metalloproteinase activities in serum of Duchenne muscular dystrophic genotypes. *Biol Chem Hoppe Seyler* 369 Suppl:277-279.

149. Steinhoff T, Moritz E, Wollmer MA, Mohajeri MH, Kins S, Nitsch RM (2001) Increased cystatin C in astrocytes of transgenic mice expressing the K670N-M671L mutation of the amyloid precursor protein and deposition in brain amyloid plaques. *Neurobiol Dis* 8:647-654.

150. Sun Q (1989) Growth stimulation of 3T3 fibroblasts by cystatin. *Exp Cell Res* 180:150-160.

151. Tanzi RE, Bertram L (2001) New frontiers in Alzheimer's disease genetics. *Neuron* 32:181-184.

152. Tanzi RE, Vaula G, Romano DM, Mortilla M, Huang TL, Tupler RG, Wasco W, Hyman BT, Haines JL, Jenkins BJ, et al (1992) Assessment of amyloid β-protein precursor gene mutations in a large set of familial and sporadic Alzheimer disease cases. *Am J Hum Gen* 51:273-282.

153. Taupin P, Ray J, Fischer WH, Suhr ST, Hakansson K, Grubb A, Gage FH (2000) FGF-2-Responsive neural stem cell proliferation requires CCg, a novel Autocrine/Paracrine cofactor. *Neuron* 28:385-397.

154. Thomssen C, Schmitt M, Goretzki L, Oppelt P, Pache L, Dettmar P, Janicke F, Graeff H (1995) Prognostic value of the cysteine proteases cathepsins B and cathepsin L in human breast cancer. *Clin Cancer Res* 1:741-746.

155. Thorsteinsson L, Georgsson G, Asgeirsson B, Bjarnadottir M, Olafsson I, Jensson O, Gudmundsson G (1992) On the role of monocytes/macro-

phages in the pathogenesis of central nervous system lesions in hereditary cystatin C amyloid angiopathy. *J Neurol Sci* 108:121-128.

156. Tizon B, Levy E (2006) Protease inhibitors and their involvement in neurological disorders. In: *Handbook of Neurochemistry and Molecular Neurobiology*, A Lajtha (eds). Springer Publishers: New York, vol 7.

157. Trabandt A, Gay RE, Fassbender HG, Gay S (1991) Cathepsin B in synovial cells at the site of joint destruction in rheumatoid arthritis. *Arthritis Rheum* 34:1444-1451.

158. Tsuchiya K, Kohda Y, Yoshida M, Zhao L, Ueno T, Yamashita J, Yoshioka T, Kominami E, Yamashima T (1999) Postictal blockade of ischemic hippocampal neuronal death in primates using selective cathepsin inhibitors. *Exp Neurol* 155:187-194.

159. Turk B, Turk D, Turk V (2000) Lysosomal cysteine proteases: more than scavengers. *Biochim Biophys Acta* 1477:98-111.

160. Uchida K, Kuroki K, Yoshino T, Yamaguchi R, Tateyama S (1997) Immunohistochemical study of constituents other than β-protein in canine senile plaques and cerebral amyloid angiopathy. *Acta Neuropathol (Berl)* 93:277-284.

161. Uchida K, Nakayama H, Tateyama S, Goto N (1992) Immunohistochemical analysis of constituents of senile plaques and cerebro-vascular amyloid in aged dogs. *J Veter Med Sci* 54:1023-1029.

162. van Duinen SG, Castano EM, Prelli F, Bots GT, Luyendijk W, Frangione B (1987) Hereditary cerebral hemorrhage with amyloidosis in patients of Dutch origin is related to Alzheimer disease. *Proc Natl Acad Sci U S A* 84:5991-5994.

163. Vattemi G, Engel WK, McFerrin J, Askanas V (2003) Cystatin C colocalizes with amyloid-β and coimmunoprecipitates with amyloid-β precursor protein in sporadic inclusion-body myositis muscles. *J Neurochem* 85:1539-1546.

164. Vinters HV (1987) Cerebral amyloid angiopathy. A critical review. *Stroke* 18:311-324.

165. Vinters HV (2001) Cerebral amyloid angiopathy: a microvascular link between parenchymal and vascular dementia? *Ann Neurol* 49:691-693.

166. Vinters HV, Gilbert JJ (1983) Cerebral amyloid angiopathy: incidence and complications in the aging brain. II. The distribution of amyloid vascular changes. *Stroke* 14:924-928.

167. Vinters HV, Nishimura GS, Secor DL, Pardridge WM (1990) Immunoreactive A4 and γ-trace peptide colocalization in amyloidotic arteriolar lesions in brains of patients with Alzheimer's disease. *Am J Pathol* 137:233-240.

168. Vonsattel JP, Myers RH, Hedley-Whyte ET, Ropper AH, Bird ED, Richardson EP, Jr. (1991) Cerebral amyloid angiopathy without and with cerebral hemorrhages: a comparative histological study. *Ann Neurol* 30:637-649.

169. Walker LC, Masters C, Beyreuther K, Price DL (1990) Amyloid in the brains of aged squirrel monkeys. *Acta Neuropathol* 80:381-387.

170. Wang Z, Wu D, Vinters HV (2002) Hypoxia and reoxygenation of brain microvascular smooth muscle cells in vitro: cellular responses and expression of cerebral amyloid angiopathy-associated proteins. *Apmis* 110:423-434.

171. Wattendorff AR, Bots GT, Went LN, Endtz LJ (1982) Familial cerebral amyloid angiopathy presenting as recurrent cerebral hemorrhage. *J Neurol Sci* 55:121-135.

172. Wei L, Berman Y, Castano EM, Cadene M, Beavis RC, Devi L, Levy E (1998) Instability of the amyloidogenic cystatin C variant of hereditary cerebral hemorrhage with amyloidosis, Icelandic type. *J Biol Chem* 273:11806-11814.

173. Wei L, Walker LC, Levy E (1996) Cystatin C: Icelandic-like mutation in an animal model of cerebrovascular β amyloidosis. *Stroke* 27:2080-2085.

174. Winkler DT, Bondolfi L, Herzig MC, Jann L, Calhoun ME, Wiederhold KH, Tolnay M, Staufenbiel M, Jucker M (2001) Spontaneous hemorrhagic stroke in a mouse model of cerebral amyloid angiopathy. *J Neurosci* 21:1619-1627.

175. Wisniewski HM, Terry RD (1973) Morphology of the aging brain, human and animal. *Prog Brain Res* 40:167-186.

176. Wisniewski T, Ghiso J, Frangione B (1991) Peptides homologous to the amyloid protein of Alzheimer's disease containing a glutamine for glutamic acid substitution have accelerated amyloid fibril formation. *Biochem Biophys Res Commun* 179:1247-1254.

177. Woo D, Sauerbeck LR, Kissela BM, Khoury JC, Szaflarski JP, Gebel J, Shukla R, Pancioli AM, Jauch EC, Menon AG, et al (2002) Genetic and environmental risk factors for intracerebral hemorrhage: preliminary results of a population-based study. *Stroke* 33:1190-1195.

178. Yamada M (2000) Cerebral amyloid angiopathy: an overview. *Neuropathology* 20:8-22.

179. Yamada M (2002) Risk factors for cerebral amyloid angiopathy in the elderly. *Ann N Y Acad Sci* 977:37-44.

180. Yamashima T, Kohda Y, Tsuchiya K, Ueno T, Yamashita J, Yoshioka T, Kominami E (1998) Inhibition of ischaemic hippocampal neuronal death in primates with cathepsin B inhibitor CA-074: a novel strategy for neuroprotection based on 'calpain-cathepsin hypothesis'. *Eur J Neurosci* 10:1723-1733.

181. Ying GX, Huang C, Jiang ZH, Liu X, Jing NH, Zhou CF (2002) Up-regulation of cystatin C expression in the murine hippocampus following perforant path transections. *Neuroscience* 112:289-298.

182. Zannis VI, Nicolosi RJ, Jensen E, Breslow JL, Hayes KC (1985) Plasma and hepatic apoE isoproteins of nonhuman primates. Differences in apoE among humans, apes, and New and Old World monkeys. *J Lipid Res* 26:1421-1430.