SERPINA3 (aka alpha-1-antichymotrypsin)

Crystal Baker, Olivia Belbin, Noor Kalsheker, Kevin Morgan

The University of Nottingham, Division of Clinical Chemistry, Molecular Medical Sciences, Institute of Genetics, University Hospital, Queen's Medical Centre, Nottingham, NG7 2UH, UK

TABLE OF CONTENTS

1. ABSTRACT

Recent advances in proteomic, transcriptomic and genomic technologies have revealed much about the ACT protein and gene. In this review, we summarize our current understanding of the structure and potential physiological roles of the ACT protein, catalogue the regulatory elements that have been implicated in expression of the *ACT* gene, describe its tissue-specific expression and list the single nucleotide polymorphisms (SNPs) within the gene that track *ACT* variability. The *ACT* gene has been implicated in a number of complex human disorders and its potential involvement as a risk factor for Alzheimer's disease has been the subject of intensive research. However, due to previous limitations in methodologies and inadequate sample numbers the data has been conflicting with many studies failing to be replicated. In this regard, we highlight some potential approaches, which may prove to be beneficial in future studies.

2. INTRODUCTION

The serine protease inhibitor, alpha-1 antichymotrypsin (*ACT, SERPINA3*; MIM 107280) has been implicated in the pathology of a number of devastating human diseases including chronic obstructive pulmonary disease (COPD), Parkinson's disease (PD), Alzheimer's disease (AD), Stroke, Cystic Fibrosis, Cerebral Haemorrhage and Multiple System Atrophy. The majority of research has concentrated on the role of ACT in AD, where ACT has not only been detected in the senile plaques and surrounding astrocytes but has been found to form a complex with toxic beta-amyloid (Abeta) $_{(1-42)}$ peptide. Transgenic mice studies have emphasised the importance of ACT in AD pathology; ACT/APP expressing mice showed increased age-related plaque deposition compared with mice expressing APP alone (1, 2). In addition, a recent microarray study (3) has revealed that ACT treatment in combination with Abeta has a more
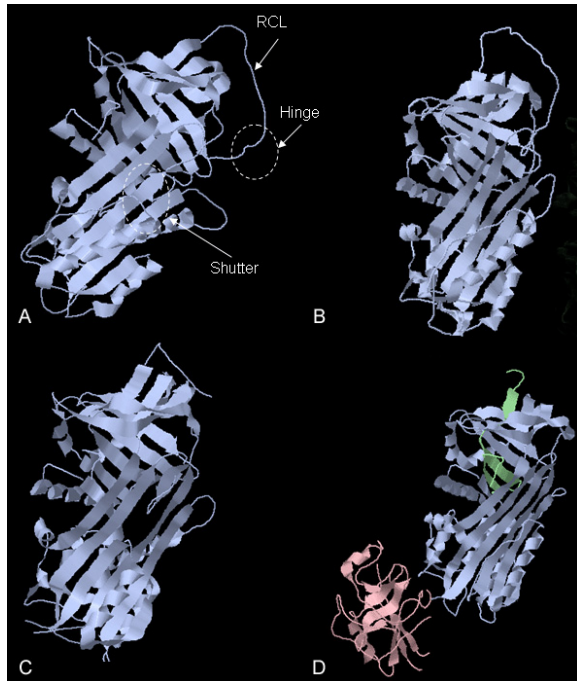
Figure 1. Serpin protein crystal structures adapted from PDB database (http://www.pdb.org). A) Intact native AAT (PDB entry 1QLP) with RCL, hinge and shutter regions labelled with open circles. B) Native mouse ACT (PBD entry 1YXA). C) Human ACT in delta conformation (PDB entry 1QMN). D) alpha-1 antitryspin (purple)/trypsin (pink) complex (PDB entry 1EZX).

pronounced effect on astrocyte gene expression compared to Abeta treatment alone.

Understanding the regulation of *ACT* is imperative to dissecting the role of ACT in disease pathology. ACT is an acute phase protein and its gene expression is stimulated by the presence of cytokines. Accordingly, functional STAT and NF-κB sites have been located in the regulatory regions of the *ACT* gene. A SNP has been identified within the *ACT* promoter that affects *ACT* gene expression both basally and under cytokine stimulation (4). This polymorphism together with one in the signal sequence region of the *ACT* gene have been associated with an increased risk of developing AD but unfortunately, like many other AD candidate gene studies, the genetic evidence remains controversial.

The development of high throughput genomic technologies has recently resulted in a wealth of information on the *ACT* gene. The aim of this review is to provide an up-to-date, thorough analysis of *ACT* gene regulation and its contribution to human disease using a number of cross-platform approaches.

3. ACT PROTEIN

ACT belongs to the serine protease inhibitor (serpin) superfamily of proteins, of which >700 have been identified to date in both eukaryotes and prokaryotes (5).

Phylogenetic analysis using protein sequences classified the human serpins into nine groups (A-I) (6). ACT protein is one of 13 extracellular proteins in group A and is comprised of 423 amino acids including a 25 residue signal peptide at the amino terminus which is cleaved from the mature protein (7, 8). The total molecular weight of ACT is approximately 55-66kDa due to heavy glycosylation at multiple sites (8). This variable glycosylation is typical of plasma serpins but is not required for serine protease inhibitor activity (8).

ACT has a typical serpin structure, comprising of three beta sheets, 8 alpha helices and an active site situated within a hypervariable reactive centre loop (RCL) (Figure 1A-C; (10, 11)). In human ACT this RCL domain is 23 amino acids long and is located near the carboxyl terminus of the protein. The active site of ACT is situated between the leucine 358 and serine 359 residues (12, 13). The length of the RCL is critical for ACT function; relocation of the active site by a single residue within the RCL has been shown to dramatically affect the serine protease inhibitor activity of ACT (14).

Serpins reside in the relaxed (native) conformation until the targeted protease binds to the RCL, which triggers a conformational change as the RCL inserts into one of the beta sheets (beta sheet A), and then migrates to the opposite pole of the serpin (Figure 1D. This process is irreversible and the serpin is no longer able to bind to additional proteases (15). The resulting complex is more stable with higher resistance to heat and SDS-PAGE than the native form. There are several key domains essential for the ability of serpins to undergo this conformational change, the sequence of which, are highly conserved amongst the inhibitory serpins. The hinge allows rapid insertion of the RCL into the beta sheet and is recognized by a consensus sequence amongst the inhibitory serpins (Figure 1A; (16)). The breach and shutter domains facilitate the opening of the incorporating beta-sheet (17). Crystal structure analysis of a Leu55 mutant within the shutter domain of ACT, led to the identification of the intermediate readily polymerised delta form (Figure 1C) with a partially inserted RCL (18).

ACT binds cognate proteases to form a serpin-protease complex, which is cleared from the circulating plasma by the liver, at a rate 10-50 times more rapidly than ACT alone (19), a process that can be blocked by competition of other serpin-protease complexes (20). In addition, formation of this serpin-protease complex increases the expression of the native serpin (21).

Recently, the complete crystal structure of the native mouse orthologue of ACT (*serpina3n*) has been elucidated (Figure 1B**;** (22)). The crystal structure for human native ACT has yet to be been published although there are crystal structures characterised for human ACT in the alternative delta (Figure 1C) and cleaved conformations (18, 23). Comparison of the mouse ACT structure with the native crystal structure of the human archetypal serpin (alpha-1 antitrypsin) (24), shows that there is complete overlay of the two tertiary structures with the exception of
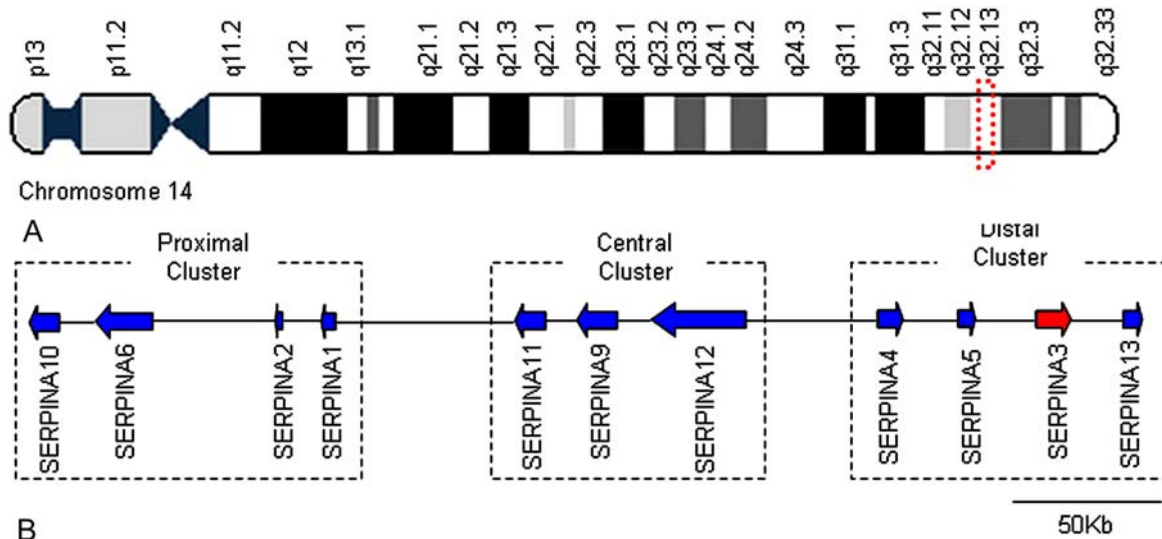
Figure 2. The serpin gene cluster on chromosome 14q32.13. A) The location of the serpin chromosome cluster on q32.13 of chromosome 14 is indicated by the red box. B) The arrangement of the serpin genes within the cluster. Each gene is represented by an arrow and the SERPINA3 (ACT) gene is coloured red. The direction of the arrow shows the DNA strand location of the gene. Arrows pointing left to right are on the forward strand whereas arrows pointing right to left are on the reverse strand. This figure was drawn to scale using the chromosomal coordinates of each gene from the ensembl database (http://www.ensembl.org).

the RCL which displays a 90 degree difference in trajectory (22).

3.1. The physiological role of ACT protein

ACT acts as an inhibitor of several serine proteases including pancreatic chymotrypsin, leukocyte cathepsin G, mast cell chymases, human glandular kallikrein 2, kallikrein 3 (prostrate specific antigen), pancreatic cationic elastase and an uncharacterised lung serum protease (25-29). The strongest association is found with cathepsin G and is thus thought to be its major target (30). Cathepsin G is contained in neutrophil granules and is released at the site of inflammation, where it kills and degrades pathogens, remodels tissues and activates pro-inflammatory cytokines and receptors (31). Excessive or prolonged activity of cathepsin G, caused by insufficient serpin regulation, can lead to tissue damage. ACT is a typical acute phase protein, with the amount of circulating protein dramatically increasing in response to inflammation (32)

In addition, ACT has the unique ability amongst serpins to bind to DNA, a property which is independent of its serine protease inhibitor activity (33). Lysine repeats within close proximity of each other in the crystal structure, (between residues 210-212 and 391-396 of ACT) have been identified as the key residues for DNA binding (33). These residues are not present in other serpins. Specific ACT recognition sequences within DNA have not been identified and any functional significance of ACT binding to DNA has yet to be clarified (33).

4. THE *ACT* GENE

4.1. *ACT* gene structure and location

The *ACT* gene is located on chromosome 14q32.1 within a serpin gene cluster containing ten other serpin genes (Figure 2). *ACT* resides within a distal sub cluster of this region containing *kallistatin* (SERPINA4), *proteinase C inhibitor* (SERPINA5) and the *kallistatin like gene* (SERPINA13). All of the genes within this distal sub-cluster, including *ACT*, are highly expressed in the liver but also have additional tissue-specific patterns of expression. Recently, microarray expression profiles have been generated for *ACT* (34) and show a wide range of expression in multiple tissue types (Figure 3). Importantly, *ACT* is the only gene within the distal serpin sub-cluster to be expressed in astrocytes (35).

Matrix attachment regions (MARs) are AT rich, highly repetitive and often intergenic regions that bind to nuclear matrices *in vitro* and are important for chromatin structure. A Mar-wiz algorithm was used to predict MARs in the distal *ACT* residing cluster (36). Ten potential MARs were identified in this distal cluster including one approximately 4kb upstream of *ACT*. DNase1-hypersensitive sites (DHSs) are more accessible to *trans*-acting factors and act as markers for regulatory regions such as promoters and enhancers. Twelve DHSs were mapped to the *ACT* distal cluster in HepG2 cells, six of which were present in astrocyte cells and all were absent in non-expressing HeLa cells (35). The six astrocyte-specific DHSs were specifically located 5' to the *ACT* gene in the region between the transcription start site and the -13kb enhancer suggesting that in astrocytes the other distal cluster members are packaged in inaccessible chromatin.

The *ACT* gene exon-intron structure is conserved amongst group A serpins and a full, detailed schematic of the gene can be seen in Figure 4A. The gene, 11.66 Kb in length, is comprised of five exons (the first of which is non-coding) and a 3' untranslated region within the final fifth exon (37). Human ACT is transcribed from a single promoter and no alternative transcripts have been reported (8).
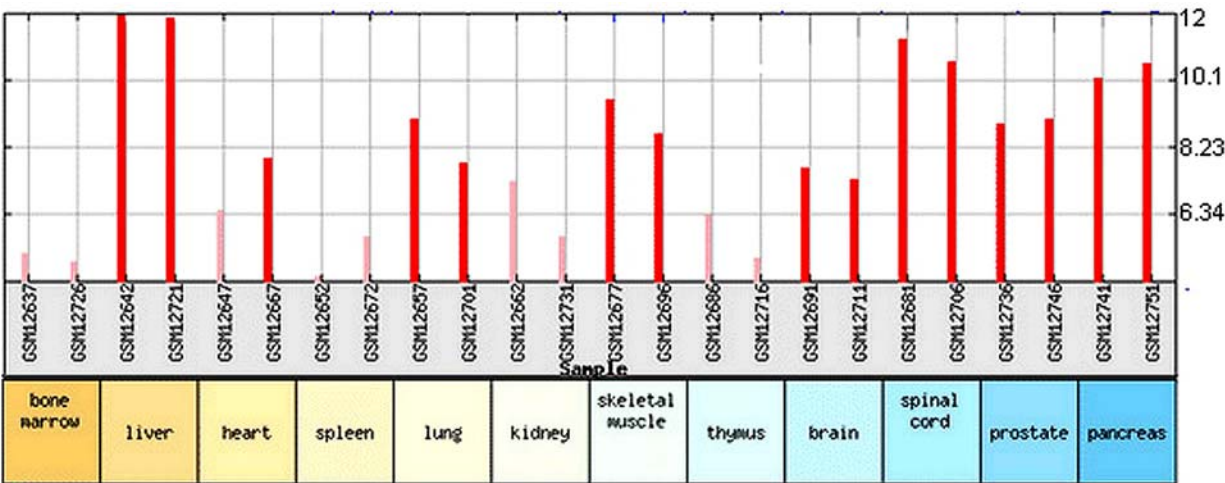
Figure 3. Gene expression profiles of *ACT*. A) *ACT* expression profiles in 12 normal tissues retrieved from Gene Expression Omnibus (www.ncbi.nih.gov); entry GDS425 (34), probe set 75248_at. Tissues were pooled from 11-25 individuals and hybridised to a U95 Affymetrix array in duplicate. The height of the bar represents the log to the base 2 of the signal value. The colour of the bar represents the detection call; red bars have present calls whereas pink bars are absent.

4.2. *ACT* Gene regulation

ACT gene regulation is principally controlled from the promoter region directly upstream from the transcription start site. The promoter elements of *ACT* have not yet been fully characterised, although there is a TATA box-like sequence at positions -23 to -29 (Figure 5) and an initiator element-like sequence at positions -2 to +6 (37). However, these sequences remain putative as there is no direct supporting experimental data. Several transcription factors influence *ACT* expression, including the liver specific transcription factor hepatocyte nuclear factor 4 (HNF4)(37), yet their exact binding sites have yet to be mapped (39).

ACT has two upstream cell specific enhancers located approximately 11.5kb and 13kb upstream from the transcription start site (Figure 5; (40, 41)). Both enhancers contain adjacent activator protein 1 (AP-1) and nuclear factor-1-X (NF1) binding sites, which are critical for basal astrocyte expression of the *ACT* gene (42).

As an acute phase protein, *ACT* gene expression is heavily modulated by cytokines. IL-6 increases the expression of *ACT* in hepatic cells (23, 43), but not in bronchial epithelial cells (44) or astrocytes (45). Although astrocytes do not appear to possess a functional IL-6 receptor, co-treatment of human astrocytes with soluble IL-6 receptor (sIL-6R) resulted in potent stimulation of *ACT* expression (45). Oncostatin M (OSM) stimulates the production of ACT in astrocytes, an effect that is lost following deletion of two STAT binding sites (mapped to positions –125 to –117 and –95 to –87 upstream of the *ACT* gene (Figure 5; suggesting that astrocytes posses a specific OSM receptor and that OSM stimulation is mediated via the JAK-STAT pathway (45).

IL-1 has been shown to increase the expression of *ACT* in hepatic cells (23, 43), bronchial epithelial cells (44)

and astrocytes (45). Functional activity mapping experiments with astrocytes revealed that the IL-1 response elements were not within or immediately 5' to the *ACT* gene. Instead, the IL-1 response is mediated through the -13kb enhancer upstream from the *ACT* gene. This enhancer contains two nuclear factor kappaB (NFkappaB) binding sites in addition to an AP-1 site (Figure 5; (40, 45)). Disruption of any of these sites results in a reduced ability to respond to IL-1 and addition of NFkappaB inhibitor (IkappaB) totally halted this IL1 response. Interestingly, the recently discovered -11.5kb enhancer also contains a putative NFkappaB site although this has yet to be shown to be functionally active (Figure 5; (41)).

An evaluation of the effect of cytokines on *ACT* gene expression has recently been evaluated in our laboratory (unpublished observations). Quantitative PCR was used to construct both dose- and time-response curves measuring the responses of HepG2 cells to the cytokines IL-6, IL-1 and OSM. All three cytokines increased expression of the *ACT* gene in a dose- and time-dependant manner, although the timing and magnitude of the effect differed between the three cytokines (Figure 6). OSM was a potent stimulator of *ACT* expression reaching a maximal 200-fold increase in gene expression after 16 hours of stimulation. IL-6 induced a maximal 100-fold effect at the slightly later time point of 24 hours. Although IL-1 stimulation significantly increased *ACT* expression 7-fold, this effect was considerably less than the IL-6 family members.

4.3. *ACT* genetic variation

Figure 4B shows the position of all single nucleotide polymorphisms (SNPs) located within the *ACT* gene and its 13kb 5' flanking region that had been submitted to dbSNP prior to July 2006 (www.ncbi.nlm.nih.gov/projects/SNP). Of these 99 SNPs, 41 have been genotyped by the International HapMap
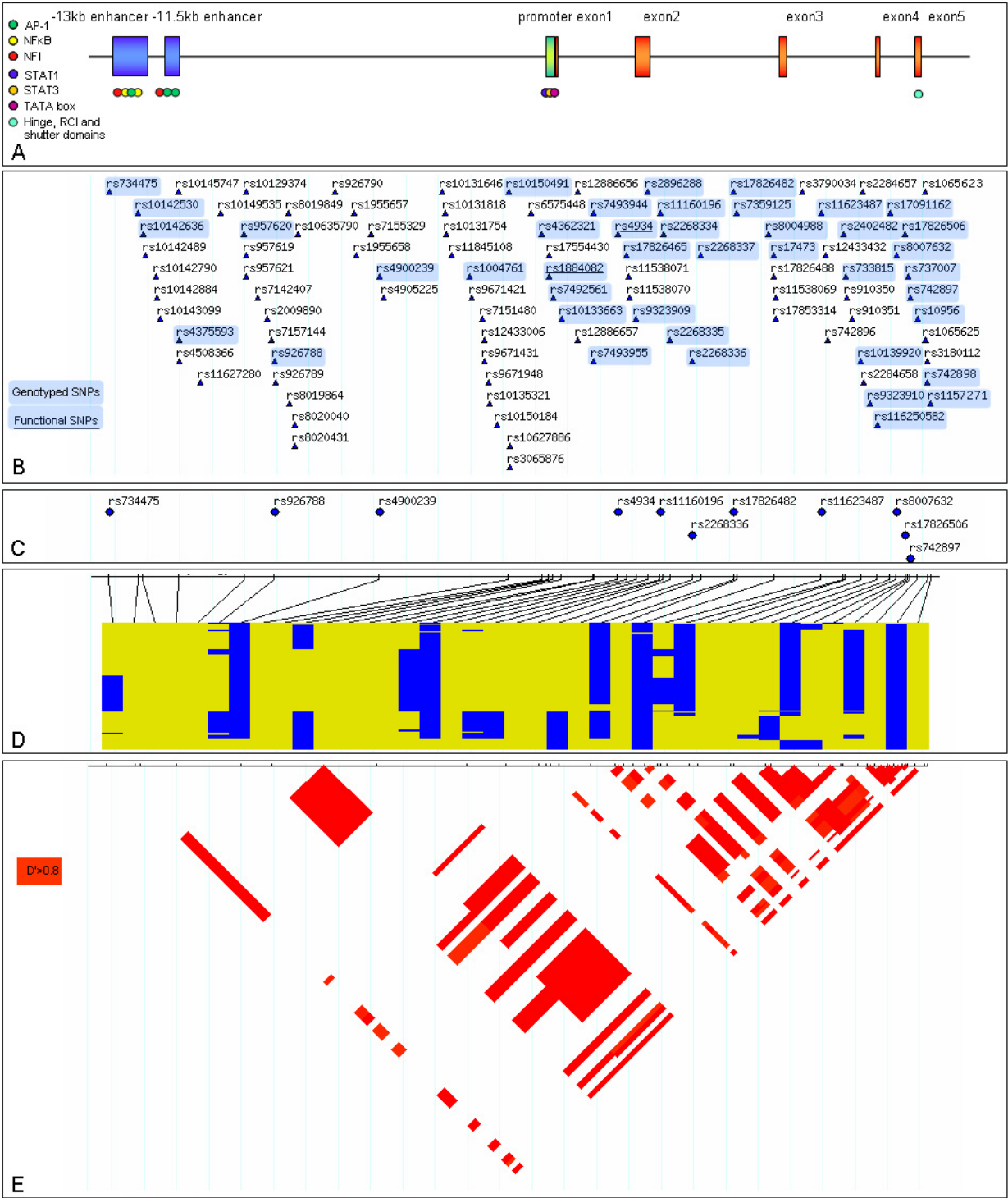
Figure 4. Scale map of the *ACT* gene and 13kb 5' flanking region . A) 5' enhancers (blue), promoter (green), exons (red) and transcription factor binding sites (HapMap database and references (40-42, 45)). B) dbSNPs in HapMap database C) Tag SNPs picked using Tag SNP picker (HapMap). D) Phased haplotypes generated from genotyped SNP data in HapMap (each chromosome of the individuals sampled by the project is represented as a line one pixel high and each SNP allele is arbitrarily coloured blue or yellow). E) LD plot (D') for each pair of genotyped SNPs in the HapMap database.

Consortium, as shown highlighted in blue. Using this genotype information, the HapMap Consortium have made information regarding linkage disequilibrium (LD) between these SNPs available on-line (www.hapmap.org). Using the 'Tag SNP picker' utility of the HapMap website, informative 'tag' SNPs for any region of interest can be

ACT promoter

```
-137    AACAAGCCCG TATTACCAGA AATTATCATC TGGTCATTTC CAGTCCGAGA ACAGAACACT
                   |  STAT  |                              |  STAT  |
-77     TGGTTGTCCT GGCATTTCCC AAGCAGGGGG AGGAGTTCTC TGCAGGAATA AATAAGCCTC

-17     AGCATTCATG AAAATCC
```

ACT enhancer -13kb

```
-13220  GAGATGCGGG AAGTCCCAGG GACAGCAGGA AAGTCCAGGC ACTGCACTGG CAGTGGGATT
              |  NFκB  |
-13160  GGGGCTTGGG TCAGAAGAGG GTCAGGGCCA CAAGGAAGGA CCCTGGGAGG CTTCCTGCTA

-13100  CATAATATGA GGGCCGGGAG CATAACAAAG GATTGCCTTA TCCATGATTG CATCATCAAA

-13040  CTGAAGCCTG AGACAGAACA TTCTGTGAGG TGGCCTTGTT CACTGCAGAG GAGCTGACTC
                                                                  |  AP1
-12980  ACACAGTTTT GTCTGGGAAG TTGCAACCTG TGCCAGGAGT AGCCAGCCTG GGAGTAACAA

-12920  AGAGGCTTTT CTACTGCAGG TTTTCCTAGA GCTCCTCTCC CTCCCTCCCC TGGCCAAAGG

-12860  TGAGCTCTGC AGGGAGGGTC CGGCTCTAGG GGACTTTGCT CCTGGGCATC CTAGCTGCAG
                                        |      NFκB      |
-12800  AGATTGGTGG GGCTTGTCCA GAAAGGGGGG TCTTCTGGAC CTGGGGATGA T
```

ACT enhancer -11.5kb

```
-11647  GATTGTATTA TGAGATTTAC TGGTTTTCTT ACTTCGTCCT CAAATTTCCC TCCTTACCCA

-11587  CTTCCCATGA TTCCTGAGAG TCAAGAGGAC GGGAGCTGTG GAATGTGTTA GTCATCTACT
                                                          |        AP1  |
-11527  AAATAGCCCA TGATTATCTC ACGGAGTTCC TGAGGGTCAG GAATCCAGGA TTGCTGTTGG

-11467  CCAGGGCAAT CCCAGAAGG
              |  NFκB  |
```

Figure 5. Location of regulatory elements within the *ACT* gene. The boxes represent regulatory elements with the corresponding transcription factors listed below. The proposed TATA box is underlined and two mapped polymorphic bases (SNPs) within the region are coloured red.

downloaded. Figure 4C shows the 11 'tag' SNPs selected by HapMap (Phase II, Jul06) covering the *ACT* gene and 5' flanking region (Chr14; position 94134265…94160508) with a linkage disequilibrium (LD) r^2 cut-off >0.8 and a minor allele frequency (MAF) >5% in the European Caucasian (CEU) population. These 11 "tag" SNPs give >80% coverage of the total common variation within this region and this knowledge will reduce the genotyping costs in future genetic association studies. It is important to note that the 'Tag SNP picker' software places all SNPs with

LD values of $r^2 > 0.8$ and MAF >5% into the same 'SNP bin' such that any one of these SNPs could be used as the 'tag' for that bin. This means that if a reliable assay cannot be designed for a particular polymorphism, a different SNP from within the same bin can be used instead.

Figure 4D demonstrates the phased haplotypes constructed from the genotyped SNPs within this region in the CEU population, as taken from HapMap. In 60 individuals tested by the International HapMap consortium,
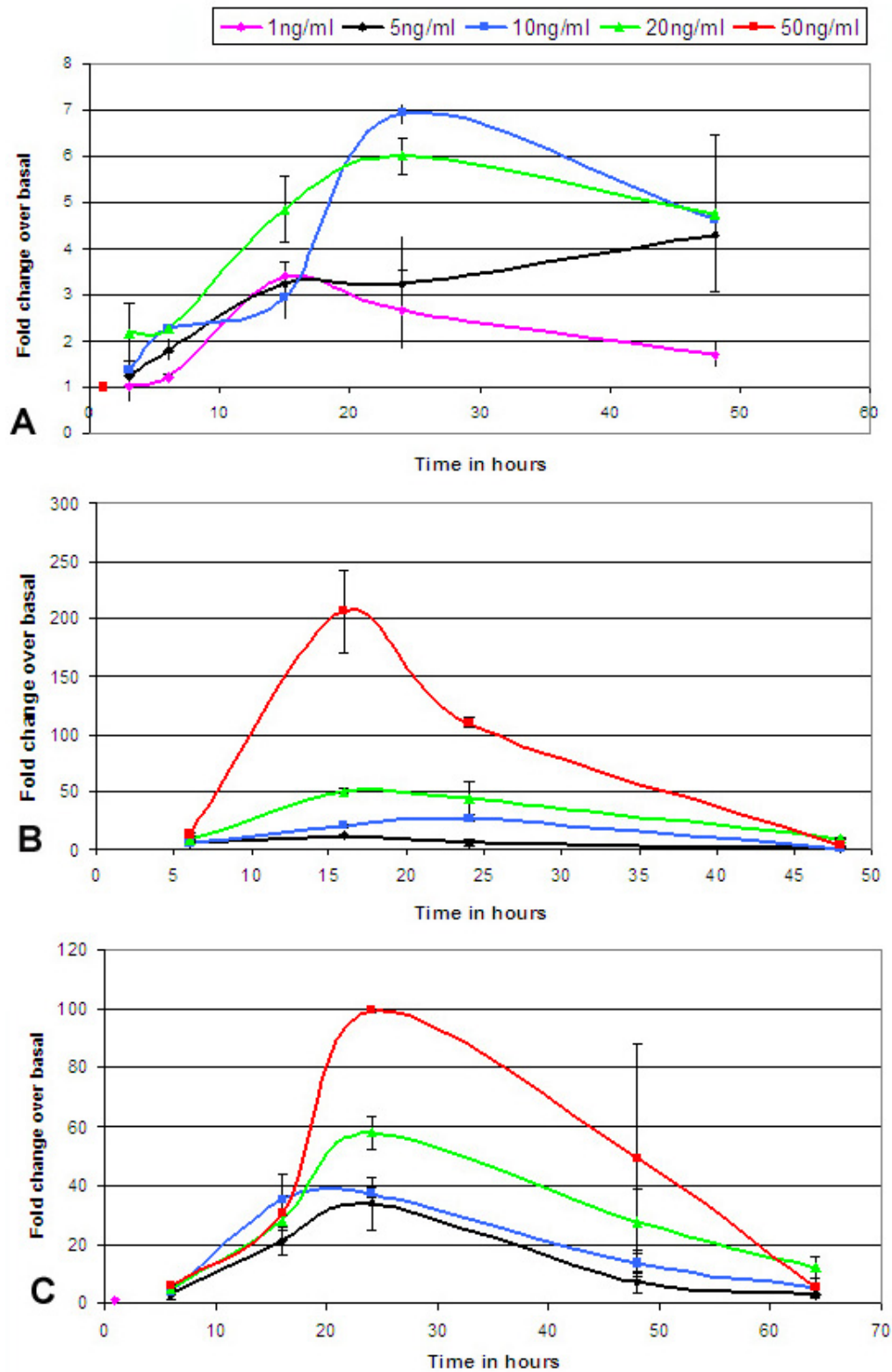
Figure 6. Quantitative PCR measurements of the effects of cytokines A) IL-1, B) OSM and C) IL-6 on *ACT* expression in HepG2 cells. Time is on the X axis and fold change over basal on the Y axis. The colour of the line indicates the cytokine dose (see key). The error bars represent the standard errors from three experiments conducted in triplicate.

genotyping of the 41 SNPs resulted in a total of 24 haplotypes, of which only five had a frequency >5%. The pairwise LD for these SNPs, represented by D', are shown in Figure 4E and demonstrate that there is limited linkage in this region.

5. *ACT* IN HUMAN DISEASE

Imbalance of the serpin-protease equilibrium can have severe consequences, leading to disease. Disease can arise from mutations, which result in deficiency or complete loss of the serpin protein product, resulting in excess uncontrolled, uninhibited target protease activity and disruption of the downstream pathway. Conversely, over-expression of the serpin can lead to increased inhibition of serine proteases, which because of their many roles in biological processes can lead to more severe phenotypes. This is well illustrated by the *C.elegans* model system where ~90% Srp2 null mutants develop normally to the adult stage, compared with only ~50% of worms expressing two Srp2 alleles (46).

SNPs in regulatory regions can potentially result in subtle variations of gene expression and may alter the serpin-protease equilibrium and therefore contribute to disease. Some serpin mutations affect key structural residues causing conformational instability, which can then lead to a group of diseases, the serpinopathies (47). The unique structure of serpins and their ability to undergo conformational change to bind their target protease renders them more susceptible to polymerisation and intracellular aggregation. Mutations in key structural regions such as the hinge, RCL or shutter domains can destabilize the structure and allow incorporation of additional RCLs from other serpin molecules (11, 47). Mutations in other less crucial regions can still lead to polymerisation but at a slower rate. The levels of ACT are elevated in the serum of individuals with malignant disease. ACT has been detected in the nuclei of pancreas, stomach, breast, hepatic and lymphatic cancers despite not being present in the nucleus of normal tissues (48-50). Within these carcinomas, ACT has been shown to bind to DNA polymerase alpha and DNA primase, suggesting a role in protection against tumorigenesis by inhibiting DNA replication (48, 50). Elevated serum ACT may be a potential biomarker for cancers of the pancreas (51), gastrointestinal tract (52) and lung (53). In prostrate cancer the levels of ACT, in a complex with prostrate specific antigen has been proposed as an aid for clinical diagnosis (54-56). Recently, ACT levels have been revealed to be a significant predictor of invasive breast cancer prognosis and reoccurrence (57).

In the context of human disease, ACT familial partial deficiency states have been identified but these are rare, and present with lung and liver disease symptoms (58). Two familial ACT mutations, Leu-55-Pro and Pro-229-Ala result in a deficiency of ACT and can lead to chronic obstructive pulmonary disease (COPD) (24). However, these mutations are rare (0%, 0.9% in 168 patients) in the total population of COPD patients (59) and this association is controversial. Interestingly, ACT deficiency has been associated with milder lung disease in cystic fibrosis patients (60).

The majority of research into the role of ACT in disease has focused on AD pathology. Alzheimer's disease, the most frequent cause of dementia, is a neurodegenerative disorder characterised by the formation of senile plaques and neurofibrillary tangles in the brain. Senile plaques result from the extracellular deposition of beta-amyloid protein (Abeta). Intact Abeta is proteolytically cleaved from amyloid precursor protein (APP), by proteases referred to as beta- and gamma-secretases (61) to produce both 40 and 42 amino acid peptides (Abeta40, Abeta42), of which Abeta42 is the principal component of plaques in human brain (62). The intracellular $Abeta_{1-42}$ isoform is a pro-amyloidogenic peptide that either directly or indirectly induces neuronal cell death (63), resulting in severe cognitive impairment at the clinical level.

ACT was first linked to Alzheimer's disease when it was identified as a major component of amyloid brain deposits (64). This finding was supported by the fact that *ACT* is highly expressed in regions of the brain associated with AD including the hippocampus (64). Furthermore, *in vitro* studies have demonstrated that ACT forms a complex with the putative neurotoxic $Abeta_{1-42}$ in a dose-dependent manner (58, 65, 66). The amino end of Abeta inserts into one β sheet (sheet C) of ACT and the carboxyl terminal into another (sheet A) (67). This transforms the ACT conformation into a stable form, similar to other serpin-protease complexes, and results in loss of ACT inhibitory activity.

The exact role of ACT in senile plaque pathology is as yet undetermined. Some studies report that ACT concentrations can destabilise fibrils as well as break down pre-formed fibrils (58, 65, 68), thus constituting part of the inflammatory response to Aβ aggregation. Stimulation of neuronal and glial cells with ACT/Abeta mixtures have shown that a specific $ACT/Abeta_{1-42}$ complex has multiple cellular effects including the activation of inflammatory transcription factors (66, 69).

A recent microarray study examined the effects of ACT treatment in combination with $Abeta_{(1-42)}$ on gene expression in astrocytes (Figure 7; (3)). Expression profiles were generated from astrocytes exposed to ACT, soluble $Abeta_{(1-42)}$, fibrillar $Abeta_{(1-42)}$, ACT/ soluble $Abeta_{(1-42)}$ and ACT/ fibrillar $Abeta_{(1-42)}$. ACT in combination with $Abeta_{(1-42)}$ had a more profound effect on astrocyte gene expression when compared to the effects of Abeta alone. The soluble and fibrillar molecular forms of the ACT/ $Abeta_{(1-42)}$ combination had opposing effects on gene expression with the majority of genes showing a decrease in expression with the soluble form compared to the majority of genes showing an increase in expression with the fibrillar form. The soluble ACT/ $Abeta_{(1-42)}$ combination decreased the expression of a number of genes involved in neurogenesis and the ubiquitin and proteasome pathways whereas the fibrillar form increased the expression of genes involved in the inflammatory and oxidative stress response. These results suggest that ACT modifies the effects of Abeta on astrocyte gene expression and provides further support for the importance of ACT in AD pathology.
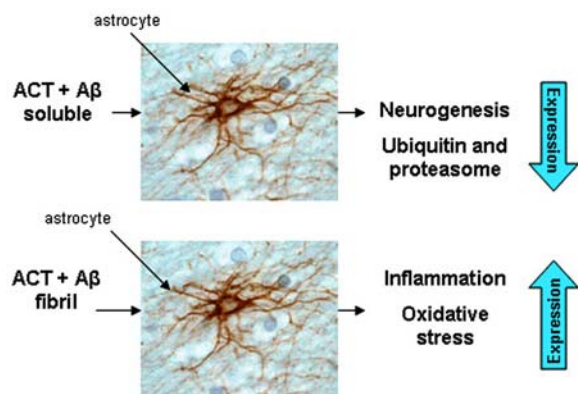
Figure 7. The effects of ACT in combination with ABeta on astrocytic gene expression. The soluble form decreased genes involved in neurogenesis and the ubiquitin and proteasome degradation pathway. The fibrillar form increased genes involved in oxidative stress and inflammation. For more detail see reference (3).

By far the most convincing evidence for a role of ACT in AD has been derived from recent *in vivo* experiments using transgenic mice expressing astrocytic *ACT* crossed with APP mutant mice. The resultant ACT/APP double transgenic mice showed an increase in plaque deposition compared with those expressing *APP* alone inferring that ACT is possibly implicated in Aβ clearance (1). In another ACT/APP mouse model, deposition of Abeta was shown to occur in an age-dependent manner (2) and more recently it has been demonstrated that cognitive impairment in the APP mouse model depends on ACT-catalysed amyloid formation (70). It is possible that different ACT structures may enhance or reduce AD pathology. In additional work, ACT and APOE mice were crossed to APP mice and all the resulting combinations studied (70). The APP/APOE mice had the poorest cognitive ability compared to APP mice with the APP/ACT displaying an intermediary performance. This suggests that ACT has a role in cognitive decline and that APOE and ACT act independently and synergistically to promote amyloid plaque formation. These transgenic mice studies provide support for the theory that ACT is behaving as a molecular chaperone of Abeta and that this may be either by increasing Aβ load or inhibiting Abeta clearance within the cell.

Supporting genetic evidence in humans for a role of ACT in AD pathogenesis has proved to be difficult to obtain and remains controversial. The AlzGene section of the Alzheimer Research Forum website (www.alzforum.org) provides an up-to-date collection of all published AD genetic association studies. Odds ratios for those studies regarding the *ACT* gene are shown in Figure 8.

In 1995, a common variant (alanine) in the *ACT* signal sequence (rs4934; base change G (alanine) to A (threonine) at position 2076 relative to the transcription start site) was found to be associated with an increased risk of AD (71). This alanine variant results in a more

hydrophobic signal sequence, which is more able to transverse the ER, resulting in increased amounts of mature glycosylated ACT secreted in a transfected rat glioma cell line (72). It has also been reported that alanine homozygotes have a lower age-at-onset of AD (73). However, following these initial observations, a number of studies refuted them (74-78) whilst others were supportive (79-81). One study even reported that the threonine and not the alanine variant was implicated in EOAD (82), increasing the risk of developing AD in APOE ε4 carriers 2-3 fold.

The alanine variant allele (G) at this locus is in strong linkage disequilibrium (D' 0.97) with the previously reported T-allele of an *ACT* promoter SNP (rs1884082; base change G-T at position -51), which has been reported to be associated with sporadic and EOAD in both a British and Italian cohort (83). The T-allele of this promoter SNP is associated with a 22% mean higher serum ACT concentration and demonstrates increased functional activity (~30%), as assessed by luciferase reporter gene assays, in hepatocytes and a mixed glial cell population (2). This higher activity T-allele has been associated with cognitive decline in an Italian AD sample collection (83) and recently we have shown that the functional effect of this SNP is more pronounced (T-allele over 200% more activity than the G-allele) in astrocytes (84).

The *ACT* gene has been screened for additional mutations, and nine SNPs were used for AD association studies and haplotype analysis (8). Two additional polymorphisms at positions 241A and 250T were associated with protective effects of AD. Interestingly, the codon 241A allele was in linkage disequilibrium with the alanine variant of the signal sequence polymorphism. A microsatellite locus in the 5' flanking sequence has also been shown to be associated with sporadic AD in *APOE* epsilon4 carriers (85) but the same cohort failed to show any association with the signal sequence polymorphism.

In addition to AD, *ACT* gene variation has been implicated in the pathology of other devastating diseases, although findings have also been somewhat conflicting. The majority of studies have concentrated on the signal sequence polymorphism (rs4934). A summary of these findings are shown in Figure 8D. The Ala variant is associated in COPD in a Japanese (86) but not an Italian (87) population. However, both of these studies were small with 55 and 66 COPD cases respectively. Recently, our laboratory performed a large case-control haplotype association study (N=1929), for five SNPs in *ACT* and reported no association between *ACT* and COPD (88).

Other neurological illnesses in which the signal sequence SNP has been implicated include Parkinson's disease (PD) (89), multiple system atrophy (90), stroke (91-93) but these findings have not been replicated in several other studies (81, 94-98).

Other diseases in which negative genetic association studies of the *ACT* signal sequence polymorphism have been published include leprosy (99),
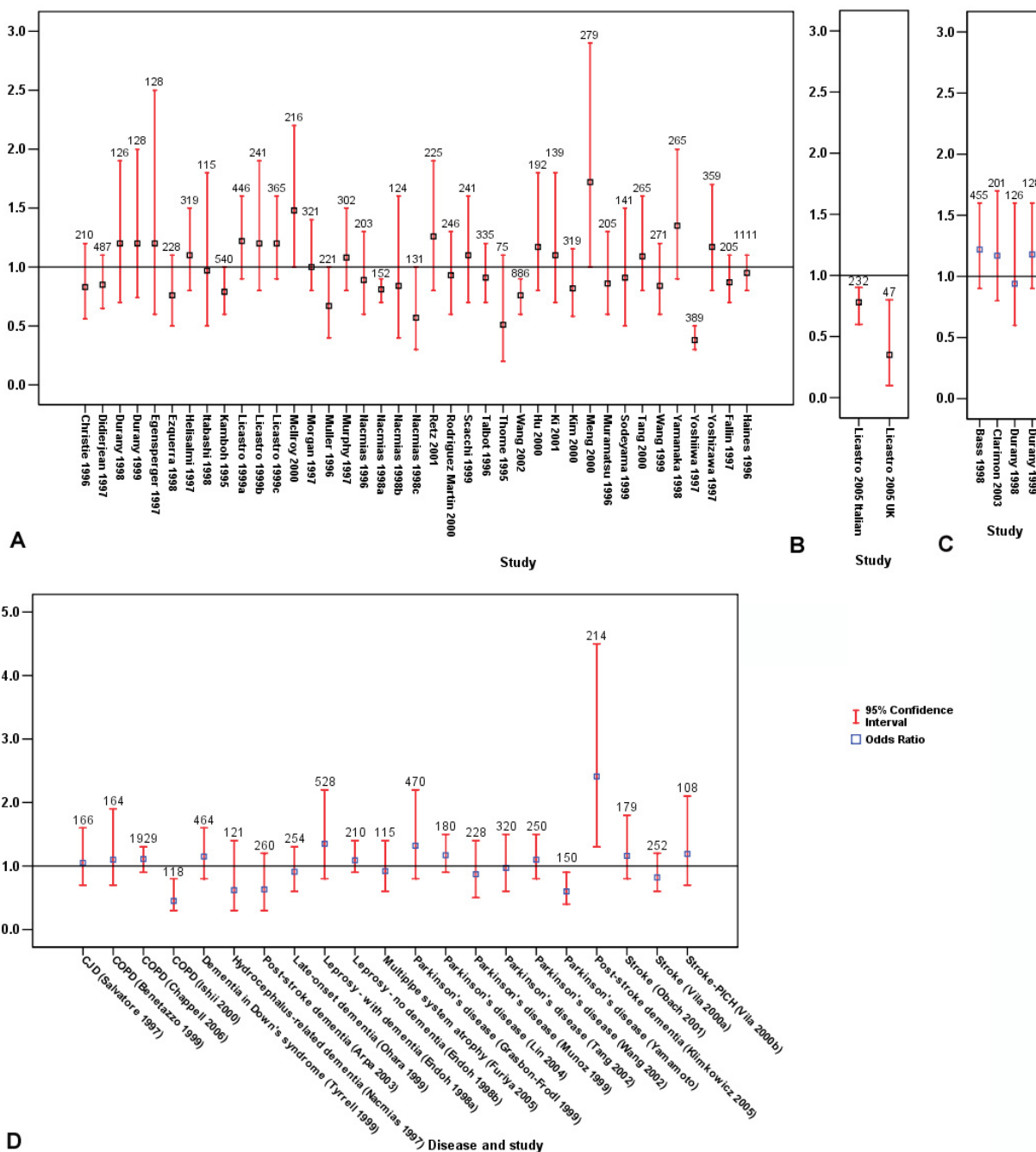
Figure 8. Odds ratios (OR) and 95% confidence intervals for genetic association studies of the *ACT* gene in Alzheimer's disease (adapted from the Alz-gene section of the Alzheimer Research Forum website). A) OR for possession of the Thr variant of the signal sequence polymrorphism (rs4934) in AD. a= All AD, b=EOAD, c=LOAD. B) OR for possession of the A10 microsatellite marker variant in AD. a= All AD, b=EOAD, c=LOAD.C) OR for possession of the G allele at the promoter SNP (rs1884082) in AD. D) OR for possession of the Thr variant of signal sequence polymorphism (rs4934) in all other diseases studied. Numbers above each bar represent total number of samples in each study.

late-onset depression (100), dementia in Down's syndrome (101), hydrocephalus-related dementia (102) and Creutzfeldt-Jakob disease (CJD) (103).

The difficulty in replication of these *ACT* genetic associations is a typical characteristic of complex disease genetics and can be due to differences in sample size, method of disease diagnosis, age-at-onset, population and gender. Furthermore, these studies concentrated predominantly on the signal sequence polymorphism within the *ACT* gene. By only looking at one SNP, additive and/or opposing associations with other SNPs are being ignored.

Such additive or opposing actions may be accounted for by multiple SNP or haplotype analysis. In order to gain enough power to detect the presumably subtle association of any of these individual polymorphisms with disease (e.g. if we assume OR = 1.2-1.4), sample sizes in the 1000s rather than the 100s will undoubtedly be required.

6. PERSPECTIVE

In this review we have catalogued how recent advances in molecular techniques have afforded us a wealth of new information on the structure of the ACT protein and also how the *ACT* gene is regulated. Currently, we have some understanding of the structure of the ACT molecule and how it compares to other closely related serpin gene family members. Recent HapMap advances have catalogued all of the SNPs within the *ACT* gene such that we are now able to describe the variation in this region in the human population. Whilst genetic association studies using single SNPs have failed to provide convincing evidence for the involvement of the *ACT* gene in a number of complex diseases, predominantly AD, it is believed by many that haplotype analysis looking at multiple SNP patterns in large cohorts might be more revealing. For example, it is conceivable that multiple SNPs within any given pathway may influence the pathology of disease. Since ACT has been proposed as a candidate, and we are now able to track its variability, it will be interesting to see if it interacts with any other genes to influence risk in a number of diseases.

7. ACKNOWLEDGEMENTS

8. REFERENCES

1. Mucke, L., G. Q. Yu, L. McConlogue, E. M. Rockenstein, C. R. Abraham & E. Masliah: Astroglial expression of human alpha (1)-antichymotrypsin enhances alzheimer-like pathology in amyloid protein precursor transgenic mice. *Am J Pathol*, 157, 2003-10 (2000)

2. Nilsson, L. N., K. R. Bales, G. DiCarlo, M. N. Gordon, D. Morgan, S. M. Paul & H. Potter: Alpha-1-antichymotrypsin promotes beta-sheet amyloid plaque deposition in a transgenic mouse model of Alzheimer's disease. *J Neurosci*, 21, 1444-51 (2001)

3. Baker, C., H. M. Nielsen, L. Minthon, H. T. Wright, S. Chappell, J. Okyere, S. May, K. Morgan, N. Kalsheker & S. M. Janciauskiene: Effects of Alzheimer's peptide and alpha1-antichymotrypsin on astrocyte gene expression. *Neurobiol Aging* (2005)

4. Morgan, K., F. Licastro, L. Tilley, A. Ritchie, L. Morgan, S. Pedrini & N. Kalsheker: Polymorphism in the alpha(1)-antichymotrypsin (ACT) gene promoter: effect on expression in transfected glial and liver cell lines and plasma ACT concentrations. *Human Genetics*, 109, 303-310(2001)

5. Silverman, G. A., P. I. Bird, R. W. Carrell, F. C. Church, P. B. Coughlin, P. G. Gettins, J. A. Irving, D. A. Lomas, C. J. Luke, R. W. Moyer, P. A. Pemberton, E. Remold-O'Donnell, G. S. Salvesen, J. Travis & J. C. Whisstock: The serpins are an expanding superfamily of structurally similar but functionally diverse proteins. Evolution, mechanism of inhibition, novel functions, and a revised nomenclature. *J Biol Chem*, 276, 33293-6 (2001)

6. Irving, J. A., R. N. Pike, A. M. Lesk & J. C. Whisstock: Phylogeny of the serpin superfamily: implications of patterns of amino acid conservation for structure and function. *Genome Res*, 10, 1845-64 (2000)

7. Chandra, T., R. Stackhouse, V. J. Kidd, K. J. Robson & S. L. Woo: Sequence homology between human alpha 1-antichymotrypsin, alpha 1-antitrypsin, and antithrombin III. *Biochemistry*, 22, 5055-61 (1983)

8. Hwang, S. R., B. Steineckert, A. Kohn, M. Palkovits & V. Y. Hook: Molecular studies define the primary structure of alpha1-antichymotrypsin (ACT) protease inhibitor in Alzheimer's disease brains. Comparison of act in hippocampus and liver. *J Biol Chem*, 274, 1821-7 (1999)

9. Potempa, J., E. Korzus & J. Travis: The serpin superfamily of proteinase inhibitors: structure, function, and regulation. *J Biol Chem*, 269, 15957-60 (1994)

10. Huber, R. & R. W. Carrell: Implications of the three-dimensional structure of alpha 1-antitrypsin for structure and function of serpins. *Biochemistry*, 28, 8951-66 (1989)

11. Janciauskiene, S.: Conformational properties of serine proteinase inhibitors (serpins) confer multiple pathophysiological roles. *Biochim Biophys Acta*, 1535, 221-35 (2001)

12. Morii, M. & J. Travis: Amino acid sequence at the reactive site of human alpha 1-antichymotrypsin. *J Biol Chem*, 258, 12749-52 (1983)

13. Mast, A. E., J. J. Enghild & G. Salvesen: Conformation of the reactive site loop of alpha 1-proteinase inhibitor probed by limited proteolysis. *Biochemistry*, 31, 2720-8 (1992)

14. Plotnick, M. I., H. Rubin & N. M. Schechter: The effects of reactive site location on the inhibitory properties of the serpin alpha (1)-antichymotrypsin. *J Biol Chem*, 277, 29927-35 (2002)

15. Huntington, J. A., R. J. Read & R. W. Carrell: Structure of a serpin-protease complex shows inhibition by deformation. *Nature*, 407, 923-6 (2000)

16. Hopkins, P. C., R. W. Carrell & S. R. Stone: Effects of mutations in the hinge region of serpins. *Biochemistry*, 32, 7650-7 (1993)

17. Whisstock, J. C., R. Skinner, R. W. Carrell & A. M. Lesk: Conformational changes in serpins: I. The native and cleaved conformations of alpha (1)-antitrypsin. *J Mol Biol*, 296, 685-99 (2000)

18. Gooptu, B., B. Hazes, W. S. Chang, T. R. Dafforn, R. W. Carrell, R. J. Read & D. A. Lomas: Inactive conformation of the serpin alpha (1)-antichymotrypsin indicates two-stage insertion of the reactive loop:

implications for inhibitory function and conformational disease. *Proc Natl Acad Sci U S A*, 97, 67-72 (2000)

19. Mast, A. E., J. J. Enghild, S. V. Pizzo & G. Salvesen: Analysis of the plasma elimination kinetics and conformational stabilities of native, proteinase-complexed, and reactive site cleaved serpins: comparison of alpha 1-proteinase inhibitor, alpha 1-antichymotrypsin, antithrombin III, alpha 2-antiplasmin, angiotensinogen, and ovalbumin. *Biochemistry*, 30, 1723-30 (1991)

20. Pizzo, S. V., A. E. Mast, S. R. Feldman & G. Salvesen: In vivo catabolism of alpha 1-antichymotrypsin is mediated by the Serpin receptor which binds alpha 1-proteinase inhibitor, antithrombin III and heparin cofactor II. *Biochim Biophys Acta*, 967, 158-62 (1988)

21. Perlmutter, D. H., J. Travis & P. I. Punsal: Elastase regulates the synthesis of its inhibitor, alpha 1-proteinase inhibitor, and exaggerates the defect in homozygous PiZZ alpha 1 PI deficiency. *J Clin Invest*, 81, 1774-80 (1988)

22. Horvath, A. J., J. A. Irving, J. Rossjohn, R. H. Law, S. P. Bottomley, N. S. Quinsey, R. N. Pike, P. B. Coughlin & J. C. Whisstock: The murine orthologue of human antichymotrypsin: a structural paradigm for clade A3 serpins. *J Biol Chem*, 280, 43168-78 (2005)

23. Baumann, U., R. Huber, W. Bode, D. Grosse, M. Lesjak & C. B. Laurell: Crystal structure of cleaved human alpha 1-antichymotrypsin at 2.7 A resolution and its comparison with other serpins. *J Mol Biol*, 218, 595-606 (1991)

24. Elliott, P. R., X. Y. Pei, T. R. Dafforn & D. A. Lomas: Topography of a 2.0 A structure of alpha1-antitrypsin reveals targets for rational drug design to prevent conformational disease. *Protein Sci*, 9, 1274-81 (2000)

25. Poller, W., J. P. Faber, S. Weidinger, K. Tief, S. Scholz, M. Fischer, K. Olek, M. Kirchgesser & H. H. Heidtmann: A leucine-to-proline substitution causes a defective alpha 1-antichymotrypsin allele associated with familial obstructive lung disease. *Genomics*, 17, 740-3 (1993)

26. Travis, J., J. Bowen & R. Baugh: Human alpha-1-antichymotrypsin: interaction with chymotrypsin-like proteinases. *Biochemistry*, 17, 5651-6 (1978)

27. Christensson, A., C. B. Laurell & H. Lilja: Enzymatic activity of prostate-specific antigen and its reactions with extracellular serine proteinase inhibitors. *Eur J Biochem*, 194, 755-63 (1990)

28. Frenette, G., D. Deperthes, R. R. Tremblay, C. Lazure & J. Y. Dube: Purification of enzymatically active kallikrein hK2 from human seminal plasma. *Biochim Biophys Acta*, 1334, 109-15 (1997)

29. Hakansson, H. O. & K. Ohlsson: Interactions in vitro and in vivo between human and porcine cationic pancreatic elastase and plasma protease inhibitors. *Biol Chem Hoppe Seyler*, 369, 309-15 (1988)

30. Beatty, K., J. Bieth & J. Travis: Kinetics of association of serine proteinases with native and oxidized alpha-1-proteinase inhibitor and alpha-1-antichymotrypsin. *J Biol Chem*, 255, 3931-4 (1980)

31. Wiedow, O. & U. Meyer-Hoffert: Neutrophil serine proteases: potential key regulators of cell signalling during inflammation. *J Intern Med*, 257, 319-28 (2005)

32. Aronsen, K. F., G. Ekelund, C. O. Kindmark & C. B. Laurell: Sequential changes of plasma proteins after surgical trauma. *Scand J Clin Lab Invest Suppl*, 124, 127-36 (1972)

33. Naidoo, N., B. S. Cooperman, Z. M. Wang, X. Z. Liu & H. Rubin: Identification of lysines within alpha 1-antichymotrypsin important for DNA binding. An unusual combination of DNA-binding elements. *J Biol Chem*, 270, 14548-55 (1995)

34. Yanai, I., H. Benjamin, M. Shmoish, V. Chalifa-Caspi, M. Shklar, R. Ophir, A. Bar-Even, S. Horn-Saban, M. Safran, E. Domany, D. Lancet & O. Shmueli: Genome-wide midrange transcription profiles reveal expression level relationships in human tissue specification. *Bioinformatics*, 21, 650-9(2005)

35. Gopalan, S., A. Kasza, W. Xu, D. L. Kiss, K. M. Wilczynska, R. E. Rydel & T. Kordula: Astrocyte- and hepatocyte-specific expression of genes from the distal serpin subcluster at 14q32.1 associates with tissue-specific chromatin structures. *J Neurochem*, 94, 763-73 (2005)

36. Namciu, S. J., R. D. Friedman, M. D. Marsden, L. M. Sarausad, C. L. Jasoni & R. E. Fournier: Sequence organization and matrix attachment regions of the human serine protease inhibitor gene cluster at 14q32.1. *Mamm Genome*, 15, 162-78 (2004)

37. Bao, J. J., R. N. Sifers, V. J. Kidd, F. D. Ledley & S. L. Woo: Molecular evolution of serpins: homologous structure of the human alpha 1-antichymotrypsin and alpha 1-antitrypsin genes. *Biochemistry*, 26, 7755-9 (1987)

38. Kalsheker, N., S. Morley & K. Morgan: Gene regulation of the serine proteinase inhibitors alpha1-antitrypsin and alpha1-antichymotrypsin. *Biochem Soc Trans*, 30, 93-8 (2002)

39. Lim, L., H. Zhou & R. H. Costa: The winged helix transcription factor HFH-4 is expressed during choroid plexus epithelial development in the mouse embryo. *Proc Natl Acad Sci U S A*, 94, 3094-9 (1997)

40. Kordula, T., M. Bugno, R. E. Rydel & J. Travis: Mechanism of interleukin-1- and tumor necrosis factor alpha-dependent regulation of the alpha 1-antichymotrypsin gene in human astrocytes. *J Neurosci*, 20, 7510-6 (2000)

41. Gopalan, S. M., K. M. Wilczynska, B. S. Konik, L. Bryan & T. Kordula: Astrocyte-specific expression of the alpha1-antichymotrypsin and glial fibrillary acidic protein genes requires activator protein-1. *J Biol Chem*, 281, 1956-63 (2006)

42. Gopalan, S. M., K. M. Wilczynska, B. S. Konik, L. Bryan & T. Kordula: Nuclear factor-1-X regulates astrocyte-specific expression of the alpha1-antichymotrypsin and glial fibrillary acidic protein genes. *J Biol Chem*, 281, 13126-33 (2006)

43. Castell, J. V., M. J. Gomez-Lechon, M. David, T. Andus, T. Geiger, R. Trullenque, R. Fabra & P. C. Heinrich: Interleukin-6 is the major regulator of acute phase protein synthesis in adult human hepatocytes. *FEBS Lett*, 242, 237-9 (1989)

44. Cichy, J., J. Potempa, R. K. Chawla & J. Travis: Regulation of alpha 1-antichymotrypsin synthesis in cells of epithelial origin. *FEBS Lett*, 359, 262-6 (1995)

45. Kordula, T., R. E. Rydel, E. F. Brigham, F. Horn, P. C. Heinrich & J. Travis: Oncostatin M and the interleukin-6 and soluble interleukin-6 receptor complex regulate alpha1-antichymotrypsin expression in human cortical astrocytes. *J Biol Chem*, 273, 4112-8 (1998)

46. Pak, S. C., V. Kumar, C. Tsu, C. J. Luke, Y. S. Askew, D. J. Askew, D. R. Mills, D. Bromme & G. A. Silverman: SRP-2 is a cross-class inhibitor that participates in postembryonic development of the nematode Caenorhabditis elegans: initial characterization of the clade L serpins. *J Biol Chem*, 279, 15448-59 (2004)

47. Lomas, D. A. & R. W. Carrell: Serpinopathies and the conformational dementias. *Nat Rev Genet*, 3, 759-68 (2002)

48. Tachikawa, H., M. Tsuda, K. Onoe, M. Ueno, S. Takagi & Y. Shinohara: alpha-1-Antichymotrypsin gene A1252G variant (ACT Isehara-1) is associated with a lacunar type of ischemic cerebrovascular disease. *J Hum Genet*, 46, 45-7 (2001)

49. Takada, S., M. Tsuda, S. Fujinami, M. Yamamura, T. Mitomi & T. Katsunuma: Incorporation of alpha-1-antichymotrypsin into carcinoma cell nuclei of human stomach adenocarcinoma transplanted into nude mice. *Cancer Res*, 46, 3688-91 (1986)

50. Takada, S., M. Tsuda, M. Matsumoto, S. Fujinami, M. Yamamura & T. Katsunuma: Incorporation of alpha-1-antichymotrypsin into human stomach adenocarcinoma cell nuclei and inhibition of DNA primase activity. *Tokai J Exp Clin Med*, 13, 321-7 (1988)

51. Koomen, J. M., L. N. Shih, K. R. Coombes, D. Li, L. C. Xiao, I. J. Fidler, J. L. Abbruzzese & R. Kobayashi: Plasma protein profiling for diagnosis of pancreatic cancer reveals the presence of host response proteins. *Clin Cancer Res*, 11, 1110-8 (2005)

52. Allgayer, H., R. Babic, K. U. Grutzner, B. C. Beyer, A. Tarabichi, F. W. Schildberg & M. M. Heiss: Tumor-associated proteases and inhibitors in gastric cancer: analysis of prognostic impact and individual risk protease patterns. *Clin Exp Metastasis*, 16, 62-73 (1998)

53. Higashiyama, M., O. Doi, H. Yokouchi, K. Kodama, S. Nakamori & R. Tateishi: Alpha-1-antichymotrypsin expression in lung adenocarcinoma and its possible association with tumor progression. *Cancer*, 76, 1368-76 (1995)

54. Leinonen, J., T. Lovgren, T. Vornanen & U. H. Stenman: Double-label time-resolved immunofluorometric assay of prostate-specific antigen and of its complex with alpha 1-antichymotrypsin. *Clin Chem*, 39, 2098-103 (1993)

55. Martinez, M., F. Espana, M. Royo, J. M. Alapont, S. Navarro, A. Estelles, J. Aznar, C. D. Vera & J. F. Jimenez-Cruz: The proportion of prostate-specific antigen (PSA) complexed to alpha (1)-antichymotrypsin improves the discrimination between prostate cancer and benign prostatic hyperplasia in men with a total PSA of 10 to 30 microg/L. *Clin Chem*, 48, 1251-6 (2002)

56. Ishibashi, M.: Standardization of prostate-specific antigen (PSA) assays: can interchangeability of PSA measurements be improved? *Clin Chem*, 52, 1-2 (2006)

57. Yamamura, J., Y. Miyoshi, Y. Tamaki, T. Taguchi, K. Iwao, M. Monden, K. Kato & S. Noguchi: mRNA expression level of estrogen-inducible gene, alpha 1-antichymotrypsin, is a predictor of early tumor recurrence in patients with invasive breast cancers. *Cancer Sci*, 95, 887-92 (2004)

58. Eriksson, S., B. Lindmark & H. Lilja: Familial alpha 1-antichymotrypsin deficiency. *Acta Med Scand*, 220, 447-53 (1986)

59. Sandford, A. J., T. Chagani, T. D. Weir & P. D. Pare: Alpha 1-antichymotrypsin mutations in patients with chronic obstructive pulmonary disease. *Dis Markers*, 13, 257-60 (1998)

60. Mahadeva, R., L. Sharples, R. I. Ross-Russell, A. K. Webb, D. Bilton & D. A. Lomas: Association of alpha (1)-antichymotrypsin deficiency with milder lung disease in patients with cystic fibrosis. *Thorax*, 56, 53-8 (2001)

61. Haass, C.: Take five-BACE and the gamma-secretase quartet conduct Alzheimer's amyloid beta-peptide generation. *Embo J*, 23, 483-8 (2004)

62. Scheuner, D., C. Eckman, M. Jensen, X. Song, M. Citron, N. Suzuki, T. D. Bird, J. Hardy, M. Hutton, W. Kukull, E. Larson, E. Levy-Lahad, M. Viitanen, E. Peskind, P. Poorkaj, G. Schellenberg, R. Tanzi, W. Wasco, L. Lannfelt, D. Selkoe & S. Younkin: Secreted amyloid beta-protein similar to that in the senile plaques of Alzheimer's disease is increased in vivo by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. *Nat Med*, 2, 864-70 (1996)

63. Kienlen-Campard, P. & J. N. Octave: Correlation between beta-amyloid peptide production and human APP-induced neuronal death. *Peptides*, 23, 1199-204 (2002)

64. Abraham, C. R., D. J. Selkoe & H. Potter: Immunochemical identification of the serine protease inhibitor alpha 1-antichymotrypsin in the brain amyloid deposits of Alzheimer's disease. *Cell*, 52, 487-501 (1988)

65. Fraser, P. E., J. T. Nguyen, D. R. McLachlan, C. R. Abraham & D. A. Kirschner: Alpha 1-antichymotrypsin binding to Alzheimer A beta peptides is sequence specific and induces fibril disaggregation in vitro. *J Neurochem*, 61, 298-305 (1993)

66. Sun, Y. X., H. T. Wright & S. Janciauskiene: Alpha1-antichymotrypsin/Alzheimer's peptide Abeta (1-42) complex perturbs lipid metabolism and activates transcription factors PPARgamma and NFkappaB in human neuroblastoma (Kelly) cells. *J Neurosci Res*, 67, 511-22 (2002)

67. Janciauskiene, S., H. Rubin, C. M. Lukacs & H. T. Wright: Alzheimer's peptide Abeta1-42 binds to two beta-sheets of alpha1-antichymotrypsin and transforms it from inhibitor to substrate. *J Biol Chem*, 273, 28360-4 (1998)

68. Evans, K. C., E. P. Berger, C. G. Cho, K. H. Weisgraber & P. T. Lansbury, Jr.: Apolipoprotein E is a kinetic but not a thermodynamic inhibitor of amyloid formation: implications for the pathogenesis and treatment of Alzheimer disease. *Proc Natl Acad Sci U S A*, 92, 763-7 (1995)

69. Sun, Y. X., H. T. Wright & S. Janciauskiene: Glioma cell activation by Alzheimer's peptide Abeta1-42, alpha1-antichymotrypsin, and their mixture. *Cell Mol Life Sci*, 59, 1734-43 (2002)

70. Nilsson, L. N. G., G. W. Arendash, R. E. Leighty, D. A. Costa, M. A. Low, M. F. Garcia, J. R. Cracciolo, A. Rojiani, X. Wu, K. R. Bales, S. M. Paul & H. Potter: Cognitive impairment in PDAPP mice depends on ApoE

and ACT-catalyzed amyloid formation. *Neurobiology of Aging*, In Press, Corrected Proof, (2004)

71. Kamboh, M. I., D. K. Sanghera, R. E. Ferrell & S. T. DeKosky: APOE*4-associated Alzheimer's disease risk is modified by alpha 1-antichymotrypsin polymorphism. *Nat Genet*, 10, 486-8 (1995)

72. Nilsson, L. N., S. Das & H. Potter: Effect of cytokines, dexamethasone and the A/T-signal peptide polymorphism on the expression of alpha (1)-antichymotrypsin in astrocytes: significance for Alzheimer's disease. *Neurochem Int*, 39, 361-70 (2001)

73. Talbot, C., H. Houlden, N. Craddock, R. Crook, M. Hutton, C. Lendon, G. Prihar, J. C. Morris, J. Hardy & A. Goate: Polymorphism in AACT gene may lower age of onset of Alzheimer's disease. *Neuroreport*, 7, 534-6 (1996)

74. Corder, E. H., A. M. Saunders, W. J. Strittmatter, D. E. Schmechel, P. C. Gaskell, G. W. Small, A. D. Roses, J. L. Haines & M. A. Pericak-Vance: Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science*, 261, 921-3 (1993)

75. Haines, J. L., M. L. Pritchard, A. M. Saunders, J. M. Schildkraut, J. H. Growdon, P. C. Gaskell, L. A. Farrer, S. A. Auerbach, J. F. Gusella, P. A. Locke, B. L. Rosi, L. Yamaoka, G. W. Small, P. M. Conneally, A. D. Roses & M. A. Pericak-Vance: No genetic effect of alpha1-antichymotrypsin in Alzheimer disease. *Genomics*, 33, 53-6 (1996)

76. Itabashi, S., H. Arai, T. Matsui, S. Matsushita, T. Muramatsu, S. Higuchi, J. Q. Trojanowski & H. Sasaki: Absence of association of alpha1-antichymotrypsin polymorphisms with Alzheimer's disease: a report on autopsy-confirmed cases. *Exp Neurol*, 151, 237-40 (1998)

77. Lamb, H., J. Christie, A. B. Singleton, A. Leake, R. H. Perry, P. G. Ince, I. G. McKeith, L. M. Melton, J. A. Edwardson & C. M. Morris: Apolipoprotein E and alpha-1 antichymotrypsin polymorphism genotyping in Alzheimer's disease and in dementia with Lewy bodies. Distinctions between diseases. *Neurology*, 50, 388-91 (1998)

78. Murphy, G. M., Jr., E. V. Sullivan, D. Gallagher-Thompson, L. W. Thompson, C. M. van Duijn, L. S. Forno, W. G. Ellis, W. J. Jagust, J. Yesavage & J. R. Tinklenberg: No association between the alpha 1-antichymotrypsin A allele and Alzheimer's disease. *Neurology*, 48, 1313-6 (1997)

79. Thome, J., A. Baumer, J. Kornhuber, M. Rosler & P. Riederer: Alpha-1-antichymotrypsin bi-allele polymorphism, apolipoprotein-E tri-allele polymorphism and genetic risk of Alzheimer's syndrome. *J Neural Transm Park Dis Dement Sect*, 10, 207-12 (1995)

80. Yoshiiwa, A., K. Kamino, H. Yamamoto, T. Kobayashi, M. Imagawa, Y. Nonomura, H. Yoneda, T. Sakai, Y. Nishiwaki, N. Sato, H. Rakugi, T. Miki & T. Ogihara: alpha 1-Antichymotrypsin as a risk modifier for late-onset Alzheimer's disease in Japanese apolipoprotein E epsilon 4 allele carriers. *Ann Neurol*, 42, 115-7 (1997)

81. Munoz, E., V. Obach, R. Oliva, M. J. Marti, M. Ezquerra, P. Pastor, F. Ballesta & E. Tolosa: Alpha1-antichymotrypsin gene polymorphism and susceptibility to Parkinson's disease. *Neurology*, 52, 297-301 (1999)

82. Licastro, F., S. Pedrini, C. Ferri, V. Casadei, M. Govoni, A. Pession, F. L. Sciacca, F. Veglia, G. Annoni, M. Bonafe, F. Olivieri, C. Franceschi & L. M. Grimaldi: Gene polymorphism affecting alpha1-antichymotrypsin and interleukin-1 plasma levels increases Alzheimer's disease risk. *Ann Neurol*, 48, 388-91 (2000)

83. Licastro, F., M. Chiappelli, L. M. Grimaldi, K. Morgan, N. Kalsheker, E. Calabrese, A. Ritchie, E. Porcellini, G. Salani, M. Franceschi & N. Canal: A new promoter polymorphism in the alpha-1-antichymotrypsin gene is a disease modifier of Alzheimer's disease. *Neurobiol Aging*, 26, 449-53 (2005)

84. Ritchie, A., K. Morgan & N. Kalsheker: Allele-specific overexpression in astrocytes of an Alzheimer's disease associated alpha-1-antichymotrypsin promoter polymorphism. *Brain Res Mol Brain Res*, 131, 88-92 (2004)

85. Morgan, K., L. Morgan, K. Carpenter, J. Lowe, L. Lam, S. Cave, J. Xuereb, C. Wischik, C. Harrington & N. A. Kalsheker: Microsatellite polymorphism of the alpha 1-antichymotrypsin gene locus associated with sporadic Alzheimer's disease. *Hum Genet*, 99, 27-31 (1997)

86. Ishii, T., T. Matsuse, S. Teramoto, H. Matsui, T. Hosoi, Y. Fukuchi & Y. Ouchi: Association between alpha-1-antichymotrypsin polymorphism and susceptibility to chronic obstructive pulmonary disease. *Eur J Clin Invest*, 30, 543-8 (2000)

87. Benetazzo, M. G., L. S. Gile, C. Bombieri, G. Malerba, M. Massobrio, P. F. Pignatti & M. Luisetti: alpha 1-antitrypsin TAQ I polymorphism and alpha 1-antichymotrypsin mutations in patients with obstructive pulmonary disease. *Respir Med*, 93, 648-54 (1999)

88. Chappell, S., L. Daly, K. Morgan, T. Guetta Baranes, J. Roca, R. Rabinovich, A. Millar, S. C. Donnelly, V. Keatings, W. MacNee, J. Stolk, P. Hiemstra, M. Miniati, S. Monti, C. M. O'Connor & N. Kalsheker: Cryptic haplotypes of SERPINA1 confer susceptibility to chronic obstructive pulmonary disease. *Hum Mutat*, 27, 103-9 (2006)

89. Yamamoto, M., I. Kondo, N. Ogawa, M. Asanuma, Y. Yamashita & Y. Mizuno: Genetic association between susceptibility to Parkinson's disease and alpha1-antichymotrypsin polymorphism. *Brain Res*, 759, 153-5 (1997)

90. Furiya, Y., M. Hirano, N. Kurumatani, T. Nakamuro, R. Matsumura, N. Futamura & S. Ueno: Alpha-1-antichymotrypsin gene polymorphism and susceptibility to multiple system atrophy (MSA). *Brain Res Mol Brain Res*, 138, 178-81 (2005)

91. Obach, V., M. Revilla, N. Vila, A. A. Cervera & A. A. Chamorro: alpha (1)-antichymotrypsin polymorphism: a risk factor for hemorrhagic stroke in normotensive subjects. *Stroke*, 32, 2588-91 (2001)

92. Vila, N., V. Obach, M. Revilla, R. Oliva & A. Chamorro: Alpha (1)-antichymotrypsin gene polymorphism in patients with stroke. *Stroke*, 31, 2103-5 (2000)

93. Klimkowicz, A., A. Slowik, T. Dziedzic, R. Polczyk & A. Szczudlik: Post-stroke dementia is associated with alpha (1)-antichymotrypsin polymorphism. *J Neurol Sci*, 234, 31-6 (2005)

94. Tang, G., H. Xie, L. Xu, Y. Hao, D. Lin & D. Ren: Genetic study of Apolipoprotein E gene, alpha-1 antichymotrypsin gene in sporadic Parkinson disease. *Am J Med Genet*, 114, 446-9 (2002)

95. Wang, Y. C., H. C. Liu, T. Y. Liu, C. J. Hong & S. J. Tsai: Genetic association analysis of alpha-1-antichymotrypsin polymorphism in Parkinson's disease. *Eur Neurol*, 45, 254-6 (2001)

96. Lin, J. J., K. C. Yueh, C. Y. Chang, C. H. Chen & S. Z. Lin: The homozygote AA genotype of the alpha1-antichymotrypsin gene may confer protection against early-onset Parkinson's disease in women. *Parkinsonism Relat Disord*, 10, 469-73 (2004)

97. Grasbon-Frodl, E. M., R. Egensperger, S. Kosel, R. Kruger, O. Riess, P. Mehraein & M. B. Graeber: The alpha1-antichymotrypsin A-allele in German Parkinson disease patients. *J Neural Transm*, 106, 729-36 (1999)

98. Arpa, A., T. del Ser, G. Goda, R. Barba & B. Bornstein: Apolipoprotein E, angiotensin-converting enzyme and alpha-1-antichymotrypsin genotypes are not associated with post-stroke dementia. *J Neurol Sci*, 210, 77-82 (2003)

99. Endoh, M., A. Ueki, K. Takahashi, H. Yamanaka, S. Izumi & T. Tabira: Alpha-1-Antichymotrypsin Is Not Associated with the Increased Frequency of Apolipoprotein-E- Epsilon-4 Allele in Elderly Non-Demented Leprosy Patients. *Dementia and Geriatric Cognitive Disorders*, 9, 26-28 (1998)

100. Ohara, K., Y. Suzuki, K. Yoshida, H. Terada & K. Ohara: Lack of association between alpha1-antichymotrypsin polymorphism and late-onset depressive disorder. *Psychiatry Res*, 88, 221-6 (1999)

101. Tyrrell, J., M. Cosgrave, J. McPherson, Z. Hawi, V. Trulock, J. Calvert, B. Lawlor & M. Gill: Presenilin 1 and alpha-1-antichymotrypsin polymorphisms in Down syndrome: no effect on the presence of dementia. *Am J Med Genet*, 88, 616-20 (1999)

102. Nacmias, B., A. Tedde, B. M. Guarnieri, C. Petruzzi, L. Ortenzi, A. Serio, L. Amaducci & S. Sorbi: Analysis of apolipoprotein E, alpha1-antichymotrypsin and presenilin-1 genes polymorphisms in dementia caused by normal pressure hydrocephalus in man. *Neurosci Lett*, 229, 177-80 (1997)

103. Salvatore, M., A. C. Seeber, B. Nacmias, R. Petraroli, S. Sorbi & M. Pocchiari: Alpha1 antichymotrypsin signal peptide polymorphism in sporadic Creutzfeldt-Jakob disease. *Neurosci Lett*, 227, 140-2 (1997)

Send correspondence to: Dr Kevin Morgan, The University of Nottingham, Division of Clinical Chemistry, Molecular Medical Sciences, Institute of Genetics, University Hospital, Queen's Medical Centre, Nottingham, NG7 2UH, UK, Tel: 0115 8230724, Fax: 0115 9709167, E-mail: Kevin.morgan@nottingham.ac.uk