LETTER

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A mutation in *APP* protects against Alzheimer's disease and age-related cognitive decline

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The prevalence of dementia in the Western world in people over the age of 60 has been estimated to be greater than 5%, about two-thirds of which are due to Alzheimer's disease[1–4]. The age-specific prevalence of Alzheimer's disease nearly doubles every 5 years after age 65, leading to a prevalence of greater than 25% in those over the age of 90 (ref. 3). Here, to search for low-frequency variants in the amyloid-β precursor protein (*APP*) gene with a significant effect on the risk of Alzheimer's disease, we studied coding variants in *APP* in a set of whole-genome sequence data from 1,795 Icelanders. We found a coding mutation (A673T) in the *APP* gene that protects against Alzheimer's disease and cognitive decline in the elderly without Alzheimer's disease. This substitution is adjacent to the aspartyl protease β-site in APP, and results in an approximately 40% reduction in the formation of amyloidogenic peptides *in vitro*. The strong protective effect of the A673T substitution against Alzheimer's disease provides proof of principle for the hypothesis that reducing the β-cleavage of APP may protect against the disease. Furthermore, as the A673T allele also protects against cognitive decline in the elderly without Alzheimer's disease, the two may be mediated through the same or similar mechanisms.

Amyloid plaques are a central pathological feature of Alzheimer's disease and largely consist of amyloid-β peptides[5,6]. Amyloid-β is formed through sequential proteolytic processing of APP, catalysed by the β- and γ-secretases[7]. The aspartyl protease β-site APP cleaving enzyme 1 (BACE1), originally identified over a decade ago[8–11], cleaves APP predominantly at a unique site, whereas the γ-secretase complex cleaves the resulting carboxy-terminal fragment at several sites, with preference for positions 40 and 42, leading to formation of amyloid-β_{1-40} ($A\beta_{1-40}$) and $A\beta_{1-42}$ peptides[7]. Alternative processing of APP at the α-site prevents the formation of amyloid-β, as the α-site is located within amyloid-β.

Over 30 coding mutations in the *APP* gene have been found. About 25 of these are pathogenic, in most cases resulting in autosomal dominant Alzheimer's disease with an early onset[12,13]. Substitutions at or near the β- and γ-proteolytic sites appear to result in overproduction of either total amyloid-β or a shift in the $A\beta_{1-40}$:$A\beta_{1-42}$ ratio towards formation of the more toxic $A\beta_{1-42}$ peptide, whereas substitutions within the amyloid-β peptide are believed to result in formation of amyloid-β with increased propensity for aggregation[14].

Until now, mutations in *APP* have not been implicated in the common, late-onset form of Alzheimer's disease, with the exception of the rare variant, N660Y, which was recently identified in one case from a late-onset Alzheimer's disease family[15]. To search for low-frequency variants in the *APP* gene with a significant effect on the risk of Alzheimer's disease, we tabulated coding variants in *APP* in a set of whole-genome sequence data from 1,795 Icelanders. Variants present in more than one individual were subsequently imputed into 71,743 chip-typed Icelanders using long-range phasing information[16–18], followed by propagation of genotypes and generation of *in silico* genotypes for 296,496 close relatives of chip-typed individuals who had not been genotyped[19].

We then investigated the association of the variants in *APP* with Alzheimer's disease (Supplementary Table 1). The control group included individuals who had lived to at least age 85 without a diagnosis of Alzheimer's disease. The most significant association was found with rs63750847. The A allele of this single nucleotide polymorphism (SNP) (rs63750847-A) results in an alanine to threonine substitution at position 673 in APP (A673T), and was found to be significantly more common in the elderly control group than in the Alzheimer's disease group (0.62% versus 0.13%; odds ratio (OR) = 5.29; P value = 4.78×10^{-7}; Table 1), and is therefore protective against Alzheimer's disease. To confirm these results, we performed Sanger sequencing of rs63750847 in 451 predicted carriers of rs63750847-A, including two predicted homozygotes. All the predicted carriers were found to have the correct copy number of rs63750847-A, confirming the results obtained with imputation. We also confirmed results by genotyping rs63750847 in 3,661 individuals (cases and controls), and found one mismatch (0.027%; Supplementary Information). Previously, the rs63750847 variant had been reported in a single individual without a history of Alzheimer's disease[20] and in one affected member of a family with late-onset Alzheimer's disease, but was deemed to be probably non-pathogenic[15]. We found the variant in 3 out of 712

Table 1 | APP A673T protects against Alzheimer's disease

Analysis	1/OR	OR	*P* value	Controls		
				Frequency (%)	N_{chip}	$N_{in\ silico}$
AD	-	-	-	0.13	2,199	849
AD versus population controls	4.24	0.236	4.19×10^{-5}	0.45	57,174	22,074
AD versus population controls aged 85 or greater	5.29	0.189	4.78×10^{-7}	0.62	7,653	1,350
AD versus cognitively intact controls at age 85	7.52	0.133	6.92×10^{-6}	0.79	827	407

The table shows association results, comparing patients with Alzheimer's disease (AD) to three different control groups (top line gives numbers for patients with Alzheimer's disease only). N_{chip}, number of individuals with chip-based genotype information; $N_{in\ silico}$, number of individuals with genealogy-based genotype information.

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Norwegian (0.21% allelic frequency), 4 out of 390 Finnish (0.51% allelic frequency) and 5 out of 590 Swedish (0.42% allelic frequency) samples. The variant was also observed in 1 out of 7,020 chromosomes by the National Heart, Lung, and Blood Institute (NHLBI) Exome Sequencing Project[21], and in 3 out of 31,714 chromosomes from a North American population using an exome SNP chip array (see Supplementary Information).

The effect of rs63750847-A is stronger when using elderly controls than when using general population controls (Table 1), which is a consequence of a greater frequency of the variant in the elderly. We estimate that the odds for carriers of rs63750847-A of reaching age 85 are 1.47-fold the odds of non-carriers.

As Alzheimer's disease is a common disease with a late onset, it is informative in association studies to use a control group that includes those who have reached old age without deficits in cognition. Therefore, we examined the frequency of rs63750847 in a control group of individuals who were cognitively intact at age 85, based on a score of 0 on the Cognitive Performance Scale (CPS), a seven-category hierarchical scale assessing cognitive function in the elderly (Supplementary Information). We found an enrichment (0.79%; OR = 7.52, $P = 6.92 \times 10^{-6}$; Table 1) of rs63750847-A in this group, consistent with a protective effect of rs63750847-A against Alzheimer's disease.

To study further the effect of the A673T substitution on cognitive decline in the elderly, we investigated cognitive function as measured with CPS in 41 carriers of A673T in the age range 80–100 as well as in 3,673 non-carriers. The Resident Assessment Instrument for Nursing Homes (RAI-NH), on which the CPS score is based, is applied on average three times per year in Icelandic Nursing Homes. Because the residency time in nursing homes in Iceland is on average 3–4 years, many determinations of CPS made at different times are available for most individuals (Supplementary Fig. 1). As expected, cognitive function declines slowly but steadily with age, both in carriers and non-carriers of A673T (Fig. 1). Analysing a total of 23,831 CPS scores for the 3,673 non-carriers of A673T without a diagnosis of Alzheimer's disease (average of 6.49 determinations per individual), and 262 CPS scores for the 41 carriers of A673T without a diagnosis of Alzheimer's disease (average of 6.39 determinations), we found on average a 1.03 unit difference between carriers and non-carriers across the 80–100 age range (Fig. 1; $P = 0.0021$), with the carriers having a score indicative of better conserved cognition. The fact that the cognitive function of non-carriers remained poorer than for carriers of A673T after removing known Alzheimer's disease cases suggests that the protective effect of A673T extends beyond the boundaries of the Alzheimer's disease phenotype.

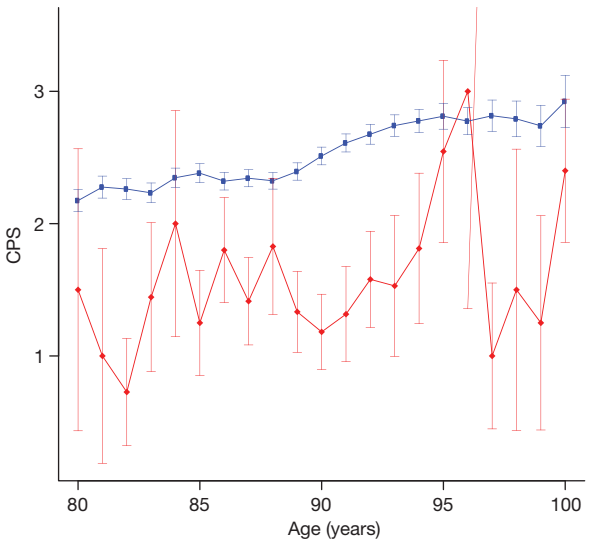

Figure 1 | Cognition measured by CPS as a function of age. Shown are CPS scores of carriers (red symbols) and non-carriers (blue symbols) of A673T as a function of age. Each symbol represents the average CPS score of individuals at the respective age (in years). Error bars represent ± 1 standard error. The jagged appearance of the graph for A673T carriers is due to the relatively small number of data points (262 in total, representing 41 individuals, as compared to 23,831 data points representing 3,673 A673T non-carriers). Individuals with a diagnosis of Alzheimer's disease were not included in the analysis.

The A673T substitution is located at position 2 in the amyloid-β peptide. Recently, an alanine to valine substitution at position 673 in APP (A673V) was reported as being recessive for Alzheimer's disease with very early onset in a single Italian pedigree[22,23]. Heterozygous carriers of A673V in this pedigree were unaffected. We identified three homozygous carriers of A673T in Icelandic samples, one of whom had died at age 88, with the other two currently living at age 67 and 83, respectively. None of these homozygous carriers had a history of dementia.

Our genetic data indicate that the A673T substitution in APP is protective against Alzheimer's disease. The proximity of A673T to the proteolytic site of BACE1 suggested to us that the variant might result in impaired BACE1 cleavage of APP in the A673T carriers.

To investigate the effect of A673T on proteolytic processing of APP, we followed the formation of extracellular APP fragments generated by APP processing at the β-site (sAPPβ) and α-site (sAPPα), respectively, as well as production of the amyloidogenic peptides $A\beta_{x-40}$ and $A\beta_{x-42}$, in 293T cells transfected with wild-type or mutant APP (Fig. 2). By western blot analysis of cell supernatants (Fig. 2a), we found that the A673T variant results in reduced production of sAPPβ with a slight apparent increase in production of sAPPα as compared to wild-type APP. We next confirmed these observations using a quantitative sandwich immunoassay approach (Fig. 2b). sAPPβ production from A673T was ~50% less than from wild-type APP, whereas sAPPα trended non-significantly towards an increase. We also found that the production of both amyloidogenic peptides $A\beta_{x-40}$ and $A\beta_{x-42}$ was ~40% less by the A673T variant than by wild-type APP (Fig. 2c, d). For comparison, we also analysed APP cleavage by the pathogenic A673V variant, which has previously been found to increase amyloidogenic processing of APP[22]. In contrast to A673T, the A673V substitution resulted in markedly increased APP processing at the β-site (Fig. 2a, b), decreased processing at the α-site (Fig. 2a, b), and greatly enhanced $A\beta_{x-40}$ and $A\beta_{x-42}$ production (Fig. 2c, d). For further reference, we also looked at $A\beta_{x-40}$ and $A\beta_{x-42}$ production by APP K670N/M671L, which has been reported to increase $A\beta_{x-40}$ and $A\beta_{x-42}$ production[24]. We confirmed that neither the A673T nor A673V substitution interfered with detection in the enzyme-linked immunosorbent assay (ELISA) (Supplementary Information). The change in the various APP cleavage products seen with A673T shows that this substitution reduces BACE1 cleavage of APP relative to wild-type APP, whereas A673V and K670N/M671L both markedly increase APP cleavage (Table 2). These results are consistent with the protective effect of A673T against Alzheimer's disease, as well as the dramatic phenotypic contrast between T and V substitution at the 673 site in APP. These data also illustrate clearly that position 673 of APP is capable of regulating proteolytic processing by BACE1.

To confirm these observations, we used an *in vitro* BACE1 cleavage assay to assess processing of a wild-type synthetic APP peptide substrate compared to a peptide bearing the A673T substitution. The A673T APP peptide was processed ~50% less efficiently than the wild-type substrate, supporting the conclusion that it codes a sub-optimal BACE1 cleavage site (see Supplementary Information). The substrate specificity of BACE1 has previously been investigated in synthetic model peptides, showing that amino acid substitutions at position 673 in APP can be tolerated[25]. Interestingly, although wild-type APP seems to be a relatively poor substrate for BACE1, most substitutions near the β-cleavage site result in an increased rate of cleavage of synthetic peptides[26]. However, consistent with our findings, a threonine substitution at position 673 of these APP peptide substrates leads to BACE1 cleavage rates that are 50-fold less than for a valine substitution at the same

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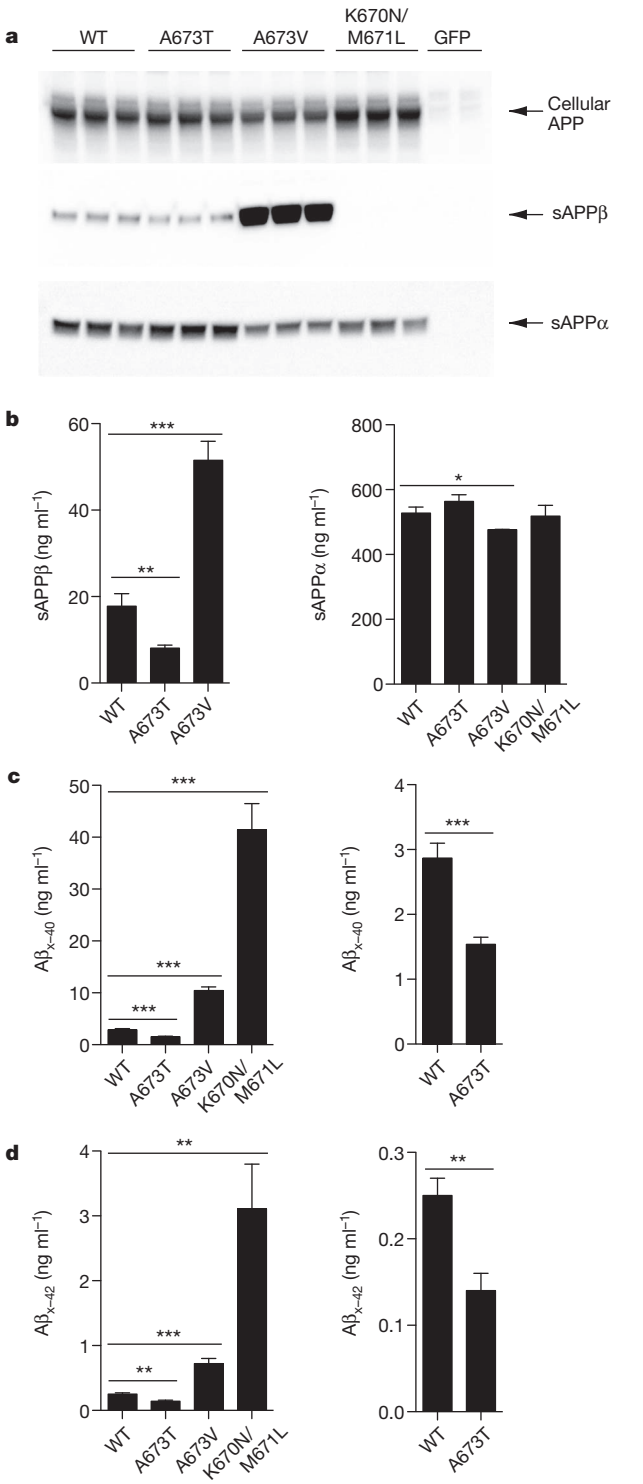

Figure 2 | A673T reduces BACE1 cleavage of APP. **a**, Western blot analysis of 293T cells transfected with wild-type (WT), A673T, A673V or K670N/M671L APP compared to GFP. Total cellular APP was compared to sAPPβ and sAPPα from cell supernatants. Note that sAPPβ is not detected from the K670N/M671L APP transfection as these mutations alter the epitope recognized by the anti-sAPPβ antibody. **b**, Immunoassay quantification of sAPPβ and sAPPα supernatants. **c**, **d**, ELISA quantification of $A\beta_{x-40}$ (**c**) and $A\beta_{x-42}$ (**d**) production from the same 293T transfected cells. $*P \leq 0.01$, $**P \leq 0.005$, $***P \leq 0.001$ (two-tailed *t*-test, compared to wild-type APP); values represent mean ± s.d. of three replicates. The experiment was repeated independently three times.

position[26]. These data further support the conclusion that the A673T substitution in APP reduces BACE1 cleavage relative to wild-type APP substrates.

Table 2 | APP cleavage products from transfected 293T cells

	Wild type	A673T	A673V	K670N/M671L
sAPPβ	17.8 ± 2.9	8.1 ± 0.7	51.5 ± 4.4	N/A
sAPPα	527 ± 18	564 ± 21	476 ± 2	518 ± 33
$A\beta_{x-40}$	2.9 ± 0.2	1.5 ± 0.1	10.4 ± 0.7	41.4 ± 5.1
$A\beta_{x-42}$	0.25 ± 0.02	0.14 ± 0.02	0.72 ± 0.08	3.11 ± 0.69

All reported values are in ng ml^{-1}. APP cleavage products were quantified from supernatants from 293T cells transfected with wild-type, A673T, A673V or K670N/M671L APP. Values represent mean ± s.d. of three replicates from a single experiment. N/A, not applicable.

Our data show that position 673 of APP is critical for amyloidogenic processing of APP by BACE1. To our knowledge, A673T represents the first example of a sequence variant conferring strong protection against Alzheimer's disease. The strong protective effect of A673T also provides further proof of principle for the idea that reducing BACE1 cleavage of APP may protect against Alzheimer's disease. Furthermore, the fact that the A673T substitution also protects against cognitive decline in the elderly without Alzheimer's disease provides indirect support for the hypothesis that the pathogenesis of Alzheimer's disease and normal cognitive decline of the elderly may be shared, at least in part. We therefore propose that Alzheimer's disease may represent the extreme of the age-related decline in cognitive function.

METHODS SUMMARY

Patients with Alzheimer's disease were enrolled through the Memory Clinic at Landspitali University Hospital. Diagnosis of Alzheimer's disease was established according to NINCDS-ADRDA criteria or according to International Classification of Diseases, 10th revision (ICD-10) code F00 criteria. Cognitive function was assessed using the CPS, which is based on the Minimum Data Set for Nursing Homes, MDS 2.0, of the RAI by InterRAI[27].

Genotype data for A673T (rs63750847) were based on whole-genome sequence data generated from 1,795 Icelanders to a depth of at least ×10. Approximately 30 million markers (SNPs and indels) were imputed based on this set of individuals. Sequencing by synthesis was performed on Illumina GAIIx and HiSeq2000 instruments using previously described methods[18]. Long-range phasing of all chip-genotyped individuals was performed using previously described methods[15,28]. SNPs that were identified and genotyped through sequencing were imputed into all Icelanders who had been phased with long-range phasing using the same model used by IMPUTE[15]. Generation of *in silico* genotypes was performed by imputing genotypes into relatives of chip-genotyped individuals, using the fully phased imputed and chip-type genotypes of the available chip-typed individuals. Association testing was performed using logistic regression, matching controls to cases based on the informativeness of the imputed genotypes. Chip-typed samples were assayed with Illumina bead chips containing from 300,000 to 2,500,000 SNPs. SNPs that did not pass a rigorous quality control test were excluded. All samples with a call rate below 97% were also excluded.

Human APP695 cDNA was cloned into pRK vector and mutagenized using QuickChange site-directed mutagenesis kit (Stratagene), followed by transfection into 293T cells. APP cleavage products were assessed both by western blots and immunoassays. $A\beta_{x-40}$ and $A\beta_{x-42}$ peptides were measured from cell supernatants with sandwich ELISAs.

For further details, see Supplementary Information.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Contributions The study was designed and results were interpreted by T.J., J.K.A., H.S., R.J.W. and K.S. Sequence data analysis was carried out by T.J., S.S., P.S., A.K., T.B., R.R.G., T.W.B. and D.G. Subject recruitment, phenotype analysis and biological material collection was organized and carried out by J.S., P.V.J., S.B., G.B., O.A.A., E.G.J. and A.P. Sequencing and genotyping was supervised by J.H., O.T.M. and U.T. Cell line experiments and BACE1 cleavage assays were carried out and analysed by J.K.A., J.M., K.H., Y. Lu, Y. Liu, A.G. and R.J.W. The paper was drafted by T.J., J.K.A., R.J.W. and K.S. All authors contributed to the final version of the paper.

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