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Systemic Mitochondrial Dysfunction and the Etiology of Alzheimer's Disease and Down Syndrome Dementia

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Abstract. Increasing evidence is implicating mitochondrial dysfunction as a central factor in the etiology of Alzheimer's disease (AD). The most significant risk factor in AD is advanced age and an important neuropathological correlate of AD is the deposition of amyloid-β peptide (Aβ_{40} and Aβ_{42}) in the brain. An AD-like dementia is also common in older individuals with Down syndrome (DS), though with a much earlier onset. We have shown that somatic mitochondrial DNA (mtDNA) control region (CR) mutations accumulate with age in post-mitotic tissues including the brain and that the level of mtDNA mutations is markedly elevated in the brains of AD patients. The elevated mtDNA CR mutations in AD brains are associated with a reduction in the mtDNA copy number and in the mtDNA L-strand transcript levels. We now show that mtDNA CR mutations increase with age in control brains; that they are markedly elevated in the brains of AD and DS and dementia (DSAD) patients; and that the increased mtDNA CR mutation rate in DSAD brains is associated with reduced mtDNA copy number and L-strand transcripts. The increased mtDNA CR mutation rate is also seen in peripheral blood DNA and in lymphoblastoid cell DNAs of AD and DSAD patients, and distinctive somatic mtDNA mutations, often at high heteroplasmy levels, are seen in AD and DSAD brain and blood cells DNA. In aging, DS, and DSAD, the mtDNA mutation level is positively correlated with β-secretase activity and mtDNA copy number is inversely correlated with insoluble Aβ_{40} and Aβ_{42} levels. Therefore, mtDNA alterations may be responsible for both age-related dementia and the associated neuropathological changes observed in AD and DSAD.

Keywords: Alzheimer's disease, amyloid-β, AβPP, control region, dementia, Down syndrome, mitochondria, mitochondrial dysfunction, mtDNA

INTRODUCTION

Alzheimer's disease (AD) is the major cause of dementia in the elderly, yet despite two decades of research, not only is there no cure but its etiology also remains unclear. Age is the greatest risk factor for AD, and AD is associated with the accumulation of amyloid-β peptide (Aβ_{40} and Aβ_{42}) containing senile plaques and intraneuronal neurofibrillary tangles in the brain. While early-onset familial AD has been linked to amyloid-β protein precursor (AβPP), presenillin1

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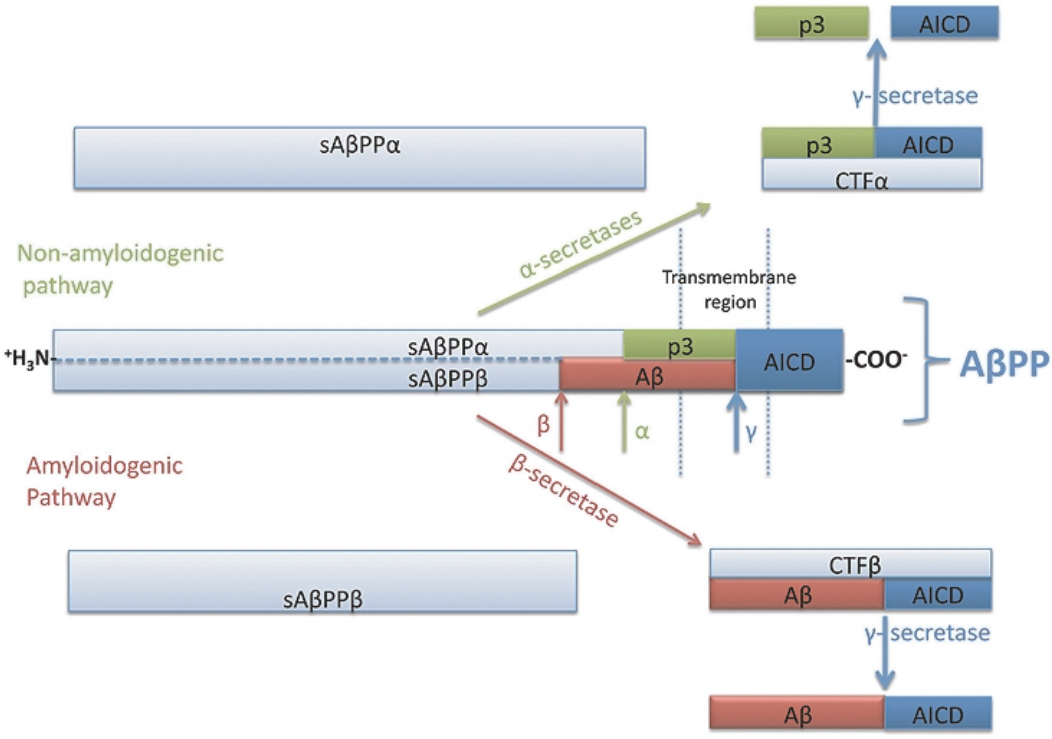

Fig. 1. Schematic representation of AβPP processing for amyloidogenic and non-amyloidogenic pathways.

(PSEN1), and presenillin2 (PSEN2) gene mutations, mutations in these genes account for less than 5% of AD cases [1–7].

Since Alois Alzheimer's [8] original report of a demented patient with amyloid deposition in the brain, it has been assumed that the accumulation of Aβ is the cause of AD dementia. Aβ peptide is the result of the processing of the AβPP, an integral membrane protein that is processed proteolytically by two pathways: amyloidogenic and non-amyloidogenic (Fig. 1). The amyloidogenic pathway is initiated by β-secretase cleavage of AβPP to yield the N-terminal soluble AβPPβ (sAβPPβ) plus a C-terminal fragment β (CTFβ). CTFβ can then be processed by γ-secretase to generate Aβ and a free AβPP intracellular domain (AICD). The non-amyloidogenic pathway is initiated by α-secretase cleavage of AβPP that produces the N-terminal soluble AβPPα (sAβPPα) and the remaining C-terminal fragment α (CTFα). CTFα can then be processed by γ-secretase to yield the small peptide p3 and AICD. Since the α-secretase cleavage of AβPP occurs within the Aβ sequence, the generation of Aβ is precluded. Hence, increased β-secretase activity and Aβ peptide are indicative of an increase in the amyloidogenic pathway [9,10].

Aβ deposition in the brains of Down syndrome (DS) patients is also associated with the development of dementia after the 4 to 5th decade of their life [11]. DS is the result of trisomy of all or part of chromosome 21, which includes the AβPP gene as well as β-site AβPP cleaving enzyme 2 (BACE2) [12–14]. In DS, AβPP is overexpressed in brain and peripheral lymphocytes throughout life [15] yet Aβ levels are similar between young DS patients and controls under 30 years. However, after 40 years, Aβ plaques begin to accumulate in AD brains [16,17]. As a result, it has been hypothesized that the rise in Aβ in DS after 40 years is due to a change in the proteolytic cleavage of AβPP toward the amyloidogenic pathway [18].

While progressive dementia and amyloid plaque deposition correlate with each other in AD, this does not necessarily indicate that amyloid plaque deposition causes the dementia. It is possible that some third factor causes both the dementia and the accumulation of Aβ. Evidence that this might be the case is seen for late onset "sporadic" AD. Late onset AD accounts for more than 90% of AD cases and has not been linked to mutations in AβPP or presenilins. Rather, the strongest genetic associations are with the ε4 allele of the apolipoprotein E gene (ApoE ε4) and closely linked short allele of the

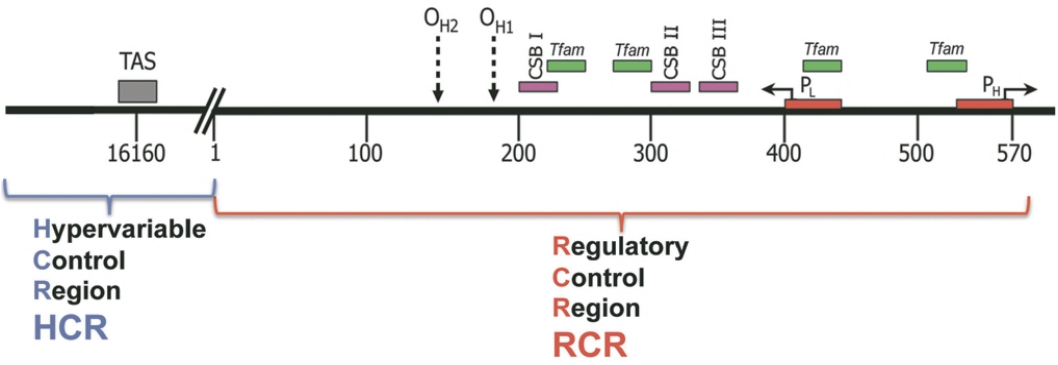

Fig. 2. Schematic representation of human mtDNA control region. Complete mtDNA control region is divided into the regulatory control region (RCR, located between nt 1 and 576) and the hypervariable control region (HCR, located between nt 16024 to 16569). O_H, the origin of the heavy (H)-stand DNA replication; HSP, the promoter for the H-strand; LSP, the promoter for the light (L)-strand; TFAM, transcription factor A mitochondrial binding sites; CSBs, conserved sequence blocks I, II and III; TAS, termination associated sequence.

TOM40 protein gene [7,19,20]. The ApoE protein is a lipid carrier, and lipids are required for the assembly of the mitochondria and are oxidized by the mitochondrion to generate energy. TOM40 is the central channel protein for the import of cytosolicly synthesized proteins through the mitochondrial outer membrane and thus into the mitochondrion [21]. Hence, late-onset AD appears to be associated to mitochondrial function.

Mitochondrial dysfunction and oxidative stress are commonly observed in AD patients [22,23]. The brain tissue, platelets, and cell lines from AD patients have been reported to manifest partial defects in mitochondrial oxidative phosphorylation (OXPHOS), specifically respiratory complex IV (cytochrome c oxidase or COX) [24–32]. Moreover, the Aβ peptide has been reported to enter the mitochondrion and inhibit COX and L-3-hydroxyacyl coenzyme Q dehydrogenase (ABAD). Inhibition of these enzymes in turn contributes to mitochondrial reactive oxygen species (ROS) production [33–38]. Similarly, the ApoE ε4 protein has been reported to be cleaved by a neuron-specific protease, with the resulting peptides also entering the mitochondrion, inhibiting OXPHOS, and increasing ROS production [39,40].

In the brains of mutant mice overexpressing AβPP, tau, and PSEN1, mitochondrial dysfunction has been detected as one of the earliest events in AD [33,41–43]. Mitochondrial defects include reduced membrane potential, an OXPHOS defect, reduced ATP production, and increased ROS production. Furthermore, one-third of the proteins that are deregulated in the triple transgenic mice are mitochondrial including subunits of complex I and complex IV [41].

Mitochondria generate the majority of cellular energy by OXPHOS, are a major generator of cellular ROS, regulate cytosolic Ca^{++}, and control cell death through activation of the mitochondrial permeability transition pore (mtPTP) [21]. Every cell contains hundreds of mitochondria and mtDNAs, with each mtDNA encoding 13 essential OXPHOS polypeptides, the rRNAs and tRNAs for mitochondrial translation, and a regulatory control region (CR) spanning nucleotides (nt) 16024 through 16569/0 to 576 (Fig. 2). The CR can be divided into the regulatory control region (RCR) and the hypervariable control region (HCR). The RCR is located between nt 1 and 576 and encompasses the origin of the heavy (H)-stand DNA replication (O_H); the promoters for the H-strand (HSP) and the light (L)-strand (LSP); transcription factor A mitochondrial, TFAM, binding sites; conserved sequence blocks (CSBs) I, II, and III. The HCR is located between nt 16024 to 16569, and has a much higher sequence diversity across the human population than do other regions of the mtDNA, and encompasses the termination associated sequence (TAS) and the transcription termination factor 3 (mTERF3) binding site [21,44] (Fig. 2).

The human mtDNA sequence variation is much greater than that seen in comparable nuclear DNA (nDNA) genes [45,46]. Furthermore, the accumulation of mtDNA mutations in post-mitotic tissues has been proposed to erode cellular energetics and thus cause the progressive decline of function perceived as aging [47, 48]. Clinical observations suggest that mtDNA variation may be important in the etiology of late onset AD comes from the observations that AD is 1.7 to 3.6 times more likely to be observed in a patient's mother than father [49,50], and from Aβ imaging studies that indicate the offspring from AD mothers have higher brain Aβ loads than the brains of offspring from AD father [51]. Certain germline mtDNA mutations and

haplotypes are also associated with AD. The mtDNA haplogroup H5a, which harbors the tRNA^{Gln} nt 4336G variant, has been repeatedly associated with late onset AD [52–61], while mtDNA haplogroups J, T, and K have been reported to be protective of AD [62–64].

Somatic mtDNA mutations have also been found to be increased in AD brains. The common mtDNA 5 kilobase (kb) deletion was found to be elevated 15 fold in AD brains [65] and somatic mtDNA CR mutations were found to be increased by 73% in AD brains. Many of the AD somatic mtDNA variants alter known CR functional elements, while CR mutations found in normal brains generally do not. The mtDNA CR T414G mutation, which accumulates with age in skin fibroblasts [66], can be detected at a low level of heteroplasmy in 65% of AD brains [67] but cannot be detected in control brains [68]. Somatic mtDNA mutation levels in AD brains also correlate with a reduction in mtDNA/nuclear DNA (nDNA) ratio and with the L-strand ND6 transcript/H-strand ND2 transcript mRNA ratios [67]. This mutation alters the binding of TFAM at its binding site associated with the LSP.

Mitochondrial dysfunction has also been implicated in DS. The triplicated region of chromosome 21 encodes not only AβPP and BACE2, but also important antioxidant genes including Cu/Zn SOD and mitochondrial genes such as NDUFV3, MRPS6 and MRPL39, HLCS, ES1, ATP5J and ATP5O [69]. Mitochondrial dysfunction and elevated oxidative stress have been observed in DS primary neuronal cultures, brains, fibroblasts, erythrocytes, and urine [70–74]. Specific DS mitochondrial alterations include decreased mitochondrial COX, complex I proteins, and heat shock proteins [75–81], and elevated mitochondrial aconitase and isocitrate dehydrogenase levels [82].

Based on these observations, we can now propose that the etiology of premature dementia is the result of an underlying mitochondrial dysfunction resulting in energetic deficiency, increased oxidative stress, altered calcium regulation, and predilection to cell death through activation of the mtPTP [21]. A systemic mitochondrial defect would preferentially affect the brain because of its very high energy demand [83]. High levels of Aβ and the ApoE ε4 degradation products can also cause dementia because they also inhibit mitochondrial function. Regardless of whether mitochondrial OXPHOS is inhibited by an inherited defect or due to a toxic peptide, inhibition of mitochondrial function will result in increased ROS production which will cause the accumulation of somatic mtDNA mutations. The resulting decline in mitochondrial function ultimately results in mtPTP activation and loss of neuronal processes and neurons. In cases where the initiating defect is a systemic mitochondrial defect, as would commonly be the case for late-onset AD, then we would expect that somatic mtDNA mutation levels would also be increased in non-brain tissues. Furthermore, the AD-like dementia of older DS patients should also be associated with increased mitochondrial dysfunction and the accumulation of somatic mtDNA mutations. In the current study, we demonstrate that these predictions are correct. Therefore, mitochondrial dysfunction appears to be a causal variable resulting in premature dementia [21].

MATERIALS AND METHODS

Sample collection

Frontal cortex samples were collected from equal proportions of male and female DSAD, DS, AD, and control subjects from the Alzheimer Disease Research Center (ADRC) at UCI and Emory University, Atlanta. These included 14 DSAD (age range 40 to 62 years old), 11 DS (newborn to 45 years old), 13 AD (55 to 90 years), and 25 Controls (newborn to 95 years). All brain samples were characterized for the presence and frequency of senile plaques and neurofibrillary tangles. This AD-like neuropathology was identified in the AD and DSAD brains but not in the DS and control samples.

Because of the influence of age on the level of accumulated somatic mtDNA CR mutations, comparisons were made between AD and DSAD samples and age-matched controls when possible. Since virtually all DS over 45 develop dementia, it was not possible to obtain older DS subjects without dementia. Studies of DS without dementia included subjects and controls from <1 to 45 years. The DSAD cases were also compared to control brains, ages 40 to 64 years of age. None of the AD cases had known AD-related nuclear gene mutations, and thus all would be classified as sporadic AD cases.

The probability of developing AD doubles every five years after the age of 65 [84]. Therefore 2–3% of individuals between ages 64–69 exhibit signs of AD, while 25–50% of people over 85 have evidence of AD. To correct for this age effect in comparisons using our AD cohort, we subdivided our AD and controls into two cohorts: young AD ages 55 to 70 years and old AD ages 81 to 90 years, with comparable age-matched controls.

Peripheral tissues from DS, DSAD, AD, and control subjects were analyzed for mtDNA somatic mutation levels using the same approaches as applied to the brain samples of these 4 groups. For six each of the AD and control cases, ages 81 to 95 years, from which brain samples were analyzed, clotted serum samples were available from which DNA was isolated, called cell free DNA samples (CF-DNA). These were analyzed for mtDNA mutation levels. Furthermore, lymphoblastoid cell lines (LCLs) were analyzed from 4 different groups of 5 to 7 subjects each of DS, DSAD, AD, and controls, all in the range of ages 45 to 62 years of age.

mtDNA CR sequence and haplotyping

The mtDNA haplogroup of each sample was determined by sequencing the CR (np-16024-570) and confirmed by testing for haplogroup specific coding region single nucleotide polymorphisms (http://www.mitomap.org). All nucleotide variants were reported relative to the modified Cambridge mtDNA Reference Sequence. To be assured that variants analyzed were not the result of amplification of nuclear DNA pseudogenes, each PCR protocol was applied to genomic DNA isolated from cells lacking mtDNA (ρ^o cells). If no PCR band was generated, then the primer set was assumed to avoid pseudogene amplification.

Detection of the T414G mutation

PNA-clamping PCR was used to detect the T414G mutation in frontal cortex DNAs. This procedure can detect one mutant mtDNA in 1000 wild-type molecules. By this procedure, the T414G mutation was detected by PCR amplification of a 334 bp PCR using a primer that overlaps with nt 414 in the presence of the wild type blocking PNA. The amplification of the mutant product was confirmed by digestion with the *FokI* restriction enzyme [85].

Identification of heteroplasmic mtDNA CR mutations by cloning and sequencing

The full array of heteroplasmic mtDNA CR mutations was identified by sequencing between 35 to 52 mtDNA CR clones in each individual case. Genomic DNA was extracted from frontal cortex using the PureGene kit (Gentra system) and the CR amplified with the high fidelity PFU Taq DNA polymerase (iStrategene UltraPFU) using primers nt 15978–15998 and nt 616–636. Purified PCR fragments were cloned into the pCR® 4Blunt-TOPO vector (Invitrogen), the cloned DNA directionally amplified with universal m13 reverse and forward primers, the products cyclically amplified using BigDye dideoxy chain terminator chemistry (Applied Biosystem), and the sequence determined using an ABI 3130 capillary sequencer and analyzed using "Sequencer v4.8" (Gene Codes Corporation).

By cloning and sequencing the full CR from nt 15978 through 16569/1 to 636, each clone included both the "regulatory control region (RCR)" (nt 1 to 600) and the "hypervariable control region (HCR)" (nt 16000to 16569). The HCR is hypervariable when comparing the mtDNAs from different individuals; however, the HCR was much less variable than the RCR when comparing mtDNAs isolated from a tissue of a single individual. Hence, in the current study, the HCR sequence provided the haplogroup signature nucleotides, thus guaranteeing that each clone is from the correct subject, while the RCR sequence permitted the detection of heteroplasmic somatic mtDNA mutations.

The sequences were aligned and deviant nucleotides identified. Unless otherwise stated, only the heteroplasmic mutations of the RCR were reported, which were assumed to have arisen within the tissue and thus be somatic in origin.

Quantification of mitochondrial transcript efficiency and mtDNA copy number

Mitochondrial L-strand/H-strand gene expression ratio was quantified from total cortical RNA extracted using TRIZOL (Gibco-BRL system). mtDNA L-Strand encodes ND6 and 8 tRNAs H-Strand encodes rest of the mRNAs and tRNAs and rRNAs. The ratio of mtDNA L-strand to H-strand transcripts was evaluated as mitochondrial L-strand/H-strand gene expression ratio. ND6 gene expression represents the L-stand transcription, and ND2 gene expression represents the H-Strand. mRNAs were quantified using quantitative reverse transcription-PCR (qRT-PCR) using Roche Light Cycler S480 Real-Time PCR system. The gene expression of ND2 and ND6 determined by absolute quantification using a reference plasmid which was derived from the ND2 and ND6 amplicones cloned in the pCR® 4Blunt-TOPO vector (Invitrogen).

The mtDNA copy number was determined in the total DNA extracted from the frontal cortices. The mtDNA ND2 gene copy number was quantified to indicate the mtDNA copy number and the 18S rDNA nuclear DNA (nDNA) copy number was estimated to determine

the nDNA copy number. The absolute copy number values were determined by comparison with quantification of reference plasmids derived with the ND2 and 18S rDNA amplicones cloned in pCR® 4Blunt-TOP vector (Invitrogen). The relative mtDNA copy number was then reported as a ratio of ND2 to 18S rDNA gene copy numbers. The primers are listed in a previous report [67].

Quantitative analysis of β-secretase activity, AβPP, and Aβ Levels

The β-secretase activity and AβPP and Aβ levels for DS, DSAD, and control brains have been reported previously [86].

Data analysis and statistics

Statistical analyses were performed using GraphPad Prism 5 software (GraphPad Software, Inc.).

RESULTS

Frequency of mtDNA RCR mutations in frontal cortices

The frequency of somatic mutations in the mtDNA RCR increases with age in normal human brain ($r = 0.57$, $p = 0.0029$) (Fig. 3A). This increase rises markedly in the majority of samples after age 65. The mean RCR mutation frequency at 0 to 23 year-old brains was 1.3×10^{-4}/nt, in 40 to 64 year old brains was 1.4×10^{-4}/nt, in 65 to 70 year-old brains was 2.7×10^{-4}/nt, and in 71 to 95 year-old brains was 3.3×10^{-4}/nt ($p = 0.01$ ANOVA) (Fig. 3B).

In AD brains, the frequency of the mtDNA RCR mutations was significantly higher at 5.8×10^{-4}/nt than in control brains at 2.3×10^{-4}/nt, in the age range of 55 to 90 years ($p = 0.002$, t-test) (Fig. 3A,C). This confirms and extends our previous observation that mtDNA CR mutations are increased in AD brains [67].

The mean frequency of mtDNA RCR mutations in DSAD brains, ages 40 to 62 years, was 2.7×10^{-4}/nt, which is also significantly higher than the 1.4×10^{-4}/nt age-matched control brains ($p = 0.012$) (Fig. 3C), though two-fold less than AD brains (2.7×10^{-4} versus 5.8×10^{-4}/nt). By contrast, the mean RCR mutation frequency of 0 to 45 year-old DS brains was 1.5×10^{-4}/nt, the same as age-matched controls at 1.3×10^{-4}/nt (Fig. 3C). Therefore, the mean mtDNA RCR somatic mutation frequency is elevated in both AD and DSAD brains, relative to age-matched control and DS brains.

Frequency of mtDNA RCR mutations in the peripheral tissues

To determine if the increased mtDNA mutation rate seen in AD brains was brain-specific or systemic, we analyzed the mtDNA RCR mutation frequency in DNA extracted from serum samples (cell free DNA, CF-DNA) collected from some of the same patients and controls as were analyzed for brain somatic mtDNA mutations. This revealed that the frequency RCR mutations in AD serum samples (ages 81 to 95 years) was 3.7×10^{-4}/nt, 2.5 times more than that found in age-matched control serum samples (1.5×10^{-4}/nt) ($p = 0.02$ Two-way ANOVA) (Fig. 4A).

The elevation of somatic mtDNA mutations in AD blood cells was confirmed by analyzing the steady state mtDNA RCR mutation frequency in lymphoblastoid cell lines derived from AD, DSAD, DS, and control subjects, derived from 45 to 62 year-old donors. The mtDNA RCR levels revealed that both the AD (3.9×10^{-4}/nt) and DSAD (3.6×10^{-4}/nt) lymphoblastoid cell lines have approximately 2.5 fold more somatic mtDNA RCR mutations than control (1.7×10^{-4}/nt) and DS (1.4×10^{-4}/nt) lymphoblastoid cell lines ($p < 0.001$ ANOVA) (Fig. 4B). Therefore, the somatic mtDNA mutation levels are consistently elevated in peripheral blood cells of AD and DSAD patients, confirming that the elevated mtDNA mutation frequency is a systemic phenomenon in patients with dementia. Since lymphoblastoid cell lines are continually replicating, the somatic mtDNA mutation levels must be maintained at a steady state level in both patients and controls. Therefore, the increased frequency of somatic mtDNA mutations in AD and DSAD lymphoblastoid cells must reflect an increased mtDNA mutation rate in the lymphoblasts from demented patients.

The nature of mtDNA somatic mutations in the brains and bloods

We previously observed that specific mtDNA RCR mutations were found in AD brains that were either rare or absent in control brains, the most striking of these being the mtDNA T414G mutation [67]. To confirm this result and determine if the T414G mutation was also found in DSAD brains, the PNA-clamping PCR procedure was used to detect the T414G mutation in AD, DS, DSAD, and control brains. The T414G mutation was found in 65% of AD brain samples but not in controls, and was also detected in 57% of DSAD brains but not in DS brains. Therefore, the accumula-

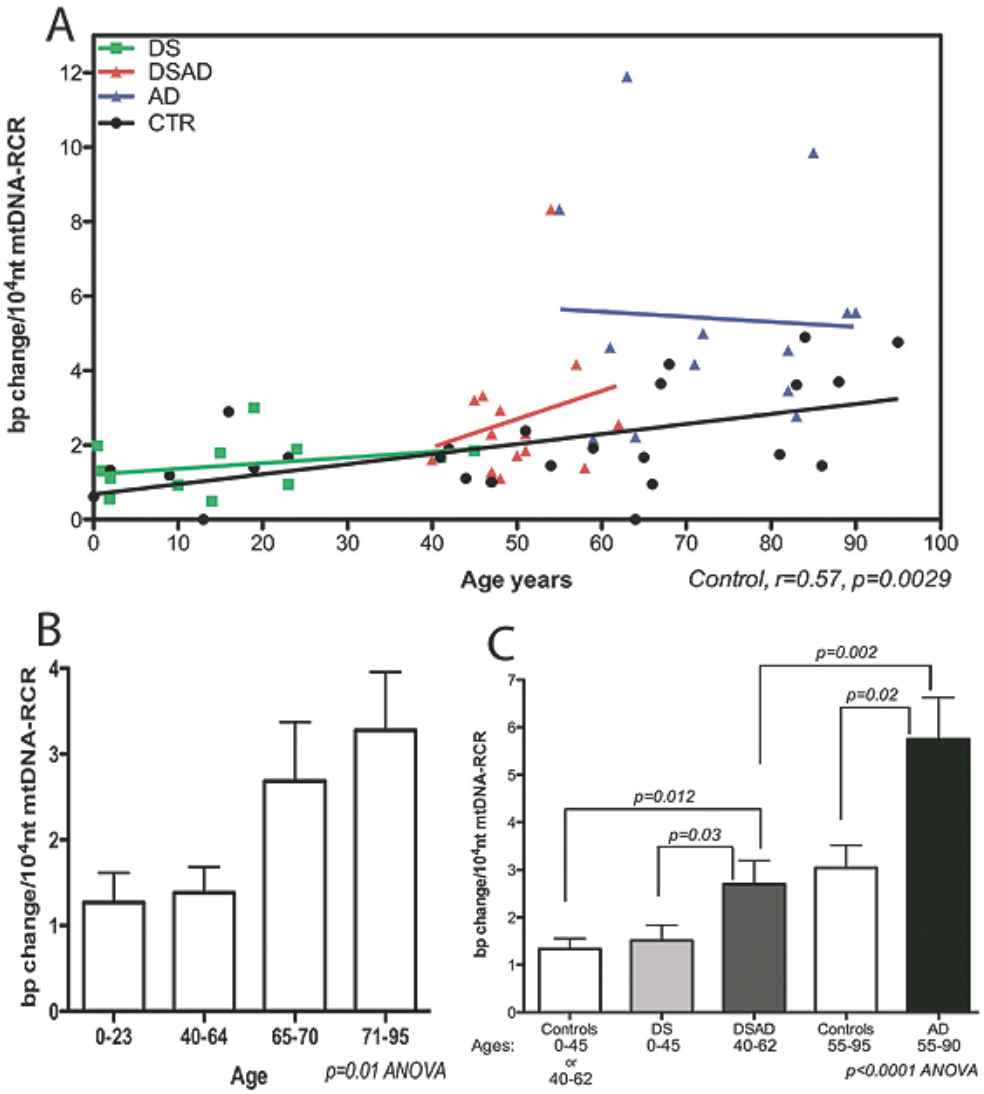

Fig. 3. mtDNA RCR mutation frequency in the frontal cortices of control, DS, DSAD and AD patients. A) The correlation of mtDNA RCR mutation frequency with aging in all four groups. B) The comparison of mtDNA RCR mutation frequency in four control brain groups for the age ranges: 0–23, 40–64, 65–70 and 71–95 years. C) The comparison of mtDNA RCR mutation frequencies of DSAD and AD brains to age-matched controls and DS brains. Because the control groups for DS (ages 0–40) and DSAD (ages 40–62) were not significantly different (see panel 3b), DS and DSAD control groups were grouped together.

tion of the T414G mutation is specific for brains with dementia.

In addition to the T414G mutation, other RCR somatic mutations were found to accumulate in AD, DS, DSAD, and control brains, documented using the mtDNA CR cloning and sequencing procedure. We considered a mutation to be somatic if different clones from the same sample had different bases at the same nucleotide position indicating that the site is heteroplasmic and thus that the nucleotide change arose recently. All variants are reported relative to the Revised Cambridge Reference Sequence. In AD brains, a heteroplasmic, somatic C mutation nt T414 was observed, suggesting that the T to C variant at nt 414 is more prevalent than the T to G variant. Additional heteroplasmic mutations that were more common in AD brains than control brains included G68A, G70A, T72C, G185A, G207A, G228A, 309delC, 309insC, T408C, T414C, C418T, and 466.2insCC (Table 1). Only the A189G/C, 309.3in CCC, A415G, and 456delC mutations were more prevalent in control brains than AD brains (Table 1).

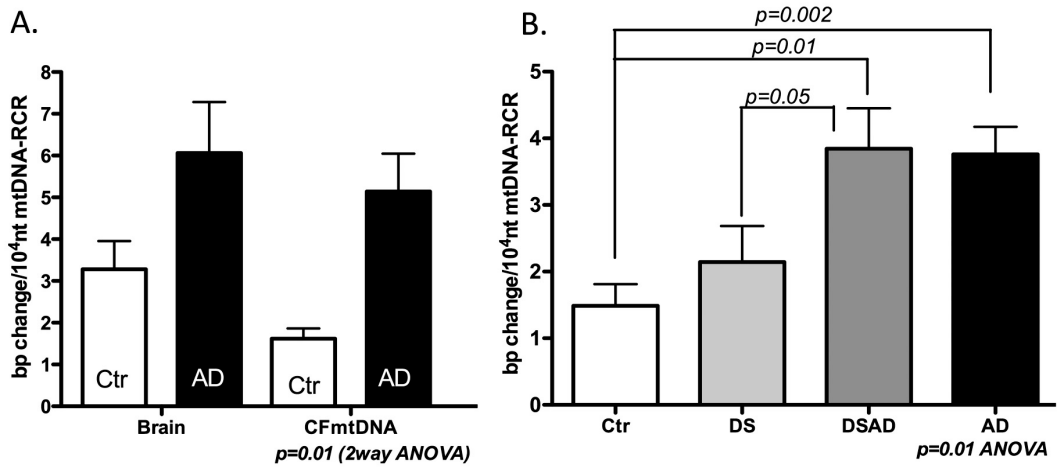

Fig. 4. mtDNA RCR mutation frequency in the peripheral tissue samples from control and dementia patient. A) The analysis of mtDNA RCR mutation frequency in the frontal cortex and serum DNA (CF-DNA) from the same AD and control cases ($n = 6$, between the ages of 81 to 95 years). B) The analysis of lymphoblastoid cell lines (LCLs) from DS, DSAD, AD, and controls ($n = 5$–7, between the ages of 45 to 62 years).

Heteroplasmic mutations were preferentially found in DSAD brains relative to controls. These included G68A, G70A, T72C, G103A, T125C, G185A, T195C, and C537T. Some of these were also elevated in AD brains (Table 1). Finally, heteroplasmic mutations that were more common in DS brains relative to controls included G70A, C114T, C150T, G185A, and 309.1-3insC (1–3).

Certain mtDNA RCR mutations were more prevalent in AD serum than control serum DNAs. These included T72C, A93G, G185A, A189G/C, G207A, T293C, T310C, and 466.2insCC. The only RCR mtDNA mutation preferentially found in control serum DNA was A234G.

Among the lymphoblastoid cell lines, six heteroplasmic variants were commonly found in AD cell lines, than control lines: A189G/C, 309.1inC, 309.2inCC, C377T, T414C, T489C, 522-523inCA, and 522-523delCA (Table 1). Three RCR mutations were elevated in control lymphoblastoid cell lines: A73G, C150T, and T199C. Several additional variants were preferentially found in DSAD lymphoblasts versus controls: G68A, G70A, C114T, A189G/C, T204C, A324G, 309.1-3insC(1–3), 377T, 456delC, 522-523inCA, and C537T. DS lymphoblasts share the A73G variant with control lymphoblasts while they more commonly have the 309.1inC, A357G, and 522–523delCA (Table 1).

Heteroplasmic somatic mutations shared by AD and DSAD brains in excess of controls were C68A, G70A, T72C, G103A, G185A, G207A, and C309del. Mutations shared by AD and DSAD lymphoblasts included A189G/C, 309.1, 309.2, C377T, and 522–523.

In addition to the actual location and proportion of cases that harbored heteroplasmic mutations, there was a striking elevation in the percentage of mutant mtDNAs (heteroplasmy level) in AD and DSAD tissues or cell types (Table 2). Surveying all casess in which the percentage heteroplasmy was greater than 10%, every case but three was observed in either AD or DSAD. The only exceptions were one DS brain which was 22% for the G70A variant, one DS brain which was 29% for the A214G variant, and one control brain that was 97% for the 522–523.1 CA insertion. Several AD and DSAD cases had mutations at remarkably high heteroplasmy levels (Table 2). Of the AD brains, individual cases with greater than 50% heteroplasmy included three cases with high G207A mutations (90%, 88%, 75%), two cases with a high proportion of 315.1 C insertion mtDNAs (68%, 84%), and three cases with the 522–523.1 CA insertion (86%, 61%, 97%). Similarly, of the DSAD brains, one case had the G68A mutation at 80%, one case of the A189G/C at 96%, one case of the A200G at 90%, and one with the G207A at 92%.

The level of heteroplasmy in lymphoblasts was generally low. However, one AD case had the 522–523.1 CA insertion in 96% of the mtDNAs.

For the serum samples, over 50% heteroplasmy levels were seen for one case at G185A (91%), and one case at G207A (94%). Remarkably, the brains from these same two patients had high levels of the same CR mutation observed in the blood. The patient with the 91% G185A mutation in blood was found to be ap-

Table 1
Specific mtDNA RCR heteroplasmic nucleotide changes reported as the percentage of the total individuals that harbor the heteroplasmic mutation

Tissue type →			BRAIN				LCL				CELL FREE DNA	
Number of cases →			24	13	14	12	5	6	6	7	6	6
np	rCRS	Change	Control	AD	DSAD	DS	Control	AD	DSAD	DS	Control	AD
68	G	A	O	15%	14%	O	O	O	17%	O	O	O
70	G	A	4%	31%	14%	8%	O	O	17%	O	O	O
72	T	C	4%	8%	14%	O	O	O	O	O	O	17%
73	A	G	4%	O	O	O	60%	O	O	71%	O	O
93	A	G	4%	O	O	O	O	O	O	O	O	17%
103	G	A	O	8%	7%	O	O	O	O	O	O	O
114	C	T	O	8%	O	8%	O	O	17%	O	O	O
150	C	T	O	O	O	17%	40%	O	O	O	O	O
152	T	C	O	O	7%	O	O	O	O	O	O	O
185	G	A	O	8%	7%	17%	O	O	O	O	O	17%
189	A	G/C	38%	23%	36%	O	O	17%	17%	O	O	17%
195	T	C	4%	O	14%	O	O	O	O	O	O	O
199	T	C	O	O	O	O	20%	O	O	O	O	O
204	T	C	4%	O	7%	O	O	O	33%	O	33%	33%
207	G	A	4%	23%	7%	O	O	O	O	O	O	17%
228	G	A	O	8%	O	O	O	O	O	O	O	O
234	A	G	O	O	O	O	O	O	17%	O	17%	O
293	T	C	O	O	O	O	O	O	O	O	O	17%
309	C	Δ	O	23%	7%	O	O	O	O	O	O	O
309.1	:	C	38%	85%	29%	58%	20%	100%	83%	43%	50%	50%
309.2	:	C	25%	38%	29%	58%	20%	50%	83%	29%	50%	50%
309.3	:	C	17%	8%	14%	33%	20%	17%	33%	O	17%	17%
310	T	C	O	O	O	8%	20%	O	O	O	O	17%
357	A	G	O	O	O	O	O	O	O	14%	O	O
377	C	T	O	O	O	O	O	33%	17%	O	O	O
408	T	A	O	31%	O	O	O	O	O	O	O	O
414	T	C	O	8%	O	O	O	17%	O	O	O	O
415	A	G	4%	O	O	O	O	O	O	O	O	O
418	C	T	4%	8%	O	O	O	O	O	O	O	O
456	C	Δ	4%	O	O	O	O	O	17%	O	O	O
466.2	:	CC	O	8%	O	O	O	O	O	O	O	17%
489	T	C	O	O	O	O	O	17%	O	O	O	O
499	G	A	O	O	O	O	O	O	O	O	O	O
522–23.1	:	CA	20%	23%	29%	8%	O	17%	17%	O	O	O
522–23	CA	Δ	O	O	O	O	O	17%	O	14%	O	O
537	C	T	O	O	7%	O	O	O	17%	O	O	O

proximately 100% mutant in the brain. This high percentage mutant was not of a germline transmission of the G185A mutation since this patient's mtDNA haplogroup was European U5a1 and U5a1 is not associated with the G185A variant. Indeed, the G185A variant is only rarely found in European mtDNAs, being more common in Asian haplogroups A and D and occasionally being seen in African L mtDNAs. Similarly, in the patient whose serum DNA was 94% for the G207A mutation, his brain mtDNA was 75% mutant. Interestingly, the G207A mutation was also present in 88% of the mtDNAs in the brain of the patient which harbored the G185A mutation in both serum and brain mtDNAs. These results indicate that certain somatic mutations arise before the separation of the neurological and blood cell lineages and become broadly distributed in cells throughout these organ systems. Subsequently selective amplification of these mutant mtDNAs would then result in the predominance of the mutation in the different tissues.

Correlation of CR mutations with functional elements

To better understand the potential significance of the AD and DSAD somatic mutations, we also compared the position of the somatic mutations relative to the location of known functional elements of the mtDNA RCR in AD, DSAD, DS, and control samples (Table 3). The most significant observation is that the frequency of somatic mutations seen in the CSBII element was significantly higher in AD brains than controls, with 10.7% of the total AD brain clones having a CSBII site mutation as compared to 4.8% of the age-matched controls ($p = 0.01$ Fisher's exact test). The increase in

Table 2
Number of cases that have $\geqslant$ 10% heteroplasmy rate. Right site of the table (under Brain, LCL and Cell free DNA (CF DNA) columns) shows all the cases – including cases reported in column 4 – that have the particular nucleotide position (np) full range of heteroplasmy in each tissue type

						Brain				LCL				Cell free DNA	
				Number of cases →		24	13	14	12	5	6	6	7	6	6
np	rCRS	Change	# of cases (% heteroplasmy)	Condition	Tissue	Ctr	AD	DSAD	DS	Ctr	AD	DSAD	DS	Ctr	AD
7	A	G	2 (12 & 80%)	DSAD	brain	1	0	2	0						
68	G	A	1 (11%)	AD	brain	0	2	2	0						
"			1 (80%)	DSAD	brain										
"			1 (13%)	DSAD	LCL					0	0	1	0		
70	G	A	1 (11%)	AD	brain	1	4	2	1						
"			2 (12 & 80%)	DSAD	brain										
"			1 (22%)	DS	brain										
"			1 (13%)	DSAD	LCL					0	0	1	0		
71	G	Δ	1 (20%)	AD	brain	6	3	4	6						
72	T	C	1 (14%)	ctr	brain	1	1	2	0						
93	A	G	1 (39%)	ctr	brain	1									
185	G	A	1 (91%)	AD	CF DNA									0	1
189	A	G/C	1 (10%)	AD	brain	10	3	5	0						
"			1 (13 & 96%)	DSAD	brain										
200	A	G	1 (90%)	DSAD	brain	0	0	1	0						
204	T	C	1 (26%)	DSAD	LCL					0	0	2	0		
207	G	A	3 (90 & 88 & 75%)	AD	brain	1	3	1	0						
"			1 (92%)	DSAD	brain										
"			1 (94%)	AD	CF DNA									0	1
214	A	G	1 (29%)	DS	brain	0	0	1	1						
234	A	G	1 (12%)	DSAD	LCL					0	0	1	0		
241	A	G	1 (10%)	DSAD	brain	0	0	1	0						
263	G	A	1 (63%)	DSAD	LCL					0	0	1	0		
490	A	G	1 (14%)	AD	brain	0	1	0	0						
537	C	T	1 (10%)	DSAD	brain	0	0	1	0						
586	A	G	1 (11%)	AD	brain	0	1	0	0						
309	C	Δ	2 (29 & 14%)	AD	brain	0	3	1	0						
315.1	:	C	2 (68 & 84%)	AD	brain	0	2	0	0						
368 1.8	:	ins	1 (30%)	AD	CF DNA									0	1
522–523.1	:	CA	1 (97%)	ctr	brain	5	3	4	1						
"			3 (86 & 61 & 97%)	AD	brain										
"			1 (96%)	AD	LCL					0	1	1	0		

CSBII mutations in AD was also seen for lymphoblast and cell free DNAs. The next most significant difference for brain samples was seen at the LSP site where 2.3% of all the AD clones had mutations versus 1.2% of age-matched control clones. A comparable increase was seen in lymphoblast when the data from all dementia subject (AD + DSAD) clones were compared with the data from all non-dementia subject (control + DS) clones. In brain, the proportion of clones with mutations in the CSBII and LSP regions was 6.3% versus 4% for the CSBII site ($p = 0.036$), and 1.4% versus 0.4% for the LSP site, ($p = 0.01$ Fisher's test) (Table 4). More mutant clones were also observed between nts 1–212 in dementia subjects than controls, 7.4% versus 3.6% ($p = 0.0009$ Fisher's test), a trend also seen in lymphoblast and cell free DNA samples (Table 3). The nt 1 to 212 RCR region contains multiple H-strand origin replication start sites [87]. Therefore, somatic mtDNA mutations in specific CR regulatory elements are significantly increased in AD and DSAD relative to controls and DS subjects (Table 4).

The copy number of mtDNA in brains

Mutations that alter the regulatory elements of the mtDNA RCR might be expected to inhibit mtDNA replication (Fig. 2), resulting in a reduction in the ratio of mtDNA to nDNA copy number. This was examined using quantitative PCR (qPCR) of the mtDNA ND2 gene versus the nDNA 18S rRNA gene in the brains of controls, AD, DS, and DSAD subjects (Fig. 5A). Overall, the mtDNA to nDNA copy number declined in control brains with age. The average copy numbers of brain samples of 0 to 23 year-old subjects was 282 mtDNAs/nDNA, that for 41 to 65 year-old subjects was

Table 3
The percentage of heteroplasmic clones that are distributed on the regulatory elements of mtDNA RCR in AD, DS, DSAD, and control brains and peripheral tissues

		Brains							Lymphoblastoid cell lines				Cell free DNA	
		All CTR	CTR	AD	CTR	DSAD	CTR	DS	CTR	AD	DSAD	DS	CTR	AD
Total clone →		658	250	262	226	377	182	470	112	164	137	172	177	135
Locus	nt span													
	1–212	4.7%	4.4%	8.4%	2.2%	6.6%	0.5%	2.1%	2.7%	5.0%	3.6%	0.6%	1.7%	3.7%
CSBI	213–35	0%	0.8%	0.4%	0%	0.3%	0%	0.2%	0%	1.0%	1.5%	0.0%	1.1%	0%
TFX	233–60	0%	0%	0.8%	0%	0.3%	0%	0%	0%	1.0%	0.7%	0%	0%	0%
TFY	276–303	1.0%	1.2%	0.8%	0.4%	0%	0%	0.2%	0%	0.0%	0%	0%	0.6%	0.7%
CSBII	299–315	4.0%	4.8%	10.7%*	2.2%	3.2%	3.8%	4.5%	3.6%	11.0%	8.8%	3.5%	4.5%	7.4%
CSBIII	346–63	0%	0%	0%	0%	0.3%	0%	0.2%	0%	1.0%	0.0%	0.6%	0.6%	0%
mt4H	371–91	0%	0.8%	0%	0%	0%	0%	0%	0%	2.0%	0.7%	0%	0%	0%
LSP	392–445	1.0%	1.2%	2.3%	0%	0.8%	0.5%	0%	1.8%	3.0%	0.7%	0.6%	0.6%	0.7%
TFL	418–45	0%	0.4%	0.4%	0%	0.3%	1.0%	0%	0%	2.0%	0.7%	0%	0.6%	0%
TFH	523–50	1.0%	1.2%	1.9%	0.9%	1.3%	0.5%	0.6%	0%	2.0%	1.5%	1.2%	0%	0%
HSP1	545–67	0%	0%	0%	0%	0.3%	0%	0%	0.9%	1.0%	0%	0%	0%	0%

*$p = 0.01$ Fisher test when AD CSBII values compared to controls. CSBI, II, III: Conserved Sequence Blocks 1, 2, 3; TFX and TFY: mitochondrial transcription factor A (TFAM) binding sites other than promoters; TFL and TFH: TFAM binding site for L-Strand Promoter (LSP) and H-Strand promoter (HSP); mt4H: H-strand control element, CTR: controls, nt: nucleotide.

Table 4
The percentage and the significance of mtDNA mutation distribution on the regulatory elements of RCR in the dementia versus non-dementia groups

Locus	nt span	Brains			Lymphoblastoid cell lines		
		Non-D	D	p	Non-D	D	p
	1–212	3.6%	7.4%	0.0009	1.8%	4.7%	0.06
CSBII	299–315	4.0%	6.3%	0.013	3.5%	7.6%	0.03
LSP	392–445	0.4%	1.4%	0.01	1.0%	1.3%	NS

Non-demented values are combination of controls and DS; demented values are AD and DSAD. Non-D: Non-Demented, D: demented, NS: non-significant, nt: nucleotide.

290 mtDNAs/nDNA, and that for 66 to 88 year old subjects was 148 mtDNAs/nDNA) (ANOVA $p = 0.01$). Hence, the mtDNA copy number declined after age 65 years in parallel with the increased mtDNA mutation rate.

The mtDNA copy number of AD brains was significantly lower than age-matched controls for 66 to 88 year-old individuals, 110 mtDNA/nDNA versus 197 mtDNA/nDNA ($p = 0.013$) (Fig. 5B). The mtDNA copy number of DSAD brains was also significantly lower than age-matched controls, 145 mtDNA/nDNA in DSAD versus 290 mtDNA/nDNA ($p = 0.016$) (Fig. 5C). By contrast, there is a trend toward increased mtDNA copy number of DS brains relative to age-matched controls, 388 mtDNA/nDNA in DS versus 282 mtDNA/nDNA in controls ($p = 0.1$) (Fig. 5D). Therefore, the brain mtDNA copy number is strikingly reduced in association with dementia.

mtDNA strand dependent gene expression ratio

Mutations in the mtDNA control region, particularly in LSP and the associated TFAM binding sites might be expected to reduce mitochondrial L-strand transcription relative to H-strand transcription. To test this prediction, we compared the relative levels of the mtDNA ND6 L-strand mRNA to that of the mtDNA H-strand ND2 mRNA by qRT-PCR for control, AD, DSAD, and DS brain samples (Fig. 6A). The brain ND6 to ND2 mRNA ratio did not change significantly with age in control brains. However, the ND6 to ND2 transcript ratio was significantly lower in both AD and DSAD brains relative to control values. Moreover, the ND6 to ND2 levels were also reduced in the DS brains (ANOVA, $p = 0.001$) (Fig. 6A,B). Hence, mental retardation and dementia were both associated with reduced mtDNA L-strand transcript levels.

Relation between mtDNA alterations and AβPP processing in DS and DSAD

AD brains typically have heightened levels of Aβ_{40} and Aβ_{42} as well as increased levels of somatic mtDNA deletions and base substitution mutations [21]. In DS and DSAD brains, the levels of AβPP protein are

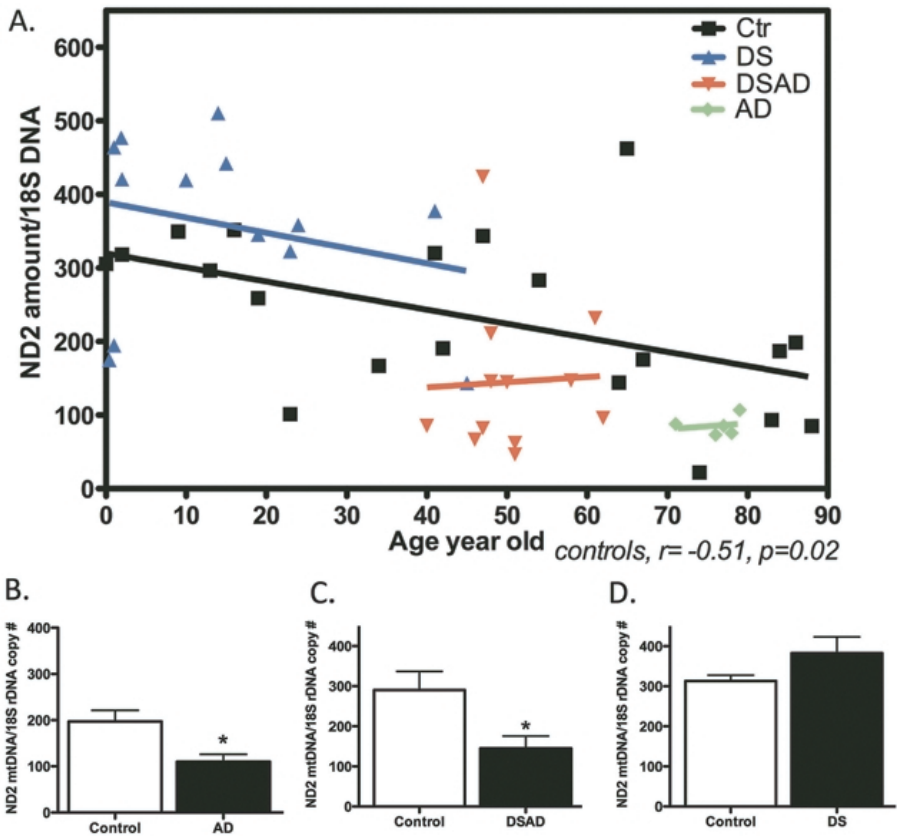

Fig. 5. mtDNA amounts in the brain samples of control, DS, DSAD and AD patients represented as ND2 DNA amount normalized to nuclear 18S rDNA. A) The correlation of mtDNA amount with age in all four groups. The control brain mtDNA amount declined with aging ($p = 0.02$). The AD and DSAD mtDNA levels were below the control brains while DS mtDNA levels were above. B-D) The comparison of AD (B), DSAD (C) and DS (D) mtDNA amounts to age-matched controls ($^*p < 0.05$).

consistently elevated relative to controls ($p = 0.0004$ t-test) (Fig. 7A), as expected for the triplication of the AβPP gene. We then compared the mtDNA alterations found with our published data on β-secretase activity and Aβ peptide levels in DS and DSAD brains.

The β-secretase activity increased in parallel with the mtDNA somatic mutation frequency in the aggregate data from all DS, DSAD, and control brains ($r = +0.55$, $p = 0.0005$) (Fig. 7B). In addition, increased β-secretase activity was correlated with decreased mtDNA amount in DSAD brains ($r = -0.57$, $p = 0.05$) (Fig. 7C). An inverse relationship was seen between mtDNA amount and Aβ levels such that the lower the mtDNA copy number, the higher Aβ_{40} in DSAD brains ($r = -0.61$, $p = 0.02$) (Fig. 7D). The proportion of Aβ_{42} also increased relative to the mtDNA content ($r = +0.72$, $p = 0.008$). Hence at low mtDNA levels, Aβ_{40} predominates (Fig. 7E). Therefore, for DSAD the decline in mtDNA function is associated with increased Aβ production.

DISCUSSION

The current study supports the hypothesis that mitochondrial dysfunction is a major factor in the age-related dementia associated with AD and advanced age DS patients. The frequency of mtDNA RCR mutations was shown to increase with age in control brains, and to be significantly elevated in AD and DSAD brains, relative to age-matched control and DS brains. The increase mtDNA somatic mutation frequency observed in AD brains was also observed in serum DNA samples collected from the same individuals and the frequency of somatic mtDNA RCR mutations was significantly elevated in lymphoblastoid cell lines from AD and DSAD patients relative to lymphoblastoid cell lines derived from age-matched controls. Therefore, for late-onset AD and DSAD individuals, the elevated brain mtDNA RCR mutation frequency is the result of a systemic elevation in the mtDNA mutation frequency.

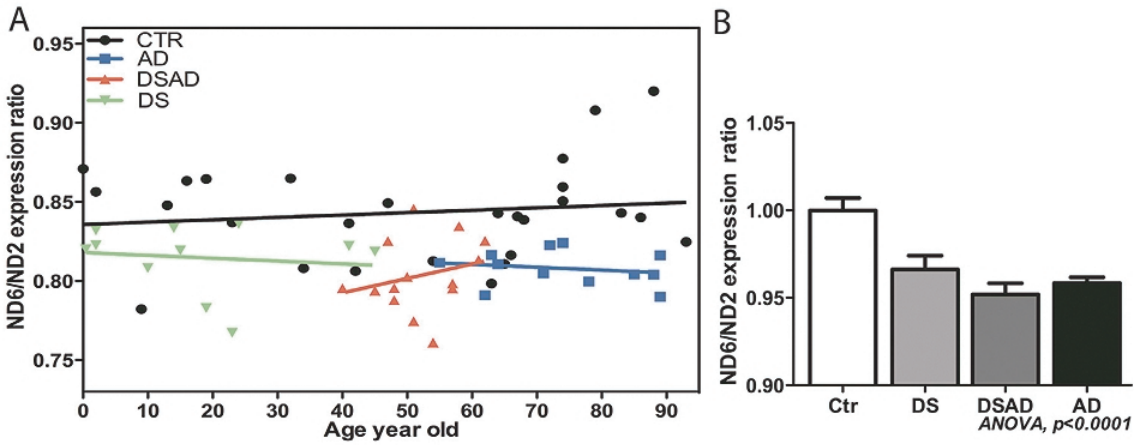

Fig. 6. The ratio of mtDNA L-strand to H-strand transcripts in control, DS, DSAD, and AD brains. A) The correlation of mitochondrial L-strand/ H-strand gene expression ratio with age in all four groups. ND6 gene expression represents the L-stand transcription, and ND2 gene expression represents the H-Strand. B) The L-strand/ H-strand gene expression ratio was reduced in DS, DSAD, and AD brains relative to controls.

The elevated somatic mtDNA mutation frequency in AD and DSAD brains is associated with the accumulation of specific mtDNA RCR mutations, a number of which are rarely if ever found in normal brains. These mutations include the T414G mutation, which accumulates in skin fibroblasts but not the brain during normal aging. While we could not detect this variant in non-demented brains, it was present in 65% of AD brains and 57% of DSAD brains. Since this mutation alters an LSP TFAM binding site [66], it should reduce L-strand transcription. Since L-strand transcription is thought to create the primer for H-strand replication, the T414G mutation should also reduce mtDNA copy number [88]. The T414C mutation was also found in AD.

Other tissue-specific somatic mtDNA mutations were also identified in AD and DSAD brains. Two somatic mtDNA mutations, T408A and A189G, have been found to accumulate in skeletal muscle [89]. Strikingly, somatic mtDNA mutations at nt 408 were found in 31% of AD brains, but not in control, DS, or DSAD brains, blood DNAs or lymphoblasts. Variation in the 408T nt would be expected to alter the mtDNA H-strand replication origins and L-strand transcription [89]. Further evidence that the somatic mtDNA RCR mutations that accumulate in AD and DSAD are functionally relevant come from the statistically significant increase in somatic mtDNA mutations found in the CSBII, LSP, and the nt 1–212 region. The CSBII directs transcriptional termination for generation of the primer for H-strand replication [90], the LSP initiates L-strand transcription which generates the L-strand encoded ND6 mRNA and multiple tRNAs but also is terminated at CBSII to generate the H-strand replication primer [88], and the 1 to 212 nt region encompasses multiple H-strand replication origins [87].

Assuming that post conception somatic mtDNA mutations are central to the pathophysiology of AD, then a high mtDNA mutation load in the brain could result from either a chronically increased mtDNA mutation rate or from the appearance of a single deleterious mutation early in development with subsequent distribution and amplification in multiple organs. The first scenario would account for the diverse array of somatic mtDNA mutations found in most AD and DSAD brains and lymphoblasts, while the second scenario would account for finding the same mutation at high heteroplasmy levels in both brain and blood.

The somatic mtDNA RCR mutations that accumulate in AD and DSAD brains would be expected to affect mtDNA replication and L-strand transcription. This prediction is supported by the fact that both the mtDNA copy number and the mtDNA L-strand transcription levels are significantly depressed in both AD and DSAD brains, relative to age-matched control brains. A reduction in the mtDNA copy number would result in an overall decline in OXPHOS and a disproportionate decrease in the L-stand ND6 transcript would specifically inhibit respiratory complex I. These reductions would result in reduced mitochondrial energy output, increased mitochondrial ROS production, reduced mitochondrial membrane potential and thus altered calcium regulation, and an increased probability for the activation of the mtPTP, predisposing neurons to loss of processes and ultimately death.

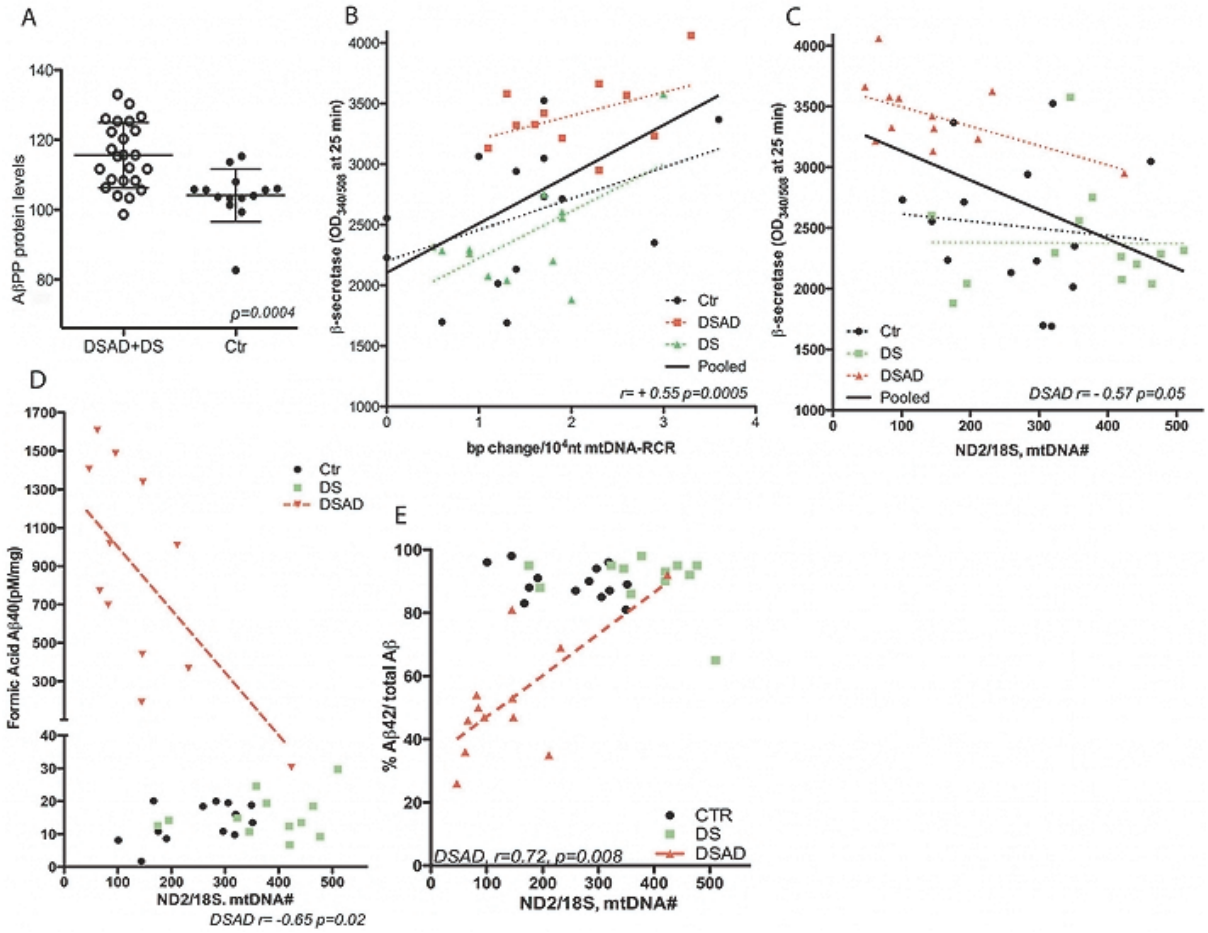

Fig. 7. The comparison of AβPP protein and processing products to mtDNA mutation frequency and mtDNA amount in DS, DSAD, and control groups. A) AβPP protein is overexpressed in DS and DSAD brains compared to controls ($p = 0.0004$, and the values are shown as mean plus SD). B) The correlation of β-secretase activity with mtDNA mutation frequency. The dashed lines show the linear regression of individual groups, whereas the solid black line shows the combination of all three groups. C) The correlation of β-secretase activity with mtDNA amount. The dashed lines show the linear regression of individual groups, whereas the solid black line shows the combination of all three groups. D) The correlation of insoluble Aβ_{40} levels with mtDNA amount. The only significant correlation was found with DSAD group ($r = -0.65$, $p = 0.02$, red dashed lines). E) The correlation of the percent of insoluble Aβ_{42} over total Aβ (i.e., Aβ_{40} and Aβ_{42}) with mtDNA amount. The proportion of Aβ_{42} increased with mtDNA levels in the DSAD group ($r = +0.72$, $p = 0.008$, red dashed lines).

The accumulation of Aβ peptides in AD brains is well-documented and has also recently been documented in DSAD [86]. Since we have shown that mtDNA somatic RCR mutations are increased in AD and DSAD and that mtDNA copy number and L-transcripts are reduced in AD and DSAD, then the increase in mtDNA mutations and decline in mtDNA function should correlate with increased Aβ. This was confirmed by comparing the brain β-secretase activity and Aβ levels determined in DS, DSAD, and control brains [86] with mtDNA parameters, which we determined from the brains of the same subjects. This revealed a direct correlation between increased mtDNA somatic mutations and β-secretase activity in all samples examined. In addition, as mtDNA copy number declined in DSAD brains, the β-secretase and the Aβ levels increased. Hence, mtDNA alterations are directly related to Aβ production. Given that increased mtDNA somatic mutation levels are systemic, this implies that they precede the development of brain dementia and Aβ accumulation. It then follows that the accumulation of Aβ may be a consequence of mitochondrial dysfunction. This would be logical if the function of the Aβ monomer is to protect neurons from mitochondrially generated oxidative stress. However, after prolonged induction, the concentration of Aβ becomes sufficiently high to form oligomers which are the toxic form of Aβ [21]. The elevated Aβ might then drive Aβ into the mitochondri-

on where it inhibits OXPHOS, ultimately activating the mtPTP and destroying the energetically compromised neuron.

This mitochondrial model for age-related dementia provides an explanation for a number of previously unexplained observations on AD patients. Multiple studies have been published in which the mtDNA from peripheral blood cells such as platelets of AD patients have been transferred into cultured human cells that lack mtDNA (ρ^o cells) by cell fusion. These AD mtDNA cybrids have been shown to exhibit mitochondrial defects and have increased Aβ production [91–96]. If AD were specifically due to the accumulation of Aβ in the brains of patients, the AD patients' blood cell mtDNAs should be no different from the mtDNAs of control subjects. However, our discovery is that the increased somatic mtDNA mutation rate is systemic and is reflected in the mtDNA of serum DNA and lymphoblastoid cells, which is consistent with mtDNA defects being transferred from AD blood cells to somatic cell cybrids.

In conclusion, we have shown that somatic mtDNA RCR mutations accumulate systemically in patients with AD and DSAD dementia, that this results in a decline in mtDNA copy number and mtDNA L-strand transcription, and that the decline in mitochondrial function correlates with increased β-secretase activity and the accumulation of Aβ peptide. Since inherited mtDNA variants such as the tRNA^{Gln} nt 4336 mutation has been repeatedly demonstrated to be associated with late-onset AD [52] and a variety of mtDNA mutations have been shown to cause a wide range neurological diseases including dementia [88], it follows logically that the underlying etiology of AD and DSAD is progressive mitochondrial decline. This mitochondrial decline can be initiated in a variety of ways: inherited germline mtDNA ancient or recent variants and mutations, genetic or environmental factors that increase the somatic mtDNA mutation rate, environmental factors that chronically inhibit mitochondrial function, or aberrant over-expression of Aβ leading to oligomerization and inhibition of OXPHOS.

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