

.The resulting cybrids then enable one to determine whether any observed defects in oxidative phosphorylation are attributable to alterations in the patient's mtDNA, since the patient's mitochondria now function in the presence of a different nuclear background. Neurofibrillary tangle bearing neurons showed increased immunostaining with antibodies to advanced glycation end products, heme oxygenase-1, malondialdehyde, 4-hydroxynonenal, protein carbonyl groups, carbonylated neurofilaments, and 3-nitrotyrosine [13,32-38]. Previous studies showed that impairment of cytochrome oxidase in vitro leads to an increase in C-terminal fragments of the amyloid precursor protein, which contain the L-amyloid peptide [39], and a decrease in non-amyloidogenic processing of the amyloid precursor protein [40]. Phosphorus magnetic resonance spectroscopy has demonstrated abnormalities in either phosphocreatine (PCr) or inorganic phosphate (Pi) in Alzheimer's disease patients as compared with elderly controls [11,12]. Familial autosomal dominant Alzheimer's disease is associated with point mutations in the amyloid precursor protein as well as in novel proteins entitled presenilin-1 and presenilin-2. Studies utilizing positron emission tomography consistently show reduced glucose metabolism in temporoparietal regions of Alzheimer's disease patients, and this appears to occur quite early in the disease course [4]. Recent studies have demonstrated that this occurs in patients at risk for Alzheimer's disease [5], and there appeared to be reduced glucose utilization in asymptomatic patients who are homozygous for the Apo E4 allele, a known risk factor for sporadic Alzheimer's disease [6]. An increase in intracellular L-amyloid A₄₂ was found after exposure of cultured guinea pig neurons to hydrogen peroxide, and oxidative stress increased L-amyloid in mammalian lens tissue [41,42]. Reduced ATP generation also leads to activation of ERK1 and ERK2 kinases which phosphorylate tau proteins into a paired helical filament-like state similar to that in AD. Although prior biochemical studies suggested that there were decreases in cytochrome oxidase activity in Alzheimer's disease platelets and cerebral tissue, it was unclear whether this was a primary or secondary effect of the disease process. The cytochrome oxidase activity shows a reduction in catalytic activity yet normal amounts of cytochrome c, suggesting that reduced complex IV activity is a consequence of abnormal catalytic activity rather than decreased enzyme levels [16]. Ethidium bromide is concentrated within mitochondria and preferentially inhibits mtDNA replication in comparison to nuclear DNA replication. The Alzheimer's disease cybrids show elevated basal cytosolic calcium concentrations as well as enhanced sensitivity to inositol-1,4,5-triphosphate mediated calcium release. A sporadic inheritance pattern with familial association and evidence for maternal transmission are characteristic features of known mitochondrial genetic diseases. Recent studies using the cybrid technique to demonstrated that the cytochrome oxidase defects in Alzheimer's disease appear to be encoded on mtDNA [20,21]. Point mutations were found in the cytochrome oxidase-1 and cytochrome oxidase-2 mtDNA encoded subunits of cytochrome oxidase, however further work needs to be done to exclude the possibility that these mutations are not present in nuclear pseudogenes. The study of Smith and colleagues demonstrated a reduction in PCr/Pi ratio in the frontal cortex of Alzheimer's disease patients [13]. Studies of post-mortem cerebral tissue of Alzheimer's disease patients concerned reduced cytochrome oxidase activity [17,18]. Cell lines from a variety of sources can be depleted of

mitochondrial DNA (mtDNA) by exposing them to low concentrations of ethidium bromide. Nuclear pseudogenes are mitochondrial DNA sequences which are randomly incorporated into the nuclear genome by unclear mechanisms, but which exist for much of the mitochondrial genome. Consistent with this possibility we found a 3-fold increase in 8-hydroxy-2-deoxyguanosine content of mtDNA in AD postmortem tissue as compared to age-matched controls [28]. Positron emission tomography studies also show increased oxygen utilization in comparison with glucose utilization in Alzheimer's disease patients [7]. Familial Alzheimer's disease accounts for approximately 5% of all cases.