

TITLE

Mitochondrial pathway polygenic risk scores are associated with Alzheimer's Disease

AUTHORS

Devashi Paliwal^{1*} | Tim W. McInerney¹ | Judy Pa³ | Russell H. Swerdlow⁴ | Simon Easteal¹ |
Shea J. Andrews^{2**} | for the Alzheimer's Disease Neuroimaging Initiative

AFFILIATIONS

¹Department of Genome Sciences, John Curtin School of Medical Research, The Australian National University, Canberra, ACT, Australia

² Department of Neuroscience, Icahn School of Medicine at Mount Sinai, New York, USA

³Mark and Mary Stevens Neuroimaging and Informatics Institute, USC Alzheimer's Disease Research Center, Keck School of USC, Los Angeles, CA 90033

⁴University of Kansas Alzheimer's Disease Center, Kansas City, KS, USA

CORRESPONDING AUTHORS:

*Devashi Paliwal, Department of Genome Sciences, John Curtin School of Medical Research, 131 Garran Road, The Australian National University, Canberra, ACT - 2601, Australia

Email: devashi.paliwal@anu.edu.au

**Shea J. Andrews, Department of Neuroscience, Icahn School of Medicine at Mount Sinai, New York, USA

Email: shea.andrews@mssm.edu

Abbreviations:

nMT-DNA: nuclear-encoded mitochondrial genome

nMT-genes: nuclear-encoded mitochondrial genes

OXPPOS: Oxidative phosphorylation

OXSTRESS: Response to oxidative stress

PRS: Polygenic risk scores

Pathway-PRS: Pathway polygenic risk scores

ABSTRACT

INTRODUCTION: Genetic, animal and epidemiological studies involving biomolecular and clinical endophenotypes implicate mitochondrial dysfunction in Alzheimer's disease (AD) pathogenesis. Polygenic risk scores (PRS) provide a novel approach to assess biological pathway-associated disease risk by combining the effects of variation at multiple, functionally related genes.

METHODS: We investigated associations of PRS for genes involved in 12 mitochondrial pathways (pathway-PRS) related to AD in 854 participants from Alzheimer's Disease Neuroimaging Initiative.

RESULTS: Pathway-PRS for four mitochondrial pathways are significantly associated with increased AD risk: (i) response to oxidative stress (OR: 2.01 [95% CI: 1.71, 2.37]); (ii) mitochondrial transport (OR: 1.81 [95% CI: 1.55, 2.13]); (iii) hallmark oxidative phosphorylation (OR: 1.23 [95% CI: 1.07, 1.41]); and (iv) mitochondrial membrane potential regulation (OR: 1.18 [95% CI: 1.03, 1.36]).

DISCUSSION: Therapeutic approaches targeting these pathways may have potential for modifying AD pathogenesis. Further investigation is required to establish a causal role for these pathways in AD pathology.

Keywords: Alzheimer's disease, Polygenic risk scores, Mitochondria, Mitochondrial dysfunction, Cognitive decline.

1. Introduction

Alzheimer's disease (AD) is a debilitating neurological condition characterized by memory deficits, cognitive and behavioural impairment [1] affecting more than 43.8 million people worldwide [2]. The classical neuropathological hallmarks of AD are the accumulation of amyloid- β peptides into extracellular neuritic plaques and hyperphosphorylated tau into intracellular neurofibrillary tangles in isocortical, subcortical and memory-associated regions of the brain [3]. The substantial attempts to develop drugs based on the role of amyloid- β and tau in AD pathogenesis have led to limited success in identifying disease modifying therapies [4]. This lack of success has led to the exploration of other potential causal mechanisms such as mitochondrial dysfunction.

Mitochondria are intracellular organelles involved in producing energy-carrying ATP molecules through oxidative phosphorylation (OXPHOS) and other cellular processes, including calcium homeostasis, response to oxidative stress (OXSTRESS) and apoptosis [5]. Each mitochondrion possesses its own ~16.5 kb circular genome (mtDNA) encoding 37 genes comprised of 2 ribosomal RNA genes, 22 tRNA genes, and 13 protein-coding genes. There are a further ~1,158 genes in the nuclear genome (nDNA) that also encode proteins involved in mitochondrial function, known as nuclear-encoded mitochondrial genes (nMT-genes) [6].

The mitochondrial cascade hypothesis of AD pathogenesis was first described in 2004 [7]. Briefly, baseline mitochondrial function is genetically determined and declines with age due to environmental and lifestyle factors [7]. This declining mitochondrial function is either the primary event initiating A β - or tau-induced toxicity (primary mitochondrial cascade) or a by-product of the amyloid cascade (secondary mitochondrial cascade) that results in AD pathology [8].

This hypothesis is supported by several lines of evidence. Early epidemiological studies reported a 3-9 fold higher AD risk associated with maternal AD history (possibly associated with maternally-inherited mtDNA) compared with paternal or no AD family history [9]. Altered mitochondrial structures and bioenergetics [10], reduced glucose utilization and functional deficits in several mitochondrial enzymes have been observed in AD brains ([11]). In transgenic *APP* mutant mice, upregulated compensatory mitochondrial mechanisms precede rather than follow amyloid- β plaque deposition and behavioural changes [12]. Cell culture studies demonstrate inhibition of mitochondrial COX enzyme activity [13], and increased mitochondrial-generated reactive oxygen species (ROS) [14] shift A β PP processing towards the amyloidogenic pathway.

Several mitochondrial pathways are dysregulated or dysfunctional in AD. ATP production is reduced due to OXPHOS dysfunction [15], mitochondrial transport is interrupted [16, 17], oxidative stress is increased [18], cellular apoptotic pathways are upregulated [19], intracellular neuronal calcium levels are increased and calcium buffering mechanisms are dysregulated [20], mitochondrial fission is increased and fusion decreased [21], mitophagy is defective [22], and mitochondrial membrane potential ($\Delta\Psi$) is reduced [23]. However, the molecular mechanisms through which the mitochondria mediate, initiate or contribute to AD-related pathology remain unknown and highly debated.

Individually, most variants in the nuclear-encoded mitochondrial genome (nMT-DNA) have sub-threshold ($p > 10^{-8}$) effects on AD risk [24] in genome-wide association studies (GWAS). Greater predictive power is obtained by investigating the combined effect of multiple SNPs as polygenic risk scores (PRS), which can be weighted by their GWAS effect sizes [25, 26]. PRSs can also be composed of genetic variants in multiple genes associated with the same biological pathway, forming a pathway-PRS. Taking this approach,

49 we recently demonstrated that PRS composed of sub-threshold variants in nMT-genes is
50 significantly associated with AD [27].

51 In this study, we use a pathway-based approach, constructing PRSs for sets of genes
52 that encode components of mitochondrial pathways, to investigate their association with AD
53 in a biologically informative way.

2. Methods

2.1 Alzheimer's Disease Neuroimaging Initiative

This study used data from the Alzheimer's Disease Neuroimaging Initiative (ADNI) [28], last accessed on 28 April 2019 (n = 2175). ADNI is a longitudinal study launched in 2004 with the objective of validating amyloid phenotyping, characterizing AD-associated biomarkers, and understanding the genetic underpinnings of AD to inform clinical trial design. ADNI's AD diagnostic criteria are based on both clinical assessments and neurophysiological tests [28]. A case-control study design was employed based on diagnosis at last assessment for each participant.

Participants were excluded if they had any of the following: (i) missing diagnoses (n = 33), (ii) diagnosis of mild cognitive impairment but not AD (n = 558), (iii) missing *APOE* genotype (n = 44) or missing genome-wide sequencing data (n = 517). Only participants with self-reported 'non-hispanic white' ancestry were included in the study to avoid bias due to population stratification, resulting in exclusion of 169 additional samples. Final study sample included 854 participants.

2.2 Genotype data

Genotype data was obtained from the ADNI database (<http://adni.loni.usc.edu>). Details of the collection, curation, processing, and quality-control of ADNI data are described in detail elsewhere [28, 29]. Briefly, nDNA was extracted from whole blood and genotyped on Illumina GWAS arrays for ADNI1 (Illumina Human 610-Quad BeadChip), ADNI GO/2 participants (Illumina HumanOmniExpress BeadChip), and ADNI3 participants (Illumina Omni 2.5M) [28]. *APOE* genotyping of the two SNPs (rs429358, rs7412) that distinguish the $\epsilon 2$, $\epsilon 3$, and $\epsilon 4$ alleles was performed separately for all individuals, and quality controlled [29].

Standard GWAS quality control [30] performed on the ADNI genotype data included: removing variants with call rate <0.95, MAF <1%; deviation from Hardy-Weinberg

equilibrium $p < 10^{-6}$ for CN and $p < 10^{-10}$ for AD cases; call rate < 0.95 ; discordance between reported and genetically determined sex; cryptic sample relatedness (π -hat threshold 0.2); and outlying heterozygosity. Genotype missingness $< 5\%$ was imputed using ADNI MAF alleles for PRS calculation. Principal component analysis (PCA) [31] was performed to correct for residual population stratification in the logistic regression model (see section 2.4).

2.3 Polygenic Risk Profiling

We constructed three whole-genome AD PRSs and 12 mitochondrial pathway-specific PRSs (Table 2) for each ADNI participant using the ‘standard weighted allele’ method implemented in PRSice2 and PRSet [32] for the whole genome and specific mitochondrial pathway gene sets, respectively.

SNPs were weighted by their GWAS effect sizes from the International Genomics of Alzheimer’s Project (IGAP) [24]. We retained GWAS SNPs with p-value threshold (P_T) ≤ 0.5 for computing the PRS as this threshold provided the best model fit for our data (see Appendix C) and is supported by published evidence as the optimum threshold for estimating AD PRSs for common variants [26]. Linkage disequilibrium (LD) clumping was performed for the whole genome (250 kb window, $r^2 < 0.1$) using PRSice-2 [32]. Set-based LD clumping was performed using PRSet [32] for pathway-specific polygenic risk scores to retain only data for SNPs in the gene-set regions (250 kb window, $r^2 < 0.1$).

We omitted loci on sex chromosomes and in the major histocompatibility complex (MHC: 28.47 Mb–33.44 Mb, Chr6, GRCh37 [33]) because estimation of polygenic risk scores in these regions is difficult due to their genomic complexity, i.e. mismapping of reads due to high sequence homology between X and Y chromosomes, and high polymorphic diversity in the MHC region [25, 34]. As a result, 40 X-linked nMT-genes (Appendix A) and 5 nMT-genes in the MHC region (Appendix B) were excluded.

2.3.1 Whole genome polygenic risk scores

Whole genome PRS was estimated in three ways: (i) Whole-genome, i.e., for all included SNPs; (ii) Excluding a 250 kb region of LD around the *APOE* gene (19:45409011 – 45412650 on GRCh37), to assess effects that are independent of the known AD-risk alleles of the *APOE* and *TOMM40* genes [35]; (iii) Excluding the *APOE* region and nMT-genes, to provide a baseline for estimating nMT-gene-specific effects.

2.3.2 Mitochondrial pathway-specific polygenic risk scores

Mitochondrial pathway-specific PRSs were constructed for (i) 12 mitochondrial pathways represented by genesets obtained from the Molecular signatures database (MsigDB) [36] (Table-2) and (ii) the nMT-DNA geneset (comprising all nMT-genes) obtained from Mitocarta 2.0 [6]. Information about the curation and selection of these pathway genesets is detailed in Appendix D. These genesets include all nuclear genes that are known to influence or to be involved in mitochondrial function (Appendix E). Only the SNPs in introns and exons defined by GRCh37 gene boundaries [33] were included for PRS calculation.

Association of *TOMM40* with increased AD risk is confounded by its high LD with the *APOE* [37]. Therefore, for the nMT-DNA and mitochondrial transport genesets, two PRSs were calculated, one that included *TOMM40* and thus the confounding effect of *APOE*, and one that excluded *TOMM40* and thus excluding both the direct effect of *TOMM40* and the confounding effect of *APOE*. All polygenic risk scores were standardized to z-scores with respect to the sample mean.

2.4 Statistical Analysis

2.4.1 Logistic Regression Modelling

Differences in the demographic characteristics of AD cases and CN controls were assessed via one-way ANOVA for continuous variables (age, years of education, Mini Mental State Examination (MMSE) score) and Fisher's exact test for categorical variables (gender, *APOE* genotype). One-way ANOVA was also performed to assess if there were significant

differences in the mean whole genome PRS and nMT-DNA PRS between the two diagnostic groups.

To evaluate the effect of the PRS on AD, a multivariable logistic regression model was run for AD cases vs CN controls. Age, sex, *APOE* ε4 allelic status (ε4 copy number = 0, 1, 2) and the first three principal components (representing population structure) were included as covariates.

All statistical analyses were performed in the R 3.4.4.

2.4.2 Multiple testing burden correction

P-value significance was calculated after correcting for multiple testing burden using two methods (a) False Discovery rate (FDR < 0.05) using the Benjamini-Hochberg procedure [38] embedded within p.adjust() R function, and (b) competitive empirical p-value approach using PRSice and PRSet [32] to compare the outcome of adjusted p-value significance for each pathway using both methods.

A competitive p-value (*Competitive – P*) was obtained for each pathway-PRS as:

$$Competitive - P = \frac{\sum_{n=1}^N I(P_{null} < P_{observed}) + 1}{N + 1}$$

where *Pobserved* is the probability of the observed difference between cases and controls for each pathway-PRS, *Pnull* is obtained for SNPs randomly selected from the background exome in numbers (N) equivalent to those in the pathway-PRSs. Comparisons were made between pseudo-case and control groups to which individuals were randomly assigned. A *Pnull* distribution was obtained from 10,000 permutations.

3. Results

3.1 Research Cohort

Descriptive statistics for ADNI participants (n = 854; CN = 355, AD = 499) are presented in Table 1. There are significant differences between the CN and AD groups for years of education, *APOE* genotype, and MMSE score. Furthermore, the mean whole-genome PRS and complete nMT-DNA PRS are significantly different between the groups, with AD cases having a higher mean PRS compared to CN controls.

3.2 Polygenic scores of the whole nuclear genome and AD risk

The whole-genome PRS is significantly associated with AD, with a 1 SD increase in the PRS associated with AD (OR: 7.42 [95% CI: 5.62, 9.99]). These results remain highly significant even after (i) exclusion of *APOE* region $\pm 250\text{kb}$ (OR: 6.37 [95% CI: 4.81, 8.62]) and (ii) exclusion of both *APOE* region $\pm 250\text{kb}$ and nMT-genes (OR: 6.40 [95% CI: 4.83, 8.67]) (Fig 1). The OR decreases when these gene regions are excluded, but the confidence intervals largely overlap (Fig 1). These ORs are substantially greater than the OR for *APOE* $\pm 250\text{kb}$ region alone (OR: 1.98 [95% CI: 1.68, 2.35]).

3.3 Polygenic scores of nMT-DNA and mitochondrial pathways and AD risk

The nMT-DNA PRS is significantly associated with AD (OR: 1.99 [95% CI: 1.70, 2.35]). In the pathway analyses, four mitochondrial pathways are significantly associated with AD: (i) OXSTRESS (OR: 2.01 [95% CI: 1.71, 2.37]); (ii) mitochondrial transport (OR: 1.81 [95% CI: 1.55, 2.13]); (iii) oxidative phosphorylation (OR: 1.23 [95% CI: 1.07, 1.41]); and (iv) mt $\Delta\Psi$ regulation (OR: 1.18 [95% CI: 1.03, 1.36]; Fig-1). For the mitophagy and regulation pathway-PRS (OR: 1.13 [95% CI: 0.98, 1.31]), the FDR-adjusted p-value is non-significant ($P_{\text{FDR}} > 0.05$), however the competitive p-value is significant ($P_{\text{competitive}} < 0.05$).

The associations of nMT-DNA PRS (OR: 1.63 [95% CI: 1.37, 1.94]) and OXSTRESS (OR: 1.57 [95% CI: 1.32, 1.88]) pathways remain significant after exclusion of *APOE*

175 $\pm 250\text{kb}$ region (Table 3). For the Mitochondrial transport pathway-PRS (OR: 1.23 [95% CI:
176 1.03, 1.48]), the FDR-adjusted p-value remains significant ($P_{\text{FDR}} < 0.05$), but the competitive
177 p-value becomes non-significant ($P_{\text{competitive}} > 0.05$) when the *APOE* $\pm 250\text{kb}$ region is
178 excluded. Omission of the *APOE* $\pm 250\text{kb}$ region was not necessary for the other pathways
179 because they do not contain genes within this region.
180 The phenotypic variance explained by the genetic contribution of each mitochondrial
181 pathway is presented in Appendix F.

4. Discussion

In this study, we investigated the association of AD polygenic risk scores composed of genetic variants located within genes associated with known mitochondrial pathways with AD risk. We found that pathway-PRS composed of the complete nuclear-encoded mitochondrial genome and genes involved in (i) response to oxidative stress, (ii) mitochondrial transport, (iii) hallmark oxidative phosphorylation, and (iv) mitochondrial membrane potential regulation were associated with increased AD risk. The results obtained by the pathway-based approach used here suggest that SNPs in nMT-genes and other nuclear genes involved in mitochondrial pathways significantly contribute to AD risk, suggesting therapeutic potential in targeting them.

Previous multi-omics studies support the role of mitochondrial pathways in AD. For instance, Mostafavi et al. (2018) built molecular networks using modules of co-expressed genes associated with AD and its endophenotypes. They found three modules enriched for gene ontology categories related to mitochondria showing a positive correlation with histopathological β -amyloid burden, cognitive decline, and clinical diagnosis of AD [39]. Similarly, Johnson et al. (2020) conducted co-expression network analysis of AD brains and found that protein co-expression families involved in mitochondrial metabolism strongly correlated with AD, and showed the strongest differences by case status [40]. Muraoka et. al (2020) performed proteomic profiling of AD brain tissues and found that 148 proteins unique to AD group belonged to gene ontology categories associated with mitochondrial metabolism [41].

Our results add to accumulating evidence from genetic, clinical, model cell and animal studies supporting the involvement of these mitochondrial pathways and nMT-genes in AD. We found that a 1 SD increase in the OXPHOS pathway-PRS is associated with 1.23 times increased likelihood of developing AD. The importance of OXPHOS pathway in

generating ATP-energy to support the high cellular energy demand in the brain and the body is well-established [5]. Reduced ATP production due to mitochondrial OXPHOS dysfunction activates a cascade of events leading to neural cell death observed in AD-associated neurodegeneration [15]. Importantly, dysregulation of gene-networks and gene-regulation can also facilitate OXPHOS dysfunction, as demonstrated for *PTCD1* using knock-out and cell-culture models [42, 43].

We found that a 1 SD increase in the mitochondrial transport pathway-PRS is associated with 1.81 times increased likelihood of developing AD. Disruption of mitochondrial transport can lead to defective communication with the nucleus and other cytosolic components. Current evidence indicates that defective mitochondrial transport in AD is primarily due to A β - and tau-interactions blocking mitochondrial channels such as TOMM40 [16], TIM23 [16], TOMM22 [44], and VDAC1 [17]. Hence, it is possible that mitochondrial transport dysfunction is a secondary effect of A β and tau toxicity. The contribution of *TOMM40* to PRS for the mitochondrial transport geneset is difficult to delineate due to confounding from its high LD with *APOE* [37].

We found that a 1SD increase in the OXTRESS pathway-PRS is associated with 2.01 times increased likelihood of developing AD. The OXTRESS pathway, which, when upregulated, activates the eIF2 α /ATF4 axis increasing expression of stress response genes [45], is strongly associated with AD pathology and causes calcium dyshomeostasis, loss of mt $\Delta\Psi$, high mutation rates, interrupted gene transcription and regulation due to high ROS-mediated cellular damage [18]. The OXSTRESS geneset contains the *APOE* gene. Increasing evidence suggests that *APOE* genotype influences mitochondrial stress-related processes in an APOE isoform-specific manner [46]. Therefore, it is likely that SNPs from the *APOE* region contribute to the higher pathway-PRS and AD association.

Finally, we found that a 1 SD increase in the mt $\Delta\Psi$ regulation pathway-PRS is associated with 1.18 times increased likelihood of developing AD. This pathway contains genes encoding proton pumps that regulate the electric charge potential across the mitochondrial membrane to maintain mitochondrial homeostasis and drive processes like the respiratory chain [47]. There is evidence for the formation of a complex through interaction of A β with cyclophilin D (CypD) that provokes mitochondrial and neuronal perturbation, as observed in transgenic *APP* mutant mice [48]. This leads to the Ca²⁺-dependent formation and opening of the mitochondrial membrane permeability transition pore (mPTP), which results in decreased mitochondrial membrane potential, and is accompanied by increased oxidative stress, compromised OXPHOS, release of cytochrome, and impaired axonal mitochondrial transport [49]. Thus, mPTP and CypD have been proposed as potential drug targets. Recent evidence showed that pharmacological blockage of mPTP with Cyclosporine A (CsA) significantly improved mitochondrial and cytosolic calcium dysregulation in AD fibroblasts [23]. Future research is required to investigate their therapeutic potential in AD brain models.

Moderate to high heritability of late-onset AD (LOAD) is indicated by SNP (~ 53%) [50] and twin studies (~ 60-80%) [51]. *APOE* is the strongest known genetic predictor of LOAD risk. It explains up to 13% of the phenotypic variance [50], which implies that at least 37% of the phenotypic variance is explained by genetic variation outside this region [50]. This could explain our result that the OR estimates for AD risk remain significant regardless of the inclusion and exclusion of *APOE* region (± 250 kb window) from the whole genome PRS (Fig-1).

The nMT-DNA, mitochondrial transport, and OXSTRESS genesets contain genes located in the *APOE* ± 250 kb region [52]. Interestingly, the OR estimates for nMT-DNA and OXSTRESS pathways are similar to the estimate for the *APOE* ± 250 kb region. Yet, the

association of nMT-DNA and OXSTRESS pathway-PRS with AD remains significant even after the exclusion of the *APOE* ± 250 kb region. Thus, reinforcing the contribution of non-*APOE* genes in conferring substantial polygenic risk for these pathways. However, the competitive p-value for mitochondrial transport is non-significant on the exclusion of *APOE* ± 250 kb region, which includes the *TOMM40* gene. This result may reflect the known high risk of *APOE/TOMM40* loci [37]. Because of high LD, the contributions of these two loci to PRS cannot be separated. Exclusion of the region may, therefore, result from either removal of a real effect of *TOMM40* variation or removal of a confounding effect of *APOE* variation.

These results should be interpreted in conjunction with some study limitations. First, the ADNI cohort is relatively small and our results require replication. Second, only participants of European ancestry were included, therefore these results may not be generalizable to other ancestrally diverse populations. Finally, our pathway-PRS may underestimate of true genetic risk conferred by mitochondrial pathways. We could not include mtDNA SNPs previously implicated with AD risk [53] since large-scale AD GWAS for mtDNA variants are currently unavailable.

The primary strength of this study is the pathway-driven, biologically informed approach to polygenic risk scoring. The pathway-PRS OR estimates presented in this paper are comparable to those previously reported for high risk single GWS variants [52]. This is because pathway-PRS effectively capture the cumulative small-effects of sub-threshold variants within genes involved in mitochondrial pathways. This results in a larger combined effect size and hence higher statistical power to detect association with AD than association testing of GWS variants individually.

In conclusion, this study demonstrated that the genetic variation within the OXPHOS, mitochondrial transport, OXSTRESS, and mt $\Delta\Psi$ regulation pathways captured by pathway-PRS significantly influences AD risk. These findings contribute to the growing evidence of a

281 mitochondrial role in AD and suggest these pathways as potential targets in ameliorating AD
 282 pathogenesis. However, it remains to be determined if mitochondrial dysfunction is the
 283 primary cause of AD pathogenesis. Further investigations are required to validate and
 284 establish the causal role of nMT-genes and pathways in AD pathology.

Table 1. Demographics of ADNI participants (n = 854) included in this study.

	Diagnosis		SMD ^c	p-value ^c
	CN	AD		
Number of individuals (N)	355	499		
Age (M (SD) years) ^b	79.45 (6.82)	78.41 (7.63)	0.143	p < 0.05
Male (%) ^a	192 (22.4)	295 (34.5)	0.102	p > 0.05
Education (M (SD) years) ^b	16.39 (2.60)	15.51 (2.89)	0.321	p < 0.001
APOE genotype (N (%)) ^a				p < 0.001
ε2+	47 (5.5.)	26 (3.0)	0.284	
ε3/ ε3	219 (25.6)	161 (18.8)	0.708	
ε4+	91 (10.6)	325 (38.0)	0.898	
MMSE (M (SD)) ^b	28.93 (1.38)	19.77 (6.09)	2.074	p < 0.001
Whole genome PRS (M (SD)) ^b	-0.47 (1.02)	0.34 (0.84)	0.864	p < 0.001
nMT-DNA PRS (M (SD)) ^b	-0.35 (0.94)	0.25 (0.97)	0.626	p < 0.001

^aNumber of individuals (N) and percentage of cohort (%) have been reported for gender and APOE genotypes.

^bMean (M) ± standard deviation (SD) have been reported for age, education, MMSE (mini-mental state examination score), whole genome PRS, and nMT-DNA PRS.

^cOne-way ANOVA was performed for continuous variables (age, years of education, MMSE score, whole genome PRS, and nMT-DNA PRS) and Fisher's exact test for categorical variables (gender, APOE genotypes). SMD (standardized mean difference) and p-values have been reported.

APOE = Apolipoprotein E. CN = Cognitively Normal; AD = Alzheimer's Disease; PRS = Polygenic Risk Score; nMT-DNA = nuclear-encoded mitochondrial DNA.

Table 2. Genomic regions and pathway genesets for which polygenic risk scores were calculated.

Polygenic Risk Score	Total genes^a	nMT-genes^a	SNPs^b
Whole nuclear genome	N/A	N/A	515164
Whole nuclear genome (excluding <i>APOE</i> $\pm$ 250 kb)	N/A	N/A	515058
Whole nuclear genome (excluding <i>APOE</i> $\pm$ 250kb and nMT-DNA ^b)	N/A	N/A	83066
nMT-DNA ^c	1158	1158	13992
Response to Oxidative Stress	352	60	5803
Hallmark Oxidative Phosphorylation	245	211	2399
Mitochondrial Transport	179	107	2394
Apoptotic mitochondrial changes	58	25	922
Mitochondrial membrane potential regulation	54	21	1124
Mitochondrial crosstalk	38	0	1061
Mitochondrial fission and regulation	24	13	652
Fatty acid beta-oxidation	21	14	211
Calcium homeostasis and transport	19	7	402
Mitochondrial fusion	19	11	262
Mitophagy and regulation	171	24	3401

^aTotal number of nuclear genes and nuclear-encoded mitochondrial genes (nMT-genes) in each geneset have been reported.

^bTotal number of SNPs included for polygenic risk score calculation for each pathway are reported.

^cnMT-DNA comprises of the complete geneset of nuclear-encoded mitochondrial genome.

APOE = Apolipoprotein E.

Table 3. Significant associations of polygenic risk scores for different genomic regions or pathways with Alzheimer's Disease.

Genomic region/ pathway	Including <i>APOE</i> region				Excluding <i>APOE</i> (± 250 kb window)			
	Beta ^a	SE ^b	FDR-adjusted	Competitive	Beta ^a	SE ^b	FDR-adjusted	Competitive
			p-value ^b	p-value			p-value ^b	p-value
Whole genome	13.70	0.14	1.36×10^{-41}	1.07×10^{-65}	12.46	0.14	9.78×10^{-35}	1.34×10^{-52}
<i>APOE</i> +/- 250 kb region	8.08	0.08	2.33×10^{-15}	9.98×10^{-19}	8.08	0.08	N/A	N/A
nMT-DNA	8.38	0.08	3.68×10^{-16}	1.23×10^{-15}	5.63	0.08	1.65×10^{-8}	5.09×10^{-7}
Response to oxidative stress	8.32	0.08	4.37×10^{-16}	2.68×10^{-17}	5.01	0.09	7.51×10^{-6}	9.02×10^{-6}
Mitochondrial transport	7.40	0.08	3.97×10^{-13}	4.75×10^{-12}	2.27	0.09	7.12×10^{-3}	6.50×10^{-2}
Hallmark oxidative phosphorylation	2.90	0.07	9.07×10^{-3}	2.41×10^{-3}	2.39	0.07	N/A	N/A
Mitochondrial membrane potential regulation	2.37	0.07	3.78×10^{-2}	1.85×10^{-2}	2.83	0.07	N/A	N/A
Mitophagy and regulation	1.80	0.07	1.34×10^{-1}	1.72×10^{-2}	1.87	0.07	N/A	N/A
Regulation of cytochrome C release from mitochondria	1.46	0.07	2.38×10^{-1}	5.49×10^{-2}	2.19	0.07	N/A	N/A
Mitochondrial fission and regulation	1.32	0.07	2.77×10^{-1}	9.13×10^{-2}	1.33	0.07	N/A	N/A

Apoptotic mitochondrial changes	1.26	0.07	2.82×10^{-1}	3.50×10^{-1}	2.10	0.07	N/A	N/A
Mitochondrial fusion	0.96	0.07	3.88×10^{-1}	6.41×10^{-1}	1.16	0.07	N/A	N/A
Fatty acid beta oxidation	1.05	0.07	3.66×10^{-1}	4.25×10^{-1}	1.73	0.07	N/A	N/A
Calcium homeostasis and transport	-0.66	0.07	5.44×10^{-1}	9.28×10^{-1}	-0.73	0.07	N/A	N/A
Mitonuclear crosstalk	-0.58	0.07	5.60×10^{-1}	4.78×10^{-1}	-0.28	0.07	N/A	N/A

^aBeta denotes beta estimates from multivariate regression between pathway-PRS and diagnosis.

^bStandard errors (SE) and competitive empirical p-value of association are reported.

APOE = Apolipoprotein E. nMT-DNA represents the complete nuclear-encoded mitochondrial geneset.

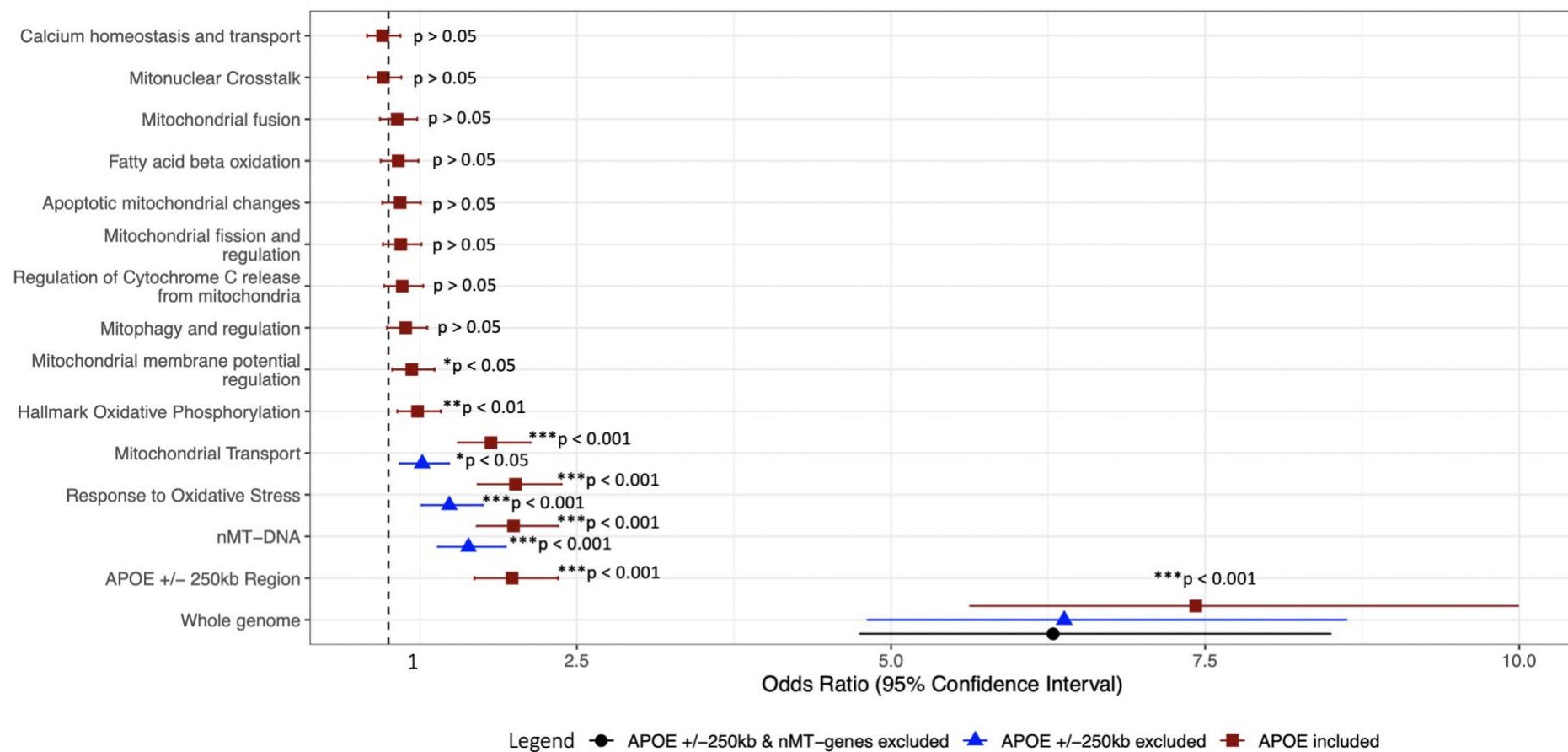


Fig 1. Odds Ratio estimates (95% Confidence intervals) for polygenic risk scores regressed with AD diagnosis. Significance of FDR-adjusted p -values reported here, are interpreted as follows: $*p < 0.05$ (significant); $**p < 0.01$ (very significant); $***p < 0.001$ (highly significant).

Red squares denote inclusion of variants in the APOE region, blue triangles denote exclusion of the APOE $\pm$ 250 kb region, and the black circle denotes exclusion of the APOE $\pm$ 250 kb region and the complete nuclear-encoded mitochondrial genome (nMT-DNA).

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Conflicts of interest

The authors have no competing interests to be disclosed.

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Appendix

Appendices A-F provided.

REFERENCES

- [1] Huang Y, Mucke L. Alzheimer mechanisms and therapeutic strategies. *Cell*. 2012;148:1204-22.
- [2] Nichols E, Szeke CEI, Vollset SE, Abbasi N, Abd-Allah F, Abdela J, et al. Global, regional, and national burden of Alzheimer's disease and other dementias, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Neurol*. 2019;18:88-106.
- [3] Thal DR, Rub U, Orantes M, Braak H. Phases of A beta-deposition in the human brain and its relevance for the development of AD. *Neurology*. 2002;58:1791-800.
- [4] Cummings J, Lee G, Ritter A, Zhong K. Alzheimer's disease drug development pipeline: 2018. *Alzheimer's & Dementia: Transl Res Clin Int*. 2018;4:195-214.
- [5] Cuperfain AB, Zhang ZL, Kennedy JL, Goncalves VF. The Complex Interaction of Mitochondrial Genetics and Mitochondrial Pathways in Psychiatric Disease. *Molecular neuropsychiatry*. 2018;4:52-69.
- [6] Calvo SE, Clauser KR, Mootha VK. MitoCarta2.0: an updated inventory of mammalian mitochondrial proteins. *Nucleic Acids Res*. 2016;44:1251-7.
- [7] Swerdlow RH, Khan SM. A "mitochondrial cascade hypothesis" for sporadic Alzheimer's disease. *Medical hypotheses*. 2004;63:8-20.
- [8] Swerdlow RH. Mitochondria and Mitochondrial Cascades in Alzheimer's Disease. *J Alzheimers Dis*. 2018;62:1403-16.
- [9] Edland SD, Silverman JM, Peskind ER, Tsuang D, Wijsman E, Morris JC. Increased risk of dementia in mothers of Alzheimer's disease cases: evidence for maternal inheritance. *Neurology*. 1996;47:254-6.

- [10] Hirai K, Aliev G, Nunomura A, Fujioka H, Russell RL, Atwood CS, et al. Mitochondrial Abnormalities in Alzheimer's Disease. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2001;21:3017.
- [11] Swerdlow RH, Khan SM. A "mitochondrial cascade hypothesis" for sporadic Alzheimer's disease. *Med Hypotheses*. 2004;63:8-20.
- [12] Reddy PH, McWeeney S, Park BS, Manczak M, Gutala RV, Partovi D, et al. Gene expression profiles of transcripts in amyloid precursor protein transgenic mice: up-regulation of mitochondrial metabolism and apoptotic genes is an early cellular change in Alzheimer's disease. *Human Molecular Genetics*. 2004;13:1225-40.
- [13] Gabuzda D, Busciglio J, Chen LB, Matsudaira P, Yankner BA. Inhibition of energy metabolism alters the processing of amyloid precursor protein and induces a potentially amyloidogenic derivative. *The Journal of biological chemistry*. 1994;269:13623-8.
- [14] Leuner K, Schutt T, Kurz C, Eckert SH, Schiller C, Occhipinti A, et al. Mitochondrion-derived reactive oxygen species lead to enhanced amyloid beta formation. *Antioxid Redox Signal*. 2012;16:1421-33.
- [15] Biffi A, Sabuncu M, Desikan R, Nick S, Salat D, Rosand J, et al. Genetic Variation within the Oxidative Phosphorylation Pathway in Stroke and Alzheimer's Disease *Neurology*. 2014;82:1956.e1-.e19568.
- [16] Devi L, Prabhu BM, Galati DF, Avadhani NG, Anandatheerthavarada HK. Accumulation of amyloid precursor protein in the mitochondrial import channels of human Alzheimer's disease brain is associated with mitochondrial dysfunction. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2006;26:9057-68.
- [17] Manczak M, Reddy PH. Abnormal interaction of VDAC1 with amyloid beta and phosphorylated tau causes mitochondrial dysfunction in Alzheimer's disease. *Hum Mol Genet*. 2012;21:5131-46.

- [18] Wang X, Wang W, Li L, Perry G, Lee H-g, Zhu X. Oxidative stress and mitochondrial dysfunction in Alzheimer's disease. *Biochimica Biophysica Acta*. 2014;1842:1240-7.
- [19] Jia D-P, Wang S, Zhang B-C, Fang F. Paraptosis triggers mitochondrial pathway-mediated apoptosis in Alzheimer's disease. *Exp Ther Med*. 2015;10:804-8.
- [20] Jadiya P, Kolmetzky DW, Tomar D, Di Meco A, Lombardi AA, Lambert JP, et al. Impaired mitochondrial calcium efflux contributes to disease progression in models of Alzheimer's disease. *Nat Comm*. 2019;10:3885.
- [21] Manczak M, Reddy PH. Abnormal interaction between the mitochondrial fission protein Drp1 and hyperphosphorylated tau in Alzheimer's disease neurons: implications for mitochondrial dysfunction and neuronal damage. *Hum Mol Genet*. 2012;21:2538-47.
- [22] Fang EF. Mitophagy and NAD(+) inhibit Alzheimer disease. *Autophagy*. 2019;15:1112-4.
- [23] Pérez MJ, Ponce DP, Aranguiz A, Behrens MI, Quintanilla RA. Mitochondrial permeability transition pore contributes to mitochondrial dysfunction in fibroblasts of patients with sporadic Alzheimer's disease. *Redox Biol*. 2018;19:290-300.
- [24] Kunkle BW, Grenier-Boley B, Sims R, Bis JC, Damotte V, Naj AC, et al. Genetic meta-analysis of diagnosed Alzheimer's disease identifies new risk loci and implicates A β , tau, immunity and lipid processing. *Nat Genet*. 2019;51:414-30.
- [25] Choi SW, Mak TS, O'Reilly PF. Tutorial: a guide to performing polygenic risk score analyses. *Nat Protoc*. 2020;15:2759-72.
- [26] Escott-Price V, Sims R, Bannister C, Harold D, Vronskaya M, Majounie E, et al. Common polygenic variation enhances risk prediction for Alzheimer's disease. *Brain : a journal of neurology*. 2015;138:3673-84.

- [27] Andrews SJ, Fulton-Howard B, Patterson C, McFall GP, Gross A, Michaelis EK, et al. Mitonuclear interactions influence Alzheimer's disease risk. *Neurobiology of aging*. 2020;87:138.e7-.e14.
- [28] Weiner MW, Veitch DP, Aisen PS, Beckett LA, Cairns NJ, Green RC, et al. The Alzheimer's Disease Neuroimaging Initiative 3: Continued innovation for clinical trial improvement. *Alzheimers Dement*. 2017;13:561-71.
- [29] Saykin AJ, Shen L, Yao X, Kim S, Nho K, Risacher SL, et al. Genetic studies of quantitative MCI and AD phenotypes in ADNI: Progress, opportunities, and plans. *Alzheimers Dement*. 2015;11:792-814.
- [30] Marees AT, de Kluiver H, Stringer S, Vorspan F, Curis E, Marie-Claire C, et al. A tutorial on conducting genome-wide association studies: Quality control and statistical analysis. *Int J Methods Psychiatr Res*. 2018;27:e1608.
- [31] Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. *Nat Genet*. 2006;38:904-9.
- [32] Choi SW, O'Reilly PF. PRSice-2: Polygenic Risk Score software for biobank-scale data. *GigaScience*. 2019;8.
- [33] NCBI GRCh37. Human Genome Region MHC. 2019.
- [34] Dawkins RL, Lloyd SS. MHC Genomics and Disease: Looking Back to Go Forward. *Cells*. 2019;8:944.
- [35] Yu C-E, Seltman H, Peskind ER, Galloway N, Zhou PX, Rosenthal E, et al. Comprehensive analysis of APOE and selected proximate markers for late-onset Alzheimer's disease: patterns of linkage disequilibrium and disease/marker association. *Genomics*. 2007;89:655-65.

- [36] Liberzon A. A description of the Molecular Signatures Database (MSigDB) Web site. *Methods Mol Biol.* 2014;1150:153-60.
- [37] Roses A, Sundseth S, Saunders A, Gottschalk W, Burns D, Lutz M. Understanding the genetics of APOE and TOMM40 and role of mitochondrial structure and function in clinical pharmacology of Alzheimer's disease. *Alzheimers Dement.* 2016;12:687-94.
- [38] Benjamini Y, Hochberg Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society: Series B (Methodological).* 1995;57:289-300.
- [39] Mostafavi S, Gaiteri C, Sullivan SE, White CC, Tasaki S, Xu J, et al. A molecular network of the aging human brain provides insights into the pathology and cognitive decline of Alzheimer's disease. *Nat Neurosci.* 2018;21:811-9.
- [40] Johnson ECB, Dammer EB, Duong DM, Ping L, Zhou M, Yin L, et al. Large-scale proteomic analysis of Alzheimer's disease brain and cerebrospinal fluid reveals early changes in energy metabolism associated with microglia and astrocyte activation. *Nature Medicine.* 2020;26:769-80.
- [41] Muraoka S, DeLeo AM, Sethi MK, Yukawa-Takamatsu K, Yang Z, Ko J, et al. Proteomic and biological profiling of extracellular vesicles from Alzheimer's disease human brain tissues. *Alzheimer's & Dementia.* 2020;16:896-907.
- [42] Fleck D, Phu L, Verschueren E, Hinkle T, Reichelt M, Bhangale T, et al. PTC1 Is Required for Mitochondrial Oxidative-Phosphorylation: Possible Genetic Association with Alzheimer's Disease. *The Journal of neuroscience : the official journal of the Society for Neuroscience.* 2019;39:4636-56.
- [43] Pa J, Andrews SJ, Swerdlow RH. Mitochondria and Alzheimer's: Is PTC1 the Smoking Gun? *Trends Neurosci.* 2019.

- [44] Hu W, Wang Z, Zheng H. Mitochondrial accumulation of amyloid beta (A β) peptides requires TOMM22 as a main A β receptor in yeast. *The Journal of biological chemistry*. 2018;293:12681-9.
- [45] Michel S, Canonne M, Arnould T, Renard P. Inhibition of mitochondrial genome expression triggers the activation of CHOP-10 by a cell signaling dependent on the integrated stress response but not the mitochondrial unfolded protein response. *Mitochondrion*. 2015;21:58-68.
- [46] Dose J, Huebbe P, Nebel A, Rimbach G. APOE genotype and stress response - a mini review. *Lipids Health Dis*. 2016;15:121-.
- [47] Zorova LD, Popkov VA, Plotnikov EY, Silachev DN, Pevzner IB, Jankauskas SS, et al. Mitochondrial membrane potential. *Anal Biochem*. 2018;552:50-9.
- [48] Du H, Yan SS. Mitochondrial permeability transition pore in Alzheimer's disease: Cyclophilin D and amyloid beta. *Biochimica et biophysica acta*. 2010;1802:198-204.
- [49] Rao VK, Carlson EA, Yan SS. Mitochondrial permeability transition pore is a potential drug target for neurodegeneration. *Biochimica et biophysica acta*. 2014;1842:1267-72.
- [50] Ridge PG, Hoyt KB, Boehme K, Mukherjee S, Crane PK, Haines JL, et al. Assessment of the genetic variance of late-onset Alzheimer's disease. *Neurobiology of aging*. 2016;41:200.e13-.e20.
- [51] Gatz M, Reynolds CA, Fratiglioni L, Johansson B, Mortimer JA, Berg S, et al. Role of genes and environments for explaining Alzheimer disease. *Archives of general psychiatry*. 2006;63:168-74.
- [52] Kunkle BW, Grenier-Boley B, Sims R, Bis JC, Damotte V, Naj AC, et al. Genetic meta-analysis of diagnosed Alzheimer's disease identifies new risk loci and implicates A β , tau, immunity and lipid processing. *Nat Genet*. 2019;51:414-30.

[53] Hahn A, Zuryn S. Mitochondrial Genome (mtDNA) Mutations that Generate Reactive Oxygen Species. *Antioxidants*. 2019;8:392.