

Mini Review

Resolving the Genetic Confounder in Preclinical Alzheimer's Models

Clarissa Cavarsan*

Department of Biomedical and Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island, Kingston, RI 02881, USA

***Correspondence:** Clarissa Cavarsan, Department of Biomedical and Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island, Kingston, RI 02881, USA, E-mail: clarissacavarsan@gmail.com; DOI: 10.1042/JCTCS.9.2.0064

Abstract

Mitochondrial dysfunction, with impaired respiration and increased reactive oxygen species (ROS), is an early feature of Alzheimer's Disease (AD) models. Characterizing Electron Transport Chain (ETC) organization in animal models is essential for translating preclinical findings. Our recent work exposes a major genetic confounder in the widely used mixed-background 3xTg-AD mouse model. We demonstrate that differences in mitochondrial super complex (mSC) organization and a unique 650 kDa Complex III (CIII) assembly are driven by segregating background strain polymorphisms in the assembly factor Cox7a2l (SCAF1), rather than transgene-induced AD pathology. This commentary synthesizes our findings, highlights implications for preclinical bioenergetic research, and proposes essential methodological corrections for the field.

Keywords: Electron Transport Chain, Mitochondrial super complex, Alzheimer's disease, Reactive oxygen species, COX7RP, SCAF1

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Introduction

Mitochondrial impairments, including altered respiration, elevated ROS, and aberrant fission-fusion dynamics, are documented in AD models and patients, often preceding plaques [1-4]. Since ETC supramolecular organization into mSCs optimizes electron transfer efficiency and reduces ROS leak [5], studying mSC dynamics is a central focus of AD research [4]. However, AD models are often maintained on mixed genetic backgrounds that introduce unanticipated genetic variables [6]. The 3xTg-AD mouse (APP^{Swe}, MAP^{Tg301L}, PSEN1^{M146V}) is classically maintained on a mixed B6;129S background and compared to B6129SF2/J control mice [6]. We recently revealed that strain-specific genetic variation can completely mask or mimic disease-associated mitochondrial phenotypes in these studies [3].

The Cox7a2l genotype discrepancy and mitochondrial phenotypes

The assembly factor Cox7a2l is a critical regulator of CIII2/CIV association and larger respirasome stabilization [3, 5]. While most mammals and mouse strains (like 129S) express a full-length, functional 113-amino acid Cox7a2l variant, C57BL/6 mice possess a shortened 111-amino acid variant featuring a two-amino acid (VP) deletion and an isoleucine-to-phenylalanine (I72F) mutation [5]. This short variant severely limits larger mSC assembly [5]. Using blue-native and clear-native PAGE, we observed that 12-15-month-old 3xTg-AD mice exhibit larger mSC assemblies and a prominent 650 kDa CIII band compared to standard B6129SF2/J control mice [3]. Our genetic sequencing revealed that the 3xTg-AD mice were exclusively homozygous for the long Cox7a2l isoform, whereas B6129SF2/J hybrid controls showed active segregation. Specifically, we found that 24 of 36 control mice were homozygous for the short C57BL/6 variant, while the remaining controls carried one (s/l) or two (l/l) long alleles (Figure 1).

Data-Independent Acquisition Mass Spectrometry (DIA-MS) of excised native gel bands confirmed that Cox7a2l was the most highly upregulated protein in the CIII super complex region and the 650 kDa band in 3xTg-AD mice. When we stratified control mice by their actual Cox7a2l genotype, those harboring the long allele (s/l and l/l) displayed a dose-dependent increase in the 650 kDa CIII band and larger mSCs

that was statistically indistinguishable from 3xTg-AD mice. Furthermore, our DIA-MS analysis of the 650 kDa band identified core complex III subunits (Uqcrc1, Uqcrc2, Cyc1, Uqcrcq, Uqcrb, Mt-Cyb, and Uqcrfs1), confirming that this structure represents a specific Complex III assembly promoted by the long Cox7a2l variant.

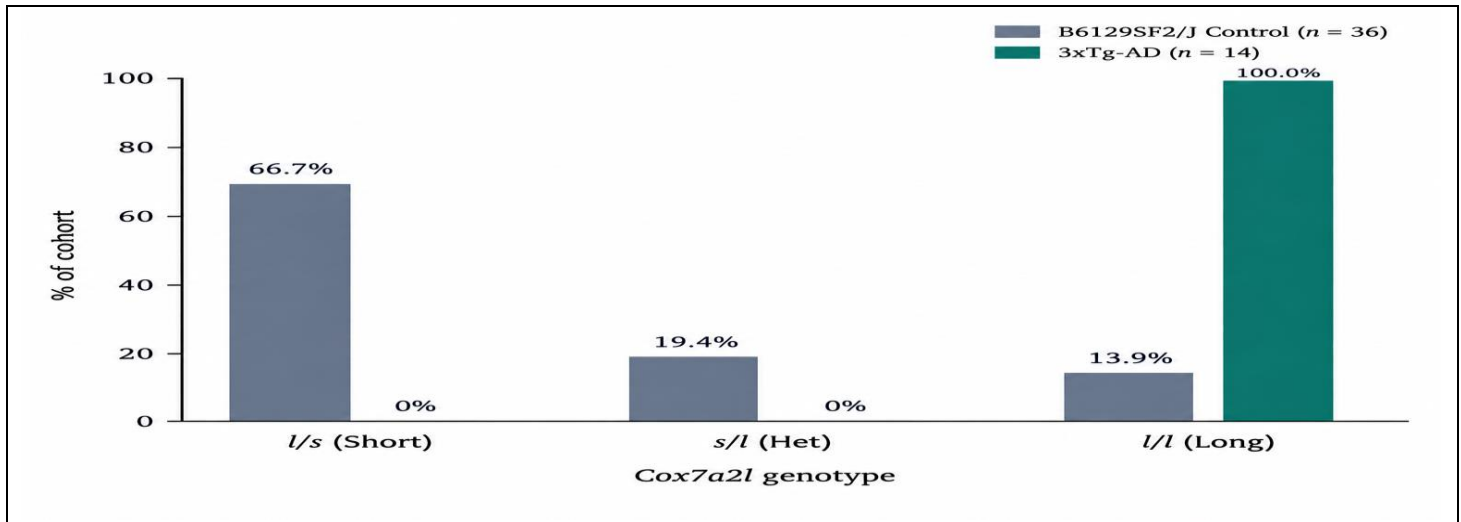


Figure 1: Genotype frequencies of *Cox7a2l* in B6129SF2/J control and 3xTg-AD mice.

Note: Distribution of *Cox7a2l* short (mutated) and long (functional) alleles between control hybrid lines and triple-transgenic AD mice, demonstrating control segregation vs. AD homogeneity.

Intriguingly, we also detected the presence of Amyloid Precursor Protein (APP) within the excised 650 kDa band. Probing duplicate gels with a C-terminal anti-APP antibody confirmed that an APP C-terminal fragment co-migrates with this Complex III assembly. Co-immunoprecipitation targeting UQCRFS1 and APP demonstrated a stable physical interaction, enriching ~40 kDa and ~15 kDa C-terminal APP fragments under denaturing conditions. While this suggests that APP overexpression might interact with ETC assembly, the sufficiency of the long *Cox7a2l* allele to drive the 650 kDa CIII band in controls indicates that this phenotype is genetically driven rather than a consequence of amyloid pathology.

Decoupling AD pathology from background strain

To definitively isolate the role of AD transgenic expression from the *Cox7a2l* genotype, we performed two comparative control experiments. First, our analysis of heart tissue (which lacks neural Thy1.2 promoter-driven mutant APP and MAPT transgene expression) showed the same 650 kDa CIII band discrepancy [6]. Control mice expressing the long *Cox7a2l* allele phenocopied the 3xTg-AD cardiac profile, demonstrating that these mitochondrial differences are independent of APP and Tau pathology. Second, we analyzed transgenic rat models of AD (TgF344 and rTgD), which express mutant APP and PSEN1 but naturally carry only the full-length, functional *Cox7a2l* variant [3, 5]. We found no significant differences in mSC or CIII organization between AD and control rats [3]. This confirms our conclusion that the mSC alterations seen in 3xTg-AD mouse brains result from the functional 129S *Cox7a2l* allele rather than AD pathology [3].

Preclinical and methodological implications

The broader impact of our findings on the preclinical AD research community is profound. A literature search reveals over 1,724 papers utilizing the mixed-background 3xTg-AD mouse model, with 212 specifically investigating mitochondrial alterations. Since many of these publications compared mixed-background 3xTg-AD mice directly to separately maintained hybrid controls without genotyping or matching for *Cox7a2l*, historical conclusions attributing mSC variations, altered respiration, and oxidative stress solely to AD pathology may be substantially confounded. Moving forward, we argue that researchers must adopt rigorous methodological changes.

Recommended methodological guidelines

Mandatory genotyping

When utilizing mixed-background models, genotyping must extend beyond the disease transgenes to include key background modifiers such as *Cox7a2l* [3].

Alternative strain selection

Investigators focusing on mitochondrial structure and bioenergetics should transition to the available 3xTg-AD line on the pure 129S4 background (JAX 031988), which uniformly carries the functional long *Cox7a2l* variant, ensuring genetic homogeneity.

Avoidance of C57BL/6 as baseline mSC models

Standard C57BL/6 mice should be avoided as default models for investigating physiological mSC regulation, as their mutated Cox7a2l variant is unrepresentative of humans and other mammals.

By addressing this genetic confounder, our field can ensure that therapeutic strategies targeting mitochondrial bioenergetics are built on genuine pathophysiological mechanisms rather than genetic background artifacts [3].

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