# Administrative Supplement Research Project

## Focus on Mexican Americans: Mitochondrial Underpinnings of Sex- and Ethnicity-based risk for Alzheimer's disease

TEXAS Alzheimer's Research and Care Consortium
a Darrell K Royal Texas Alzheimer's Initiative

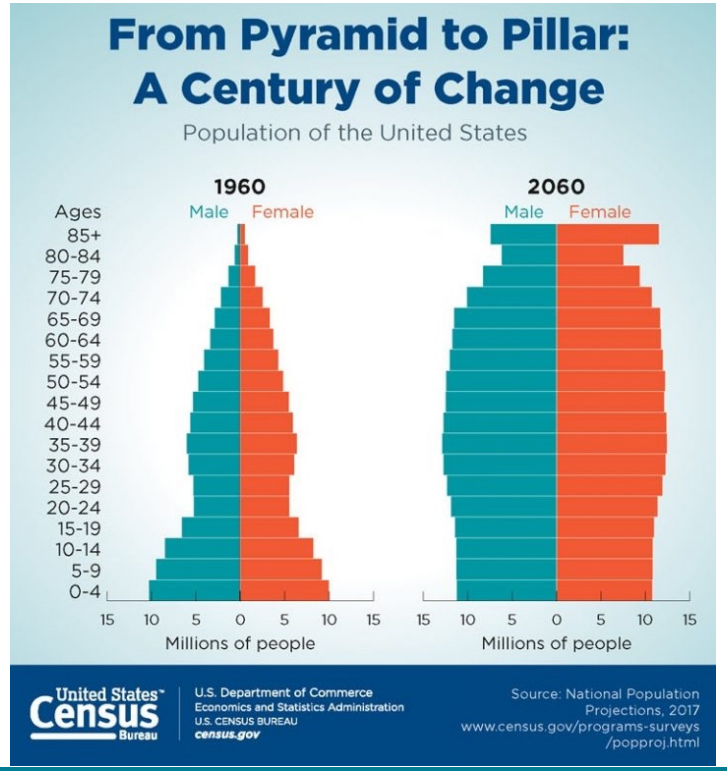
hsc™ THE UNIVERSITY *of* NORTH TEXAS HEALTH SCIENCE CENTER *at* FORT WORTH

Nicole Phillips, Ph.D., M.S.
Assistant Professor
Microbiology, Immunology & Genetics

# The US population is changing

## Age shift…

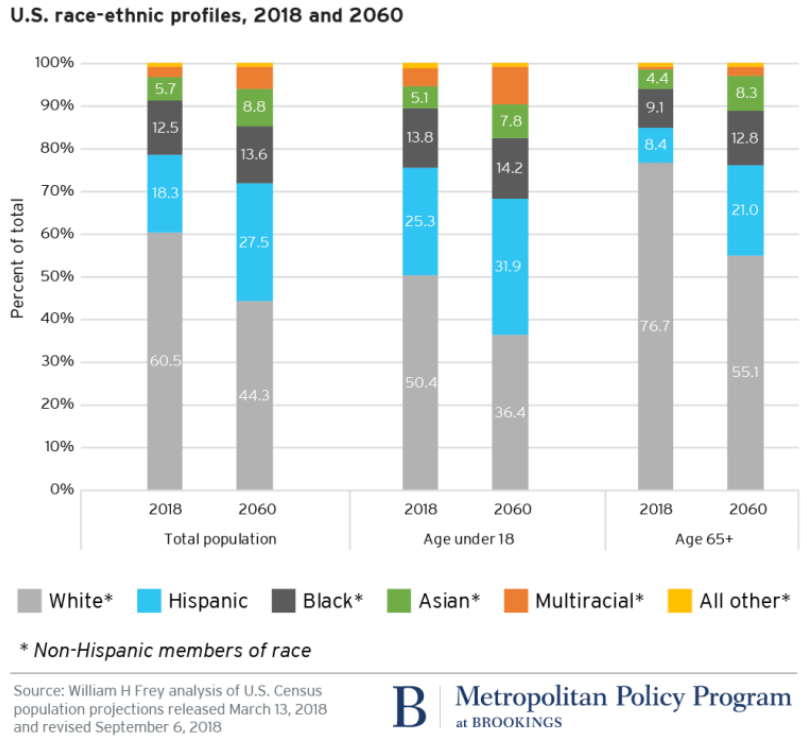
- Living longer
- Large, Baby Boomer generation is now aged 55-73 years




Increased burden of age-related disease, namely **Alzheimer's Disease (AD)**

## Race/ethnicity shift…

- Diversification
- Fast-growing Mexican American population



# Mexican American-AD Health Disparities

*J Alzheimers Dis.* 2013 January 1; 33(2): 373–379. doi:10.3233/JAD-2012-121420.

**Characterization of Mexican Americans with Mild Cognitive Impairment and Alzheimer's Disease**

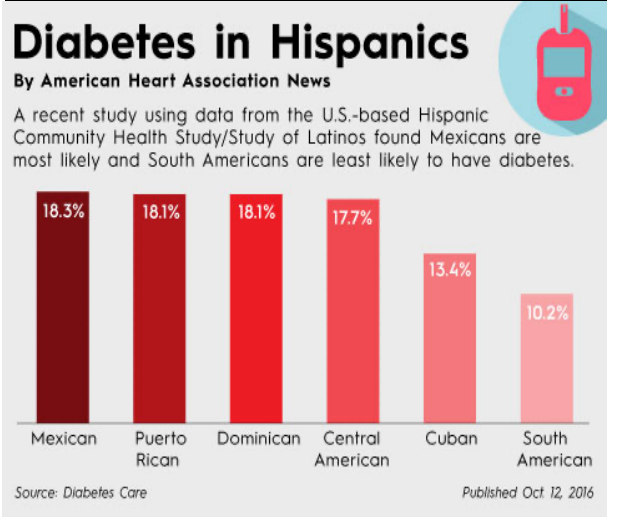
**Sid E. O'Bryant[1,2], Leigh Johnson[1,2], Valerie Balldin[3], Melissa Edwards[1,4], Robert Barber[2,5], Benjamin Williams[6], Michael Devous[7], Blair Cushings[1], Janice Knebl[1], and James Hall[2,8]**

| | Mexican American | | | Non-Hispanic White | | |
|---|---|---|---|---|---|---|
| | AD (n=35) | MCI (n=67) | NC (n=337) | AD (n=160) | MCI (n=97) | NC (n= 376) |
| **Age (years)** | 73.6 (9.1) | 61.9 (12.3) | 58.7 (9.9) | 79.4 (7.0) | 74.4 (10.6) | 65.6 (11.5) |
| **Education (years)** | 5.9 (4.5) | 6.6 (4.2) | 8.1 (4.2) | 13.2 (3.2) | 12.4 (2.5) | 14.3 (2.8) |
| **Gender (%male)** | 45% | 38% | 29% | 39% | 33% | 32% |
| **MMSE** | 18.5 (5.0) | 24.7 (3.6) | 27.5 (2.8) | 21.6 (4.6) | 26.1 (2.7) | 29.0 (1.3) |
| **CDR SB** | 5.5 (3.6) | 0.8 (1.0) | 0.1 (0.4) | 5.4 (3.3) | 1.2 (1.1) | 0.1 (0.4) |
| **GDS** | 9.8 (5.5) | 9.3 (1.5) | 6.1 (5.6) | 5.9 (4.4) | 5.6 (0.7) | 4.4 (4.7) |
| **Depressed (%yes)** | 46% | 44% | 21% | 18% | 29% | 10% |
| **ApoEε4 positive** | 38% | 26% | 19% | 60% | 37% | 23% |
| **Diabetes** | 46% | 51% | 35% | 14% | 29% | 16% |
| **Obese** | 27% | 45% | 47% | 13% | 16% | 25% |

**Mexican Americans and AD Health Disparities**

- **Earlier onset** of cognitive impairment/AD
- Hispanics have **higher incidence** rate of AD-related comorbidities
- Diagnosis **later** and at **more advanced** stage of disease progression
- Women in general have **higher risk** for AD and MA women bear the care-giving burden

2x the prevalence rate compared to non-Hispanic whites



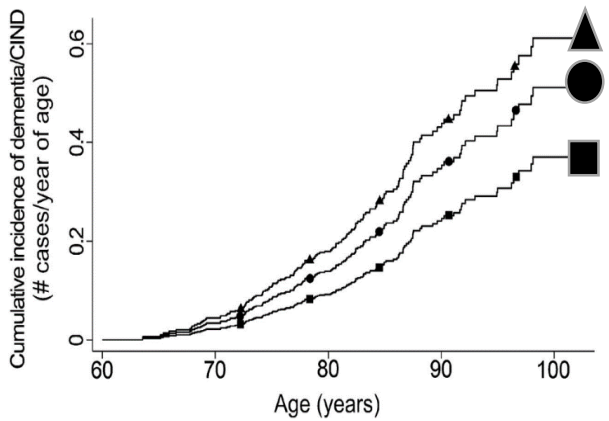



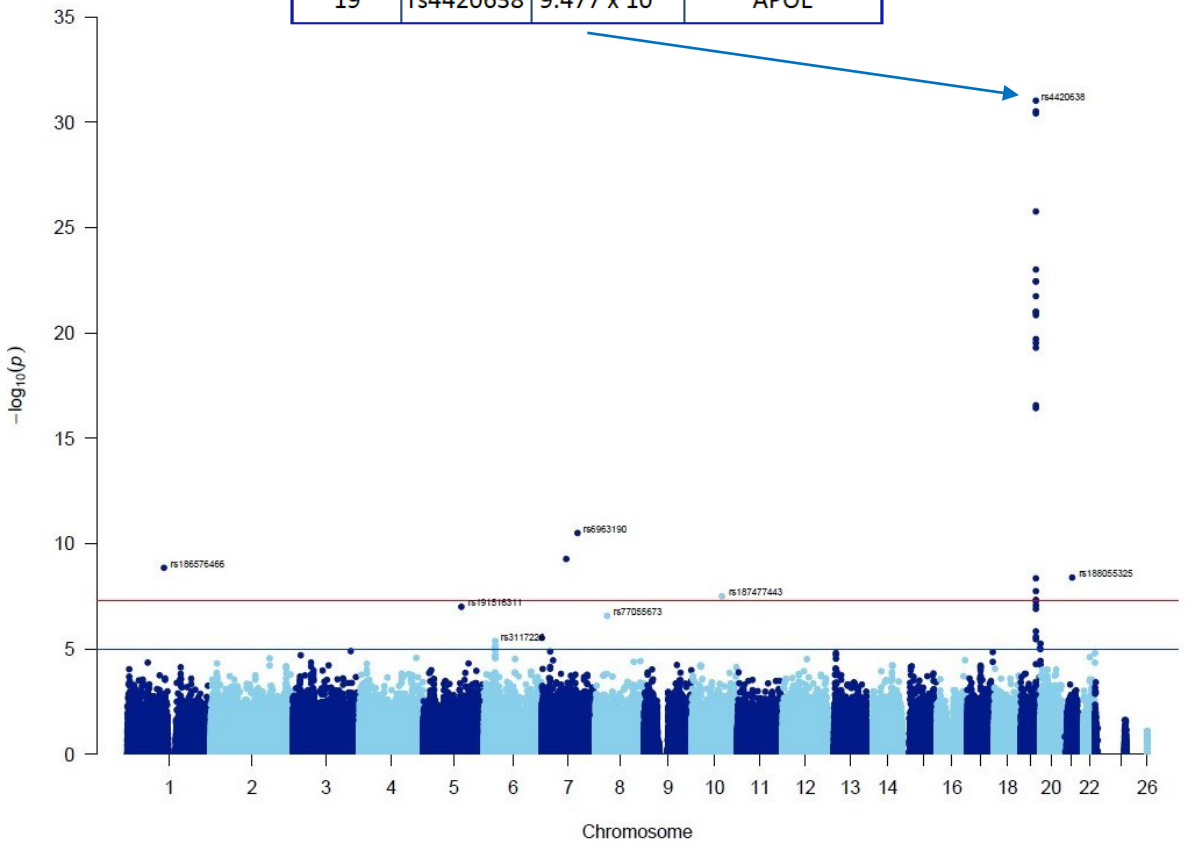

*Figure 1. Cumulative incidence of dementia (CIND) by diabetes status. Squares, no diabetes; circles, diabetes untreated; triangles, diabetes treated. From Mayeda et al., 2013.*

# Biology of MA-AD Health Disparity

- Unique genetic risk factors for dementia…

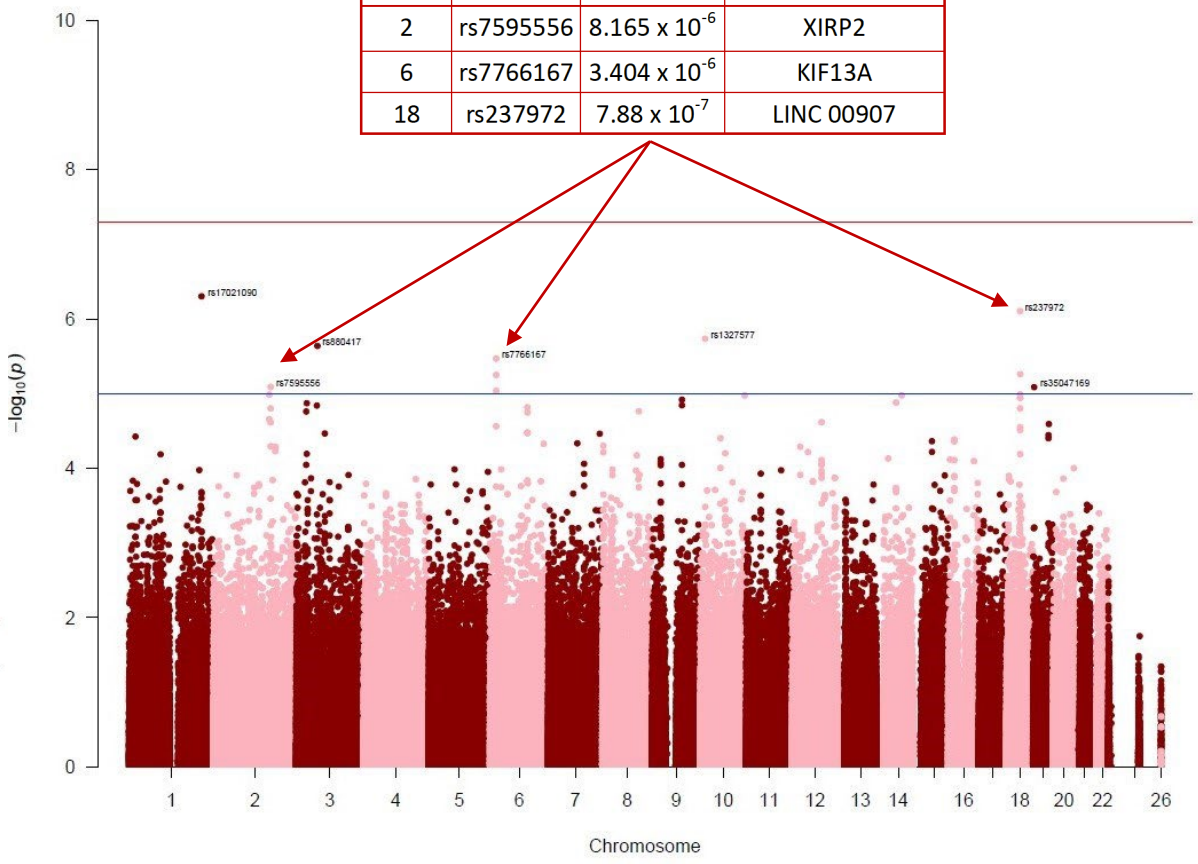
Non-Hispanic Whites

| Chr | Lead SNP | P-value | Gene |
|---|---|---|---|
| 19 | rs4420638 | $9.477 \times 10^{-32}$ | APOE |




Mexican Americans

| Chr | Lead SNP | P-value | Gene |
|---|---|---|---|
| 2 | rs7595556 | $8.165 \times 10^{-6}$ | XIRP2 |
| 6 | rs7766167 | $3.404 \times 10^{-6}$ | KIF13A |
| 18 | rs237972 | $7.88 \times 10^{-7}$ | LINC 00907 |




Colmenarez, M., Barber, R., Phillips, N. R. Genomic Architecture of the latent variable 'D' in Mexican Americans and Non-Hispanic Whites; (2020, In preparation).

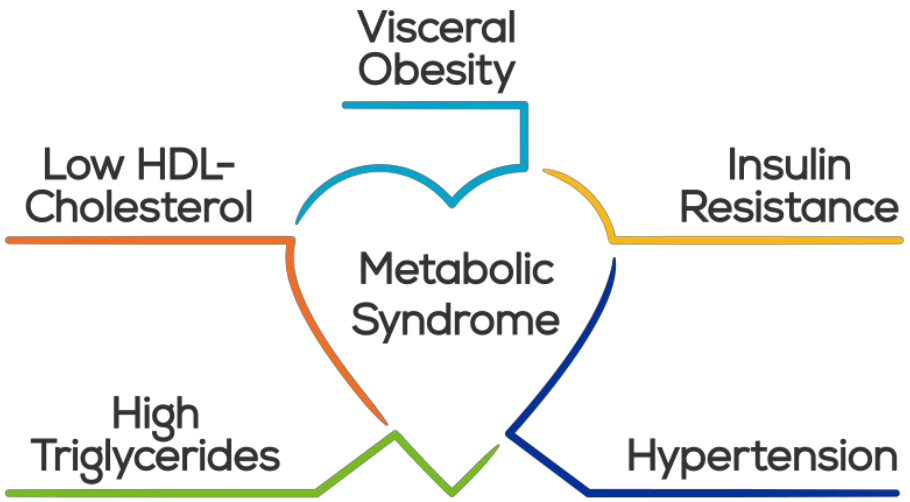
# Biology of MA-AD Health Disparity

- Unique blood-based AD signature…

*J Alzheimers Dis*. 2013 January 1; 34(4): 841–849. doi:10.3233/JAD-122074.

**Biomarkers of Alzheimer's Disease Among Mexican Americans**

**Sid E. O'Bryant[a,b], Guanghua Xiao[c], Melissa Edwards[a,d], Michael Devous[e], Veer Bala Gupta[f,g], Ralph Martins[f,g], Fan Zhang[h], Robert Barber[b,i], and for the Texas Alzheimer's Research and Care Consortium (TARCC)[*]**




Top 30 markers associated with AD among Mexican Americans as compared to non-Hispanic whites

| Top 30 biomarkers of AD among Mexican Americans | Fold Change of Mexican American biomarkers | Top 30 biomarkers previously identified among non-Hispanic whites[22] | Fold Change among non-Hispanic whites |
|---|---|---|---|
| 1. Fatty Acid Binding Protein | 1.70 | Thrombopoietin | 2.18 |
| 2. CD40 | 1.29 | MIP1α | 0.70 |
| 3. Glucagon like peptide 1 | 1.21 | Eotaxin 3 | 1.26 |
| 4. IgM | 0.67 | TNFα | 0.74 |
| 5. Beta 2 Microglobulin | 1.34 | Creatine kinase | 0.80 |
| 6. IGF BP2 | 1.61 | Tenascin C | 1.60 |
| 7. IL8 | 1.09 | FAS | 1.03 |
| 8. Peptide YY | 1.69 | Fibrinogen | 0.87 |
| 9. Macrophage-derived chemokine | 1.09 | IL 10 | 0.76 |
| 10. MIP1α | 1.31 | IL 7 | 1.02 |
| 11. Pancreatic polypeptide | 1.50 | Cancer antigen 19 9 | 1.09 |
| 12. TNF RII | 1.30 | Prostatic acid phosphatase | 0.78 |
| 13. IL18 | 0 98 | Apolipoprotein CIII | 1.12 |
| 14. Myoglobin | 1.34 | Fas ligand | 0.85 |
| 15. CRP | 0.75 | CRP | 0.86 |
| 16. α1-antitrypsin | 1.16 | Pancreatic polypeptide | 1.33 |
| 17. Super oxide dismutase | 1.24 | TIMP 1 | 0.99 |
| 18. Migration inhibitory factor | 1.50 | Angiopoietin 2 | 0.95 |
| 19. Thyroxine binding globulin | 0.95 | Stem cell factor | 0.74 |
| 20. EGF | 1.06 | IL 5 | 0.92 |
| 21. VCAM1 | 1.17 | Lipoprotein a | 1.07 |
| 22. Carcinoembryonic antigen | 1.34 | α2-macroglobulin | 2.45 |
| 23. Glutathione S transferase | 0.69 | ACE CD143 | 1.01 |
| 24. Prostatic acid phosphatase | 1.04 | MCP 1 | 0.85 |
| 25. Tenascin C | 1.23 | Ferritin | 0.97 |

# Biology of MA-AD Health Disparity

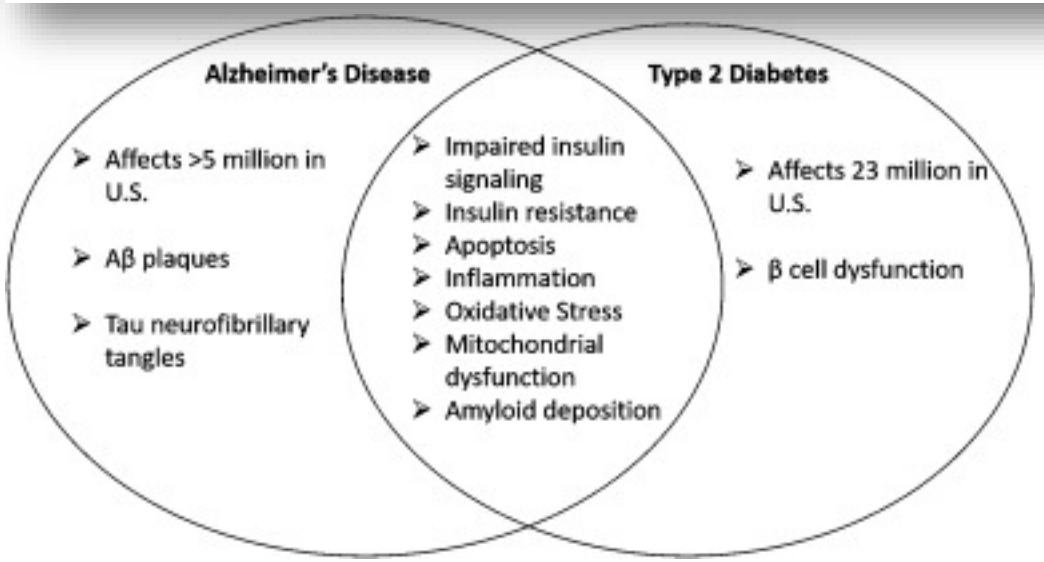
- A role for mitochondrial function…

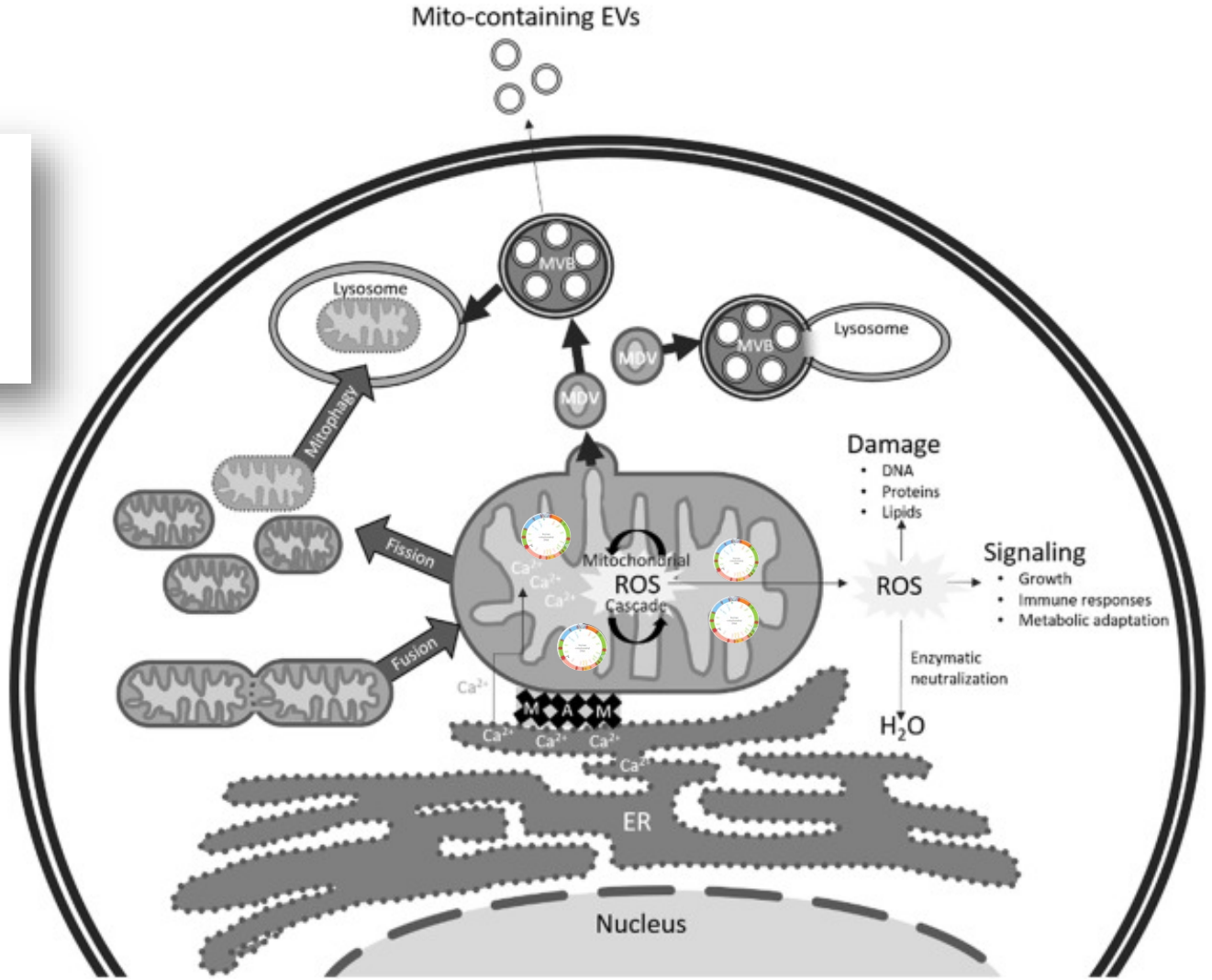
Review

**Etiology of type 2 diabetes and Alzheimer's disease: Exploring the mitochondria**

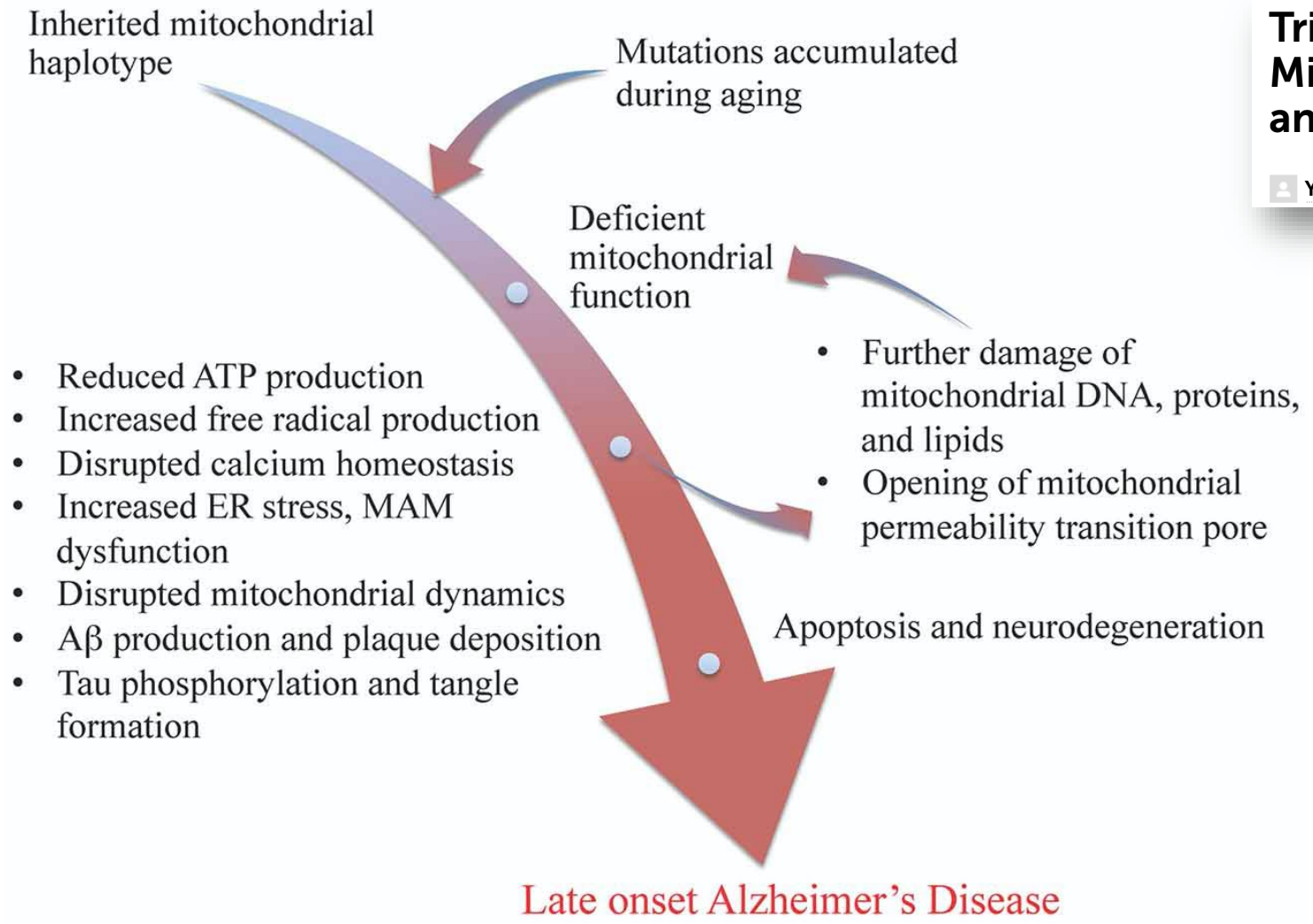
Talisa K. Silzer[a], Nicole R. Phillips[a,b,*]

[a] Department of Microbiology, Immunology and Genetics, University of North Texas Health Science Center, Fort Worth, TX, USA
[b] Center for Alzheimer's and Neurodegenerative Disease Research (CANDR), University of North Texas Health Science Center, Fort Worth, TX, USA



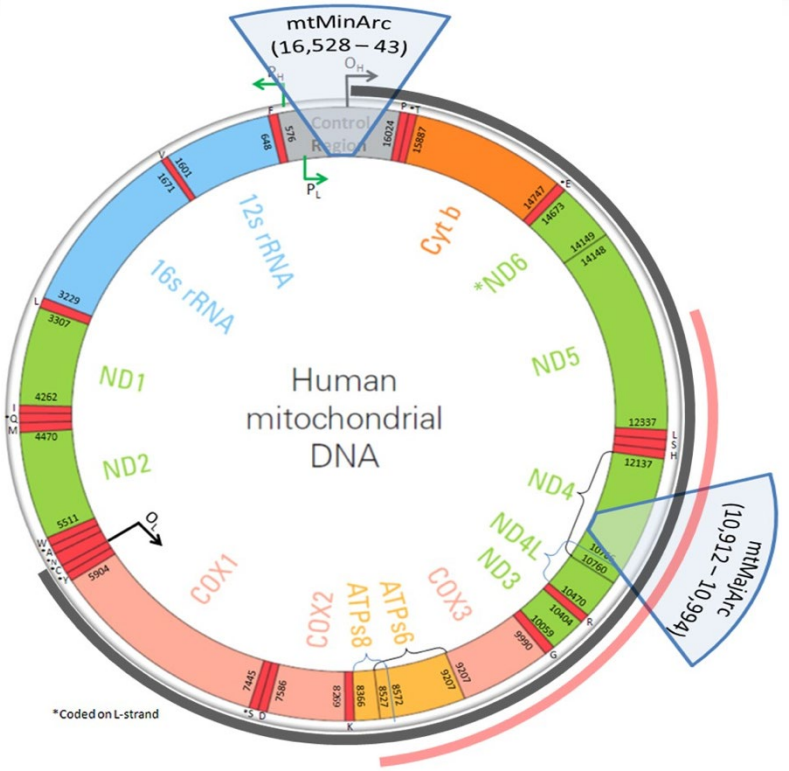



# Mitochondrial DNA



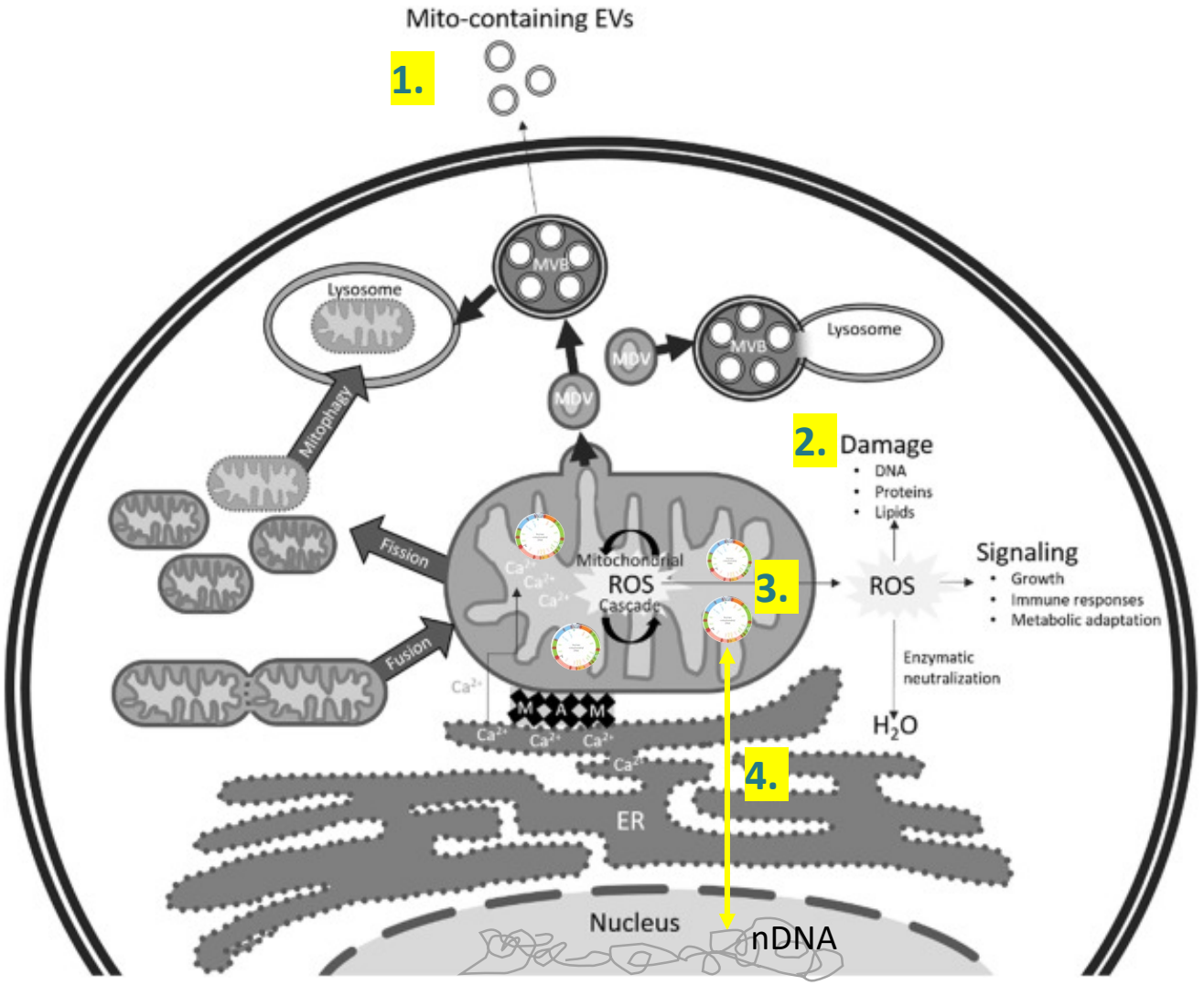

**Triad of Risk for Late Onset Alzheimer's: Mitochondrial Haplotype, APOE Genotype and Chromosomal Sex**

Yiwei Wang[1] and Roberta D. Brinton[2*]



# Key Mitochondrial DNA Phenotypes

1. CFmtDNA- amount of mtDNA in the cell-free space of peripheral blood (i.e., plasma or serum)
2. MLmtDNA- degree of mtDNA damage or mutational load
3. CNmtDNA- mtDNA copy number
4. MitoType and MNC- mitonuclear compatibility



# Our Overarching Hypothesis

**We hypothesize** that blood-based indices of mitochondrial function will reveal sex-and ethnicity-specific mitochondrial dysfunction which is positively associated with T2D and cognitive impairment (AD or MCI) using samples collected as part of the Texas Alzheimer's Research and Care Consortium.

# TARCC Participants

- State-wide research collaboration between the Top 9 biomedical research institutions in Texas, focused on improving diagnosis, prevention, and treatment of AD patients
- Research strategy includes longitudinally-collected biological samples; ~3000 NHW and MA patients

*Table 1. TARCC Enrollment Demographic Summary (updated 2016). Accessed 2/01/2019 at www.txalzresearch.org /.*

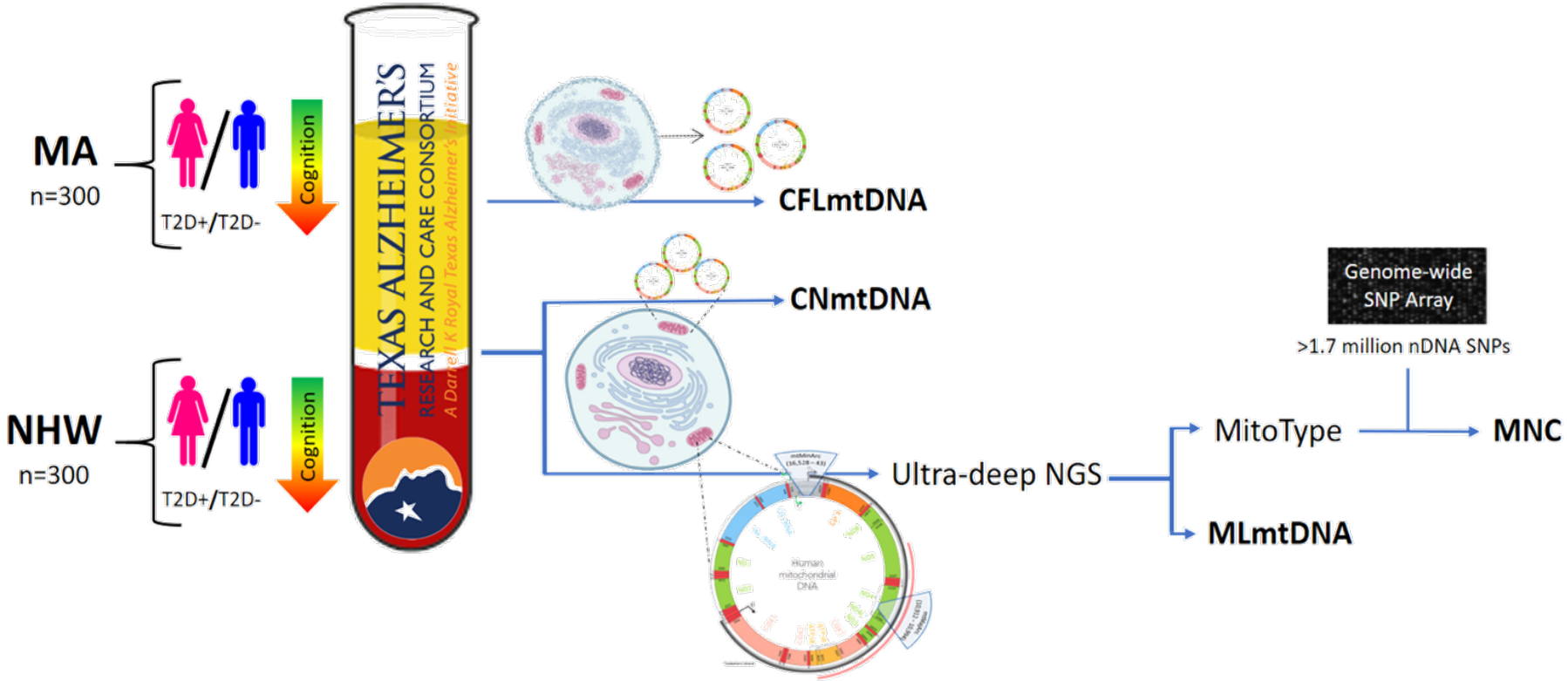
| Total Enrollment Count | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Women | | | | Men | | | | All | |
| Diagnosis | | Hispanic | non-Hispanic | Total | | Hispanic | non-Hispanic | Total | | Hispanic | non-Hispanic | Total |
| AD | | 112 | 603 | 715 | | 62 | 497 | 559 | | 174 | 1100 | 1274 |
| MCI | | 214 | 205 | 419 | | 114 | 190 | 304 | | 328 | 395 | 723 |
| NC | | 528 | 457 | 985 | | 198 | 259 | 457 | | 726 | 716 | 1442 |
| All | | 854 | 1265 | 2119 | | 374 | 946 | 1320 | | 1228 | 2211 | 3439 |
| | | | | | | | | | | | | |
| Total Enrollment Percent | | | | | | | | | | | | |
| | | | Women | | | | Men | | | | All | |
| Diagnosis | | Hispanic | non-Hispanic | Total | | Hispanic | non-Hispanic | Total | | Hispanic | non-Hispanic | Total |
| AD | | 16% | 84% | 56% | | 11% | 89% | 44% | | 14% | 86% | 100% |
| MCI | | 51% | 49% | 58% | | 38% | 63% | 42% | | 45% | 55% | 100% |
| NC | | 54% | 46% | 68% | | 43% | 57% | 32% | | 50% | 50% | 100% |
| All | | 40% | 60% | 62% | | 28% | 72% | 38% | | 36% | 64% | 100% |

TEXAS Alzheimer's Research and Care Consortium
a Darrell K Royal Texas Alzheimer's Initiative

# Overview of Approach

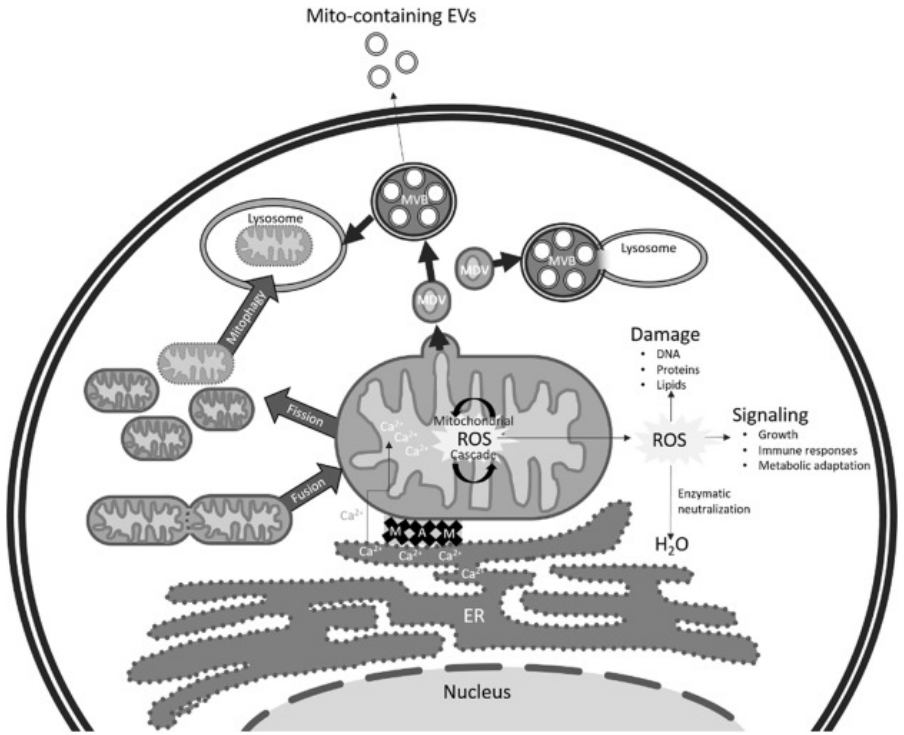
**Specific Aim 1 Research Q:** What are the primary markers of mitochondrial function that indicate cognitive impairment and/or comorbidity in Mexican Americans?

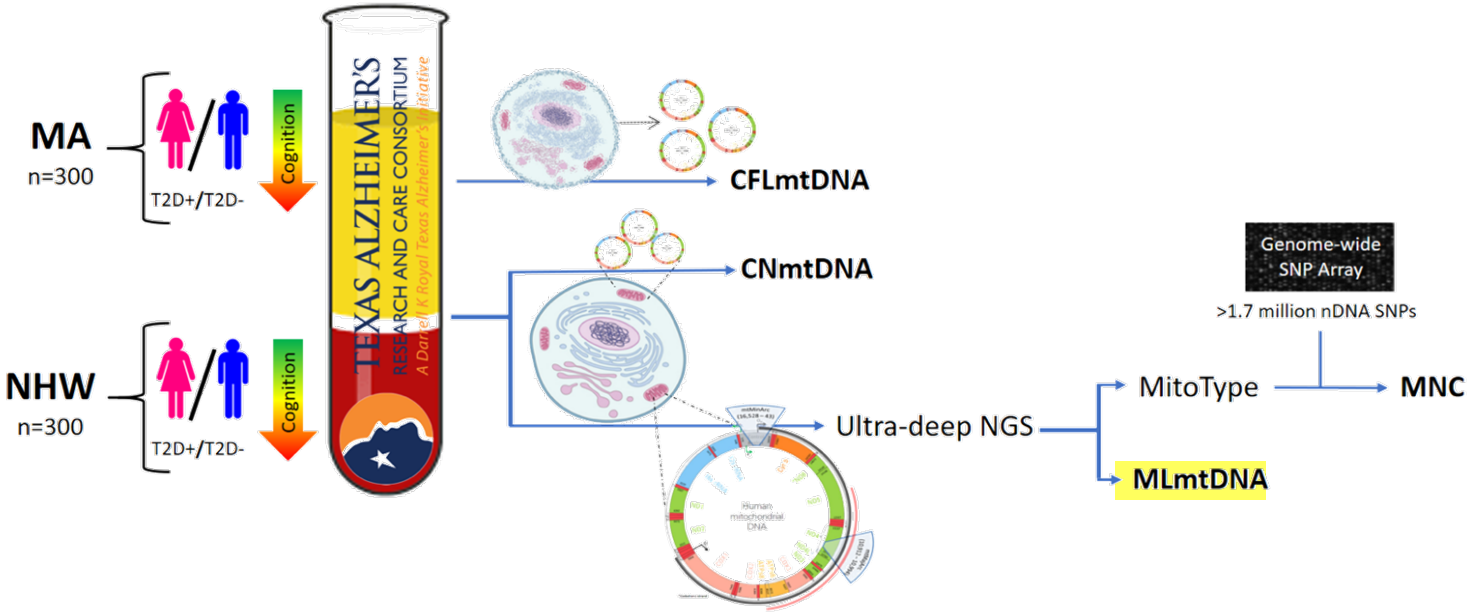
**Specific Aim 2 Research Q:** Can markers of mitochondrial function combined with genetic risk predict cognitive impairment and/or comorbidity in Mexican American women?



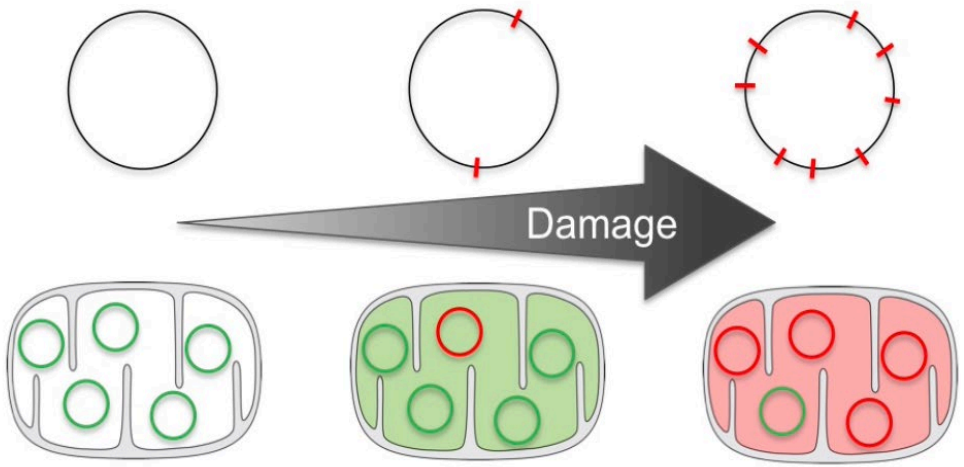
# Specific Aim 1

What are the primary markers of mitochondrial function that indicate cognitive impairment and/or comorbidity in Mexican Americans?



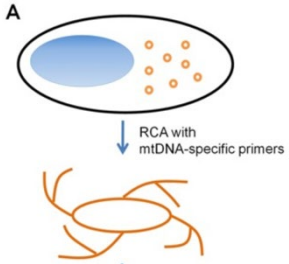



# Background- MtDNA Mutational Load

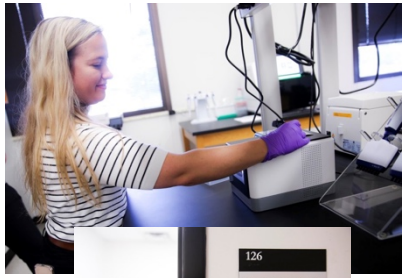


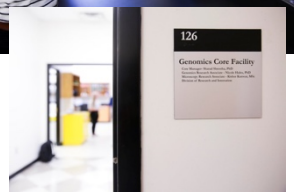

http://www.mrc-mbu.cam.ac.uk/

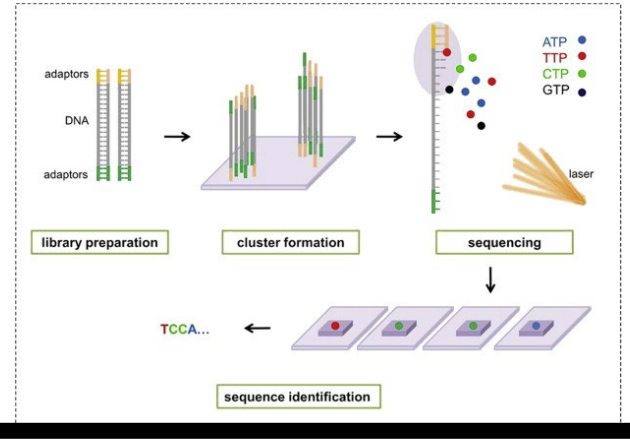
Step 1



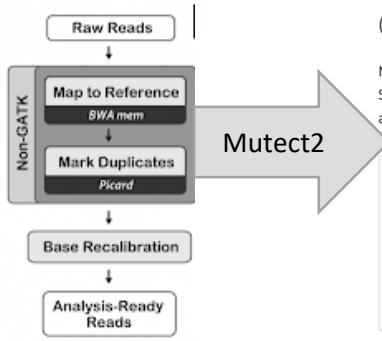

Step 2






Step 3



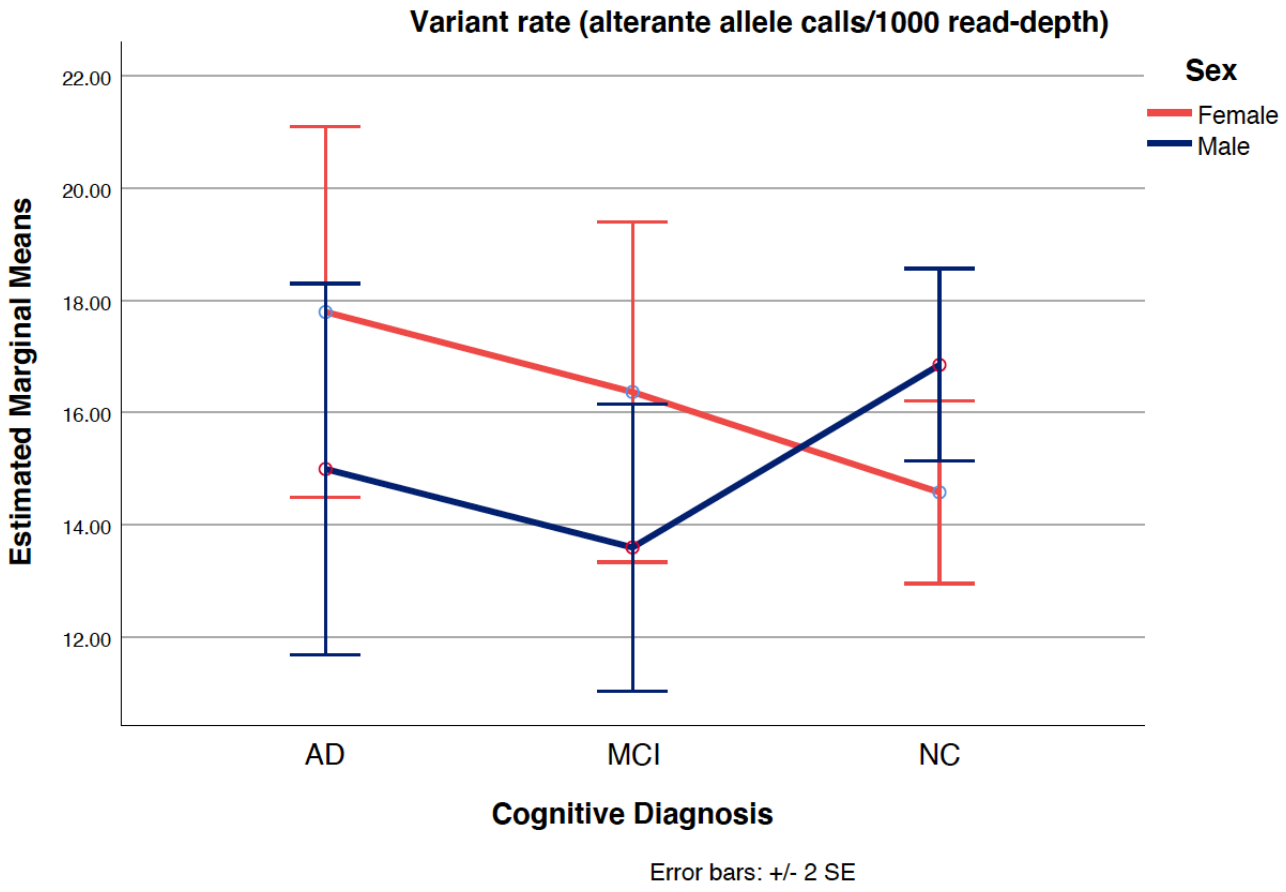

Mutect2

(iii) Mitochondrial mode

Mutect2 automatically sets parameters appropriately for calling on mitochondria with the --mitochondria flag. Specifically, the mode sets --initial-tumor-lod to 0, --tumor-lod-to-emit to 0, --af-of-alleles-not-in-resource to 4e-3, and the advanced parameter --pruning-lod-threshold to -4.

```
gatk Mutect2 \
-R reference.fa \
-L chrM \
--mitochondria \
-I mitochondria.bam \
-O mitochondria.vcf.gz
```

# MtDNA Mutational Load



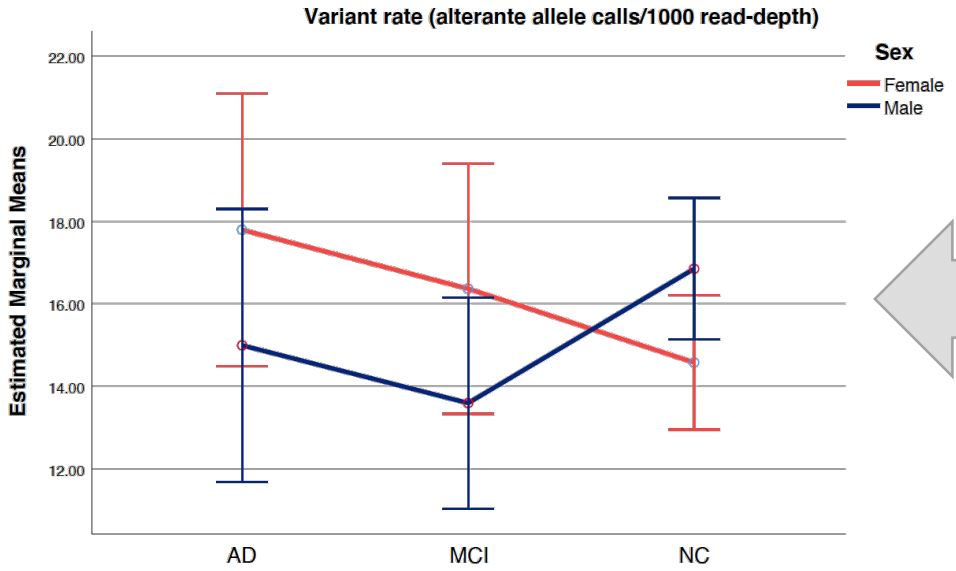

Potential sex-disease status interaction in MLmtDNA

Dependent Variable: Per1000depth_variants

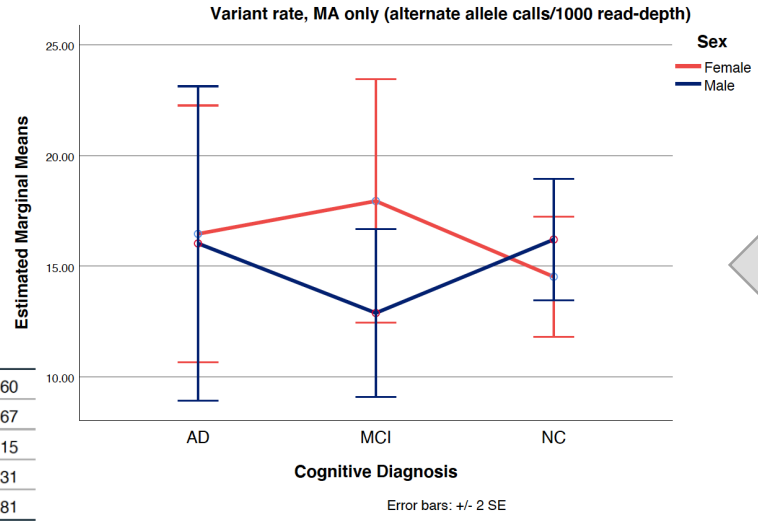
| Source | Type III Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Corrected Model | 476.864[a] | 5 | 95.373 | 1.658 | .145 |
| Intercept | 47122.357 | 1 | 47122.357 | 819.433 | .000 |
| Sex | 57.940 | 1 | 57.940 | 1.008 | .316 |
| vlookup_Diagnosis_Cog | 49.998 | 2 | 24.999 | .435 | .648 |
| Sex * vlookup_Diagnosis_Cog | 398.474 | 2 | 199.237 | 3.465 | .033 |
| Error | 15009.073 | 261 | 57.506 | | |
| Total | 80146.291 | 267 | | | |
| Corrected Total | 15485.937 | 266 | | | |

a. R Squared = .031 (Adjusted R Squared = .012)

# MtDNA Mutational Load

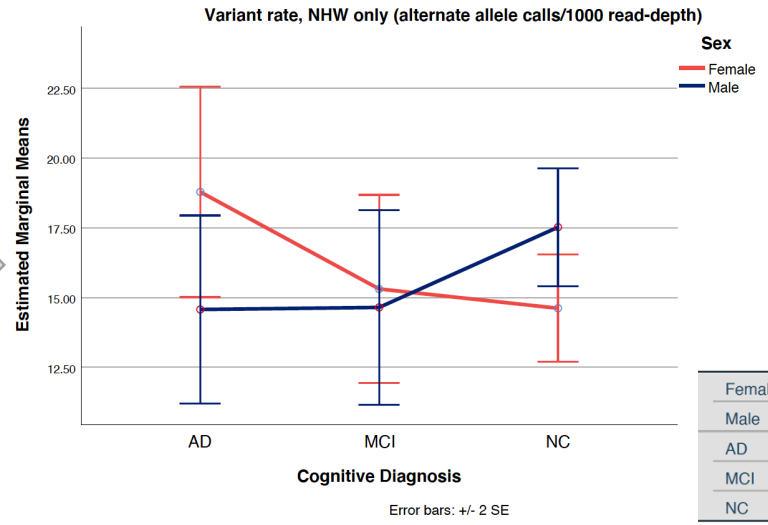



Potential sex-disease status interaction in MLmtDNA




| | N |
|---|---|
| Female | 60 |
| Male | 67 |
| AD | 15 |
| MCI | 31 |
| NC | 81 |

Trends are potentially different in NHW compared to MA.



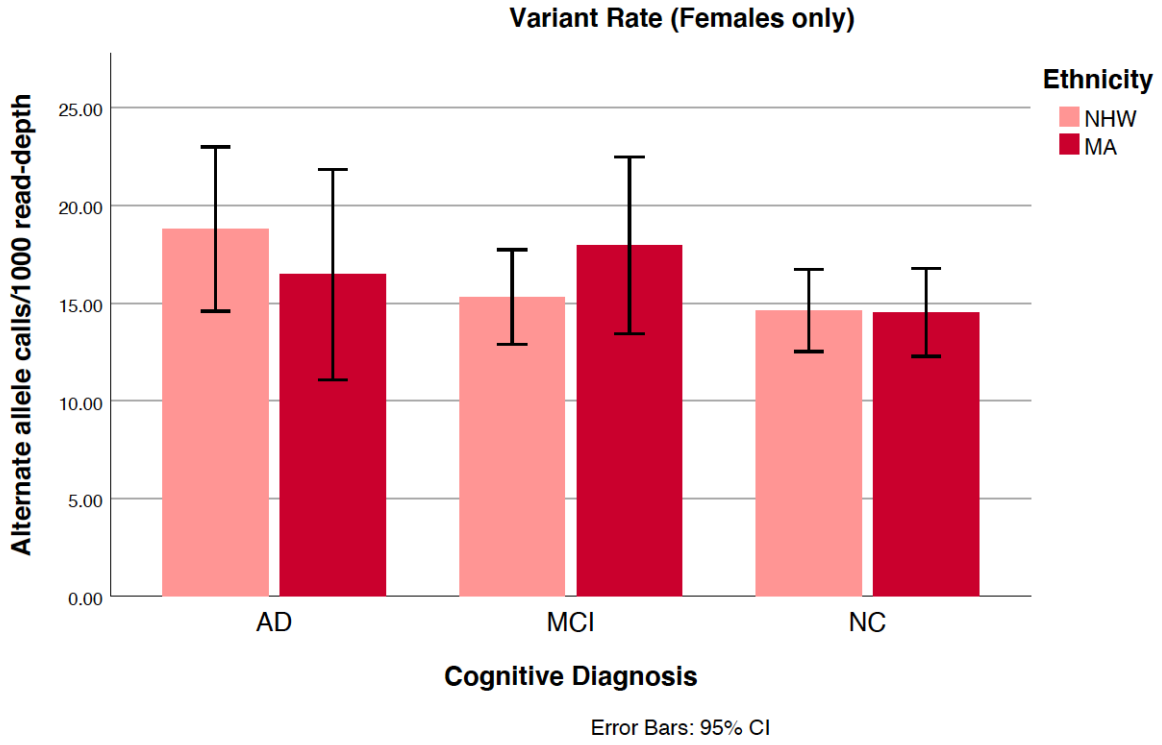

| | N |
|---|---|
| Female | 73 |
| Male | 67 |
| AD | 27 |
| MCI | 29 |
| NC | 84 |

# Mutational Load- by sex and ethnicity

**ANOVA**

Per1000depth_variants

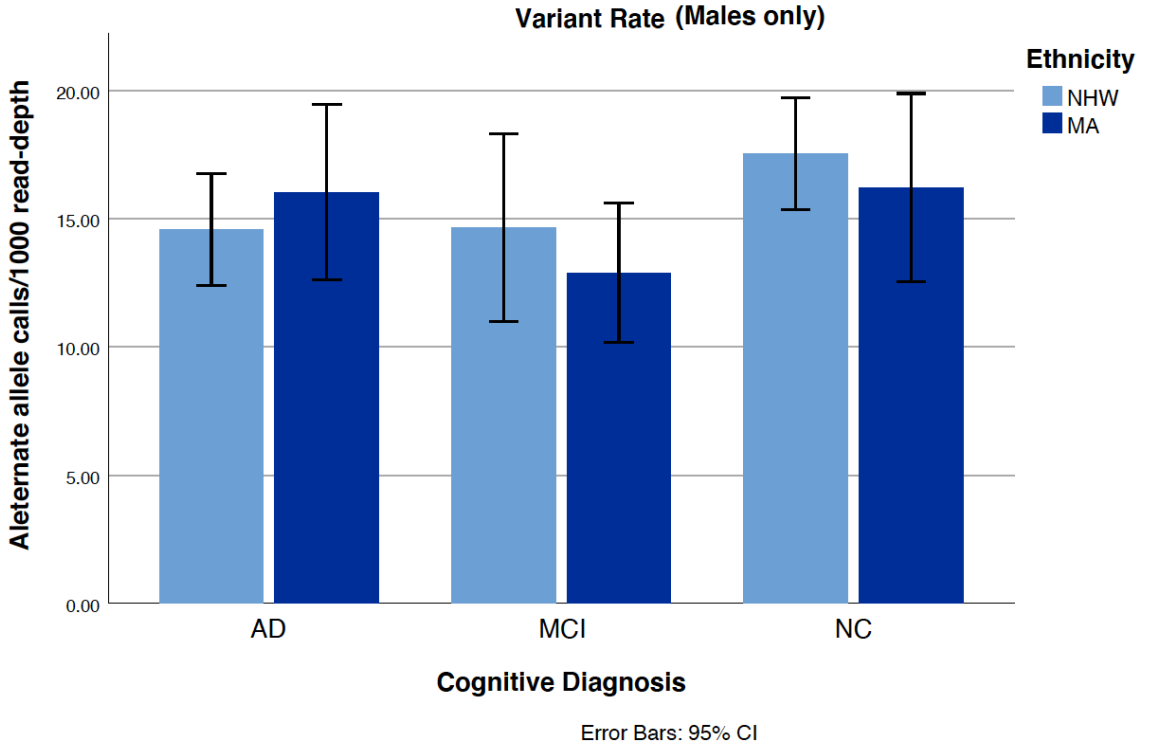
| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 202.556 | 2 | 101.278 | 2.088 | .128 |
| Within Groups | 6305.394 | 130 | 48.503 | | |
| Total | 6507.950 | 132 | | | |




**ANOVA**

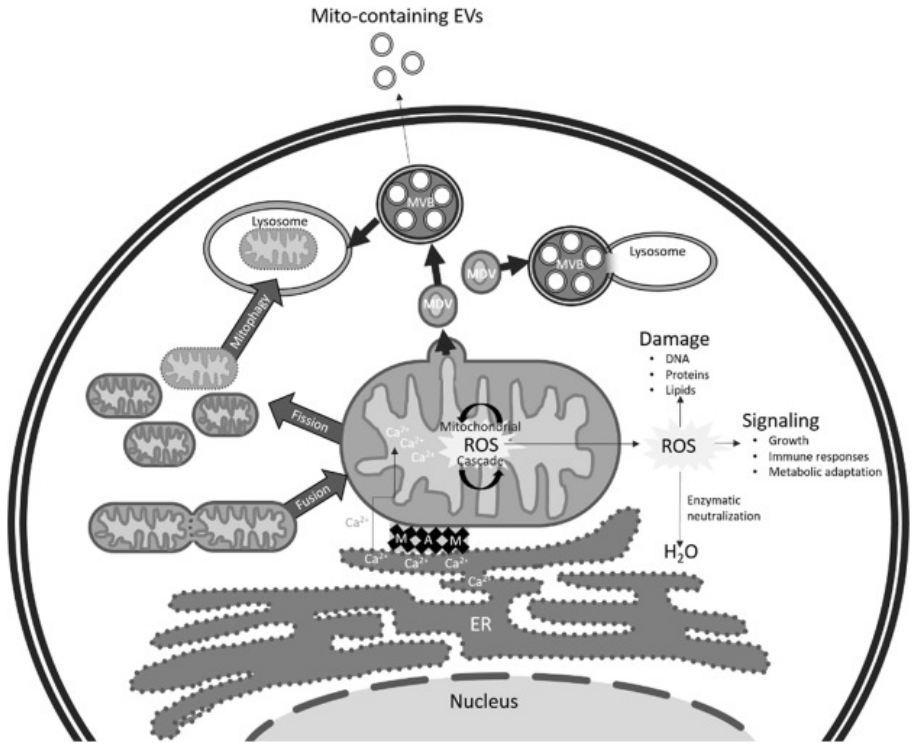
Per1000depth_variants

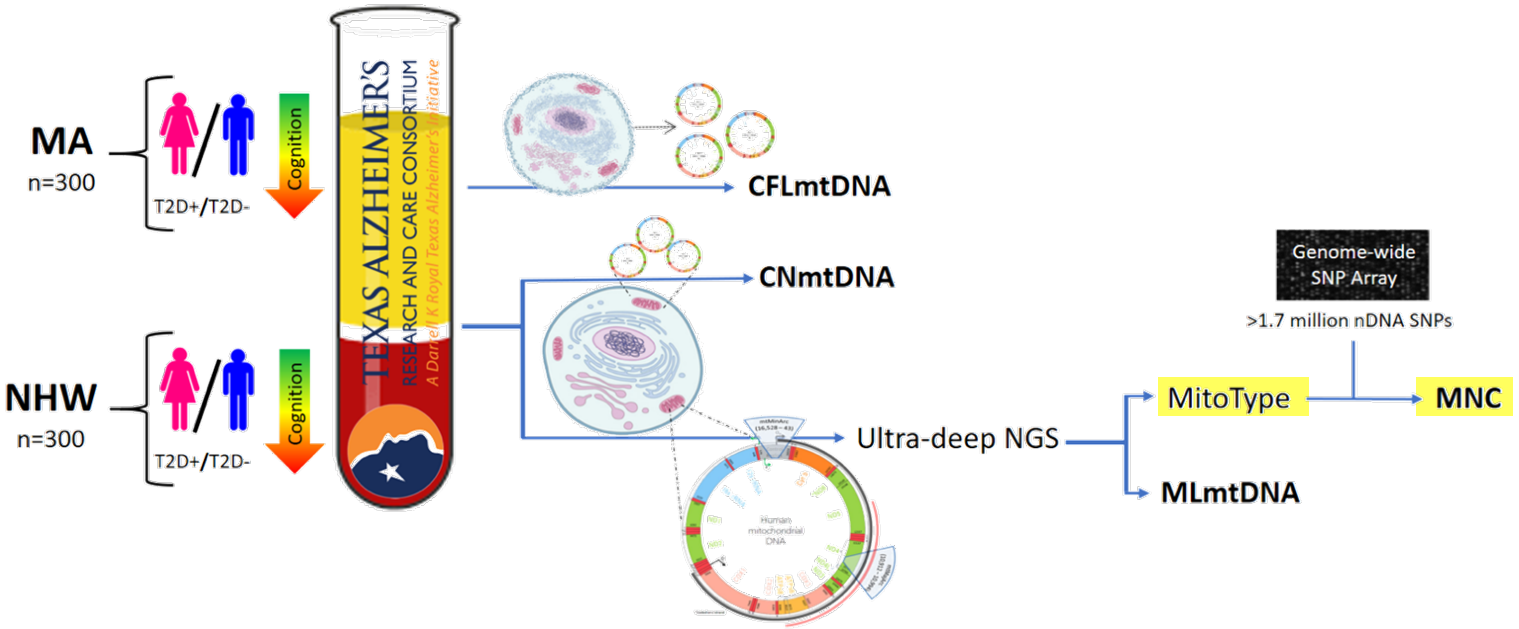
| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 268.818 | 2 | 134.409 | 2.023 | .136 |
| Within Groups | 8703.679 | 131 | 66.440 | | |
| Total | 8972.497 | 133 | | | |



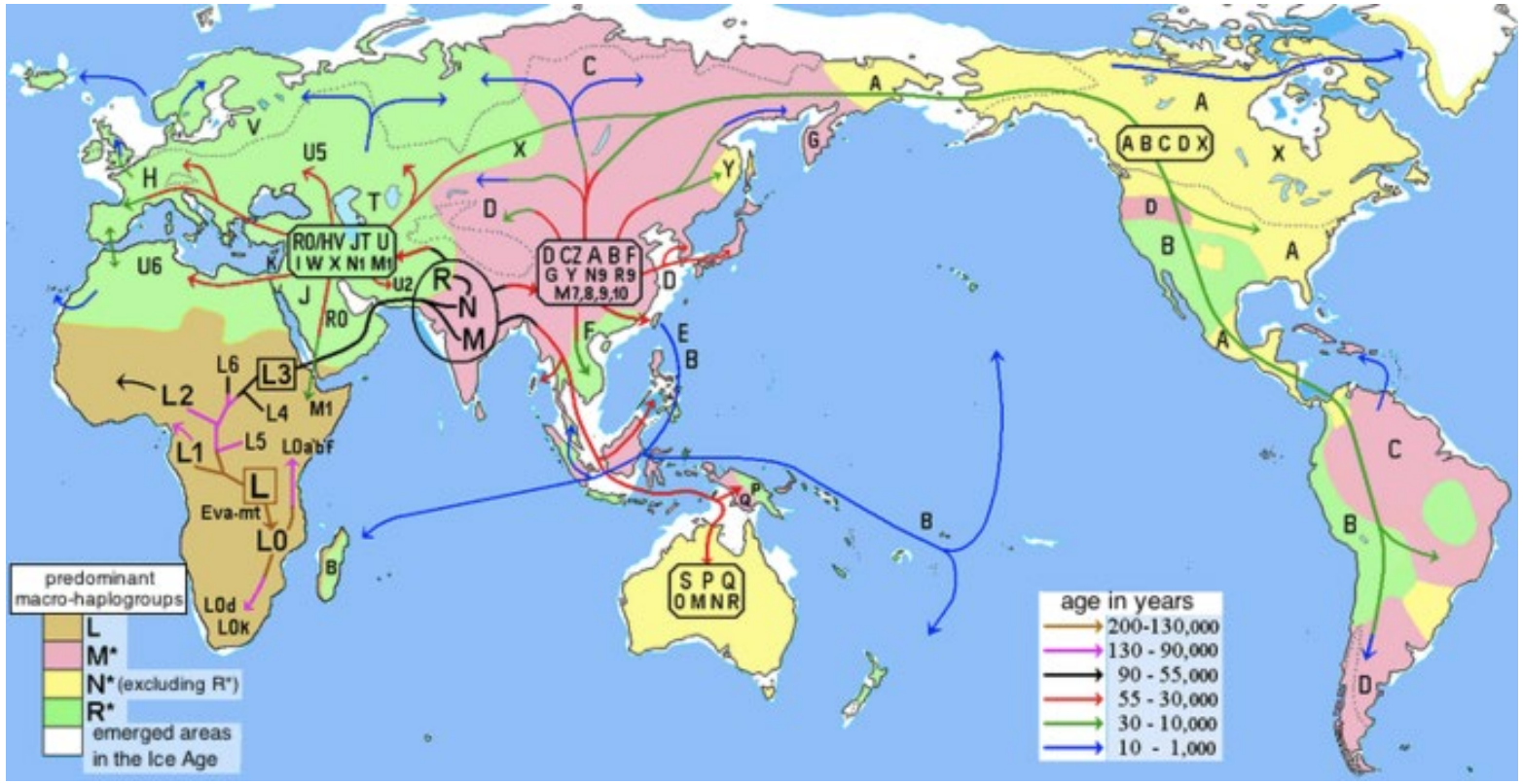
# Specific Aim 2

Can markers of mitochondrial function combined with genetic risk predict cognitive impairment and/or comorbidity in Mexican American women?

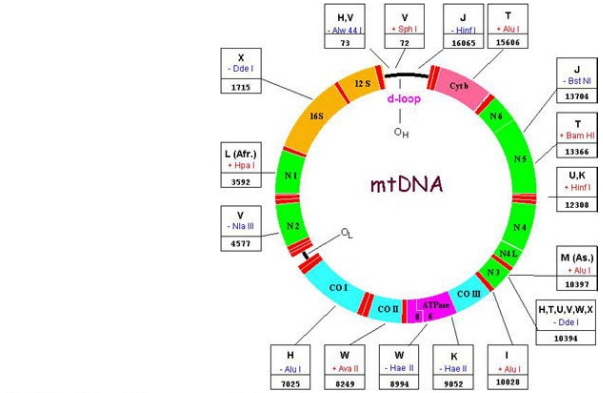


# Background- Mitochondrial Genetics and MNC

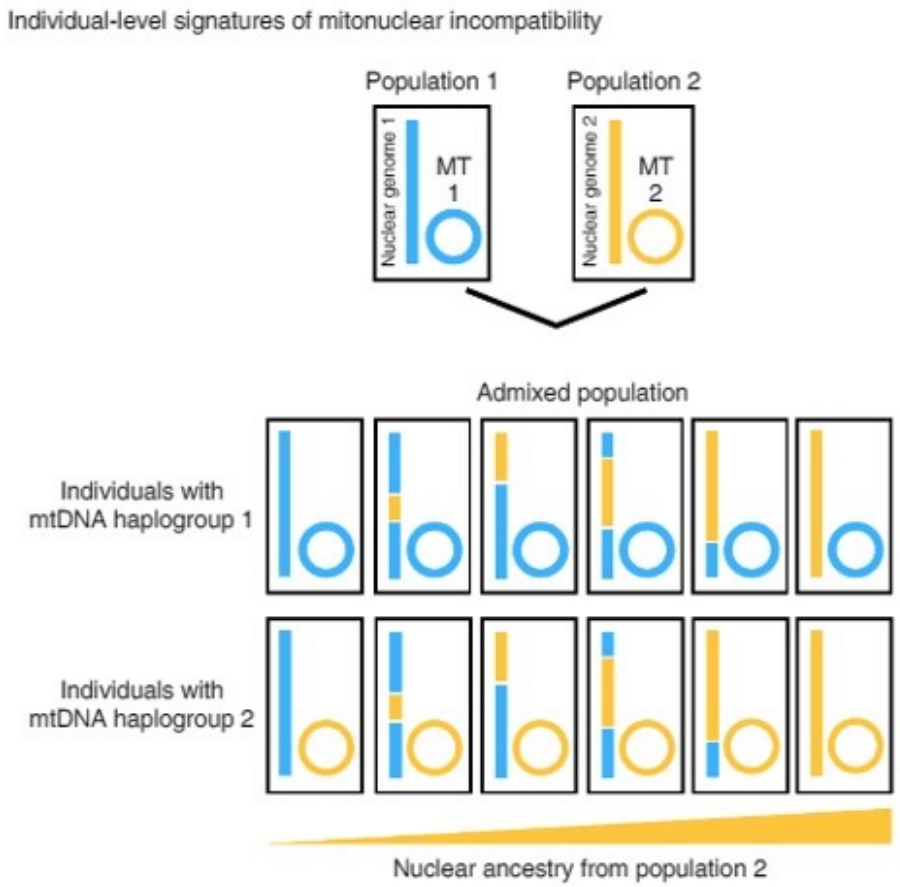



Detailed map of mitochondrial haplotype migration patterns and conceptual schematic describing the role of nDNA admixture in mitonuclear compatibility (MNC) (Zaidi et al 2019).

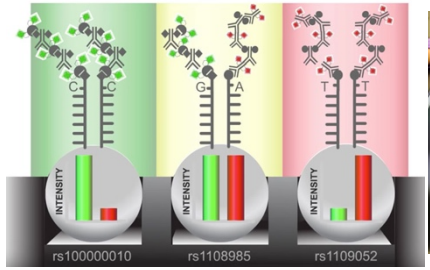


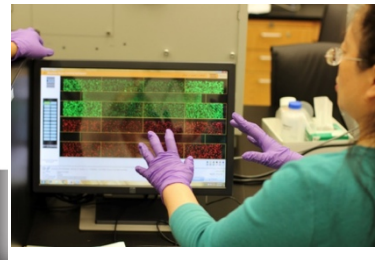

Zaidi A.A., K.D. Makova. “Investigating mitonuclear interactions in human admixed populations”. *Nature Ecology and Evolution.* 2019; 3: 213-222; doi: 10.1038/s41559-018-0766-1

# Specific Aim 2- MtDNA genetics and MNC

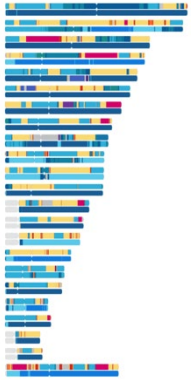



Step 1



Step 2



**SNP type selection (PLINK)**
- Linkage disequilibrium (LD) calculation
- Minor allele frequency (MAF) calculation

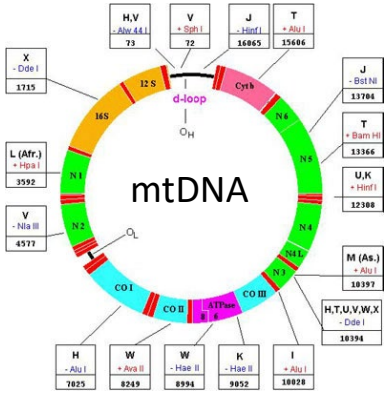
**AIMs Ranking**
$I_n$-statistic
(~ 5000 AIMs Candidate)

**AIM panel**

**Human Genome Diversity Project**

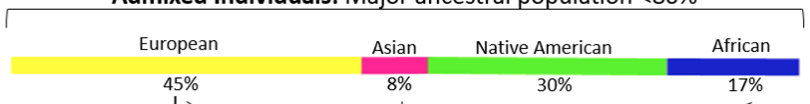
**Admixture Estimation**
(Structure or Admixture)
Estimation individuals' population ancestral proportions

Extract mtDNA variants


Step 3

**Admixed Individuals:** Major ancestral population <80%




Haplogroup **A**

**Native American**

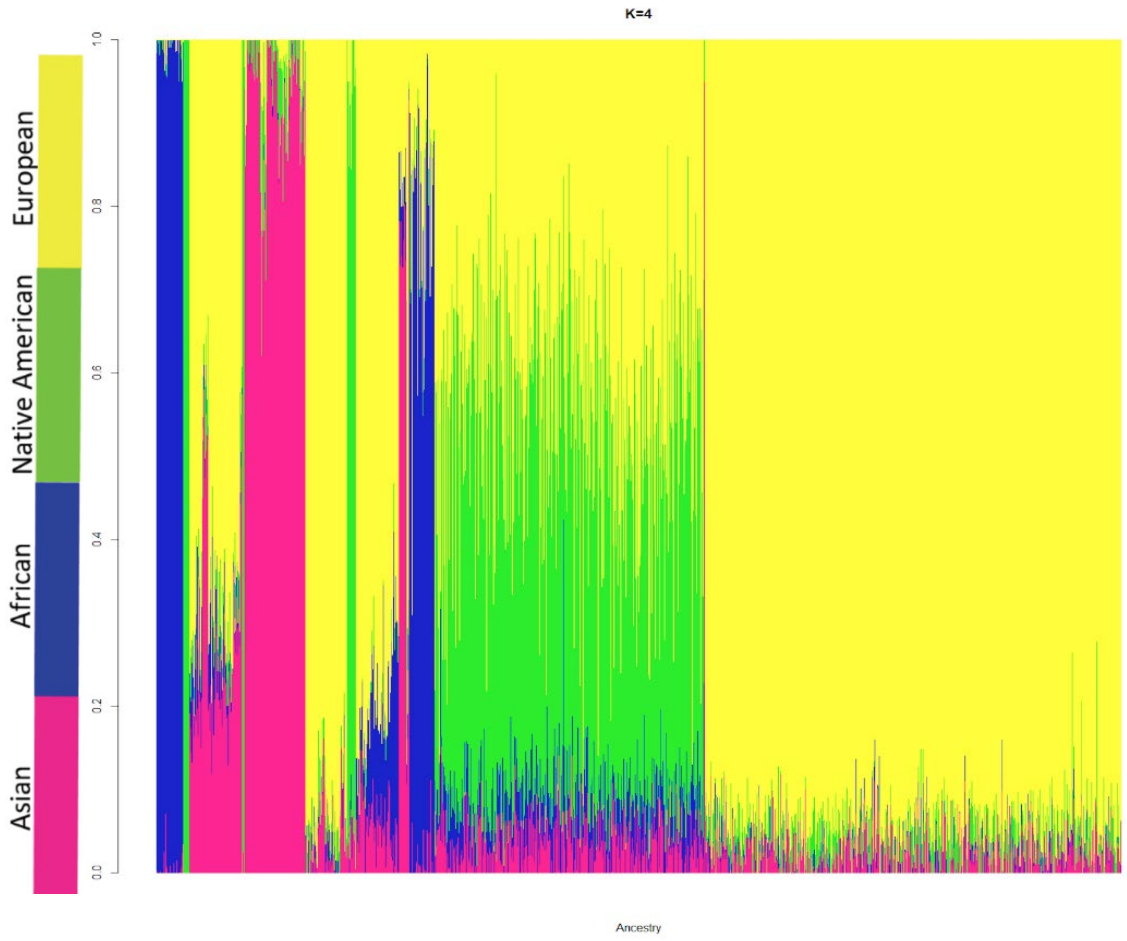
Discordant

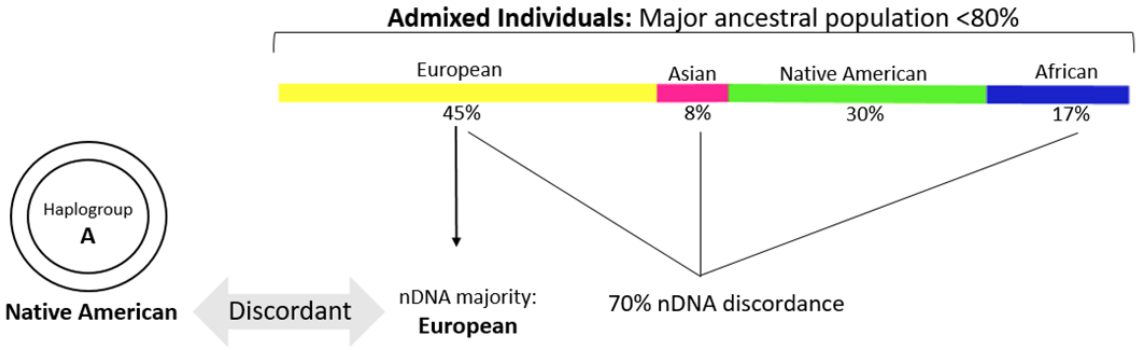
nDNA majority: **European**

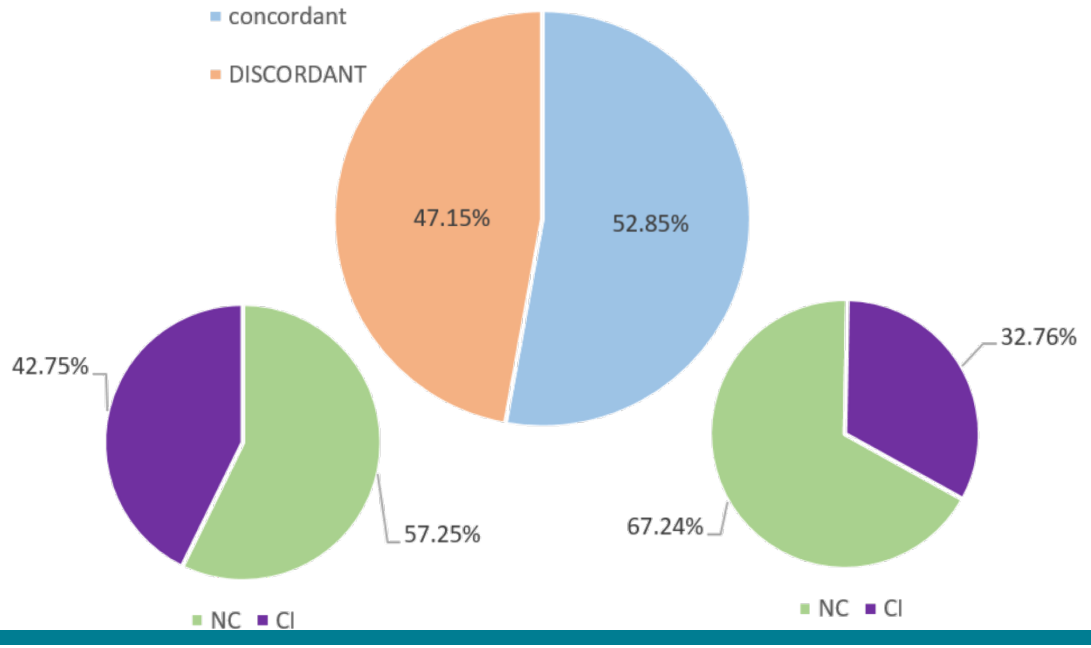
70% nDNA discordance

# Mitochondrial Genetics and MNC in MAs

- Mito-Nuclear Compatibility (MNC)

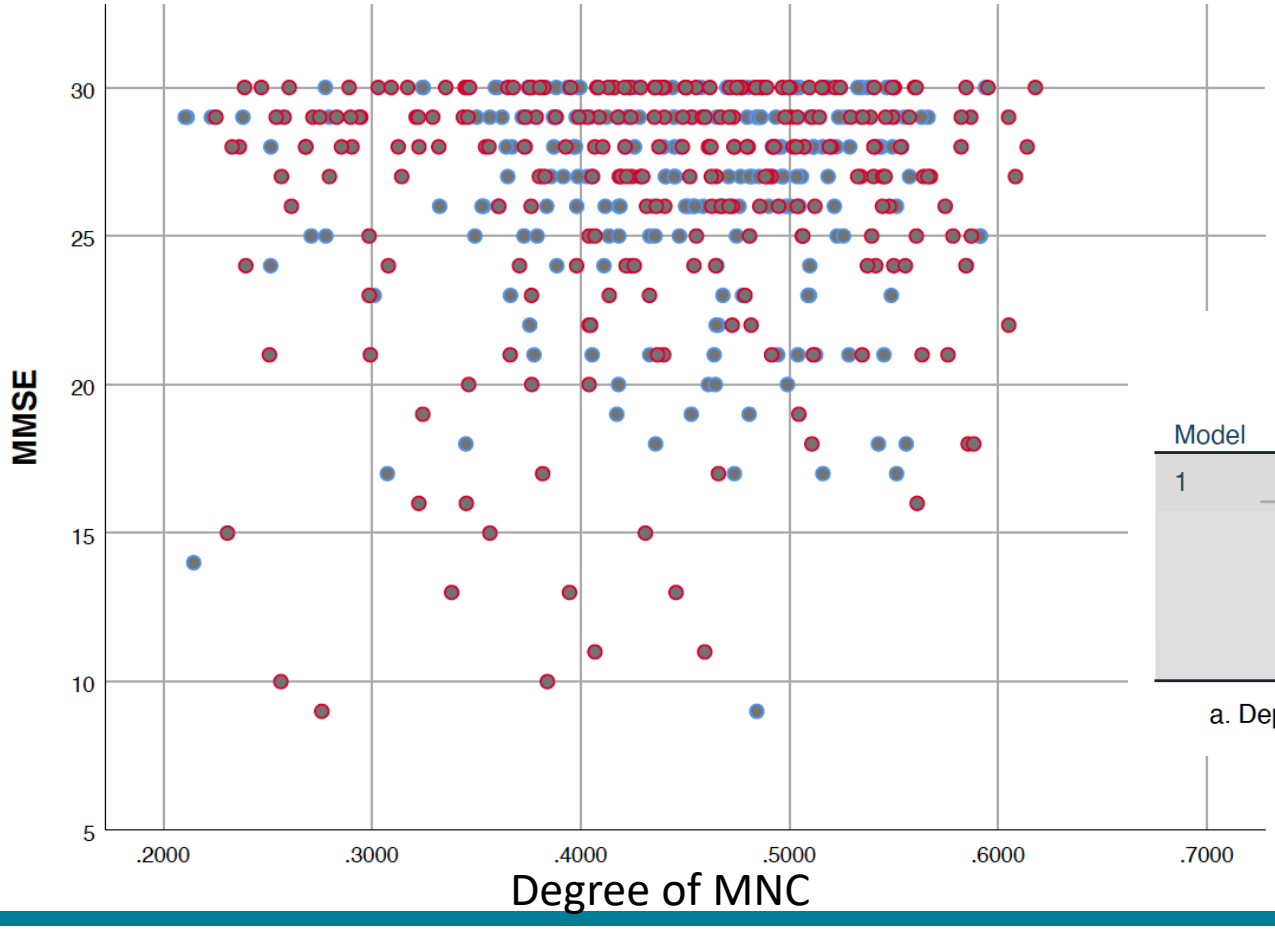


# Mitochondrial genetics- MNC

- In MA with concordant global mtDNA:nDNA types




Degree of MNC -vs- MMSE

Partial correlation, controlling for sex and education

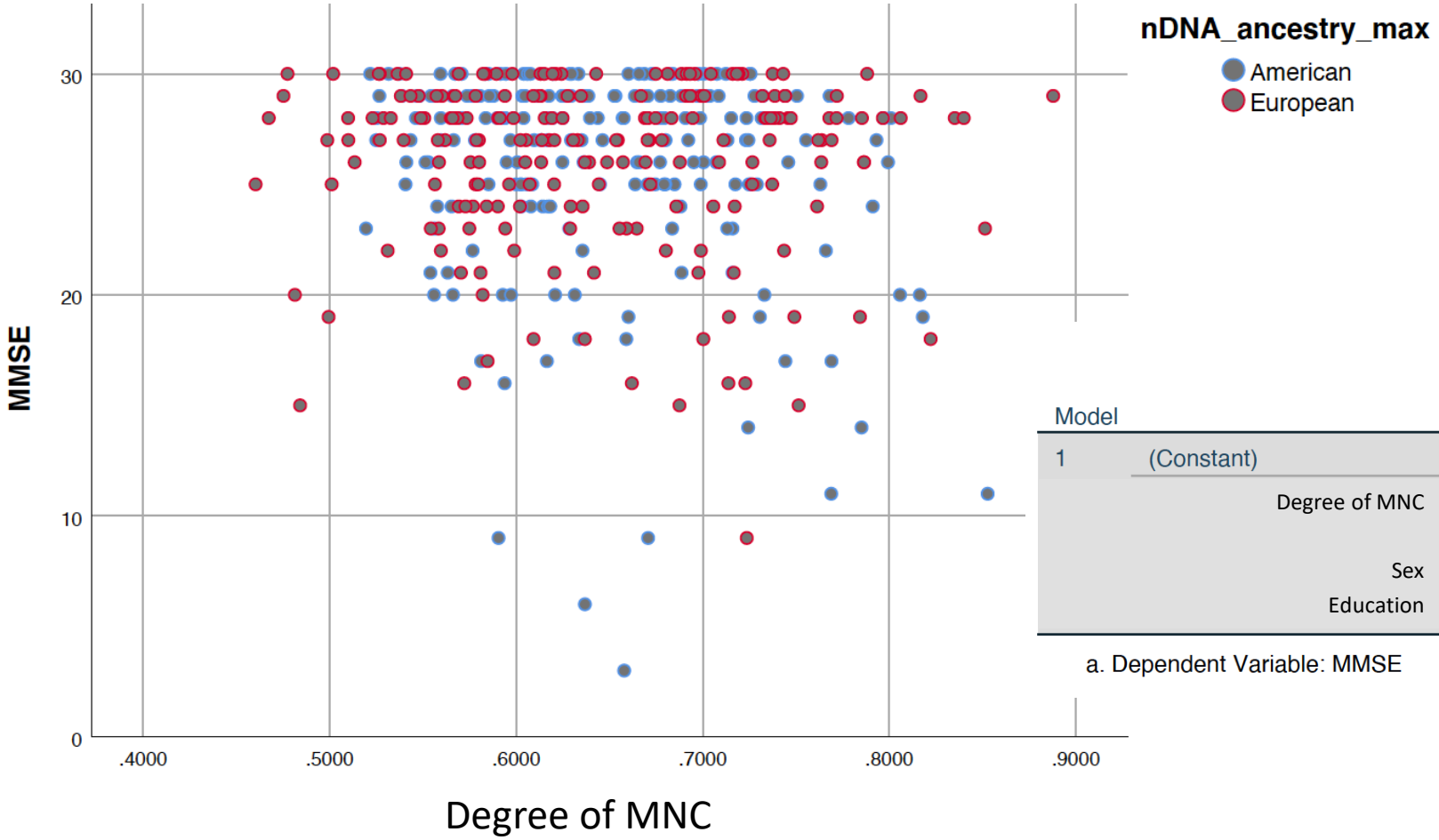
| | |
|---|---|
| Correlation | .099 |
| Significance (2-tailed) | .234 |
| df | 145 |

| Model | | Unstandardized Coefficients B | Unstandardized Coefficients Std. Error | Standardized Coefficients Beta | t | Sig. |
|---|---|---|---|---|---|---|
| 1 | (Constant) | 19.918 | 1.228 | | 16.221 | .000 |
| | Degree of MNC | 4.145 | 1.992 | .090 | 2.081 | .038 |
| | Sex | .721 | .383 | .081 | 1.882 | .061 |
| | Education | .338 | .037 | .395 | 9.030 | .000 |

a. Dependent Variable: MMSE

# Mitochondrial genetics- MNC

- In MA with discordant global mtDNA:nDNA types




Degree of MNC -vs- MMSE

Partial correlation, controlling for sex and education

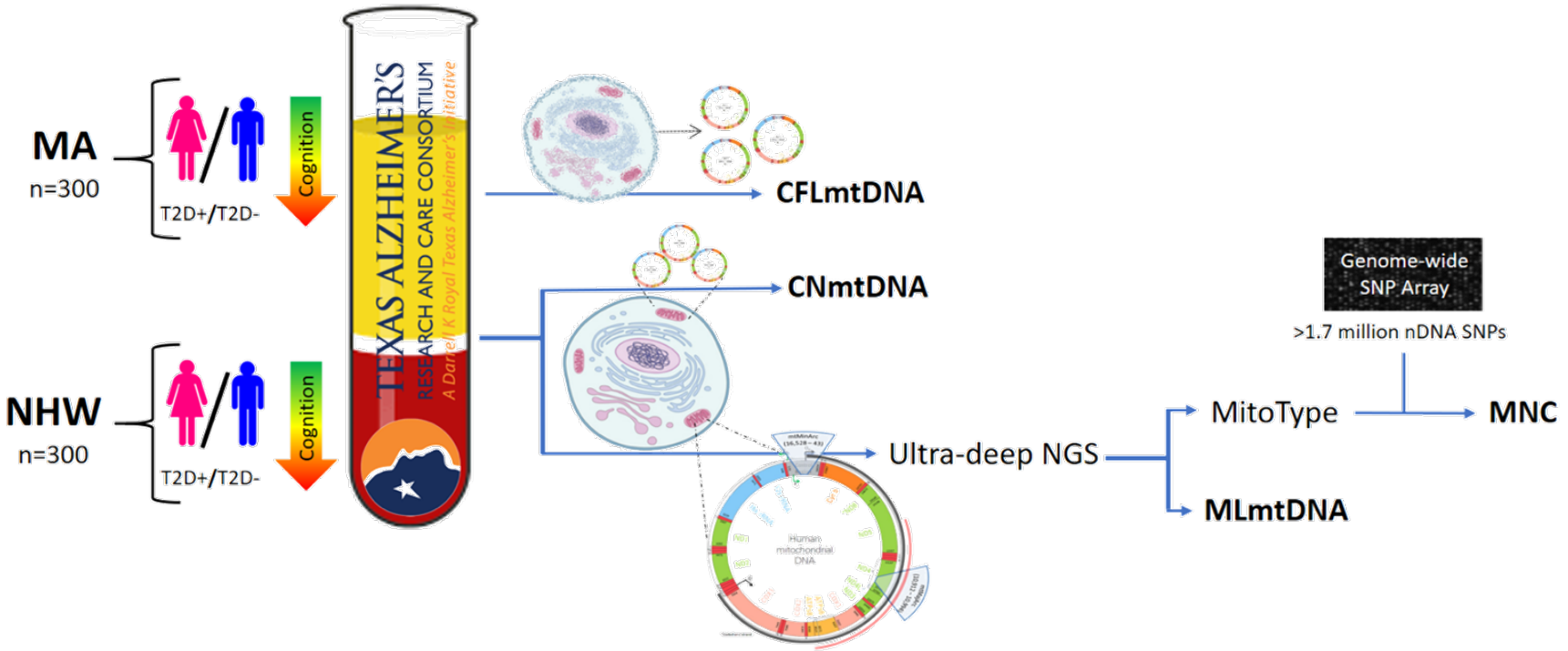
| | |
|---|---|
| Correlation | -.194 |
| Significance (2-tailed) | .017 |
| df | 148 |

| Model | | Unstandardized Coefficients B | Unstandardized Coefficients Std. Error | Standardized Coefficients Beta | t | Sig. |
|---|---|---|---|---|---|---|
| 1 | (Constant) | 24.206 | 1.721 | | 14.066 | .000 |
| | Degree of MNC | -5.229 | 2.358 | -.098 | -2.217 | .027 |
| | Sex | .788 | .397 | .087 | 1.984 | .048 |
| | Education | .403 | .040 | .446 | 10.116 | .000 |

a. Dependent Variable: MMSE

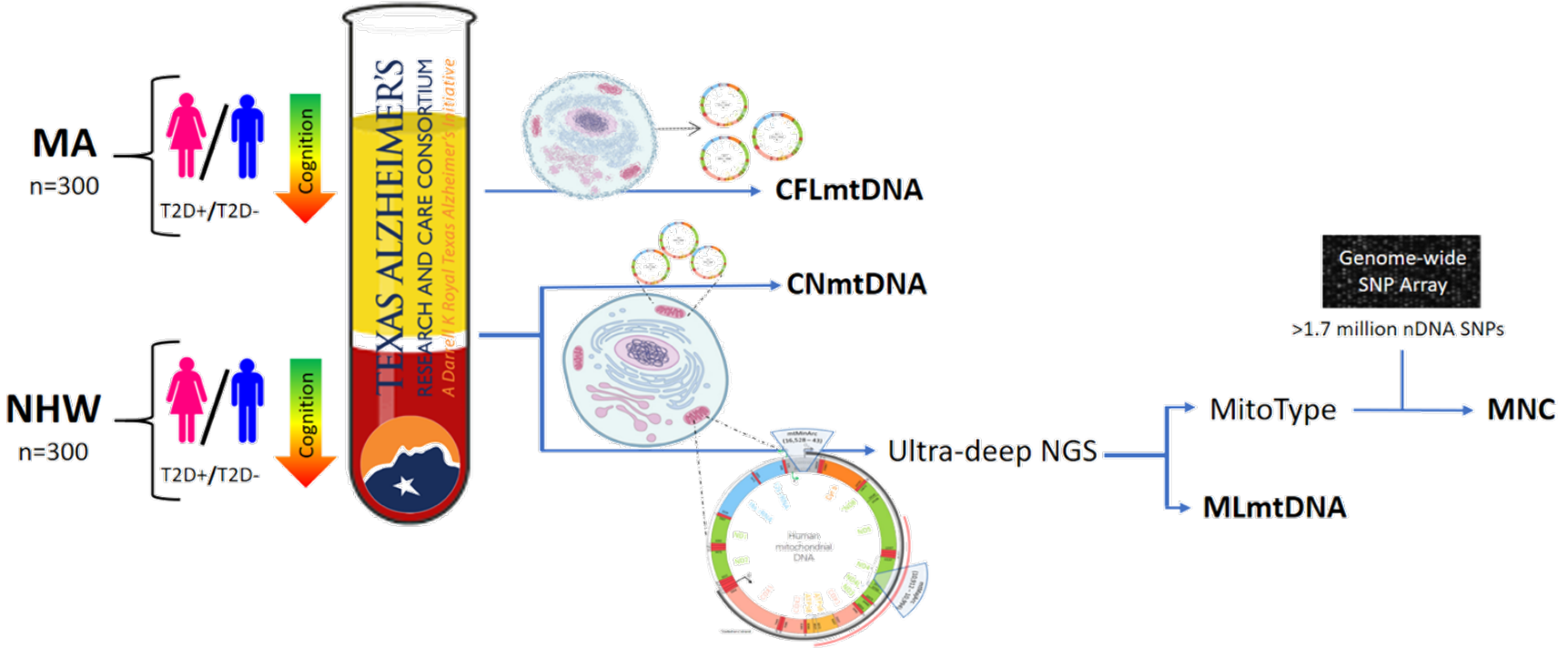
# Next Steps and Long-term Goals

- Preliminary results point to several potentially interesting trends, particularly with respect to sex interactions; the full data set will be needed to power the study.



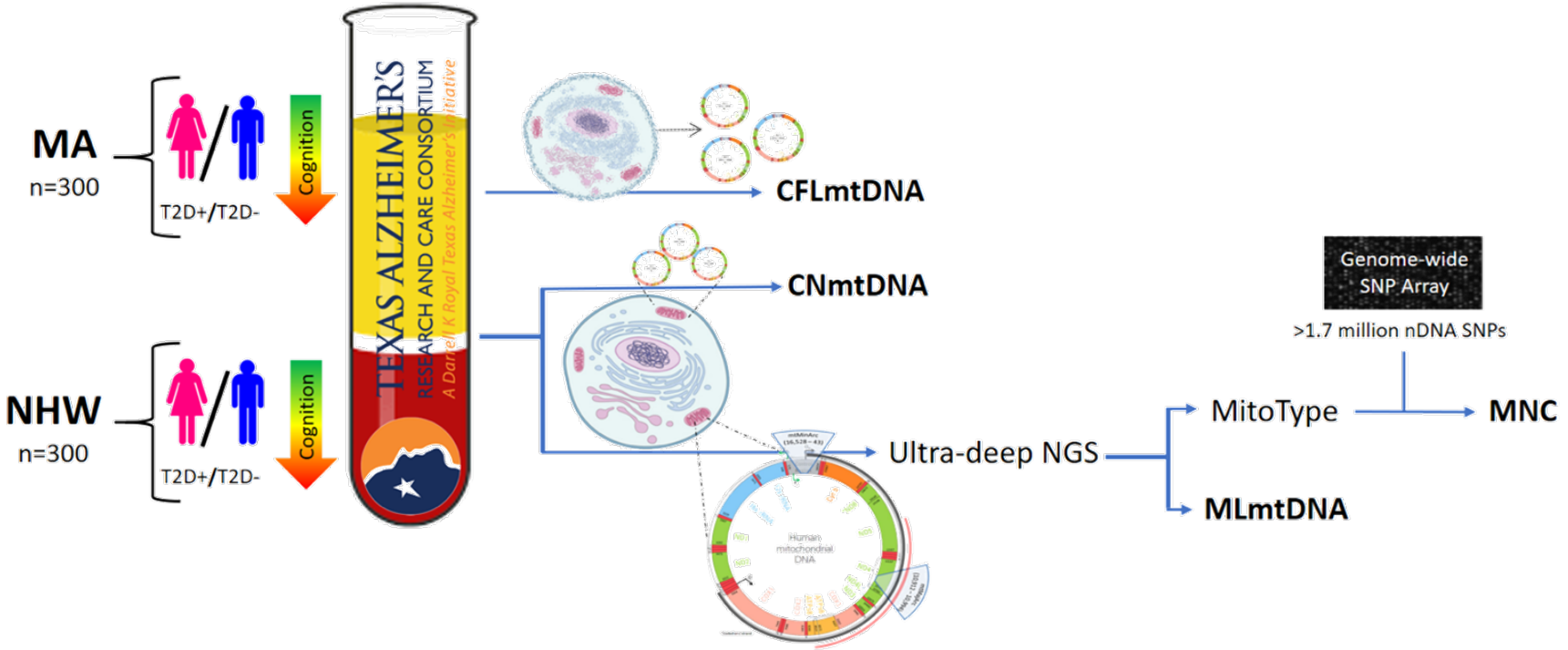
# Next Steps and Long-term Goals

- A major goal of this research is to integrate multi-dimensional mtDNA phenotypic data which captures both genetic risk and environmental stressors in Mexican American women; copy number and cell-free data collection are underway as well has higher-resolution mtDNA typing.



# Next Steps and Long-term Goals

- Additional analyses to include metabolic phenotypes (e.g., T2D, hypertension, etc.) will be conducted, along with comprehensive predictive modeling.



# Thank you!  Questions??