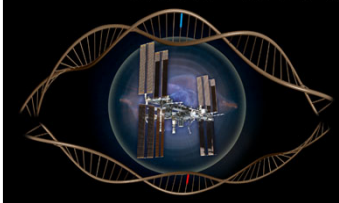
One-Carbon Metabolism and SANS: 2022 Update



SM Smith, MT Bhatti, AY Chang, JJ Chen, CR Gibson, GS Ginsburg, JF Gregory, TH Mader, RA Myers, SH Zeisel, SR Zwart

Nutritional Biochemistry NASA

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utmb

Coastal Eye Associates

DUKE CENTER for APPLIED GENOMICS & PRECISION MEDICINE

MAYO CLINIC

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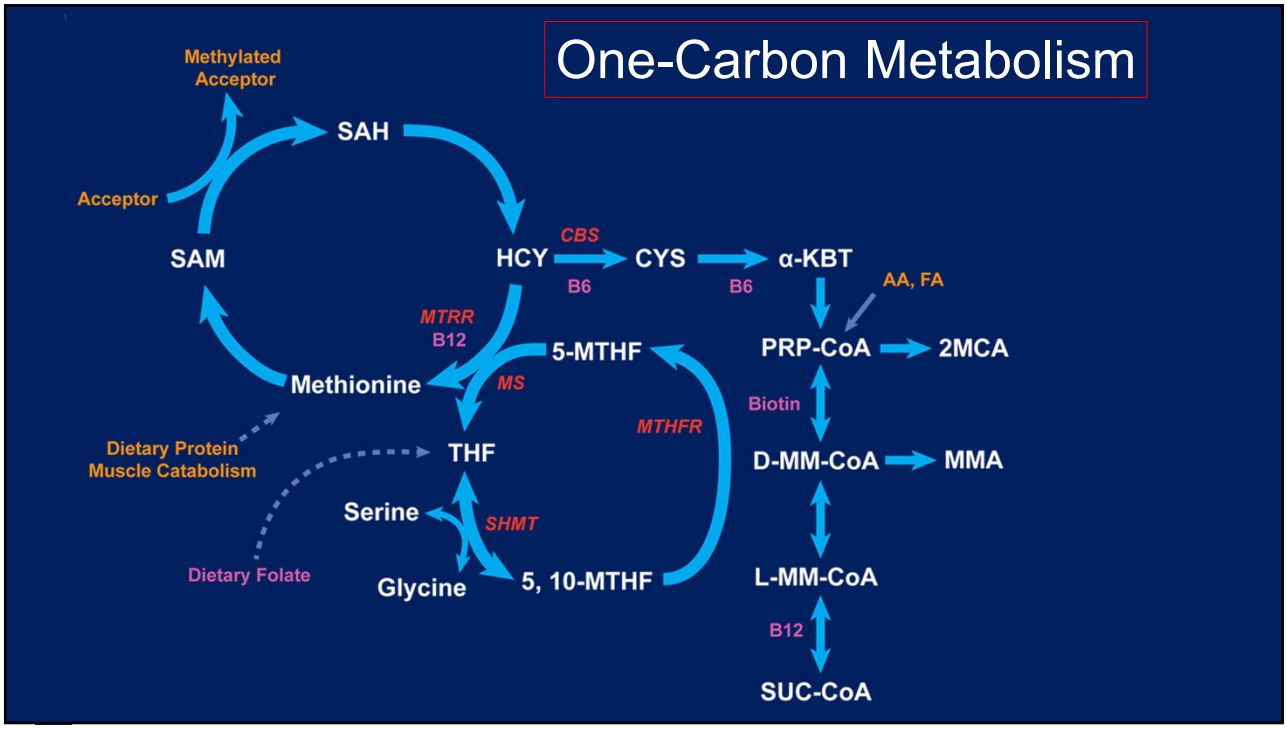
One-Carbon Metabolism



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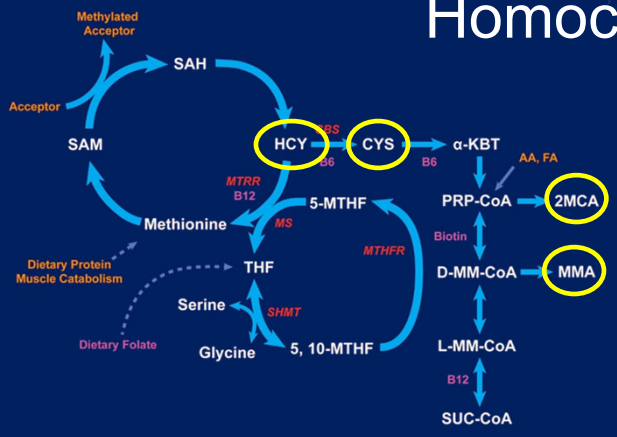
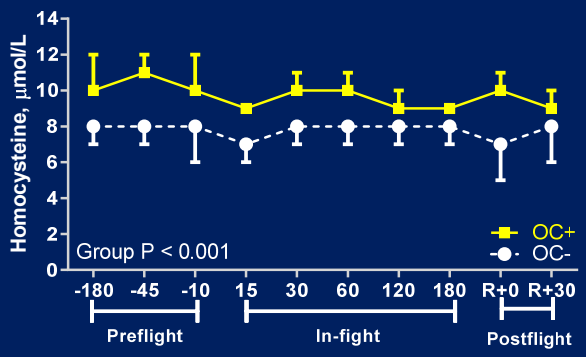
Homocysteine





Astronauts with ocular changes had higher serum homocysteine concentration than astronauts without ocular changes. *Before* flight.

Zwart et al., *J Nutr*, 2012

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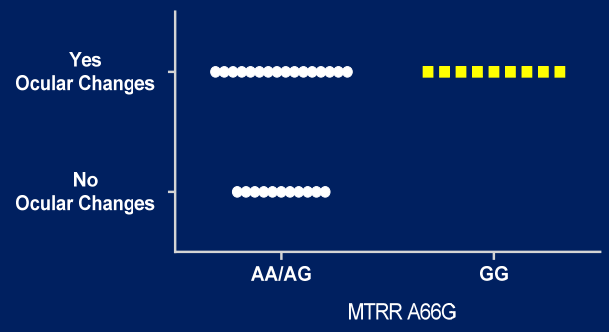
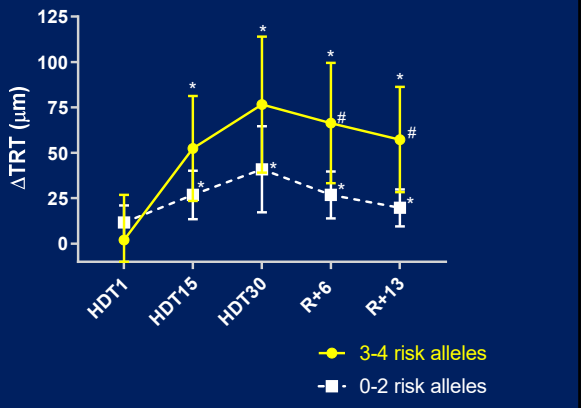
Genetics and B-vitamin status predispose some individuals to develop ocular pathologies in spaceflight and ground analogs.

Astronauts



Zwart et al., *FASEB J*, 2016

Bed Rest Subjects



Zwart et al., *JAMA Ophthamology*, 2019

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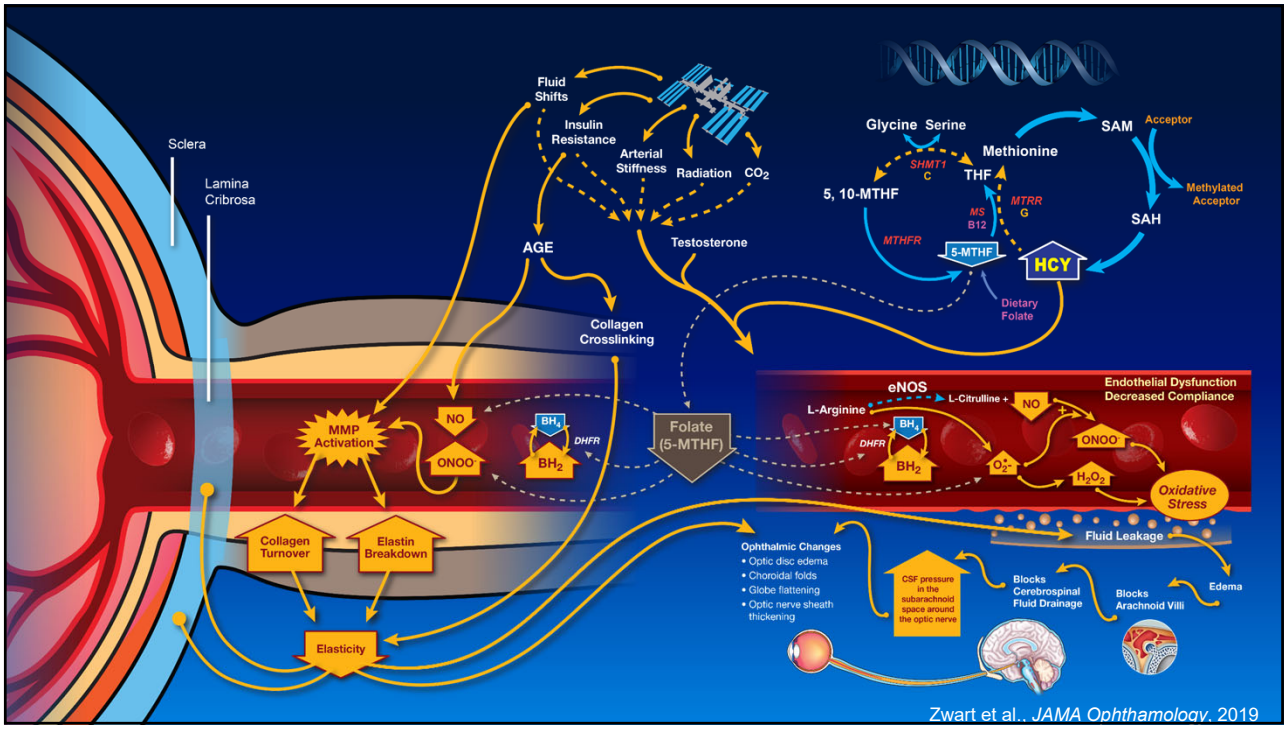
Sclera
Lamina Cribrosa
Fluid Shifts
Insulin Resistance
Arterial Stiffness
Radiation
CO_2
AGE
Testosterone
Collagen Crosslinking
Glycine Serine
Methionine
SAM
Acceptor
Methylated Acceptor
SAH
HCY
5-MTHF
5, 10-MTHF
THF
SHMT1
C
MS
B12
MTRR
G
MTHFR
Dietary Folate
MMP Activation
NO
ONOO
BH_4
BH_2
DHFR
Folate (5-MTHF)
eNOS
L-Arginine
L-Citrulline +
O_2^-
H_2O_2
Endothelial Dysfunction
Decreased Compliance
Oxidative Stress
Fluid Leakage
Collagen Turnover
Elastin Breakdown
Elasticity
Ophthalmic Changes
• Optic disc edema
• Choroidal folds
• Globe flattening
• Optic nerve sheath thickening
CSF pressure in the subarachnoid space around the optic nerve
Blocks Cerebrospinal Fluid Drainage
Blocks Arachnoid Villi
Edema
Zwart et al., JAMA Ophthalmology, 2019

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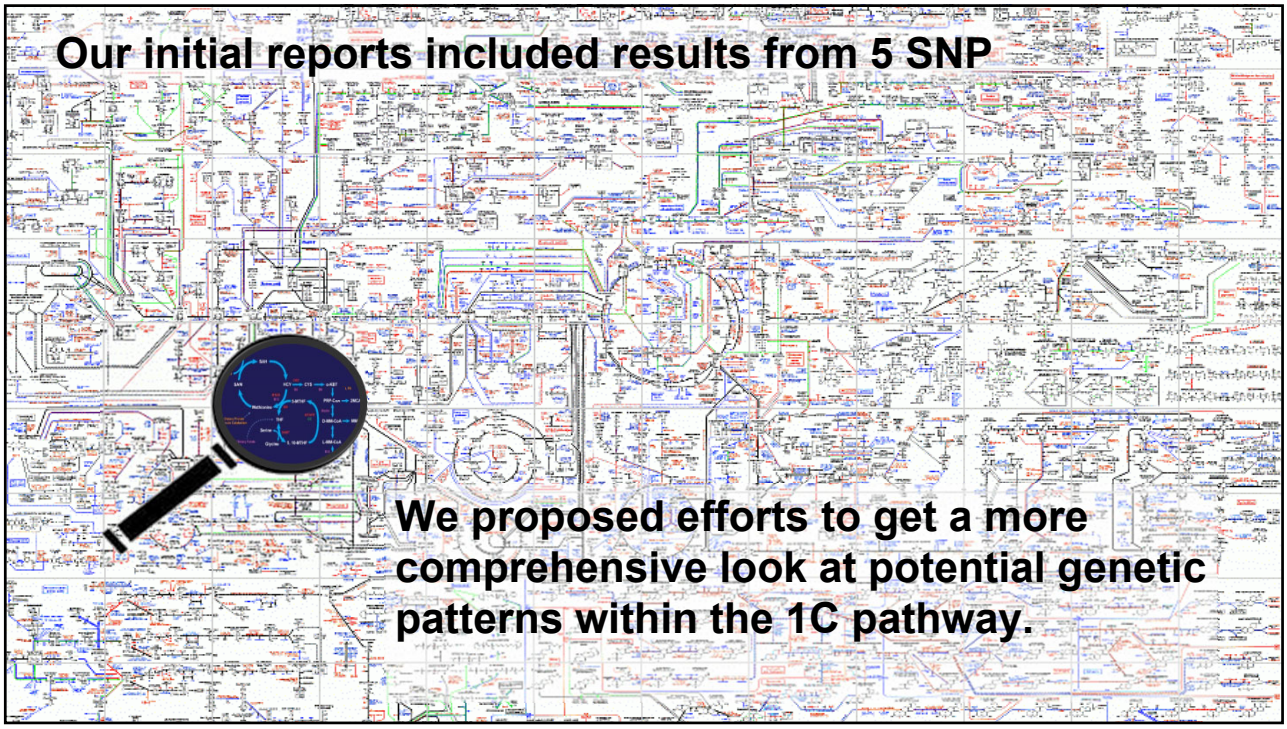
Our initial reports included results from 5 SNP
We proposed efforts to get a more comprehensive look at potential genetic patterns within the 1C pathway.

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Expanded Genetic Testing

- One Carbon Expansion study: 500+ SNPs, looking for predisposing genetic profiles.
  - Original subjects reconsented for the additional analyses
  - In 2021, ATP to recruit 8 additional flown astronauts (1 complete)
- Study of PCOS and/or IIH as SANS analog
  - Working with Mayo Clinic Endocrinologist and Neuroophthalmologist
  - Subject recruiting stopped due to pandemic
- Bed Rest: “VIIP and Psychological :envihab Research” (VaPER).
  - Extension of the original findings with two key SNPs

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Expanded Genetic Testing Cohorts

- Flight Cohort (n = 60)
  - 36 with genotypes and ocular traits
  - Up to 35 with genotypes and biochemistry traits
- PCOS Cohort (n=55)
  - 50 with genotypes and ocular traits
  - 54 with genotypes and biochemistry traits
- VaPER Cohort (n=11)
  - 11 with genotypes and ocular traits
  - 11 with genotypes and biochemistry traits

413 variants across ~90 genes / genomic regions called and passed QC

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Expanded Genetic Testing Traits

- Flight Cohort
  - Pre, in, and postflight measurements
  - Ocular traits: TRT, RNFL, Disc edema, choroidal folds, choroidal thickness, globe flattening grade, BMO measures, change in diopters, cotton wool spots
  - Biochemistry traits: Homocysteine, MMA, 2MCA, Cystathionine, RBC and serum folate, bioavailable and total serum testosterone, and many others
- PCOS Cohort (n=55)
  - Single measurement
  - Ocular traits: TRT, RNFL
  - Biochemistry traits: Homocysteine, folate, testosterone, and many others
- VaPER Cohort (n=11)
  - 11 with genotypes and ocular traits
  - Ocular traits: TRT, RNFL, Disc edema
  - Biochemistry traits: Homocysteine, folate, testosterone, and many others

* OD, OS, and average of OD and OS were each analyzed; participants with multiple measurement series (i.e. multiple missions) are down-selected to one measurement series per participant

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Expanded Genetic Testing Association Testing Overview

- For each trait in each cohort, two association models were tested:
  - No covariate model: Trait ~ genotype + device*
    - *if more than 1 device was used to measure the trait
  - Covariate model:
    - (flight) Trait ~ genotype + device + prior LDSF + PC1 + PC2 + PC3 + PC4 + PC5
    - (Mayo/PCOS) Trait ~ genotype + age + PC1 + PC2 + PC3 + PC4 + PC5
    - (flight) Trait ~ genotype + gender + PC1 + PC2 + PC3 + PC4 + PC5
- Meta-analysis (across cohorts)
  - “inverse variance weighted mean” method
  - Meta effect = sum (study effects * study weight)
  - Study weights = 1/sigma2 from each study
- Significance threshold
  - Unadjusted p value < 0.05 in ***both*** the covariate and no-covariate model meta-analysis

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Meta-Analysis Results: MTHFD1L

Methylenetetrahydrofolate dehydrogenase-1L
- Mitochondrial protein responsible for interconversion of multiple forms of tetrahydrofolate

- rs803456, 6:150924852, MTHFD1L intronic variant
  - Variant has multiple robust associations with Δ TRT and RNFL
    - 2.9- 3.2 increase in pre – post flight/bedrest Δ RNFL per G allele
    - 12.7-20.8 increase in pre-post flight/bed rest Δ TRT per G allele
  - Variant has a robust association with homocysteine

- Additional MTHFD1L variant (rs6910267) with multiple robust associations with post-flight TRT

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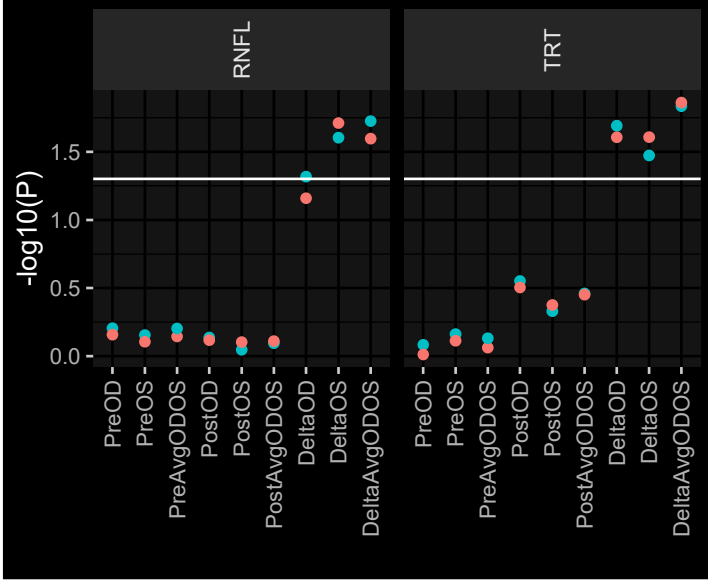
Meta-analysis results: rs803456 (MTHFD1L)



Location 6:150924852
Annotation: MTHFD1L

G allele is associated with 2.9- 3.2 increase in pre – post flight/bedrest changes in RNFL

12.7-20.8 increase in pre-post flight/bed rest changes in TRT

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Meta-analysis Results: FMO3

Flavin containing monooxygenase-3

- Protein responsible for conversion of trimethylamine to trimethylamine N oxide (TMAO)

- rs3754491, 1:171090482, FMO3 2K upstream variant
- Variant has multiple robust associations with pre and post TRT
  - 63.1-71.5 decrease in post-flight TRT per C allele
  - 45.0- 65.0 decrease in pre-flight TRT per C allele
  - Variant has a robust association with homocysteine
  - Variant has a robust association with bioavailable testosterone during flight
- Additional FMO variant w/ multiple robust TRT associations: rs909530

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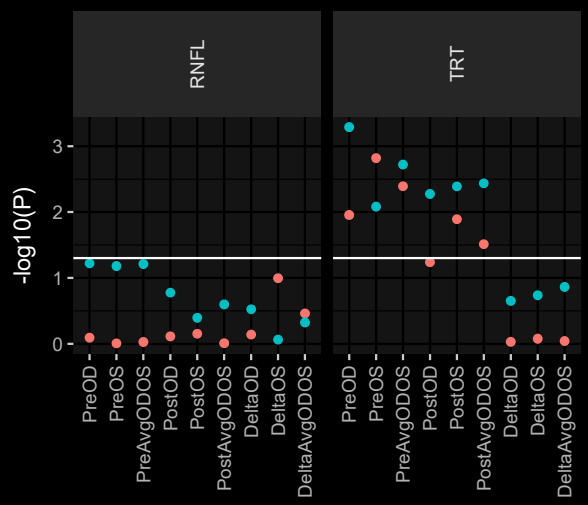
Meta-analysis results: rs3754491 (FMO3)



Location 1:171090482
Annotation: FMO3, 2K upstream variant

C allele is associated with
- 63.1-71.5 decrease in post-flight TRT
- 45.0- 65.0 decrease in pre-flight TRT

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Ongoing Work

- B-Complex study (SR Zwart, PI) to assess B-vitamins as a SANS countermeasure during bed rest.
  - Bed rest underway
  - Countermeasure had to be pulled

- AGBRESA bed rest study
  - Omnibus proposal selected in January 2021 with two aims:
    - Determine AGBRESA 1C genetics and biochemistry
    - Examine GCX markers in VaPER and AGBRESA samples

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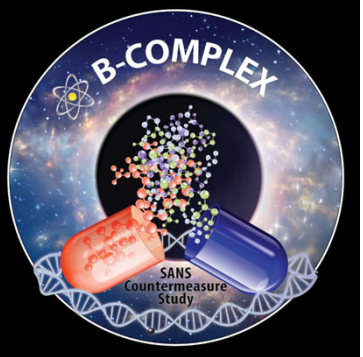
Future Work

The “B Complex: A Nutraceutical SANS Countermeasure” study was granted ATP on December 22, 2021.



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Summary

We continue to analyze flight, bed rest, and PCOS study data.

- The preliminary findings reported here support our overarching hypothesis.
- Additional flight and bed rest data will augment this work

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Summary

- Genetics and B-vitamin status predispose some individuals to develop ocular pathologies in flight and in ground analogs.
- Whether B-vitamin supplementation can override genetics, alter biochemistry, and counteract the response to (CO_2 and/or fluid shift and/or 0g and/or ??) in at-risk individuals needs to be tested.

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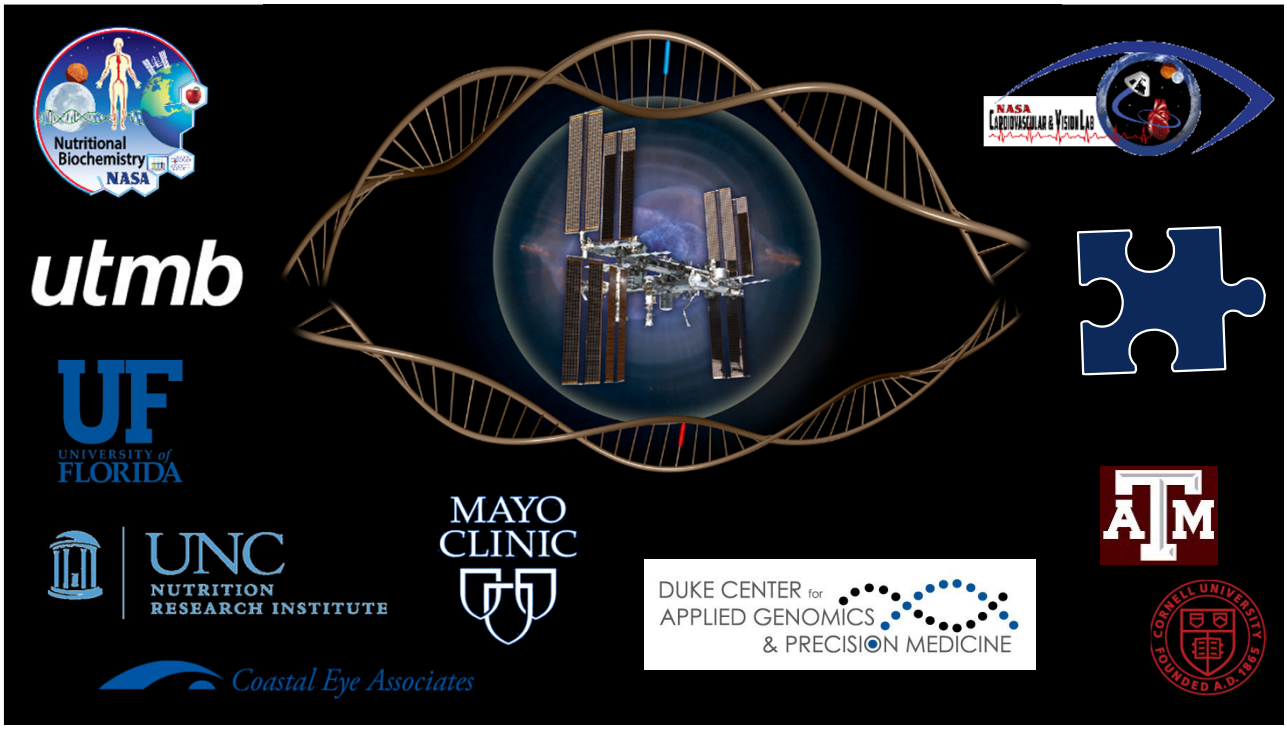
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NASA CARDIOVASCULAR & VISION LAB
CORNELL UNIVERSITY FOUNDED A.D. 1865

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