Human Research Program

The Twins Study: NASA’s First Foray into 21st Century Omics Research

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ISS Crew: Scott Kelly, Mikhail Kornienko Sign On for One-Year Mission

A veteran NASA space commander and Russian cosmonaut have signed on for the ultimate space voyage: a yearlong trip on the International Space Station.

American astronaut Scott Kelly and Russian cosmonaut Mikhail Kornienko will launch on the one-year space station flight in spring 2015 and return to Earth in spring 2016, NASA officials announced today (Nov. 26). They will begin their mission training in early 2015.

The mission will help NASA understand how the human body adapts to extremely long space missions, such as voyages around the moon, to an asteroid and ultimately to Mars, NASA officials said.
A Chance in a Lifetime Opportunity for a Twins Study
Great Value in n = 1 Omics Study Over Time
One Notion

Flight Subject

pre-flight  in-flight  post-flight
time

Ground Subject

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www.nasa.gov/content/twins-study
“To capitalize on this unique opportunity,

NASA’s Human Research Program (HRP) and the National Space Biomedical Research Institute (NSBRI) are initiating

a *pilot demonstration project focused on the use of integrated human -omic analyses* to

better understand the biomolecular responses to

the physical, physiological, and environmental stressors

associated with spaceflight.”
Differential effects on Telomeres and Telomerase in Twin astronauts associated with spaceflight

Specific Aims

The rate at which telomeres shorten provides an informative biomarker of aging and age-related pathologies (e.g., cardiovascular disease and cancer) that captures the interplay between genetics and lifestyle factors.

We propose that for the astronauts telomere maintenance is particularly relevant, as it reflects the combined exposures (e.g., radiation) and experiences (nutritional, psychological and physical stressors) encountered during space travel. The Twins study provides the extraordinary opportunity to control variables of individual genetic differences, susceptibilities and lifestyle factors, making differential effects observed between the twins space-flight specific.

Comparisons with unrelated astronauts (separate study), will allow evaluating role of genetics/individual susceptibilities.

Implications of Research for Space & Earth

Space: This twins study will identify space-flight specific factors that influence telomere length and telomerase activity, informative biological indicators of aging and age-related degenerative diseases (e.g., cardiovascular disease and cancer). Our mechanistic investigations will begin to establish relevant relationships and suggest potential mitigation strategies for future study and to improve astronaut overall health.

Earth: Aging and age-related diseases like cardiovascular disease and cancer are an everyday concern on earth as well, therefore this study also seeks to make comparisons with unrelated astronauts (and controls) that will serve to identify individual susceptibility factors that influence telomere length and telomerase activity. Taken together with our mechanistic studies, mitigation strategies will be improved and applicable to all.

Our goal is to assess changes in telomere length and telomerase activity associated with the upcoming yearlong ISS mission in the space- and earth-bound twin astronauts.

We hypothesize that accelerated telomere shortening and elevated telomerase activity will be associated with space flight as compared to ground based control, in a duration and severity dependent manner.

- Blood samples will be taken pre-flight (to establish baseline), in-flight (to evaluate short-term/temporary changes) and post-flight (to evaluate long-term/permanent changes)
- Data sharing for other endpoints will also inform this effort
- In vitro studies will investigate potential mechanisms (e.g., oxidative stress) and mitigation strategies (e.g., antioxidants)

Telomere length will be assessed using TELO-FISH

Florescence in situ Hybridization (FISH) with telomere probe on chromosomes (and interphase nuclei) is evaluated as Relative Fluorescence Intensity (RFI) distributions.

Telomerase activity will be assessed using qRT-PCR TRAP

quantitative Real Time-PCR Telomere Repeat Amplification Protocol
Epigenomics

Specific Aims
Aim 1. We will measure DNA methylation and chromatin at a genome-wide level in biological samples obtained from the space traveler at quarterly intervals, pre- and post-flight, and at times of unexpected exposures such as radiation events, or spacecraft environmental contamination. We also obtain measurements of the ground-based twin.
Aim 2. We will integrate epigenomic data with exposure to spaceflight conditions, looking for exposure-linked changes, and by comparison to the ground-based twin, determine whether these are transient or long-lived effects. We will also determine whether DNA mutations arise secondarily to these epigenetic changes.

Implications of the Research for Space & Earth

Space: Identify reversible causes of genomic damage in space, e.g. radiation or toxin induced epigenomics change; quantify aging and genomic exposure.

Earth: First human study of the epigenome over time in a defined/controlled environment.

- DNA methylation
- Histone modifications (>200 known)
- Chromatin factor complexes
- Chromatin structure
Landscape of DNA and RNA Methylation

**Specific Aims**

#1 – Genome-wide epigenetic profiles of DNA methylation changes

#2 – A comprehensive catalog of coding and noncoding, small and large RNA

#3 – Transcriptome-wide maps of RNA methylation sites

**Implications of the Research for Space & Earth**

**Space:** (1) Establish the genetic networks and expression patterns activated by space travel, (2) trace clonality of epigenetic changes, (3) examine the methylation of RNA

**Earth:** Aid research on aging, cancer, RNA biology, and circadian rhythm, all of which show differences at the (epi)genome & (epi)transcriptome

**Epigenome**

- DNA transcription

**Epitranscriptome**

- RNA translation

**Epiproteome**

- Protein

**Δ in Epigenetics:** Loci, regions, and clones

**Δ in Transcriptome:** Genes, Isoform, Edits, Allele, SNVs, ncRNAs, Fusions, & Methylation

**Sequencing Data**

- RNA-seq gives many views of biology. We use r-make.

**Remaining Reads**

- Viruses/Bacteria/Other
  - Use: BLAST, MetaPhlAN
  - Predict polyA sites & gain/loss of miRNA binding sites
    - Algorithms: r-make, BAGET, AlexaSeq, TargetScan
  - Gene fusion detection
    - Algorithms: r-make, Snowshoes
  - Find ncRNAs and new genes
    - Algorithms: r-make, Aceview
  - Differential expression by gene, exon, splice isoform, allele, & transcript
    - Algorithms: STAR, r-make, ASE, limma-voom, RSEM
  - Genetic variation (SNVs and indels)
    - Algorithms: STAR/GATK, r-make

**References**

- Adapters
- rRNA
- miRBase
- RefSeq
- hg19
Biochemical Profile: Homozygous Twin control for a 12 month Space Flight Exposure

Specific Aims
To provide a database of biochemical analyses from blood and urine samples. The analyses reflect a broad set of nutritional and physiological variables that may be altered as a result of the space flight environment (including diet, stress, weightlessness). Collecting data on the Ground twin will allow for a more direct comparison of the effects of space flight on human biochemistry and physiology.

Blood and urine collections
Preflight:
L-180, L-45, L-10
In-flight:
FD15, 30, 60, 120, 180, 240, 300, 360
Post flight:
R+0, R+30

Implications of the Research for Space & Earth

Space:
Improve understanding and time course of biochemical changes during flight and how the changes relate to diet during flight.

Earth:
Improve understanding of how diet can impact different biological systems.
**Immunome studies in space**

**Specific Aims**
- Study how long term space travel affects the immune system
- We will study how parameters of the immune system change at baseline and after a seasonal flu vaccination
- To do so, we study baseline and post flu parameters before, during and after a one year space flight

**Implications of the Research for Space & Earth**

**Space:**
Will ensure that astronauts keep a healthy immune system during long flight, and stay protected against infections from earth when visitors are coming.

**Earth:**
Understand how immune response to vaccination differ in twins

Cytokine production changes in flu reactive T cells following vaccination

Changes in T cell receptors clones after vaccination

Emmanuel Mignot, MD, PhD
Stanford University
Specific Aims

• To study the effects of long-duration spaceflight on the cardiovascular system independent of genotype
• To investigate relationships between gene expression, metabolomic profiles, biomarkers in blood and urine, and arterial structure and function using the space-flown and the ground-based identical twin

Implications of the Research for Space & Earth

Space: Determine if the spaceflight environment perturbs genomic and metabolomic profiles and accelerates development of atherosclerosis (occupational health)

Earth: Develop novel insights of how longitudinal changes in genomic and metabolomic profiles are related to risk factors for atherosclerosis

Pre- and Post-flight Testing

Inflight Operations
PROTEOMIC ASSESSMENT OF FLUID SHIFTS AND ASSOCIATION WITH VISUAL IMPAIRMENTS AND INTRACRANIAL PRESSURE IN TWIN ASTRONAUTS

Specific Aims
To explore proteomic and genomic biomarkers underlying space flight-induced fluid shifts and visual impairment & intracranial pressure (VIIP) symptoms.

The proteome is the set of proteins produced by the genome at a given time. Proteomics captures the state of molecular and cellular processes at a specific time point.

Implications of the Research for Space & Earth

Space: Discovery of molecular pathways involved in the evolution of spaceflight adaptations related to fluid redistribution in-flight and the etiology of visual acuity and ocular changes in-flight and post-flight.

Earth: This project has broader impact on Earth-based clinical areas such as traumatic brain injury-induced elevations of intracranial pressure, hydrocephalus, and glaucoma.

Pre- and Post-flight Testing
All in-flight operations and:
Tissue hydration MRI

In-flight Operations
Blood Plasma proteins
Blood Plasma collection
Ultrasound measures of fluid shifts
Intracranial Pressure
Intraocular Pressure
Ocular Structure
Blood Pressure
Heart Rate
Vascular Resistance

In-flight Operations

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Cognitive Performance in Spaceflight

Specific Aims
There are a number of environmental stressors unique to the spaceflight environment that may affect cognitive performance, which is crucial for mission success. Our main objective in the TWINS study is to investigate whether cognitive performance is affected by initial and prolonged exposure to the spaceflight environment and after return to Earth. We will use the Cognition test battery, which consists of 10 brief neuropsychological tests that were specifically designed for high performing astronauts. We will compare data within subjects, between twins, relative to astronauts flying 6-month missions, and relative to normative data gathered in astronauts on the ground. The cognitive data will be correlated with markers derived from biological samples taken before, during, and after the 12-month mission.

Implications of the Research for Space & Earth

Space: Exploration-type missions will require humans to spend unprecedented durations in space, yet our knowledge on the effects of prolonged exposure to the spaceflight environment is very limited. After the study, we will have an initial understanding of whether and to what extend prolonged ISS missions are associated with changes in cognitive performance, and how these relate to biologic markers.

Earth: The results have direct implication for other high performing populations exposed to stressful environments for prolonged periods of time on Earth.

The Cognition Test Battery
Cognition was specifically designed for astronauts and is currently used during 6-month ISS missions and in multiple space analog environments (including Antarctica, HI-SEAS, and HERA).
The Bacteriome in the Gastrointestinal Tract

Specific Aims

The GI tract of humans is populated by a diverse “ecosystem” of micro organisms, mostly bacteria: the bacteriome. The bacteriome can help--contributing to digestion and immune system function--or harm--overgrowth of some types accompanies illness or stress. This project will examine what changes occur to the bacterial populations over a year in space, that are different from the changes over time on Earth. Are particular types of bacteria susceptible to the space environment, and if so, which types?

Implications of the Research for Space & Earth

Space: Knowing how the bacteriome changes over time in space can help us make plans to protect astronauts’ health for longer-term space flights. For example, adjustments to diet could help maintain beneficial bacterial types.

Earth: Observing how the bacteriome changes in relation to health and environmental changes, (such as those studied in other Twin Projects) can provide insights into how the bacteriome may respond to challenges and contribute to the human host’s health.

Relative abundance of different families of bacteria. Will there be systematic changes in the twin in space not seen in the twin on Earth?

Fred Turek, Ph.D.
Specific Aims

Our main objective in the twin study is to perform a complete analysis of all biomedical and molecular data collected during the mission to produce the singular most comprehensive portrait of the human biophysical response to the rigors of spaceflight. We are at an unprecedented era in genomic medicine, allowing for the sensitive and precise measurement of billions of biochemical molecules, which will allow us to detect the subtlest of changes in Scott and Mark’s physiology over time. By integrating these data, we can follow alterations in their cellular systems to both better understand the effects of space travel on human health, and how an astronaut’s genome may contribute to his/her own unique physiologic response to microgravity.

Implications of the Research for Space & Earth

**Space:** We will generate a detailed benchmark for how human physiology changes in space in great molecular detail. This wealth of data will be essential for any future planning of long duration space exploration missions, and provide a proof-of-principle for better monitoring and managing astronaut health.

**Earth:** With this study, Scott and Mark Kelly will be the most thoroughly profiled twins in history, and the resultant data will offer new insights into how two siblings with nearly-identical genomes respond to different conditions.

Risk-o-gram

- Dyslipidemia
- Coronary artery disease
- Basal cell carcinoma
- Type 2 diabetes
- Hypertriglyceridemia
- Osteoarthritis
- Age related macular degeneration
- Hypertension
- Obesity
- Asthma
- Stroke
- Psoriasis
Scott Kelly – ISS for one year

Mark Kelly – Earth control

Telomere Length
Bailey

B-cells / T-cells
Mignot

Antibodies
Mignot/Snyder

Cytokines
Mignot

Vasculature
Lee

Cognition
Basner

Microbiome
Turek

Targeted and Global Metabolomics
Lee/Rana, Mignot/Snyder & Smith

Chromatin
Feinberg

DNA Mutations
Feinberg

DNA Hydroxy-methylation
Mason

DNA Methylation
Feinberg & Mason

large/small RNA
& RNA Methylation
Mason

Proteomics
Lee/Rana

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Timeline

- One Year Mission Announced
- Solicitation Released
- Grants Announced
- Baseline Data Collection Begins
- One Year Mission
- Last Major Post-flight Data Collection


Now

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Progress to Date

• Pre-flight, baseline data collections complete
  - Most protocols already established
  - New protocols developed for Cell Preparation Tubes (CPTs)
    • To sort or not to sort?

• In-flight collections have begun
  - CPTs processed and frozen
  - CPTs processed and en route to Houston right now!
**Issues Associated with Omic Research**

- **Research ethics**
  - The primary risks involved in genetic research are risks of social and psychological harm, rather than risks of physical injury
    - Could provoke anxiety and confusion about disease risk
    - Uncover unwanted information about heritage, ancestry, and family relationships
  - **De-identification** of genomic information is difficult
    - Astronauts are public figures
  - **Information given to subjects**
    - Individual genome sequence data?
    - Interpretation of the genome sequence and/or genetic counseling?
    - Option to decline to receive all or part of the results (Right Not to Know)?
  - **Research community’s access** to genomic information
  - Interim policy on genetic research **JID 1800.4**
  - NASA-wide policy under development

- **Medical care**
- **Occupational health**
- **Insurance (health, disability, life)**
- **Employment activity**
• The Twins Study (Scott and Mark Kelly) is NASA’s first foray into 21st-century omics research
  - Built around Scott Kelly’s one year mission
• The Twins Study will examine
  - Genome, telomeres, epigenome
  - Transcriptome and epitranscriptome
  - Proteome
  - Metabolome
  - Physiology
  - Cognition
  - Microbiome
• NASA is addressing
  - Protections for research participants
  - Use of data in medical care, occupational medicine, mission planning
Acknowledgements

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[Image of astronauts with the text: Meet the Twins Unlocking the Secrets of Space by Jeffrey Kluger.]

time.com/meet-the-twins-unlocking-the-secrets-of-space/

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