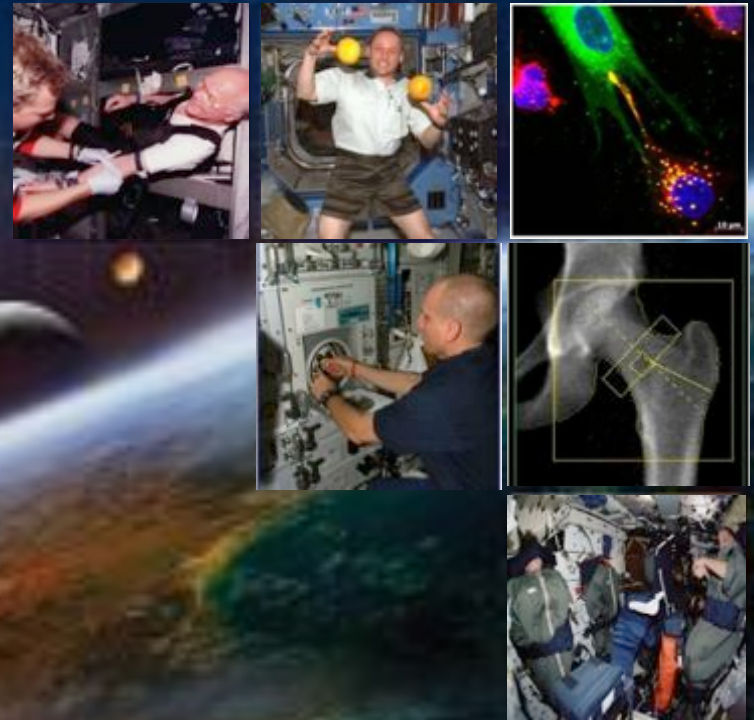




Human Research Program

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

AsMA
14 May 2015



Craig E. Kundrot, Ph.D.
Deputy Chief Scientist, HRP
SA2/NASA JSC



The One-Year Mission



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

Posted: 11/26/2012 9:28 am EST Updated: 11/26/2012 9:46 am EST

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By: Tariq Malik
Published: 11/26/2012 09:27 AM EST on SPACE.com

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 2013.

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.

Astronaut Scott Kelly Preparing for Unprecedented One Year in Space; Mission to Experiment on His Bone Mass, Vision, Immune System

By Leah Feller Staff Writer, Dec 07, 2012 05:00 PM EST

0 Comments

Tags: ISS, Space



A Chance in a Lifetime Opportunity for a Twins Study



Great Value in n = 1 Omics Study Over Time

Resource

Cell

Personal Omics Profiling Reveals Dynamic Molecular and Medical Phenotypes

Rui Shen,¹ George L. Miao,^{1,2} Jennifer Li-Ping Han,^{1,3} Li-Ping Han,^{1,3} Hugo Yik, Li-Ping Han,^{1,3} Wang Chen,^{1,4} Bao-Min Wang,¹ Kunal J. Manjivani,¹ Sheng-Hua Wang,¹ Richard E. Green,¹ Frank Zhang,¹ Michael A. Clark,¹ Benjamin Van Der Valk,¹ J. Gregory Kuznetsov,^{1,5} Mounir Ghannouchi,¹ and Y. Shirley Yang,¹ *et al.*

¹Department of Genetics, Stanford University School of Medicine, Stanford, California, USA
²Department of Systems Medicine and Institute of Translational Genomics, Department of Pediatrics, Chinese Academy of Medical Sciences, Beijing, China
³Department of Pediatrics, Department of Medicine, Department of Biostatistics, Stanford University, Stanford, California, USA
⁴Program in Computational Biology and Mathematical Genomics, Department of Molecular, Cellular and Developmental Biology, Department of Computer Science, Princeton University, Princeton, New Jersey, USA
⁵Department of Pediatrics, National Children's Medical Center, Beijing, China



Mike Snyder

Summary
 Personalized medicine is expected to benefit from combining genomic information with regular monitoring of physiological states by multiple high-throughput methods. Here, we present an integrative personal omics profile (POP), an analysis that combines genomic, transcriptomic, proteomic, metabolomic, and microbiome profiles from a single individual over a 16-month period. Our POP analysis revealed various medical risks, including type 2 diabetes. It also uncovered extensive dynamic changes in diverse molecular components and biological pathways across healthy and diseased conditions. Extensively high-coverage genomic and transcriptomic data, which provide the basis of our POP, revealed extensive interatomic changes during healthy and diseased states and an unexpected RNA editing mechanism. This study demonstrates that longitudinal POP can be used to interpret healthy and diseased states by connecting genomic information with additional dynamic omics activity.

Introduction
 Personalized medicine aims to assess medical risks, monitor disease and treat patients according to their specific genetic constitution and molecular phenotype. The extent of genome-wide genotyping in the context of personalized medicine is restricted to a handful of genetic variants. Research recently published indicates that integration of diverse omics and medical information is now critical. Much of the genome is difficult to sequence and many complex elements, such as clusters, repetitive elements and centromeres, have been a large number of different genes and biological pathways (Shen *et al.*, 2015; Shen *et al.*, 2015; Li *et al.*, 2015), as well as environmental conditions that can be difficult to assess. As a result, the combination of personal phenotypic data with deep-coverage omics data of samples will be essential for predicting, diagnosing and treating diseases as well as for understanding disease progression and prevention of disease states (Snyder *et al.*, 2016). Presently, healthy and diseased states are typically followed using a limited number of assays that sample a small number of markers of disease types. With the advancement of next-generation sequencing, it is now possible to analyze several of 10⁹ molecular components. For example, DNA microarrays have allowed the interrogation of hundreds and thousands

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Cell

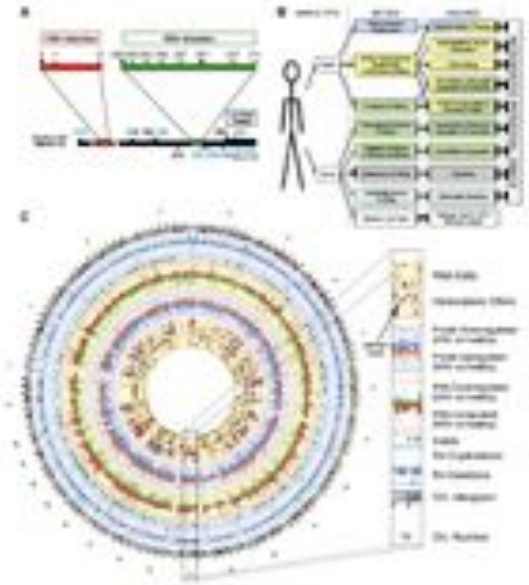
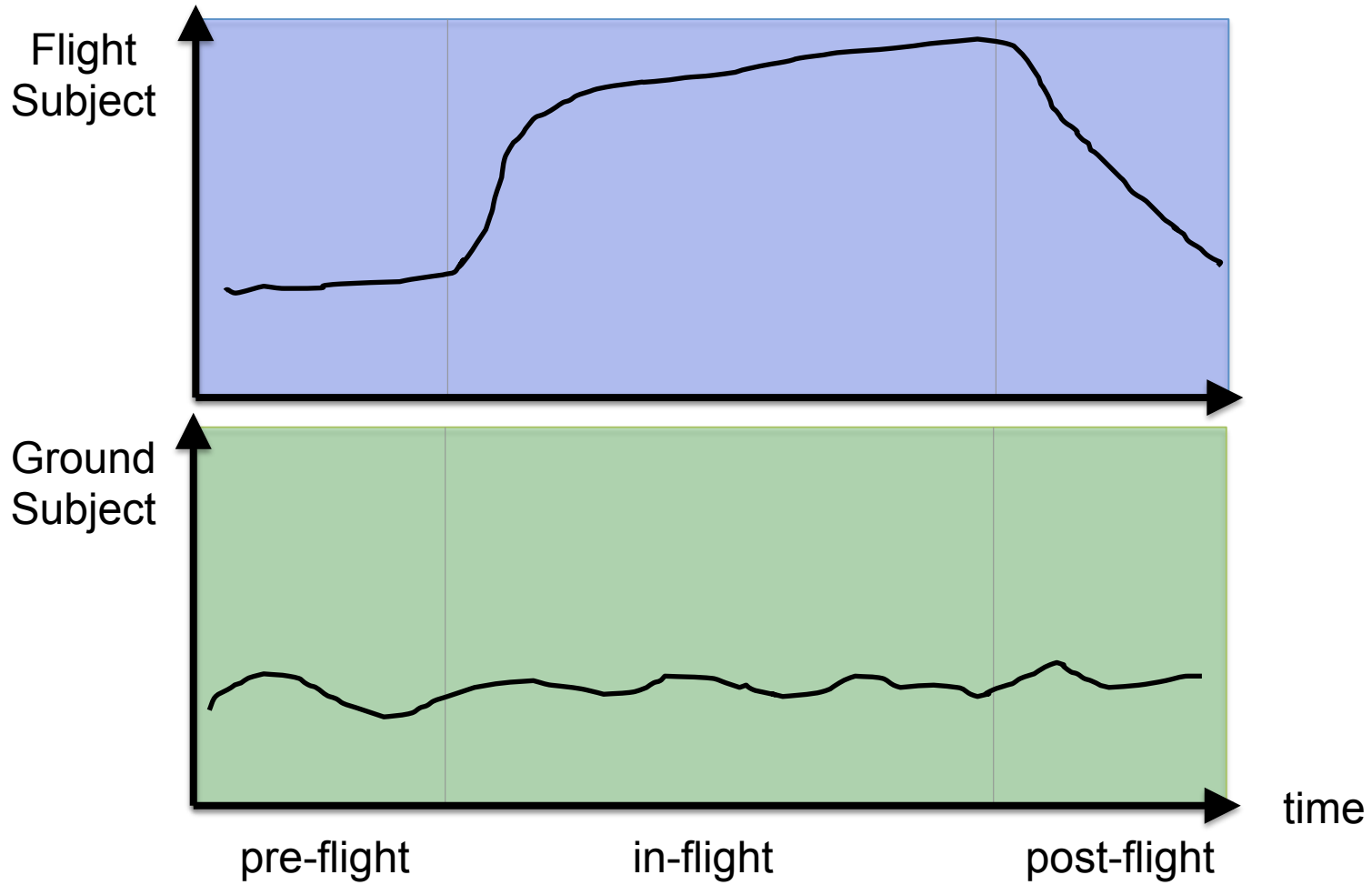


Figure 1. Personal Omics Profile (POP) analysis.
 (A) POP pipeline. The subjects in the study were followed for 16 months. (B) Personal Omics Profile (POP) analysis. The subjects in the study were followed for 16 months. (C) POP analysis. The subjects in the study were followed for 16 months. (D) POP analysis. The subjects in the study were followed for 16 months.

POP-based Disease Risk Evaluation
 We sought to identify genes to be prioritized with increased susceptibility to disease (Shen *et al.*, 2015). The list of top candidate genes and their associated pathways is shown in Figure 2. The list of top candidate genes and their associated pathways is shown in Figure 2. The list of top candidate genes and their associated pathways is shown in Figure 2.

Cell 148:1293-1307 March 19, 2015 ©2015 Elsevier Inc. 1299

One Notion





National Aeronautics and Space Administration
Johnson Space Center
Human Exploration and Operations Mission Directorate
Human Research Program
Houston, TX 77058

Human Exploration Research Opportunities (HERO)

Appendix D

Differential Effects on Homozygous Twin Astronauts Associated with Differences in Exposure to Spaceflight Factors

Response Period: July 30, 2013 – September 17, 2013
Proposals Due: September 17, 2013, 5 PM Eastern Time
Estimated Selection Announcement: January 2014

Appendix D - 1

“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.”



Buccal & Saliva



Urine



Blood

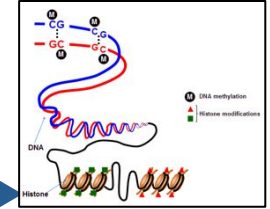


Stool

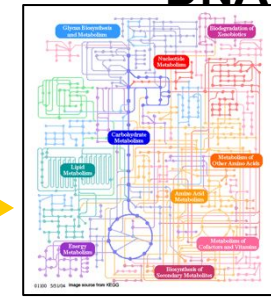


Epigenome

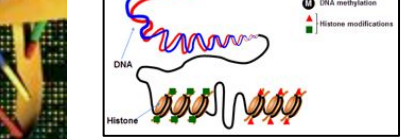
DNA



Proteins



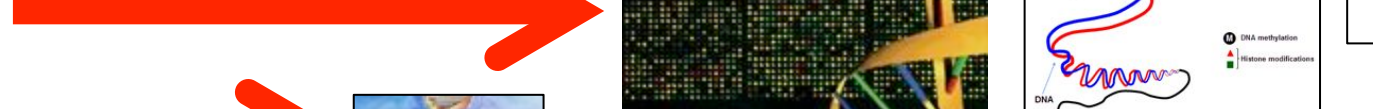
Metabolites



Epigenome



Proteins



DNA



RNA



Metagenome

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



Susan Bailey, Ph.D.
Colorado State Univ



Kerry George
Wyle Labs/JSC

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.

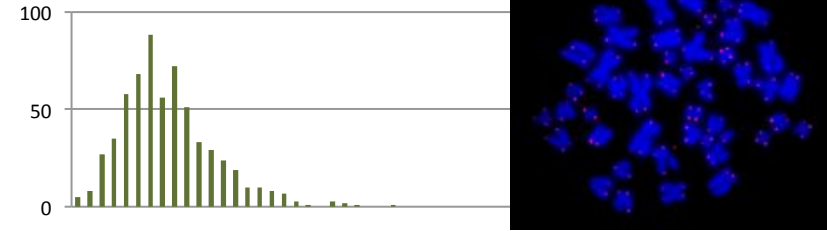
Comparisons with unrelated astronauts (separate study), will allow evaluating role of genetics/individual susceptibilities.

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.

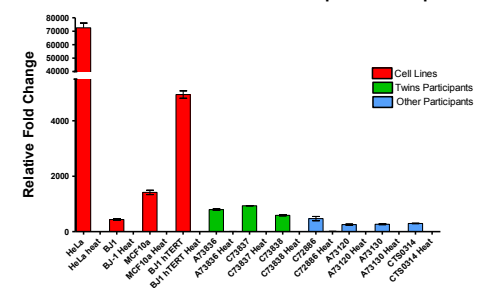
We hypothesize that accelerated telomere shortening and elevated telomerase activity will be associated with space flight as compared to ground based control, in a duration and severity dependent manner.

- 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)

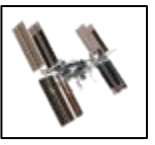
Telomere length will be assessed using TELO-FISH
 Florescence *in situ* Hybridization (FISH) with telomere probe on chromosomes (and interphase nuclei) is evaluated as Relative Fluorescence Intensity (RFI) distributions.



Telomerase activity will be assessed using qRT-PCR TRAP
 quantitative Real Time-PCR Telomere Repeat Amplification Protocol



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.

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.



Andrew Feinberg, M.D., M.P.H., and Jason Feinberg

Sample Collection and Analysis



- Whole genome DNA sequencing prior to launch and post-recovery
- Whole genome bisulfite sequencing at several time points, 450K between
- ChIP-seq at all time points
- RNAseq at several time points, arrays between

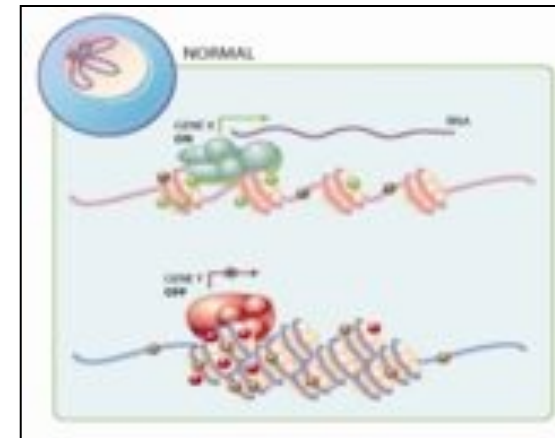
Implications of the Research for Space & Earth



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

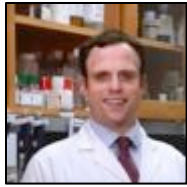


Earth: First human study of the epigenome over time in a defined/controlled environment.



- DNA methylation
- Histone modifications (>200 known)
- Chromatin factor complexes
- Chromatin structure

Landscape of DNA and RNA Methylation



Christopher Mason, Ph.D.

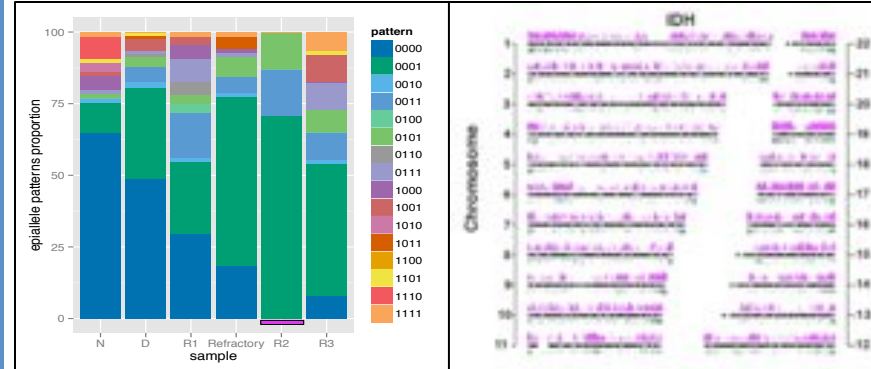


Francine Garrett-Bakelman, M.D. Ph.D.



- #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

Δ in Epigenetics : Loci, regions, and clones



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

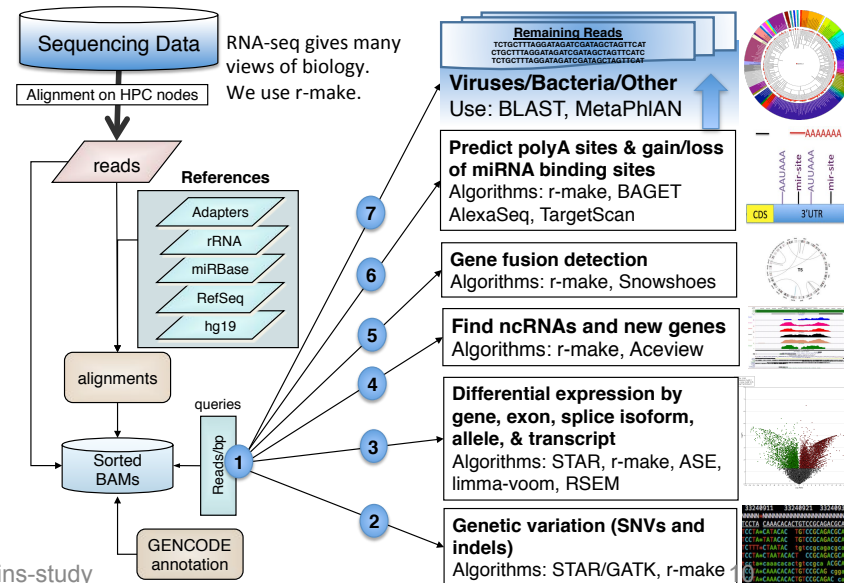
Implications of the Research for Space & Earth



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



Biochemical Profile: Homozygous Twin control for a 12 month Space Flight Exposure



Scott M.
Smith, Ph.D.

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



Emmanuel Mignot, MD, PhD



Stanford University

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



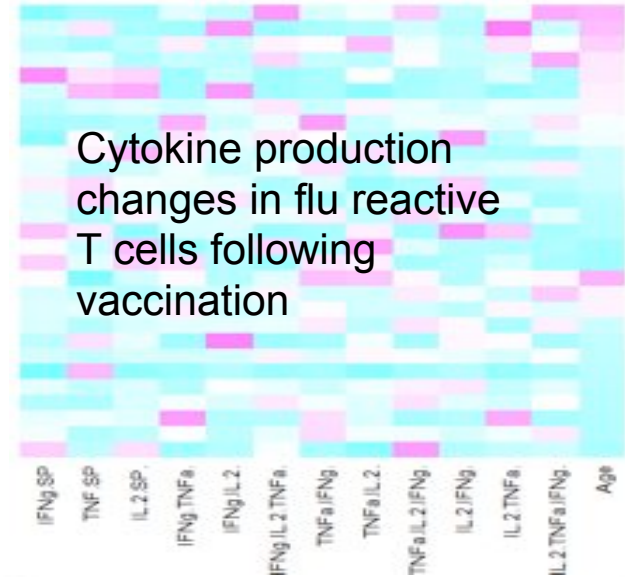
Space:

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

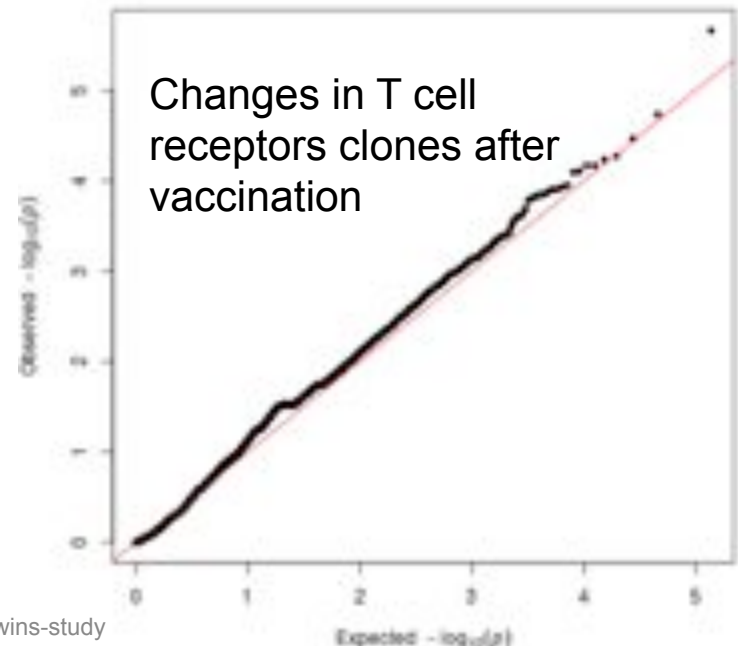


Earth:

Understand how immune response to vaccination differ in twins

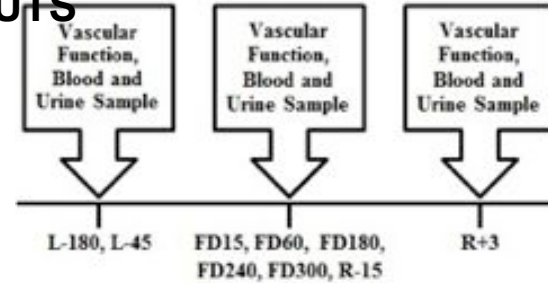
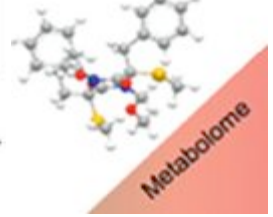


Cytokine production changes in flu reactive T cells following vaccination



Changes in T cell receptors clones after vaccination

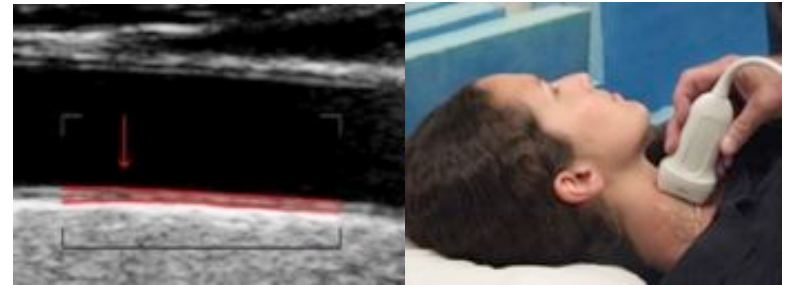
METABOLOMIC AND GENOMIC MARKERS OF ATHEROSCLEROSIS IN TWIN ASTRONAUTS



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

Pre- and Post-flight Testing



Inflight Operations



Stuart Lee

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



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



Brinda Rana, PhD
Mike Stenger, PhD
Vivian Hook, PhD

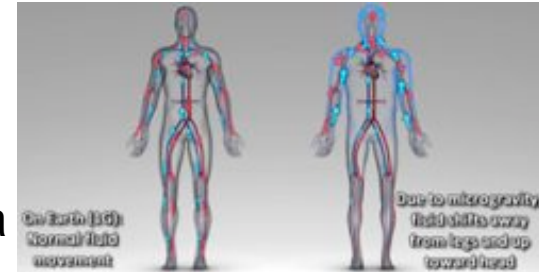
Specific Aims

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.



Blood Plasma proteins



In-flight Operations



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

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

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.



Mathias Basner, M.D., Ph.D.



Ruben C. Gur, Ph.D.

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 extent 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.

	Test	Cognitive Domain	Brain Regions (from fMRI studies)	Avg. Time (Min)
	Motor Praxis (MPT)	Sensory-motor ability	Sensorimotor Cortex	0.51
	Visual Object Learning (VOLT)	Visual object learning and memory	Medial Temporal Cortex - Hippocampus	1.69
	Fractal 2-Back (F2B)	Attention and working memory	Dorsolateral prefrontal Cortex, Cingulate, Hippocampus	1.93
	Abstract Matching Task (AMT)	Abstraction and mental flexibility	Prefrontal Cortex	2.33
	Line Orientation (LOT)	Spacial orientation	Right Temporo-Parietal Cortex, Visual Cortex	2.07
	Emotion Recognition (ERT)	Emotion recognition	Cingulate Cortex, Amygdala, Hippocampus, Fusiform Face Area	2.03
	Matrix Reasoning (MRT)	Abstract reasoning	Prefrontal Cortex, Parietal Cortex, Temporal Cortex	2.09
	Digit Symbol Substitution (DSST)	Complex scanning, visual tracking, attention	Temporal Cortex, Prefrontal Cortex, Motor Cortex	1.60
	Balloon Analog Risk (BART)	Risk decision making	Orbital frontal Cortex, Amygdala, Hippocampus, Anterior Cingulate Cortex	2.39
	Psychomotor Vigilance (PVT)	Vigilant attention and psychomotor speed	Prefrontal Cortex, Motor Cortex, Visual Cortex	3.17

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



Fred Turek,
Ph.D.

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?

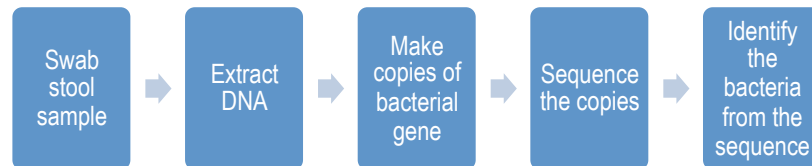
Implications of the Research for Space & Earth



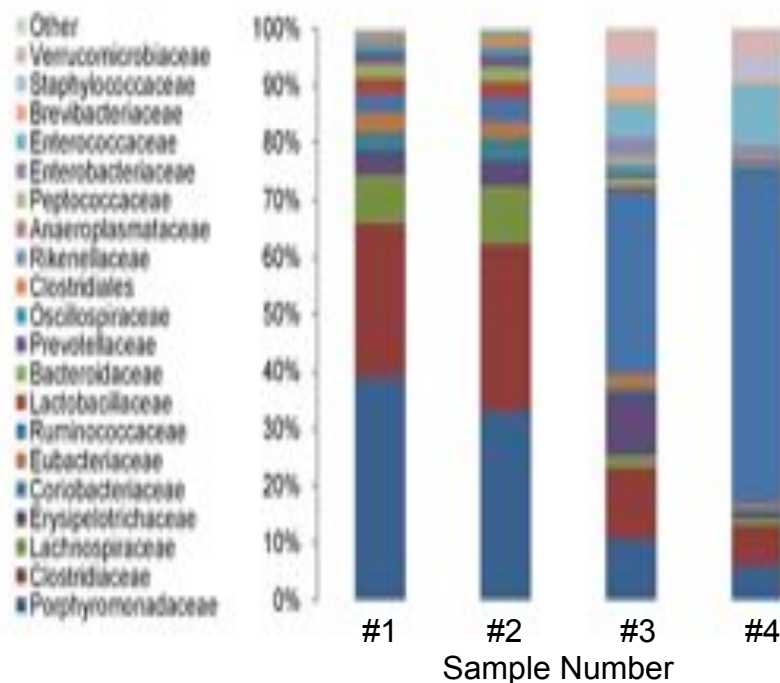
Space: Knowing how the bacteriome changes over time in space can help us make plans to protect astronauts' health for longer-term space flights. For example, adjustments to diet could help maintain beneficial bacterial types.



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.



Classify bacteria from each sample



Integrated Omics: Mike Snyder, Ph.D.



Michael Snyder, Ph.D.



Juliane Winkelmann, M.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.

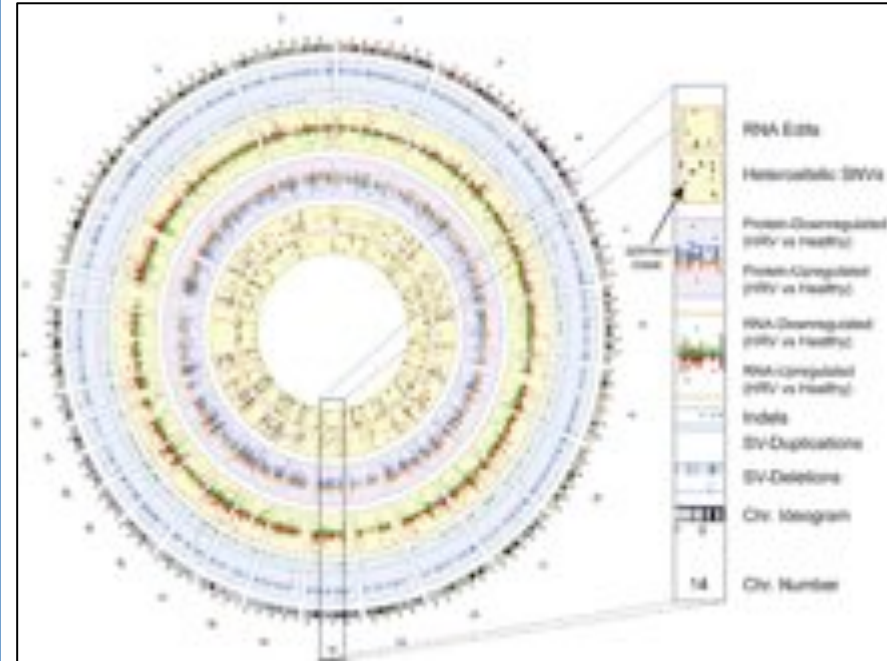
Implications of the Research for Space & Earth



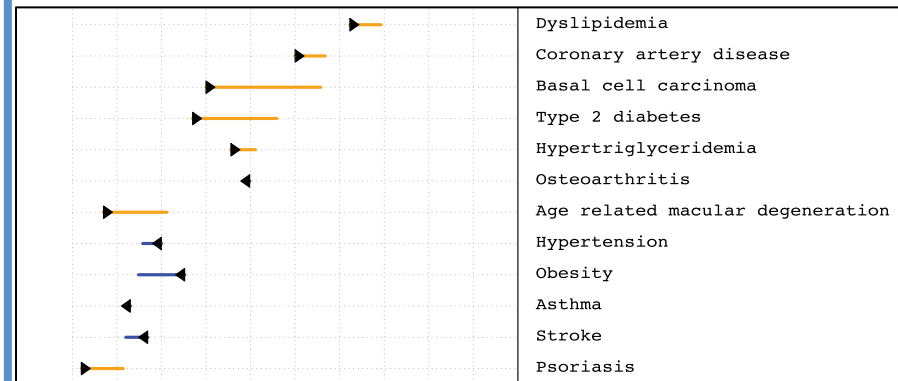
Space: We will generate a detailed benchmark for how human physiology changes in space in great molecular detail. This wealth of data will be essential for any future planning of long duration space exploration missions, and provide a proof-of-principle for better monitoring and managing astronaut health.



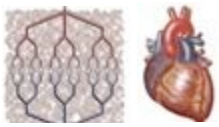
Earth: With this study, Scott and Mark Kelly will be the most thoroughly profiled twins in history, and the resultant data will offer new insights into how two siblings with nearly-identical genomes respond to different conditions.



Integrative multi-omic model



Risk-o-gram



Vasculature
Lee



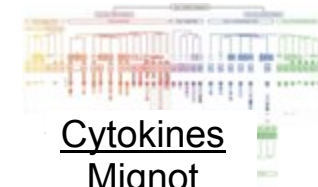
Cognition
Basner



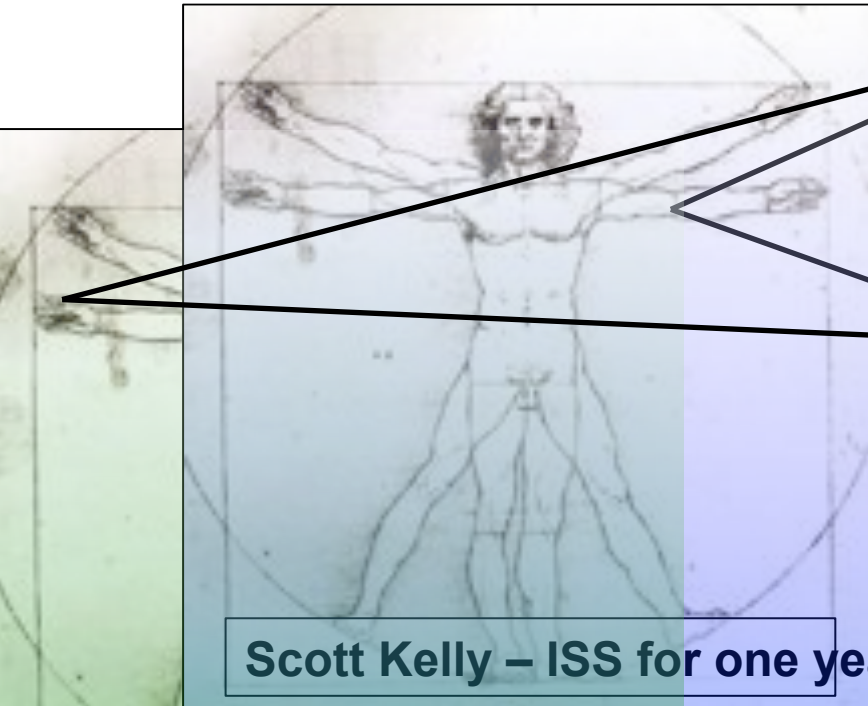
Microbiome
Turek



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

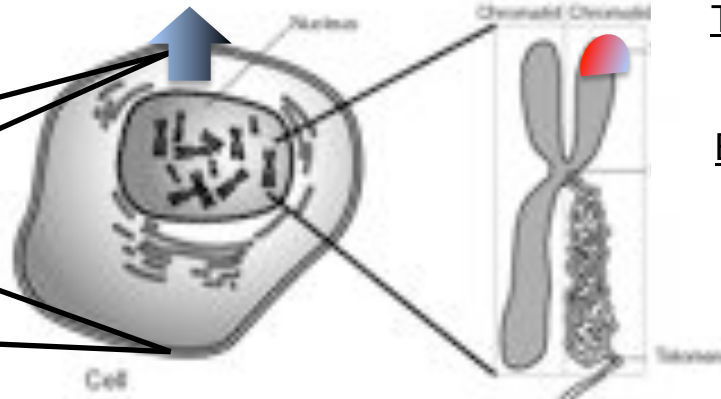


Cytokines
Mignot



Scott Kelly – ISS for one year

Mark Kelly – Earth control

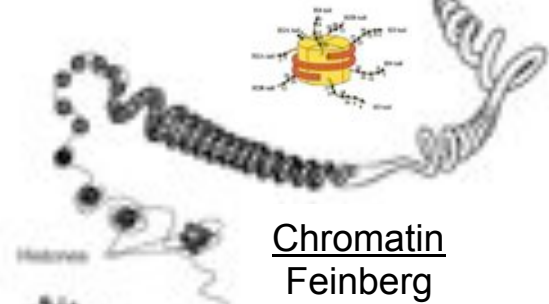


Telomere Length
Bailey

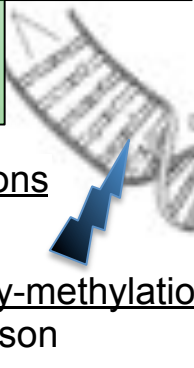
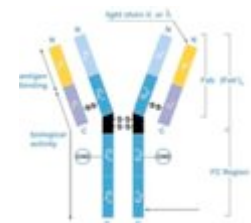
B-cells / T-cells
Mignot



Antibodies
Mignot/Snyder



Chromatin
Feinberg



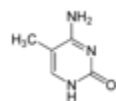
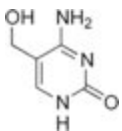
DNA Mutations
Feinberg

DNA Hydroxy-methylation
Mason

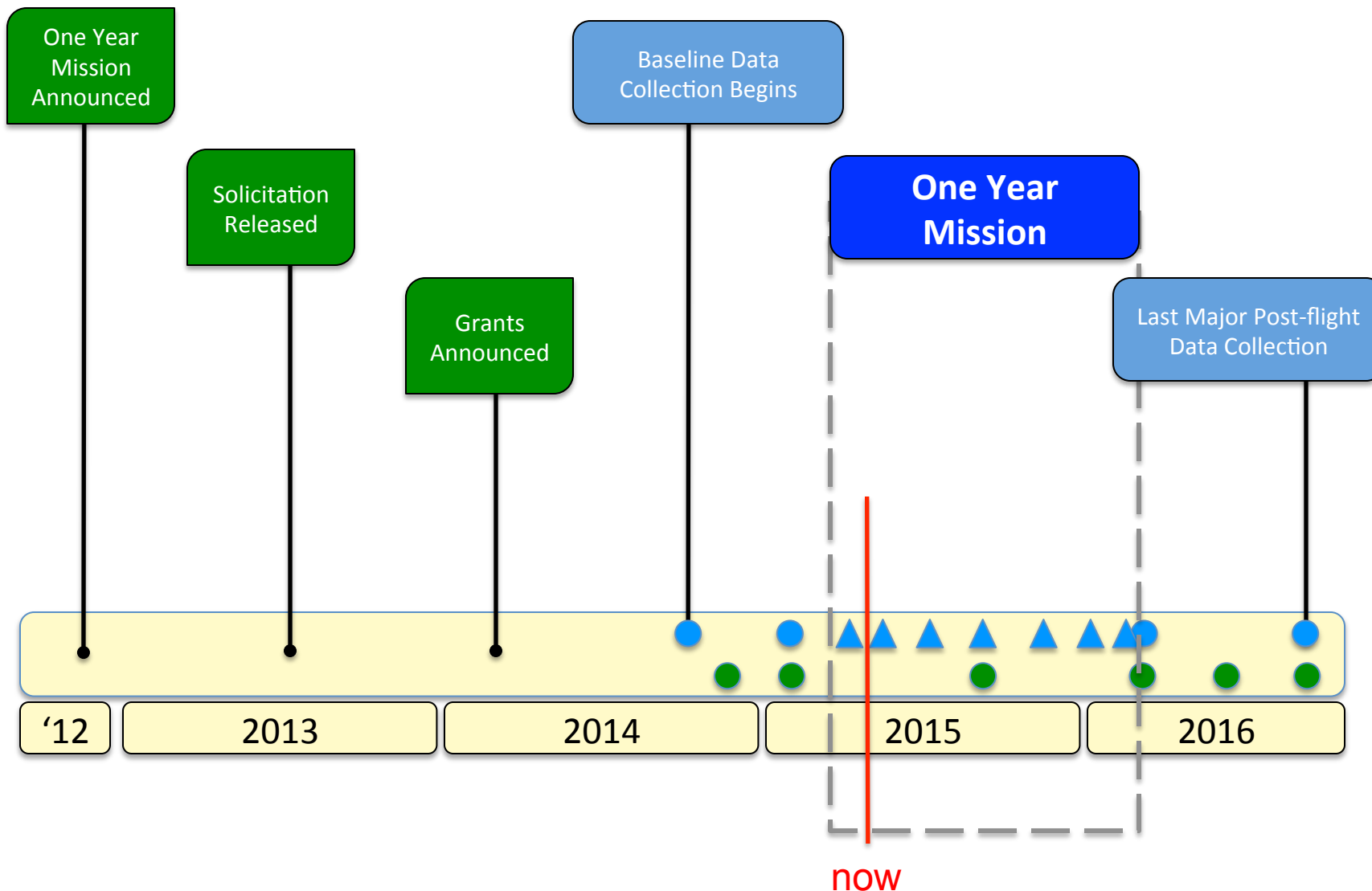
DNA Methylation
Feinberg & Mason

large/small RNA & RNA Methylation
Mason

Proteomics
Lee/Rana



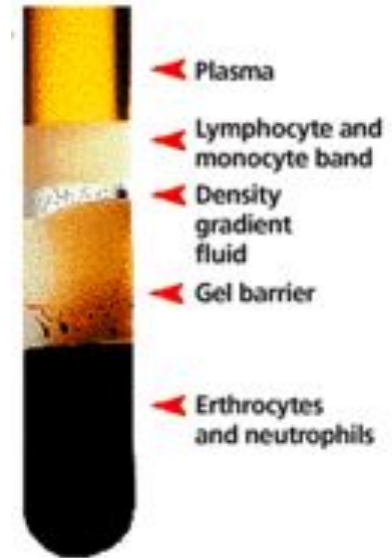
Timeline



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**



Conclusion

- 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



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The Twins Study Investigator Team



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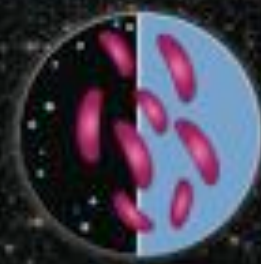
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time.com/meet-the-twins-unlocking-the-secrets-of-space/



www.nasa.gov/content/twins-study