



# Human Research Program

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

Grand Rounds  
23 September 2014



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Deputy Chief Scientist, HRP  
SA2/NASA JSC



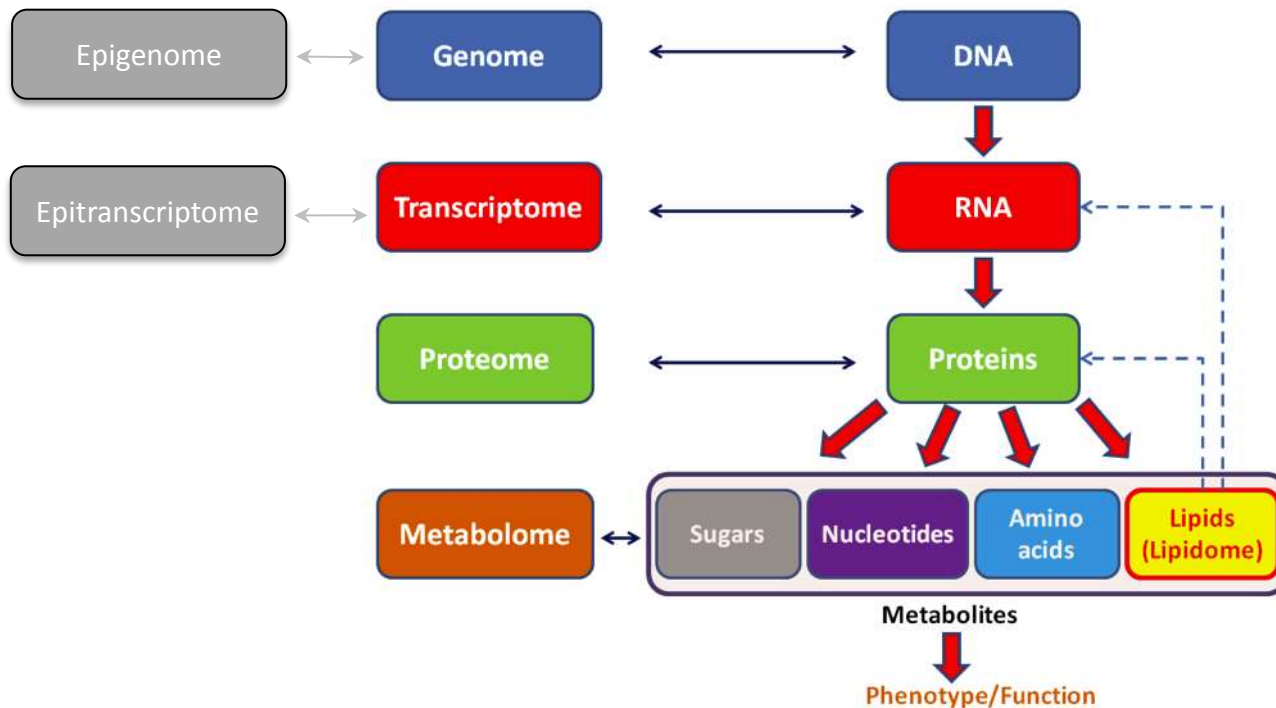


- What is “omics” and what can we learn from an omics investigation?
- What is the Twins Study?
- What issues is NASA grappling with as it undertakes omic research?



Omics: A neologism for the constellation of an organism's “-omic” information, which includes the genome itself (genomic), transcription products (transcriptomic), protein products (proteomic) and metabolic products (metabolomic).

[medical-dictionary.thefreedictionary.com/omics](http://medical-dictionary.thefreedictionary.com/omics)





# Example: The "Snyderome"



Resource

Cell

## Personal Omics Profiling Reveals Dynamic Molecular and Medical Phenotypes

Rui Chen,<sup>1,11</sup> George L. Mias,<sup>1,11</sup> Jennifer Li-Pook-Than,<sup>1,11</sup> Lihua Jiang,<sup>1,11</sup> Hugo Y.K. Lam,<sup>1,12</sup> Rong Chen,<sup>1,12</sup> Elnaz Mirami,<sup>1</sup> Konrad J. Karczewski,<sup>1</sup> Manoj Harthanan,<sup>1</sup> Frederick E. Dewey,<sup>3</sup> Yong Chang,<sup>1</sup> Michael J. Clark,<sup>1</sup> Huijiao Im,<sup>1</sup> Lukas Habegger,<sup>4,5</sup> Sagartha Balasubramanian,<sup>6,7</sup> Masaru O'Huillachain,<sup>1</sup> Joel T. Dudley,<sup>7</sup> Sara Hilariswayer,<sup>1</sup> Rajni Huskikhin,<sup>1</sup> Donald Sharon,<sup>1</sup> Ghia Euskirchen,<sup>1</sup> Phil Lacroute,<sup>1</sup> Keith Baldinger,<sup>1</sup> Alan P. Boyle,<sup>1</sup> Maya Kasowski,<sup>1</sup> Fabian Gruber,<sup>1</sup> Scott Sokol,<sup>2</sup> Marco Garcia,<sup>2</sup> Michelle Whitt-Carrillo,<sup>1</sup> Mercedes Gallardo,<sup>8,10</sup> Maria A. Blasco,<sup>9</sup> Peter L. Greenberg,<sup>4</sup> Phyllis Snyder,<sup>1</sup> Teri E. Klein,<sup>1</sup> Russ B. Altman,<sup>1,2</sup> Afaf J. Butte,<sup>2</sup> Euan A. Ashley,<sup>2</sup> Mark Gerstein,<sup>4,12</sup> Karl C. Nadeau,<sup>2</sup> Hua Tang,<sup>1</sup> and Michael Snyder<sup>1,6</sup>

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 DOI:10.1016/j.cell.2012.02.009



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 (iPOP), an analysis that combines genomic, transcriptomic, proteomic, metabolomic, and autoantibody profiles from a single individual over a 14-month period. Our iPOP 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. Extremely high-coverage genomic and transcriptomic data, which provide the basis of our iPOP, revealed extensive heterallelic changes during healthy and diseased states and an unexpected RNA editing mechanism. This study demonstrates that longitudinal iPOP 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, diagnose and treat patients according to their specific genetic composition and molecular phenotypes. The advent of genome sequencing and the analysis of physiological states has proven to be powerful (Cancer Genome Atlas Research Network, 2015). However, its implementation for estimation of disease risk and medical intervention is less clear. Much of the genome is difficult to interpret and many complex diseases, such as diabetes, neurological disorders and cancer, likely involve a large number of different genes and biological pathways (Ashley et al., 2010; Grayson et al., 2011; Li et al., 2011), as well as environmental contributors that can be difficult to assess. As such, the combination of genomic information along with a detailed molecular analysis of samples will be important for predicting, diagnosing and treating diseases as well as for understanding the onset, progression, and prevalence of disease states (Snyder et al., 2008). Presently, healthy and diseased states are typically followed using a limited number of assays that analyze a small number of markers of distinct types. With the advancement of many new technologies, it is now possible to analyze upward of 10<sup>6</sup> molecular constituents. For example, DNA microarrays have allowed the subcategorization of lymphomas and gliomas

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Cell

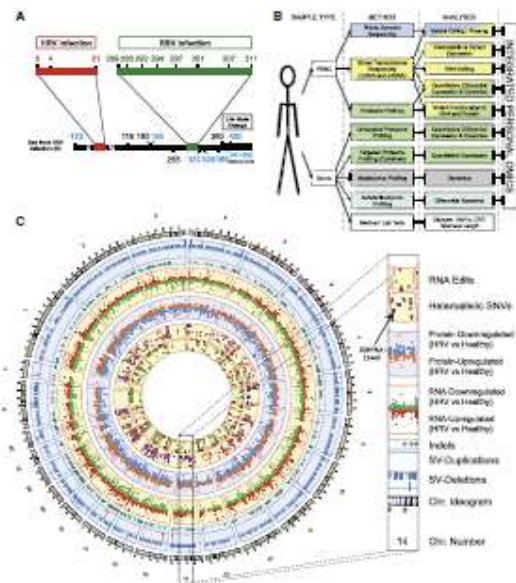


Figure 1. Summary of Study

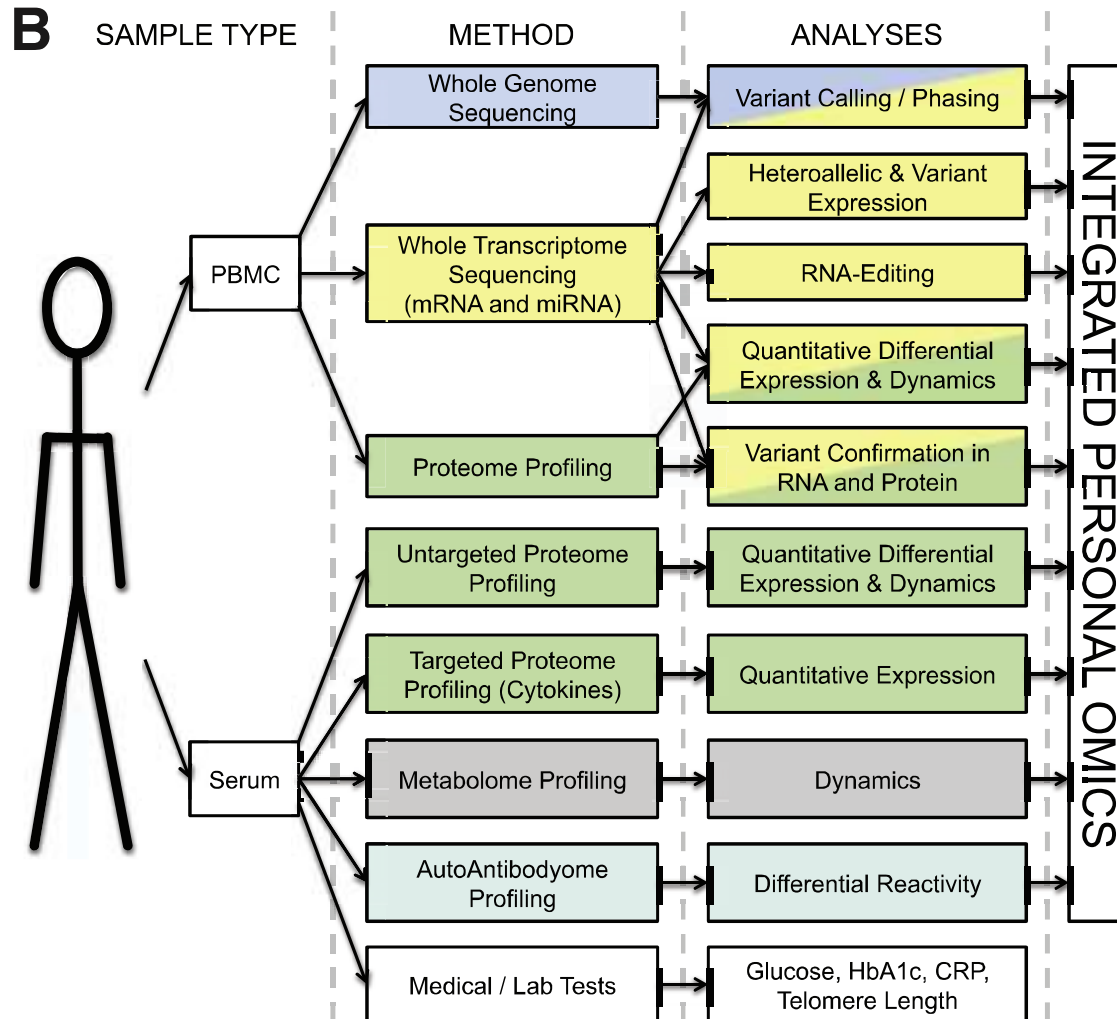
(A) Time course summary. The subjects were monitored for a total of 720 days, during which there were two injections (red bar, IRV; green bar, RDV). The black bar indicates the period when the subjects (1) increased exercise, (2) ingested 0.1 mg of acetylsalicylic acid and 0.5 mg of protein tablets each day (the latter only during the first 6 weeks of this period), and (3) substantially reduced sugar intake. Blue numbers indicate blood draw points.  
 (B) iPOP experimental design indicating the tissues and analyses involved in this study.  
 (C) Circos (Zeylanli et al., 2009) plot summarizing iPOP. From outer to inner rings: chromosome ideogram; genetic data (pink/blue ring); structural variants > 50 bp (4 directions) (blue/black); duplications (red/black); indels (green/orange ring); transcription data (yellow ring); expression ratio of IRV injection to healthy state; proteomic data (light purple ring); ratio of protein levels during IRV injection to healthy state; transcriptomic data (yellow ring); differential heterallelic expression ratio of alternative allele to reference allele for missense and synonymous variants (purple dots) and candidate RNA editing and synonymous edits (red triangles, purple dots, orange triangles and green dots, respectively). See also Figure S1.

### WGS-Based Disease Risk Evaluation

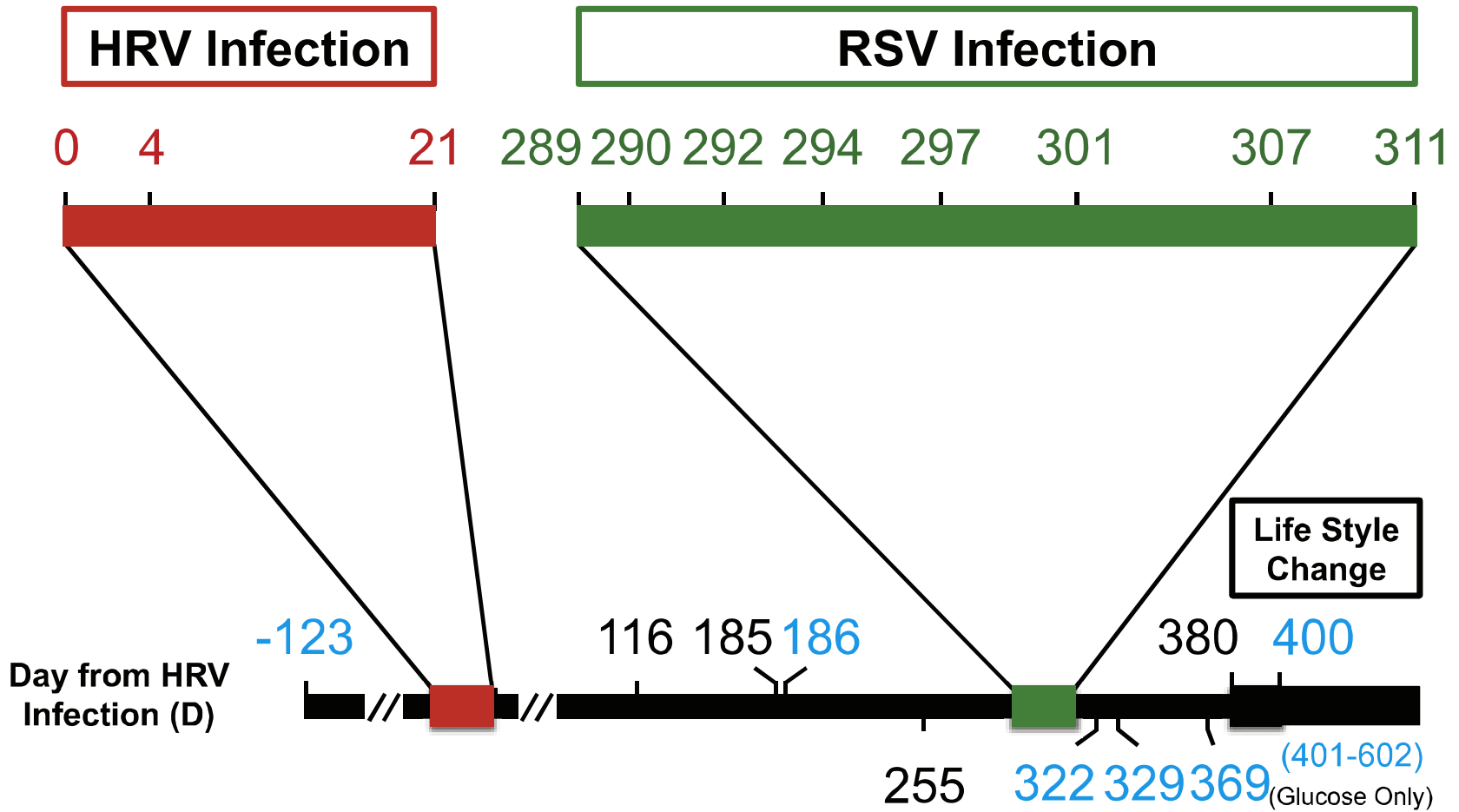
We identified variants likely to be associated with increased susceptibility to disease (Dewey et al., 2011). The list of high confidence SNVs and indels was analyzed for rare alleles (<5% of the major allele frequency in Europeans) and for changes in genes with known Mendelian disease phenotypes (data summarized in Table 2), revealing that 51 and 4 of the rare coding SNV and indels, respectively, in genes present in OMIM are predicted

to lead to loss-of-function (Table S2A). This list of genes was further examined for medical relevance (Table S2A); example alleles are summarized in Figure 2A, and 11 were validated by Sanger sequencing. High interest genes include: (1) a mutation (E58K) in the *SERPINA1* gene previously known in the subject, (2) a damaging mutation in *TERF1*, associated with acquired aplastic anemia (Yamaguchi et al., 2005), and (3) variants associated with hypertriglyceridemia and diabetes, such as GGN7

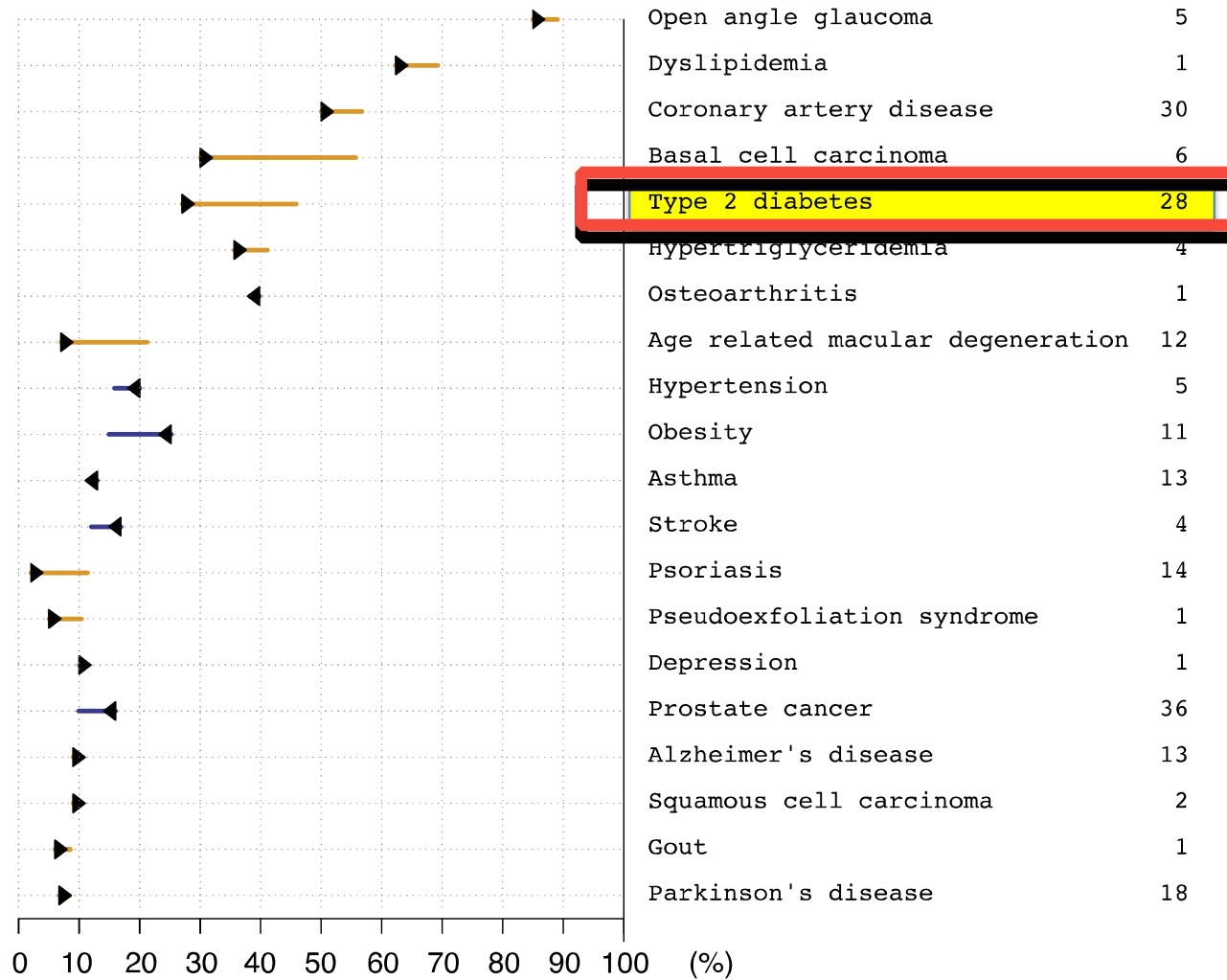
Cell 148, 1293–1307, March 16, 2012 ©2012 Elsevier Inc. 1295



# Timeline



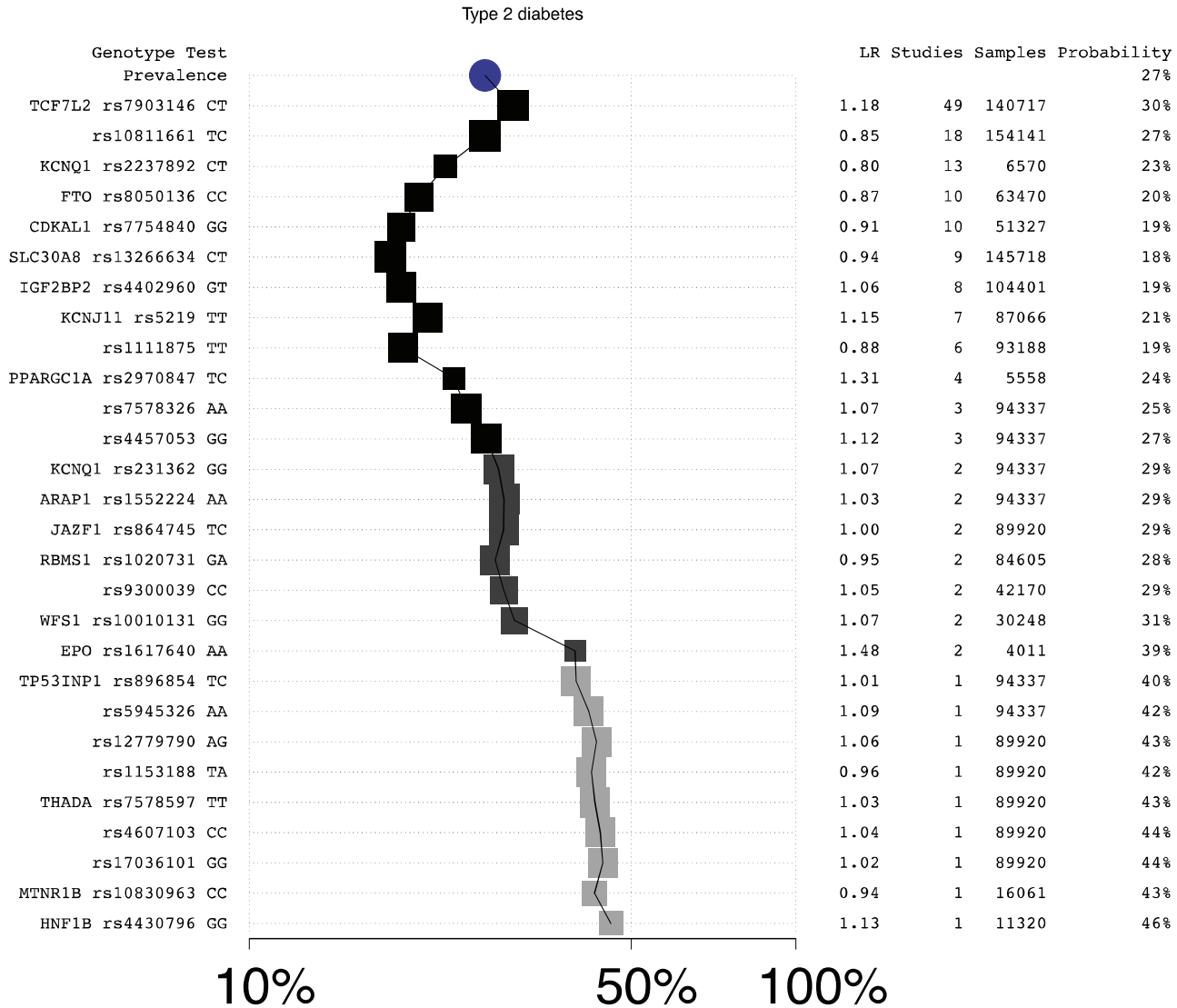
# Genome: Quantitative Risk Estimates



# Decomposition of the Risk Estimate



C





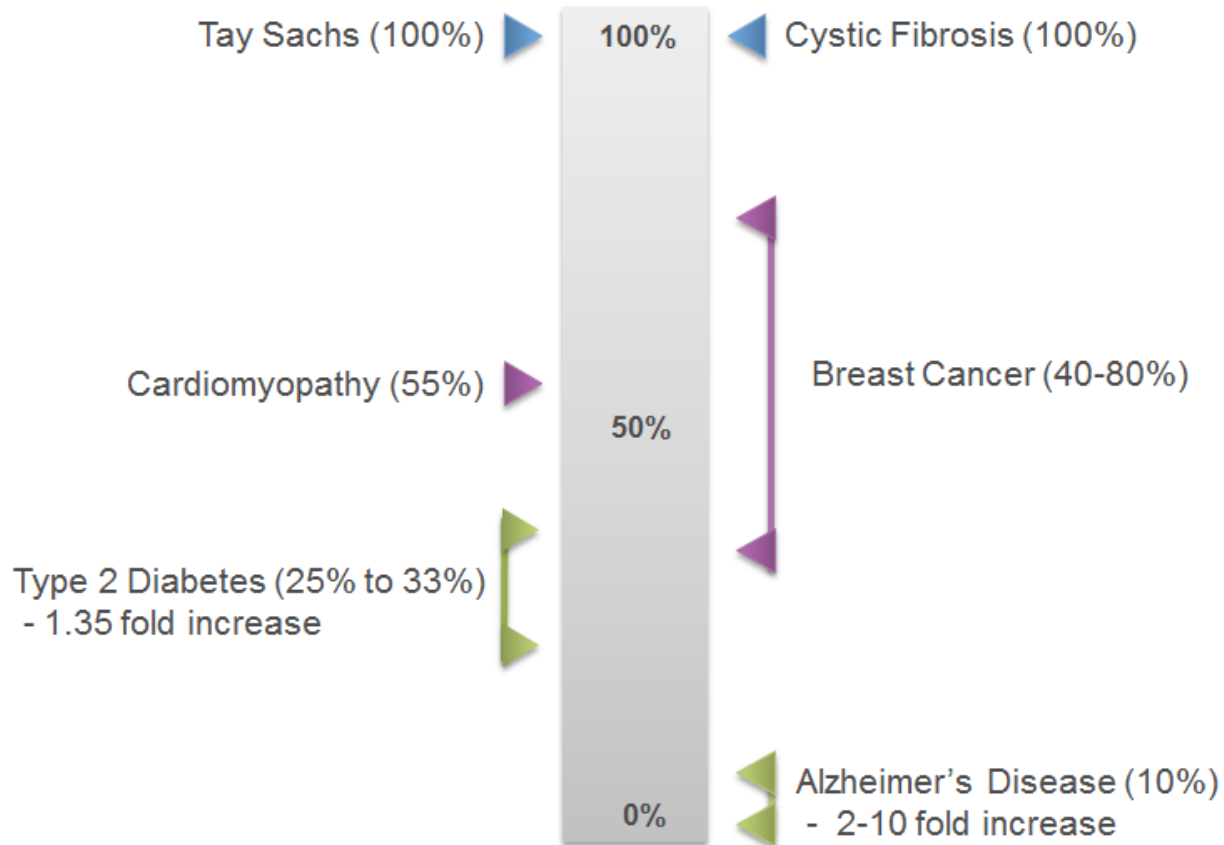
# Wide Range of Predictive Power



## Genomes are complex

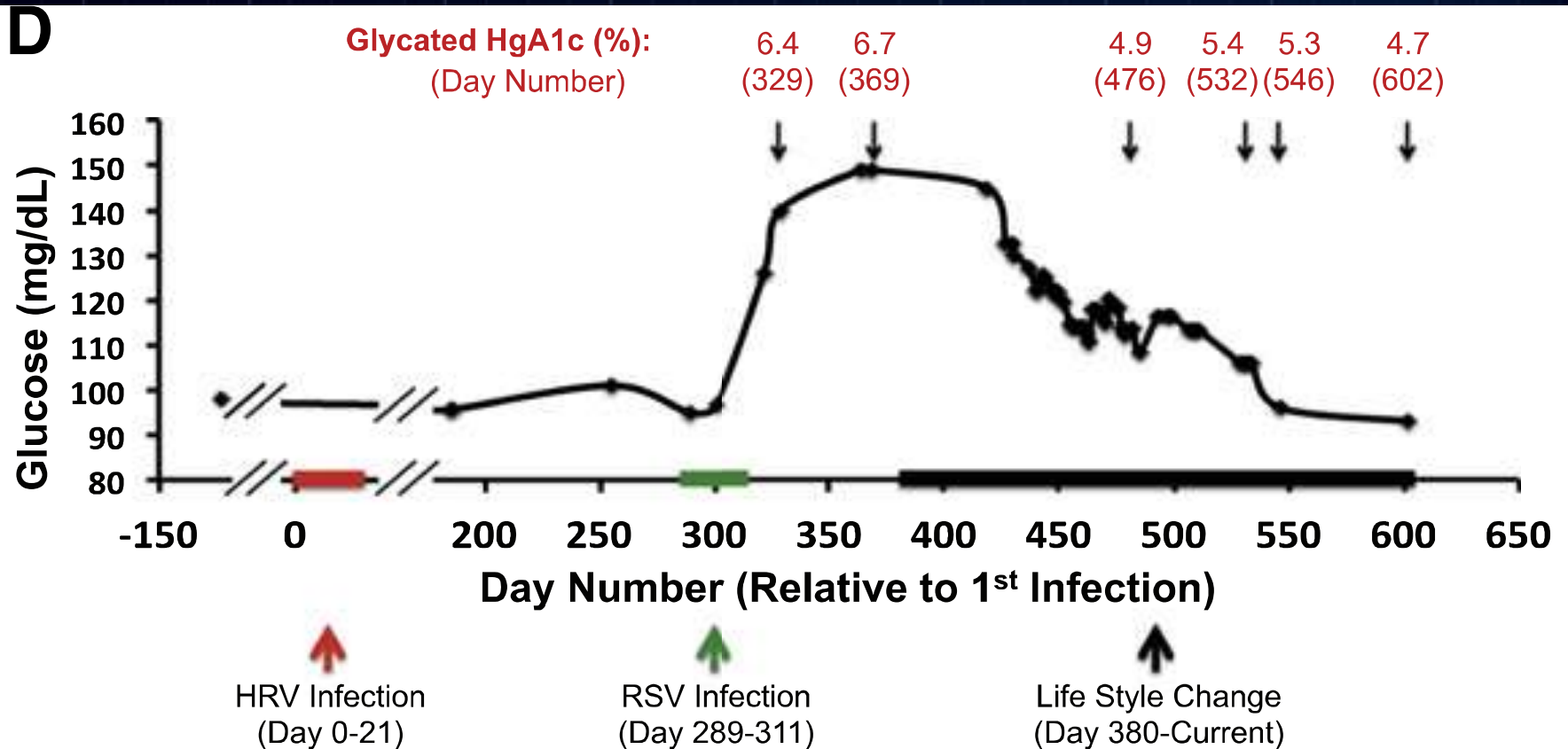
- ▲ Monogenic diseases
- ▲ Majority of disease risk by single gene
- ▲ Epigenetic disease(>1 gene + environment)

### 11% of the genes for clinical interpretation





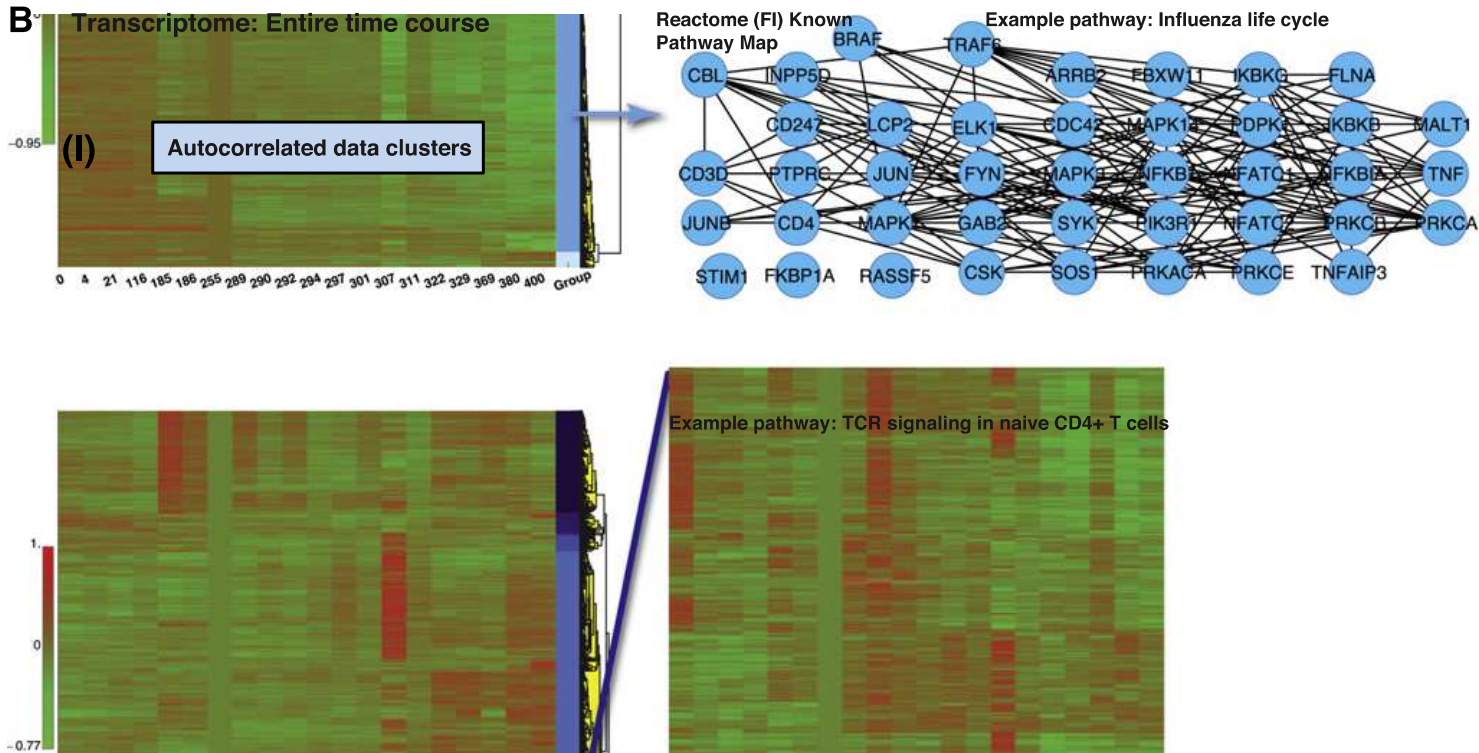
# (Targeted) Metabolome: Glucose



“After a dramatic change in diet, exercise and ingestion of low doses of acetylsalicylic acid a gradual decrease in glucose (to ~93 mg/dl at day 602) and HbA1c levels to 4.7% was observed.”

“These results indicate that a genome sequence can be used to estimate disease risk in a healthy individual, and by monitoring traits associated with that disease, disease markers can be detected and the phenotype treated.”

# Transcriptome: Unexpected Activations



“A large number of genes with a coexpression pattern common to both infections in the time course have yet to be implicated in known pathways and provide possible connections related to immune response.”



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# The One-Year Mission



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

Posted: 11/26/2012 9:25 am EST Updated: 11/26/2012 9:40 am EST

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FOLLOW: Video, Scott Kelly, International Space Station, IIS Crew, IIS Mission, Mikhail Kornienko, International Space Station, Science News

By: Tariq Malik  
Published: 11/26/2012 08:12 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.

HOME > SCIENCE

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

By Latin Times Staff Writer, Dec 07, 2012 08:00 PM EST

0 Comments 0 Like 0 Tweet 0 +1 0 Share Text Size - +

Tags: NASA, Space







SCIENCE

30  
COMMENTS

## NASA will separate twin brothers for a year: one on Earth, one in space

By Carl Franzen on August 5, 2013 09:34 pm [Email](#) [@carlfranz](#)

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141 Of all the members of NASA's current crop of distinguished astronauts, only two have the unique distinction of being identical twin brothers. And now NASA is using an idea by the brothers, Mark Kelly and Scott Kelly, to perform a study that's been an act-up until now. Beginning in March 2015, the space agency will be comparing the biological states of both twin brothers over the span of a year, with a twist: Scott will be aboard the International Space Station for the duration of that period, while Mark, who retired from NASA back in 2011, will remain back here on Earth.

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New Oculus Rift cover shooter is so realistic, players warned not to lean on virtual objects

Sea level and temperatures hit record highs last year, report says

Microsoft launches Windows Phone web tool to let anyone create apps

US government considers creation of a cyber...

THE VERGE



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

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## 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

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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.”

# Selections

# NASA Funded 10 Research Proposals In Response to its “Twins” Solicitation



- 2 Subjects

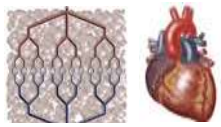
- Scott Kelly
- Mark Kelly

- 10 Selections

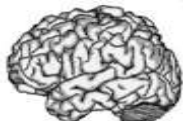
1. Susan Bailey, Colorado State University, Differential effects on telomeres and telomerase in twin astronauts associated with spaceflight
2. Andrew Feinberg, Johns Hopkins University School of Medicine, Comprehensive whole genome analysis of differential epigenetic effects of space travel on monozygotic twins
3. Christopher Mason, Weill Medical College of Cornell University, The Landscape of DNA and RNA Methylation Before, During, and After Human Space Travel
4. Scott Smith, NASA Johnson Space Center, Biochemical Profile: Homozygous Twin control for a 12 month Space Flight Exposure
5. Emmanuel Mignot, Stanford University School of Medicine, HERO Twin Astronaut Study Consortium (TASC): Immunome Changes in Space
6. Fred Turek, Northwestern University, HERO Twin Astronaut Study Consortium (TASC) Project: Metagenomic Sequencing of the Bacteriome in GI Tract of Twin Astronauts
7. Stuart Lee, Wyle Laboratories, Metabolomic And Genomic Markers Of Atherosclerosis As Related To Oxidative Stress, Inflammation, And Vascular Function In Twin Astronauts
8. Brinda Rana, University of California, Proteomic Assessment of Fluid Shifts and Association with Visual Impairment and Intracranial Pressure in Twin Astronauts
9. Mathias Basner, University of Pennsylvania School of Medicine, HERO Twin Astronaut Study Consortium (TASC) Project: Cognition on Monozygotic Twin on Earth
10. Michael Snyder, Stanford University, HERO Twin Astronaut Study Consortium (TASC) Project: Longitudinal integrated multi-omics analysis of the biomolecular effects of space travel







Vasculature  
Lee



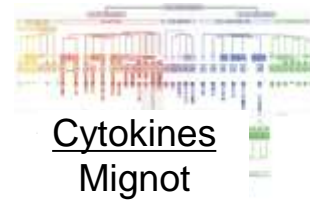
Cognition  
Basner



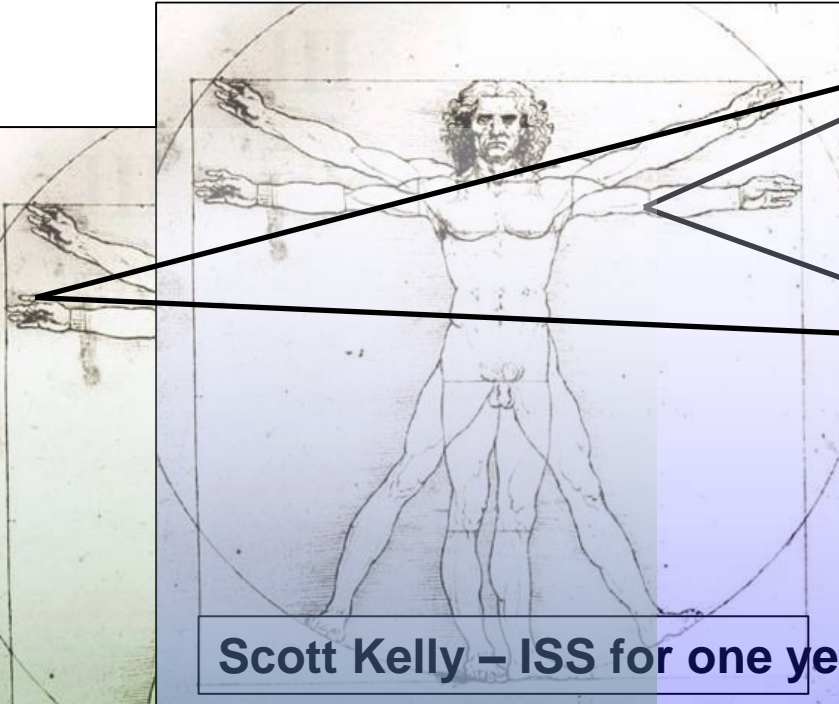
Microbiome  
Turek



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

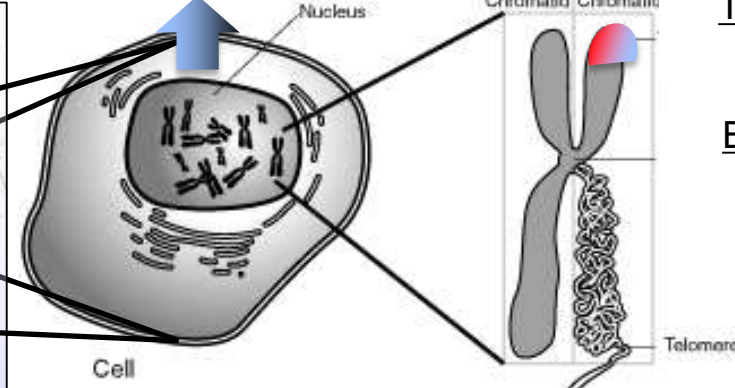


Cytokines  
Mignot



**Scott Kelly – ISS for one year**

**Mark Kelly – Earth control**

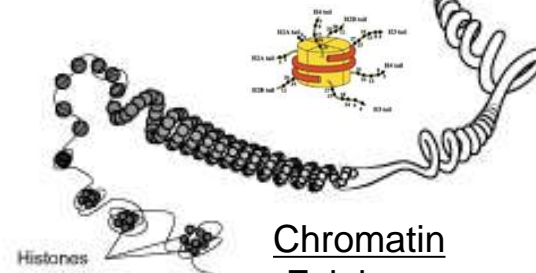


Telomere Length  
Bailey

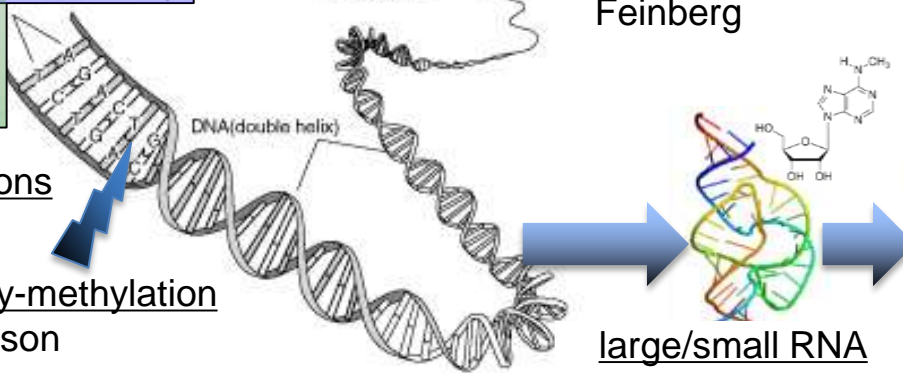
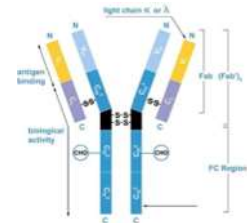
B-cells / T-cells  
Mignot



Antibodies  
Mignot/Snyder



Chromatin  
Feinberg



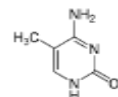
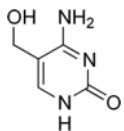
DNA Mutations  
Feinberg

DNA Hydroxy-methylation  
Mason

DNA Methylation  
Feinberg & Mason

large/small RNA & RNA Methylation  
Mason

Proteomics  
Lee/Rana





**Buccal**



**Urine**



**Blood**



**Stool**



**Epigenome**



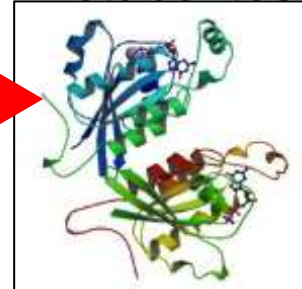
**DNA**



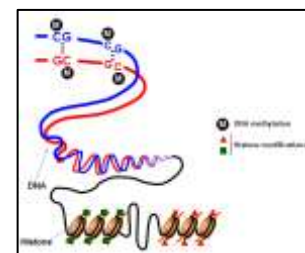
**Proteins**



**Metabolites**



**Proteins**



**Epigenome**



**DNA**



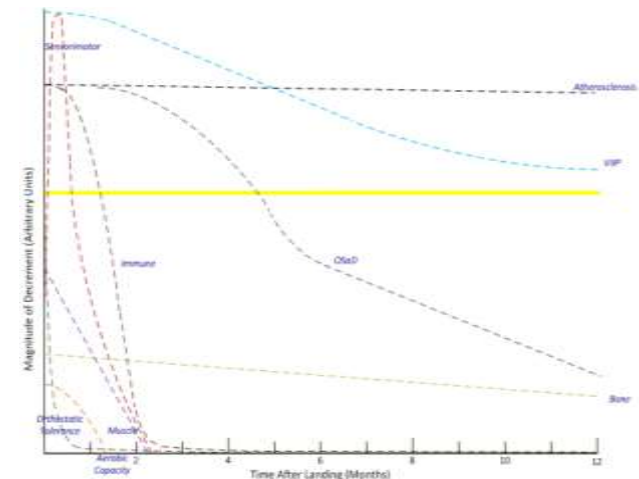
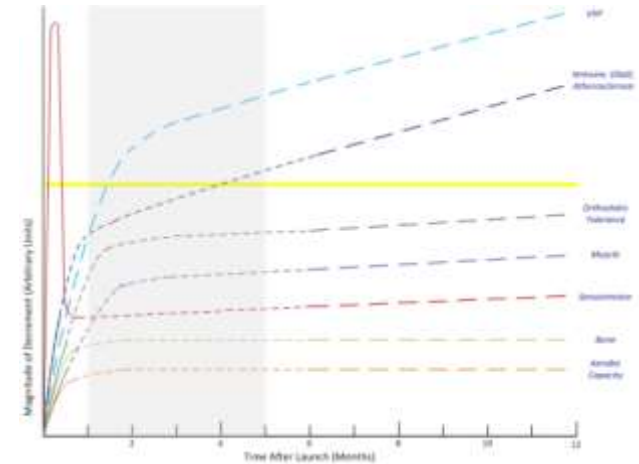
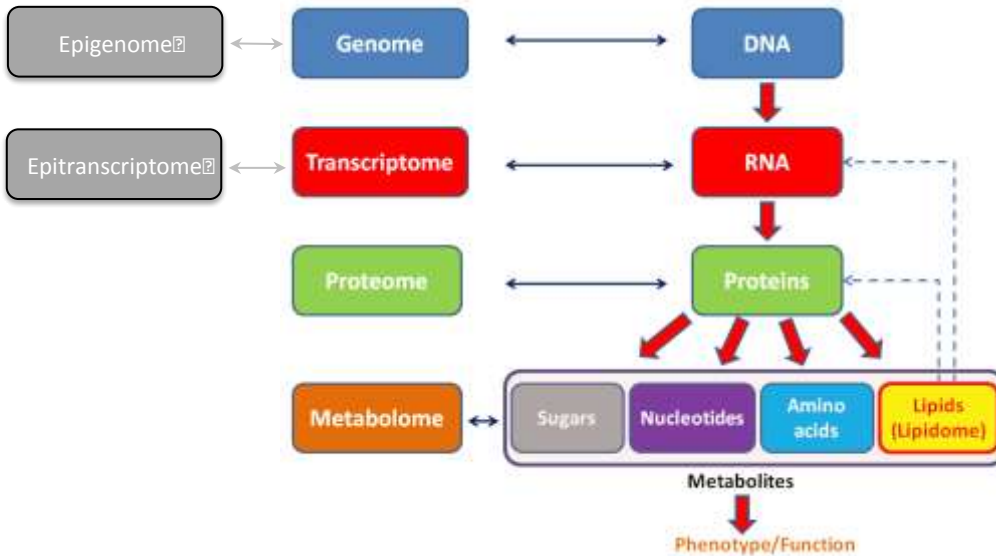
**RNA**



**Metagenome**



# Measuring the Temporal Response to Space Flight



- 2 major sample collections pre-flight
- 10 major sample collections in-flight
- 2 major sample collections post-flight
- 6 major sample collections ground

Notional Time Courses



- What is “omics” and what can we learn from an omics investigation?
- What is the Twins Study?
- What issues is NASA grappling with as it undertakes omic research?

# Issues Associated with Omic Research



- Protect the Research Subject
- Medical care
- Occupational health
- Employment activity

# Protecting the Human Subject



- Interim policy on genetic research JID 1800.4
  - Applies to the NASA Flight IRB
  - “For purposes of this policy, the term ‘genetic analysis’ includes research involving human DNA, RNA, chromosomes, proteins, or metabolites that detects genotypes, mutations, or chromosomal changes. It excludes the analysis and collection of bio-specimens that will not be submitted to genetic analysis.”

## • Changes to the Informed Consent Form

The **Substitution**  
FROM: **44/00000**  
SUBJECT: **NASA Flight Institutional Review Board (Flight IRB) Review of Proposals Involving Genetic Analysis**

**5. Purpose and scope**

IRBs will not have primary focus on genetic research. Their main purpose is to protect the individual subjects of the research and to ensure that the research is conducted in a manner that is consistent with the ethical principles of the Declaration of Helsinki. IRBs will not have primary focus on genetic research. Their main purpose is to protect the individual subjects of the research and to ensure that the research is conducted in a manner that is consistent with the ethical principles of the Declaration of Helsinki.

**6. Flight IRB limitations**

**1. IRB cannot review any research involving the collection, storage, analysis, or dissemination of genetic information that is not approved by the Flight IRB.**

**2. The Flight IRB shall categorize all studies involving the collection, storage, analysis, or dissemination of genetic information as either "approved" or "not approved."**

**3. In addition to meeting all the requirements of the Common Rule and the Federal Acquisition Regulation (FAR), all studies submitted to the Flight IRB shall comply with the following requirements:**

**a. The genetic data shall include the genetic data that will be collected or stored as part of the study.**

**b. The genetic data shall include the genetic data that will be collected or stored as part of the study.**

**NASA INSTITUTIONAL REVIEW BOARD (IRB)  
CONSENT TO BE A PART OF A RESEARCH STUDY**

**ONE YEAR MISSION - NASA TWENTY FIFTY**

**NOTE: Any alterations to this consent document will invalidate the form unless consented to by the IRB.**

**ABOUT THIS RESEARCH CONSENT FORM**

You may be eligible to take part in a research study.

This NASA IRB Consent Form contains the information about the research study that you are being asked to participate in. It includes information about the purpose, procedures, and potential risks and benefits of the study. It also includes information about your rights as a research subject, including your right to refuse to participate or to stop participating at any time.

**1. GENERAL INFORMATION**

1.1 Your study title:

1.2 Your study team includes a Principal Investigator, Co-Investigator, Key Personnel, and other staff members.

1.3 This study is approved by the IRB.

**2. PURPOSE OF THE STUDY (Purpose and Background)**

2.1 The following is a brief description of the study:



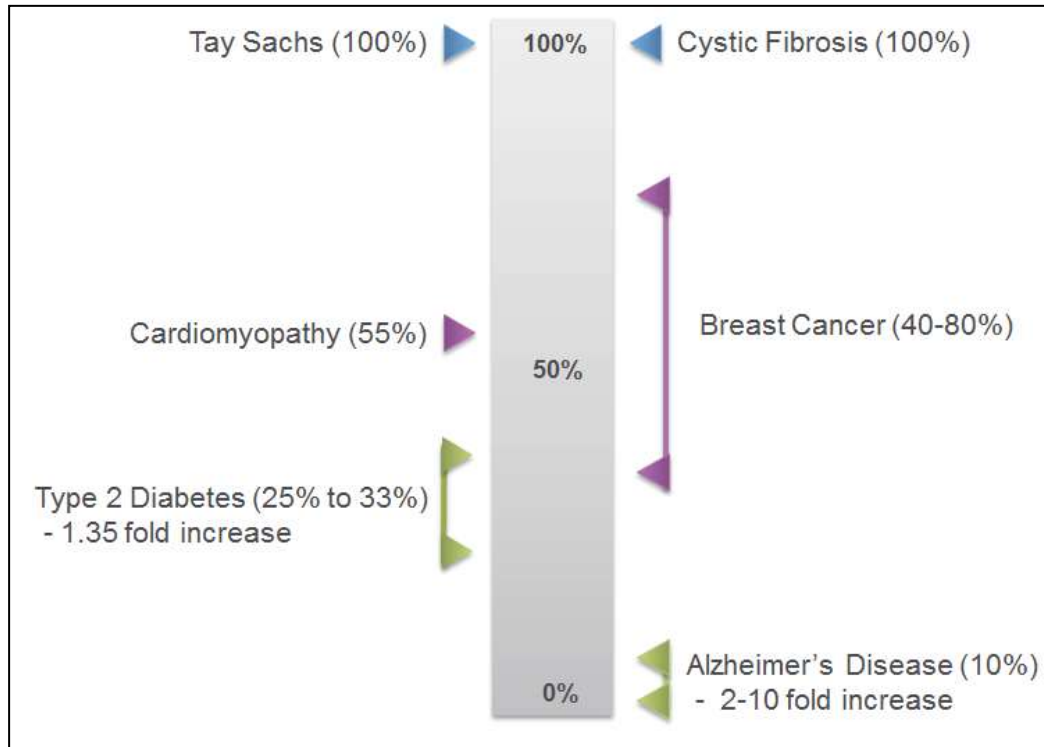
- The primary risks involved in genetic research are risks of social and psychological harm, rather than risks of physical injury
- Genetic studies that generate information about subjects' personal health risks
  - Could provoke anxiety and confusion
  - Damage familial relationships
  - Uncover unwanted information about heritage, ancestry, and family relationships

## II.C. Additional Informed Consent Requirements

1. Any study involving genetic data shall provide test subjects with genetic counseling as appropriate to the study objectives and when requested by the Flight IRB.



- 56 genes might lead to medically actionable results
- American College of Medical Genetics and Genomics (ACMG) 2013 – (<http://goo.gl/C888BY>)



## II.C. Additional Informed Consent Requirements

1. Any study involving genetic data shall provide test subjects with genetic counseling as appropriate to the study objectives and when requested by the Flight IRB.

# Sharing Information with the Research Subject



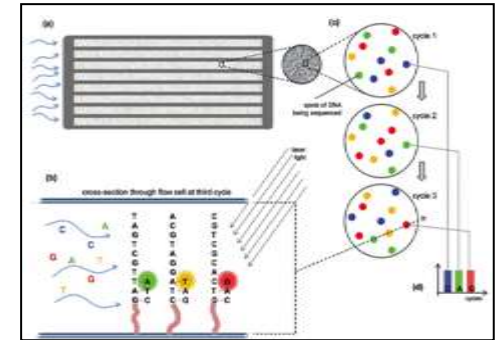
- Will the subject have the option to receive individual genome sequence data?
- Will the investigators interpret the results of the genome sequence and will that result be disclosed to the research subject?
- If the genome data are given to research subject will he/she have the option to decline to receive all or part of the results?  
(Right Not to Know)
- If there are medically actionable results will the investigators provide expert counseling or referral?

## II.C. Additional Informed Consent Requirements

1. Any study involving genetic data shall provide test subjects with genetic counseling as appropriate to the study objectives and when requested by the Flight IRB.

# Concern: Identifiable Data

- There are several kinds of gene sequencing
  - Whole Genome
  - RNA-seq
  - CHIP-seq
  - Methyl-seq
  
- All generate identifiable data
  - Deidentified sequences can be re-identified





- Individual genome sequences are unique and therefore uniquely identifiable
- Genome sequence placed in the public domain, may enable others to infer health information about the individual and his/her relatives

## II.B.2. Use of genetic data

- a) Data **shall not be disseminated** beyond the immediate control of the individual investigators documented in the protocol approved by the Flight IRB.
- b) Genetic data **shall not be data-mined or cross-referenced with other databases** of any kind unless approved in advance by the Flight IRB.
- c) Investigators **shall not attempt to identify individual participants** within de-identified data sets or pooled specimens, or to otherwise "reverse engineer" or "disassemble" data sets for bio-specimens involving NASA research subjects.

## II.B.3. Security and Storage of data

- a) Genetic data shall be encrypted and stored on secure servers. All IT systems used to store, process, or analyze genetic data shall comply with NASA's IT security standards for systems containing Privacy Act-protected information. In addition, no genetic data may be stored on mobile devices such as tablets, smart phones, or on removable media.
- b) Genetic data stored on laptops shall be limited to the minimum amount required at any one time for current research purposes.
- c) Once a study is completed, attributable data shall be archived in a secure manner by the investigator. **Investigators may be required to archive original study data at NASA or elsewhere at NASA's direction, and to destroy all copies of the original study data after the study is complete.**

## II.B.4. Release of data

- a) **No genetic sequence data may be posted online or otherwise published or made public.**
- b) The IRB may waive this prohibition for the release of limited sequence data that is non-attributable. **The IRB must approve such a limited release in advance. The informed consent of the affected research subjects will be sought prior to such release.**
- c) The **privacy** of genetic information will be **protected** to the full extent of the law, **including after the death of the subject** to avoid the unwarranted invasion of personal privacy of surviving family members.

# The General Levels of Confidentiality and Privacy



1. The study may retain genome sequences and not allow any data sharing to any third party
2. The study may share with qualified third parties conducting related research
3. The study may share the data using a secure server like the NIH dbGAP
4. The study may deposit the data in a publically accessible database

## II.B.3. Security and Storage of data

c) Once a study is completed, attributable data shall be archived in a secure manner by the investigator. Investigators may be required to archive original study data at NASA or elsewhere at NASA's direction, and to destroy all copies of the original study data after the study is complete.

## II.B.4. Release of data

- a) No genetic sequence data may be posted online or otherwise published or made public.
- b) The IRB may waive this prohibition for the release of limited sequence data that is non-attributable. The IRB must approve such a limited release in advance. The informed consent of the affected research subjects will be sought prior to such release.
- c) The privacy of genetic information will be protected to the full extent of the law, including after the death of the subject to avoid the unwarranted invasion of personal privacy of surviving family members.

**II.C.3. Subjects have the right to review presentation slides prior to public presentation and to review manuscript drafts and final publications before public release. Research subjects have the right to have their identifiable information removed from the presentation or publication.**





- What assurances can be made about health insurance, disability insurance, life insurance, and employment?

From the Informed Consent Form (ICF)

12.9 Confidentiality and release of protected health information for genetic research studies

- We will not convey your individual research results from this study to your medical record.
- We will not give your results to anyone else including your doctors. If we find something in your research testing that we believe can be used to directly help you with medical decisions, we will give that information to you.

# Conclusion



- The Twins Study (Scott and Mark Kelly) is NASA's first foray into 21<sup>st</sup>-century omics research
  - Built around Scott Kelly's one year mission
- The Twins Study will examine
  - Genome and epigenome
  - Transcriptome and epitranscriptome
  - Proteome
  - Metabolome
  - Microbiome
  - Physiology
  - Cognition
- NASA is addressing
  - Protections for research subjects
    - Interim Genetic Research Policy JID 1800.4
    - Agency-level policy expected by summer 2016
  - Use of data in medical care and occupational medicine
  - Use of data in mission planning



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