COVER PAGE

Research Campaign:
Twin Orbit – A Revolutionary Long-Duration Spaceflight Twins Study

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Executive Summary

The commercialization of space (e.g. SpaceX, Axiom Space, Blue Origin) brings new opportunities to study astronauts, including identical twins, on industry-led space stations with unprecedented detail. I propose a new, long-duration Twins Study (building from the NASA Twins Study), which will accomplish four main goals:

1. Conduct Scientific Research for Biomedical Discovery and Testing New Hypotheses
2. Develop and Deploy New Technologies for Crew Health and Mission Maintenance
3. Develop Crew Health Operating Procedures Applicable for Long-duration and Deep Space Missions
4. Inspire the Next Generation of Explorers

Extremely long-duration microgravity will be the new normal when we travel into deep space, starting with Mars, and our success will be a test of health endurance in several respects. First, given the great distances, there will be very limited support from Earth once the mission begins. Second, the space weather (and radiation) is an order of magnitude more challenging. Third, the behavioral and cognitive challenges for such long missions will place an unprecedented strain on the crew. Although NASA, ESA, JAXA, ROSCOSMOS, and others are building a large knowledge base to address these challenges through missions on the International Space Station, very few crew have been studied in weightlessness for durations over six months, and more data from longer missions is critically needed.

The original NASA Twins Study (www.nasa.gov/twins-study) was a seminal study of microgravity effects at the molecular, systemic, and behavioral level. Two identical twins, for the first time ever, showed how a single human body adapts to space. Unfortunately, there is limited confidence with a sample size of N=1, and we simply can't say if the rest of humanity would adapt the same way. Studying more identical twins is critical because it will add more data to support or refute hypotheses from previous studies.

Microgravity research will continue to discover fundamental molecular behavior in DNA damage, oxidative stress, mitochondrial dysregulation, epigenetic changes (including gene regulation), telomere length changes, and microbiome shifts. These lead to improved theories which can be tested over several months in orbit. Additionally, the technology for analyzing crew health must be transitioned to take place almost exclusively in a simulated deep-space environment, and the operating procedures for monitoring and administering their health must be developed without the advantage of medical communication from Earth.

Finally, this study will serve as an inspiration to future space explorers. We're taking serious steps towards landing humans on Mars for the first time in history, and this new Twins Study will help us get there sooner and enable safer travel for the crew.

A Revolutionary New Twins Study

Making a cislunar human spaceflight to low-Earth orbit (LEO) or the moon takes anywhere from a few minutes to a few days. The physiological adaptation begins soon after orbit insertion in the free-falling weightless environment.[1-4] Human physiological adaptation is well-described in

which longer spaceflights result in longer disruptive effects.[4-13] The longer duration missions onboard the International Space Station (ISS) last many months. Only a few have been studied for prolonged durations over six months.

Missions to Mars will take significantly longer[14] and encounter a much more hostile interplanetary environment. The radiation problem alone is formidable, and our attempts to understand the human body's behavior to extended (> 6 months) spaceflight is still in the early stages.[9-11,15-17] At the present time, our collective knowledge is scant, and thus significantly more data is needed to understand the risks and mitigation strategies for long-duration spaceflight.[18] In addition to the quantity collected, the correct type of data is equally as important. We need more and better data to integrate the simultaneous effects on multiple body systems and data types in the same subject.[18]

Advances in scientific research are finally revealing unprecedented insights about how the body responds and adapts during extended spaceflight, and how to accelerate countermeasures and treatments.[19] The essential task is to understand the genetics, which begins with the human genome. The best case scenario, by an overwhelming margin, is to measure the real-time behavior of exact copies of DNA. Identical twins provide a means for researching exact genome differences rather than researching the variation itself between unrelated healthy individuals.[17,20,21] Comparison with a biological test standard, such as a genetically identical twin, maximizes scientific value and is, from a statistical and biological standpoint, a superior formalism for a human research program.

The Uniqueness and Medical Benefit of Studying Twins

Nature provides this very rare opportunity to investigate identical twins in the context of human spaceflight and weightlessness. The rapidly emerging field of *epigenetics* investigates how behaviors and environmental factors cause changes that affect gene expression, but not changes in the DNA sequence itself. The significance of identical twins having the same genetic material, and in the same sequence, is critical to discerning genetic vs. environmental differences in health and disease.[22] The number of noncoding genes that are clearly defined is continuously increasing, and thus some of the most critical genes for adaptation to spaceflight may still be awaiting discovery.[20]

The mathematics clearly demonstrate that using paired twins reduces the required sample size by an order of magnitude or more. In general, hundreds of twin pairs are required to reject false theoretical models. A similar requirement for singletons would be in the thousands or more.[21,23-28] At the moment, it is not feasible to collect hundreds of twins for human spaceflight studies, but the major theme is resounding that twins greatly enhance the validity of research findings and take much of the guesswork out of research planning.[21,29] It is possible to lay the long-term foundation for such a study due to rapid advances in commerical and private spaceflight missions. Powerful identical twins studies can begin to address some of the most essential knowledge gaps as we prepare for Mars.[18]

Epigenetics and the twin design can potentially make the greatest discoveries as it relates to the fundamental problem of radiation and its many effects.[30,31] There is a clear link between

exposure to high doses of ionizing radiation and the long-term development of cancer, including cardiovascular disease and degenerative heart changes.[30-34] The first indicators of radiation exposure may be altered DNA methylation, histone modifications, or modulation of non-coding RNA expression.[31-34] Epigenetic changes are now recognized as critical aspects of the emerging picture of the cell response to ionizing radiation.[33,34]

Genetic Engineering as a Biological Defense Mechanism

NASA identified radiation as one of the the largest risk factors for astronauts and whole body damage.[35] The effectiveness of our "genetic armor" is crucial, yet it is not likely sufficient for deep space radiation exposure. Thus, we will need to deploy all the known technological, physical, pharmacological, and medical protective measures that are safe and effective, but we can also (for the first time), deploy genetic measures of defense. Today, we know enough to be able to modify, tweak, and engineer life to improve the odds of survival or to create new adaptive features and mechanisms.[20]

We only recently had the ability to map base-level, cell-specific, and high-resolution genetic mutations and understand their potential impact for astronauts. This is made possible by next generation DNA sequencing (NGS) methods which manifest powerful predictive methods. Indeed, mutations could forecast the development of cancer and cardiovascular disease years before they occur.[36] As an example, a future astronaut could have the expression of the MC1R gene increased within melanocytes to help control free radicals from skin-damaging radiation, which could modify the expression of TP53 and Dsup proteins to ensure proper cellular response. The engineered system to facilitate such changes would only have three genes, but each has a separate function critical to preserving astronaut health.[20]

Beginning a New Twins Study

We are now in the genomic era. Numerous changes were observed from NASA Astronaut Scott Kelly's comprehensive and molecular genetic data during his 340 consecutive days in space.[17] Ninety-one percent (91%) of the gene expression changes returned to normal within six months of returning to Earth. Yet, there was still a "molecular echo" from his time in space, wherein the cells in his body could be seen actively working to maintain DNA stability. Some genes were still disrupted in their expression while adapting to life back on Earth. Numerous indicators gave us a guide as to which genes may need to be accelerated, decelerated, or otherwise altered to help response to spaceflight in future astronauts.

A second Twins Study will begin a new era in monitoring and addressing real-time astronaut health conditions. There are groundbreaking improvements for inflight data and sample collection strategies (while in orbit). We have the technology to perform continual genetic-based monitoring of twins at the same time regardless of their location. The advent of comprehensive molecular profiling allows us to begin developing real-time personalized countermeasures to most accurately address the risk factors mentioned earlier.

Dr. Christopher Mason at Weill Cornell Medicine developed a Standard Omics Processing pipeline that is currently being deployed for SpaceX private astronauts, and which subsumes and

expands upon the battery of methods and molecular tools used in the original NASA Twins Study. Figure (1) below shows the types of samples and the associated analyses for each, as well as the sampling intervals. Actual studies will take multiple years to finish, and the greatly anticipated results and discoveries will not be fully known for a year or more after returning to Earth.

Better sequence-based DNA monitoring techniques are now possible which will allow for early detection of a variety of diseases. Cell-free sequencing of DNA and RNA essentially provides a whole-body molecular scan to yield information about the types of tissues experiencing stress or damage, as well another means by which one can track mutational changes. Also, as shown with the Biomolecular Sequencer Mission in 2016, NGS in space is now flight-validated and can be used to better track microbial changes and dynamics during long-term missions.[37]

Spaceflight crews consist of two or more members and it's likely disease or sickness biomarkers will be recognized at different times, thereby requiring a strategy to individually administer countermeasures. In the context of radiation damage this may include any combination of physical shielding, pharmacological protection (radioprotectors, radiomodulators, and radiomitigators), drugs, and antioxidants.[32]

There are fundamental spaceflight concerns focused on body mass and depletion of body nutrients.[38] Astronauts are generally not amply nourished, and in particular the nutrients which support the immune system. The causes of these may include food shortage, ineffective food intake, metabolism function, and modifications of nutrients in the food supply. Biomarkers for measuring the accuracy of biological aging are continuing to improve. Genetics and other impact factors need to be considered to develop a personalized and optimized nutrition plan for maximized immune response.[39-41]

Twins provide a superior basis from which to understand how long-duration spaceflight affects the genotype and molecular phenotype. Evolutionary approaches using the essential, conditionally essential, and nonessential inputs can be applied and studied in-depth and in real-time.[19] This actionable strategy will lead to even more insights and groundbreaking discoveries. Applications include treating and minimizing SANS, drug detoxification, reducing the risk of liver damage using acetaminophen, vaccines, environmental chemicals (xenobiotics), nonessential food components, radiation, water, air, and heavy metals.

Summary

Mars is the goal. We have the technology to get there, but we need to understand the best science and the most efficient methods. The proposed second Twins Study will encompass nearly 30 types of analyses from across multiple cell types, tissues, genotypes, and phenotypes. Major focus areas include gene function and immune system response, microbiome changes, telomere elongation behavior, intravascular fluid behavior, vision-related problems, cytokine behavior, genome instability and rearrangement, genome expression, cognitive behavior, and epigenetic analyses.

Real-time collection methods and multi-omics monitoring tools in orbit will revolutionize how we obtain astronaut health information and deploy associated countermeasures. DNA sequencing in orbit permits for predictive methods to understand impacts before they occur, including possibly cancer and cardiovascular disease. Data will provide an additional comparison with super armor species, which will lead to insights on how human cells might be genetically modified for improved radiation resistance using revolutionary DNA sequencing methods. The science of personalized countermeasures will ultimately improve the health, performance, and safety of astronauts.

Significant medical advances in the past decade permit, for the first time, groundbreaking strategies for protecting astronauts and ensuring their survival in even the most hazardous of missions. We just need the means, the deployment of the tools, and the continual accretion of new twins, new data, and new targets to refine the therapies for the decades of exploration to come.

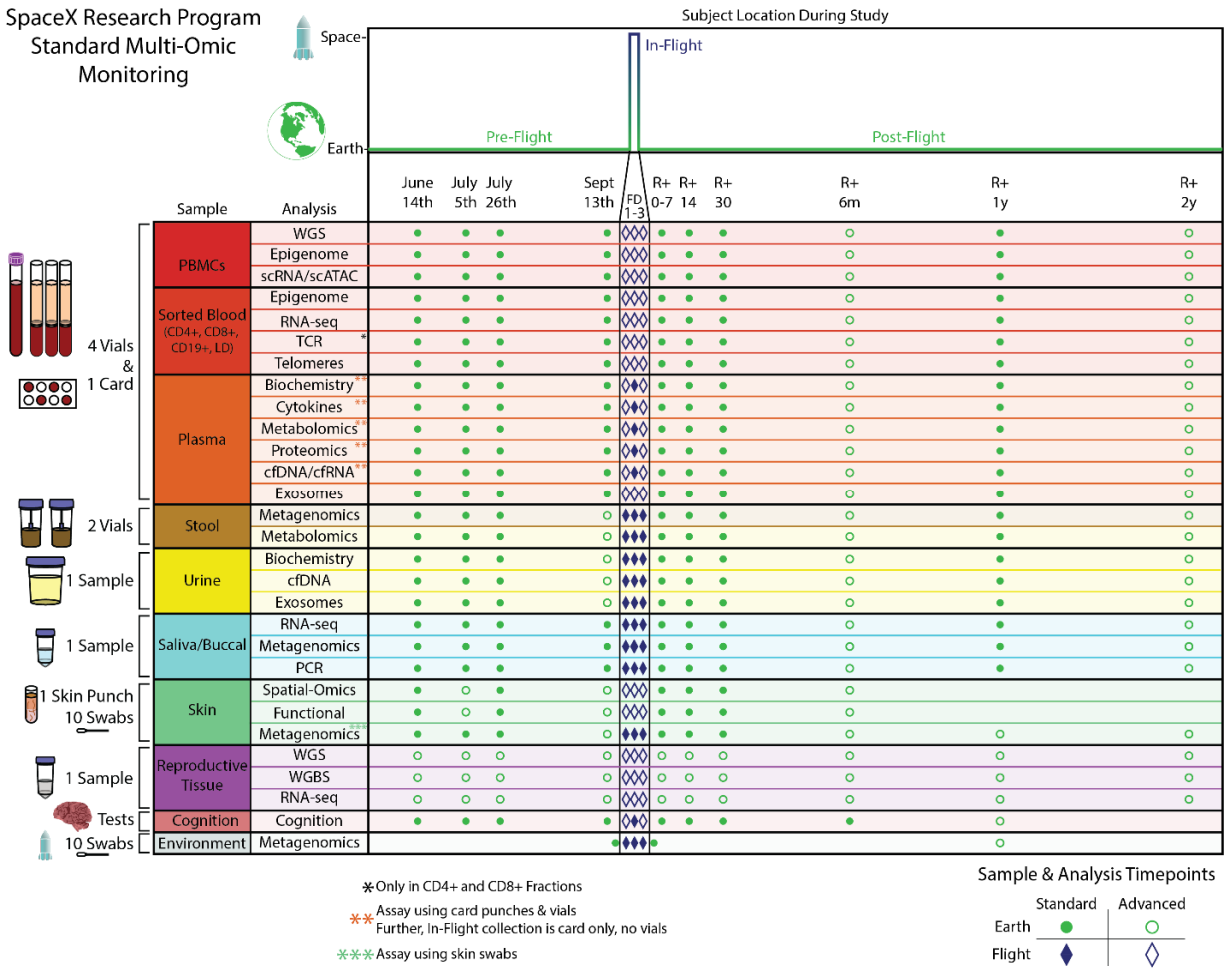

Figure 1. An improved Multi-Omic Monitoring program to collect twin samples. This set of samples was developed by Dr. Christopher Mason at Weill Cornell Medicine.

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