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Post-terrestrial Vision, Pomegranate Dreams (ft. Tardigrade Genes) Part I

Apollo 8's "Earthrise" (NASA) aside surrealist cosmic genoscape (Hotpot AI, edited).

Posted 5 September 2023: https://www.linkedin.com/pulse/post-terrestrial-vision-pomegranate-dreams-ft

In the news: Spaceflight associated neuro-ocular conditions, gene editing for ocular pathology, and pomegranate extract for anti-inflammatory effects in corneal epithelial cells. This two part article summarises the main points of some recent studies and offers a few additional tangents and synthesis.

At the end of July 2023, a congressional hearing in the U.S. on 'unidentified anomalous phenomena' took place while as of August, it seems an age of extreme (and extremely expensive) space tourism is upon us. Inevitably, even Poland can into space. Along with stratospheric balloon restaurants, bioethical legislation, dystopian laments, necro-futurological fables and cosmic pessimism, it would be timely to mention some physiological consequences of human inter-planetary travel.

First, and somewhat horrifying to contemplate (so here lie some diagrams and a video to fully visualise) the brain reacts to spaceflight conditions with a mechanical upward shift of itself and the optic chiasm (Roberts *et al.* 2017; Shinojima *et al.* 2018; Ong *et al.* 2023). Next, it adapts via expansion of its own fluid-filled spaces, the ventricles and the choroid, with the former hitting an anatomical ceiling at around 6 months. Besides choroidal expansion and ventricular enlargement, there are changes in the location and volume of gray matter and white matter microstructure, some of which return to baseline after an adjustment period (See McGregor *et al.* 2023).

Overall, numerous aspects of the nervous, glymphatic, sensory, and visual system are affected. Several decades worth of missions and focused research in other areas like bone resorption and muscle loss from microgravity have enabled governments to better prepare astronauts for a future of longer missions and further exploration. There may be significant protective benefits from penguin suits, medication, specialised nutrition plans and fitness routines. However, as of now, they can't address everything including what happens to molecular pathways or brain fluids and tissue… or to long-haul tourists.

Part 1: Post-Terrestrial Genes & CRISPR Vision

Spaceflight Associated Neuro-Ocular Syndrome (SANS)

According to Ong *et al.* (2023) and data assembled by NASA, a constellation of pathophysiological findings are observed in astronauts during and after extended spaceflights that may last months or years, or even end up permanent. Ocular changes include optical disc oedema, posterior globe flattening, retinal thickening, chorioretinal folds, and refractive error. Altered fluid movement and vascular remodelling in spacecraft environments can affect tissues like the optic nerve, hence are implicated in SANS pathogenesis. There are slightly trickier to addess molecular and genetic pathways that develop as well. Examples listed in the paper include those for B12 carbon transfer, oxidative stress, and ATP generation. Risk for SANS through the one-carbon metabolism pathway may be genetically linked to increased MTRR 66G and 1420C alleles, acting in combination with low vitamin B2, B6 and B9 status.

Countermeasures are being explored at this stage, with recommendations such as lower body compression bands and eye goggles, Vitamin B nutrition supplements, and profiling the genetic risk of space travellers for SANS. What will happen to that genetic information or how it will be used for astronaut recruitment, training, or tourism is unclear.

There have been other suggestions for making moves into deep space that include human enhancement through techniques like genome editing, building on earthly analogues and biomedical applications. Makes sense. Instead of going through the trouble and expense of terraforming another planet, why not do it the old fashioned way: Genetic change of the invading organism. Except this time, instead of bothering with dated techniques like meiotic reproduction and generations of ecological pressure, we cheat!

Gene Editing for Eye Disease (and Space Migration)

Inherited Retinal Disorders (IRDs) & Ocular Pathology

Testing

It is estimated that 1/1000 people worldwide are affected by an 'ocular disorder', some symptomatic, some genotypically determined by a single or dozens of loci, with differences in eventual medical manifestations (Sundaresan *et al.*, 2023). Inherited retinal disorders (IRDs) like Leber's Congenital Amaurosis and Retinitis Pigmentosa are degenerative conditions that can result in total blindness. A few of their subtypes (according to underlying pathogenic gene variants) are targets of potentially revolutionary therapies and practice of genomic medicine.

The field is still new, but governmental and institutional supports along with media attention may be able to raise the level of knowledge, advisory capacity, and routinised platforms and resources, even for neigbourhood clinics around the world. This is easier in developed economies such as Europe and North and East Asia. Without routine genetic risk assessments, IRDs and other gene-based disorders are detected via check-ups. If the clinic is not equipped with appropriate imaging machines, detection only happens after vision loss has begun. Patients may be referred to special institutes and charities, or relocate to participate in clinical trials. Even as more gene therapies become commercially available, the costs are some of the highest in the healthcare market.

Luxturna is the only one approved for eye disease so far, price-listed in Japan as of 30th August this year. The product and service by Novartis requires genetic testing prior to therapeutics to ensure candidate suitability. Testing is a major aspect of the nascent gene therapy market in Korea and Japan with a front-runner for the industry: Sysmex's PrismGuide IRD Panel System, based on blood samples. Another approach involves 'deep phenotyping' and harmonisation of medical diagnoses in the Human Phenotype Ontology (HPO), meant not just for treatment but long-term to pave the way for collaborative datasets in genetic research.

Yet another data-driven innovation was announced in June. Eye2Gene is an Artificial Intelligence (AI) device based on 30 years of work and approximately 4000 retinal scans matched to genetic profiles. The team of developers from Moorfields Eye Hospital stated that the results from Eye2Gene matched or exceeded the accuracy of a HPO-only approach 70% of the time. According to the team, using three different imaging modalities Eye2Gene is able to generate a fairly accurate gene-level prediction score for 36 individual IR genes, just from the retinal scans. From an organisational perspective, this could save a huge amount of time and specialist resources. Also, given the worldwide internet penetration rate of around 64%, the potential scope of the tool through its invitation-based app is large.

Furthermore, a database of 3749 is still small, considering the global estimate of >5 million IRD cases. As external validation via international collaborations continue, and more control and IRD patient data is added, accuracy could increase for a variety of sub-groups. They hope the device will improve accessibility and efficiency in the gathering of genetic information through routine eye tests, spurring a 'genetic diagnostic odyssey' (Pontikos *et al.*, 2022) 'to accelerate and democratise the IRD diagnosis service' (Nguyen *et al.*, 2023).

CRISPR Techniques

The pace is clearly rapid, but medical applications of gene technology as a whole are still in the early stages, several trials focusing on ocular pathologies and CRISPR-based gene editing.

The eye reportedly serves as an 'ideal system to assess and validate novel genetic therapies' (Sundaresan *et al.* 2023; See also Choi *et al.*, 2023) with an expanding array of techniques. Foremost among them is use of the CRISPR-Cas9 system. CRISPR stands for 'clustered regularly interspaced short palindromic repeats', and 'Cas' for CRISPR-associated systems and proteins, capable of editing DNA. Some, like CRISPR-Cas13, are capable of editing both DNA and RNA. The latter target is considered less risky, as no permanent changes to the genome are involved (Kumar *et al.* 2023).

CRISPR-Cas *au natural* is a highly adaptive defence system of bacteria and single-celled organisms, coding for a set of search & cleave enzymes that detect and excise foreign genetic fragments, preserving the integrity of the host DNA sequence and its expression. It is not just a static immune arrangement, but a tool of bacteria to enhance their virulence, i.e. strategically & tactically evolve, against the hostile environment and molecular defences of other species (Shabbir *et al.*, 2019).

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Type II Cas9 protein is the simplest but strongest weapon in the bacterial arsenal, translating to enormous biomedical potential. The Cas9 system consists of a protein with endonuclease (covalent bond-cutting) activity and single guide RNA, widely used in genome editing and research. Cost, simplicity, and versatility of this system eventually spawned the techniques of base followed by prime editing, newest additions to the repertoire that use an impaired enzyme to cut one instead of both strands of DNA, making them much safer for patient cells.

Double-strand breaks (DSBs) are some of, if not the most dangerous DNA lesions, implicated in apoptosis and cancer. DSBs are a major consequence of circumstances like severe dehydration and exposure to ionising radiation (IR). We encounter IR in polar and spaceflights, from solar flares, the explosive activity of distant stars, and x-rays. In cosmic territory, for astronauts this means that without protective measures not only are their brains transfigured (Ong *et al.*, 2023), but their DNA phosphodiester backbones are cracked by careening photons and reactive oxygen species (ROS) (Cannan & Pedersen, 2016).

Using single-strand breaks (SSBs), a type of prime editing named CRISPR PE^{SpRY}, has just been used to protect and restore photoreceptor health in a mouse model of retinitis pigmentosa (Qin *et al.* 2023, image in Hutton, 2023). Some advantages of this technique are in terms of precision & safety, versatility, and scope, with applications for up to 89% of known human disease-causing gene variants (Labant, 2023). Base editing is able to irreversibly switch individual cytosine and adenine (CBE & ABE) nucleobases, though with restrictions in terms of what and where. However, it still offers higher precision, safety, and potential than traditional Cas9 for addressing point mutations (Choi *et al.* 2023; Labant, 2023; Regalado, 2023), perhaps some of those posing risk for SANS (spaceflight associated neuro-ocular syndrome) and a long list of autosomal and X-linked diseases.

As for human ocular applications, there have been recent reviews by Choi *et al.* (2023) on genome editing techniques, Sundaresan *et al.* (2023) on CRISPR-Cas9, and Kumar *et al.* (2023) on RNA-targeting strategies, utilising Type VI CRISPR-Cas13. The RNA knockdown route using Cas13, which is reversible with lower risk of permanent off-target effects, is suggested for glaucoma and neovascular disease, widening the range of therapeutic targets if available in addition to genome(coding; regulatory)- and protein-targeting methods (Kumar *et al.*, 2023).

CRISPR-Cas9 is highlighted for primary open-angle glaucoma (POAG) and granular corneal dystrophy (GCD), as well as for extermination of herpes simplex virus type 1 (HSV-1) in corneal infections plus its viral reservoirs in the trigeminal ganglia. POAG therapies could involve myocilin (MYOC gene) knockdown, or reducing the elevated levels of protein transforming growth factor beta 2 (TGFβ2) found in 50% of POAG cases. GCD treatments would instead address a point mutation (R124H) to enable normal expression of transforming growth factor-β-induced protein (TGFBIp) (See Choi *et al.* 2023; Sundaresan *et al.*, 2023). As an important complement to anti-vascular endothelial growth factor (anti-VEGF) treatment in ocular vascularisation, altered expression or ablation of the 'highly pathological' VEGF-A is suggested by Sundaresan *et al.* (2023). Most of these proposals are still in theoretical or experimental stages.

Luxturna targets mutant variants of the RPE65 gene, replacing them with code for a functional and crucial visual cycle enzyme, though the improvement, unfortunately, seems to be temporary. If left untreated or when retinal degeneration resumes, the RPE65-mutant proteins can lead to progressive blindness in certain types of Leber's Congenital Amaurosis. Research for other genome editing technologies is ongoing for IRDs, albinism-related vision difficulties, ocular neovascular disease, Parkinson's, blood disorders, cancer, antibiotic-resistant pathogens, and even the creation of albino squid lines for neuro-imaging purposes.

Unlike the difficult financing situation for rare inherited disorders (Regalado, 2023), there would be enormous private market and government interest in subsidising R&D in the event that gene therapy is applicable to refractive errors,

where risk is partially heritable and cases are expected to reach a global figure of 4.8 billion by 2050. Myopia risk is being rigorously traced to specific gene loci as well as epigenetic mechanisms and environmental influences. The past few years of enthusiastic genome-wide association studies (GWAS) and complex aetiological approaches – as seen in HPO – have been able to refine diagnoses, enabling better disease classification, causal analysis, and treatment success.

As an example, when investigating the role of gene PDE4B in high myopia (Chinese), researchers were able to observe that myopia and high myopia may in fact have entirely different genetic mechanisms (Zhao *et al.*, 2021), with major implications at the translational level. Polygenic risk scores (PGS) for high myopia have since been refined in other ancestral groups (European), but the accuracy recommended for clinical utility has not been met yet and interestingly, there was no advantage over traditional methods in prediction of myopic macular degeneration (MMD) (Clark *et al.* 2023).

Assuming PGSs are refined to the extent that they meet criteria for accuracy in practice, findings in the sphere of refractive error may cause legal and ethical issues. Genome editing if interpreted as an 'enhancement', while palatable for adults, is not yet widely accepted for child and embryonic use outside of the scientific community. Severe diseases and blindness-causing IRDs are thus the industry fore-runners and exceptions, with the minimum age for Luxturna at 12 months old in the United States.

Aside from CRISPR-babies and CRISPR-research mice, rabbits, and minipigs, or the delivery of functional *human* proteins to target cells, there's an organism of interest that covers multiple biomedical domains and novel terrains.

Continued in Part II: Of Pomegranate Dreams & Tardigrade Genes

Disclaimer: *The material presented is for informational and entertainment purposes only, in summary of recent news and events. It neither reflects the views nor constitutes professional advice of the organisation. The major sources used are referenced below.*

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