CRC: Project Proposal

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A. Study Purpose and Rationale

The purpose of this study is to determine the functionally conserved and variable regions of human rhinovirus (HRV) within the viral swarm infecting human subjects.

Human rhinovirus (HRV) is a single-stranded RNA virus, which causes upper respiratory infections and exacerbations of asthma and chronic obstructive pulmonary disease. It is the most frequent cause of the common cold and asthma exacerbations, costing billions every year in medications, hospital stays, and missed work and school days (1-8).

HRV has > 100 strain or lineages within 3 different species and design of vaccines and anti-viral medications largely unsuccessful (9, 10). With the numerous HRV strains with differing immunogenic capsids and host cell receptor tropism, targeting the virus is a challenge. However, even well-designed and targeted medications have largely failed to limit or stop infection. The failure of these past medications is likely due to an RNA viral evolutionary strategy called quasispecies (11-13).

Human rhinovirus (HRV) is theorized to infect human hosts as a quasispecies, which enables an evolutionary advantage. The main or 'consensus' virus mutates with every replication cycle generating a swarm of mutated, but related viral genomes. The host places pressures on the swarm, rather than on individual virus particles thus enabling functional advantages to a swarm of genetically different viral genomes vs. identical viral genome copies (14-17).

Over the last 5 years, methods have been established to investigate quasispecies within natural HRV infections. The protocols enable visualization of hundreds to thousands of copies of the viral swarm (18, 19). Using next generation sequencing, a small sample of 15 natural HRV infections have shown variability as well as conserved regions within the swarm of individual infections. However, this study has been limited by the small number of samples and that all the viruses were of the same strain of HRV.

My project will analyze ~200 HRV samples for conserved and variable regions across swarms representing multiple HRV lineages. I hypothesize that despite numerous and genetically variable lineages, that the swarms will reveal conserved regions for functional experiments and ultimately, better targets for anti-viral development.

B. Sample Ascertainment and Characteristics

1. Collected from groups around the world with URI/respiratory symptoms of varying severity and no restrictions on race, age, gender, or medical problems. [Requirements were simply HRV viral load high enough to be considered infectious and nasal swabs or lavage that had not been cultured.]
2. Samples were sequenced via 454 and Illumina next generation sequencing by Institute for Genome Sciences at the University of Maryland School of Medicine.
3. The sequences have been assembled into consensus sequences and are publicly available on NCBI databases.

C. Study Design and Statistical Analysis

1. Swarm variant detection with gsVariant and illumina software
2. Alignment of 'master' swarm variant file against relative consensus HRV strain [hrv39 swarm against HRV 39 consensus…] [Programs → HMMER and Se-Al]
3. Location of all swarm variants [insertions, deletions, recombinations, and SNPs] along a master genome alignment [Programs → excel and R]

4. Distribution of all variants across 200 viral infection samples, determination of 99th and 1st percentile values [Prism]
5. Apply 99th and 1st percentile values to graph created in Step 3 above, and identify regions that are hyper-variable and conserved [greater than 99% and less than 1%]
6. Assess these regions for function associated with conservation or hypervariability [RNA folding, protein on capsid, polymerase, etc.]
7. Test region(s) of interest in functional in vitro experiments [mutate virus un region of interest and compare viral replication/survival/swarm mutations to control virus]

D. Limitations

1. Samples are already sequenced and many only cover 80-90% of genome [may not have swarm data for entire HRV genome]
2. The 99th and 1st percentile cut-offs may be too stringent or too liberal to have a reasonable number of regions to investigate [if this happens, then the cut off can be adjusted and/or regions with a greater number of conserved variants screened]
3. More sequences may be needed to define areas of conservation, may just be a level of noise covering a large portion of genome

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