NATIONAL CENTER FOR CASE STUDY TEACHING IN SCIENCE

The Evolving Genetics of Disease Resistance

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Part I – Introduction

HIV/AIDS continues to be one of the most serious public health threats across the globe. The World Health Organization (WHO) estimates that 1 million adults and 150,000 children die from AIDS-related causes each year globally, and over 35 million adults and 2.5 million children are currently living with HIV (World Health Organization, <http://www.who.int/hiv/en/>). Although recent medical advances and distribution of antiretroviral drugs have improved HIV treatment, HIV continues to occur, and research into new HIV prevention and treatment is ongoing.

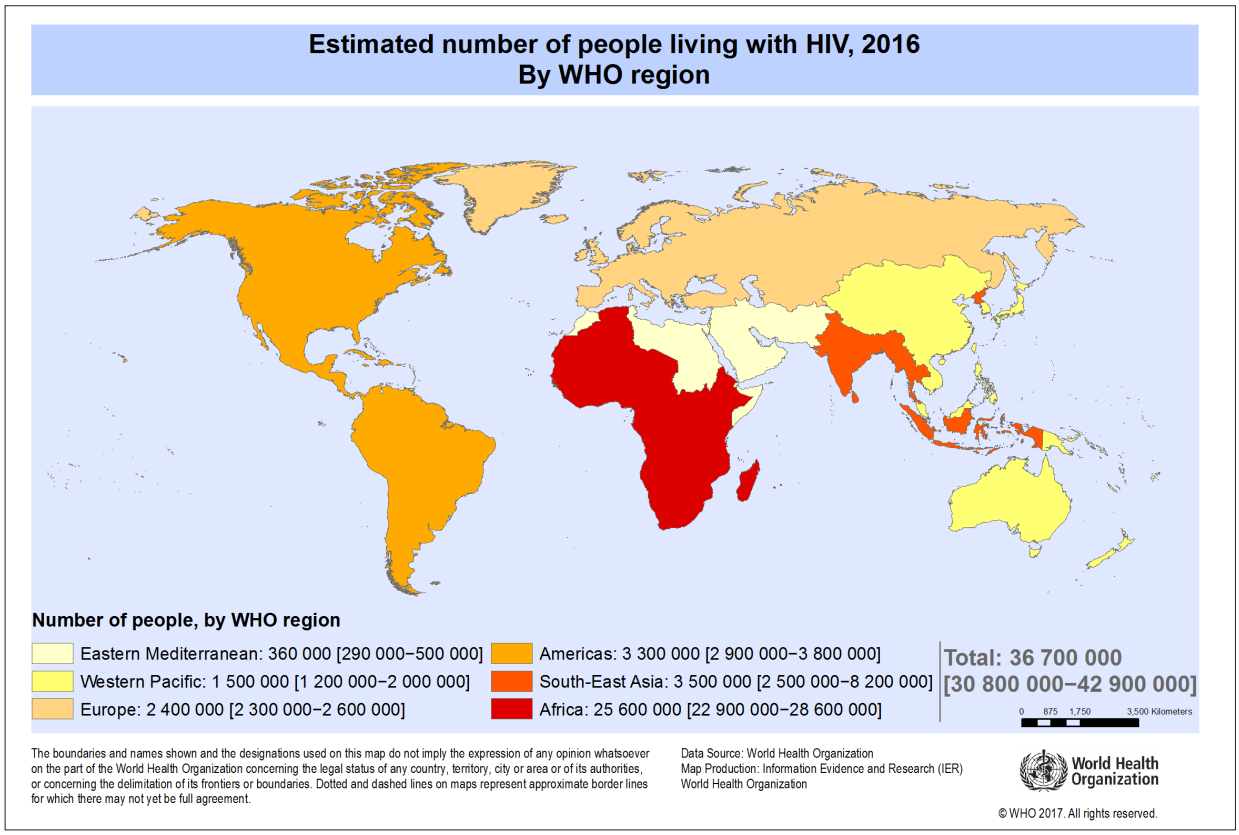

Figure 1. Adults and children estimated to be living with HIV in 2016 by WHO region. <http://gamapserver.who.int/mapLibrary/app/searchResults.aspx>

HIV damages immune system function by infecting and destroying CD4 immune cells (a type of T-helper white blood cell). HIV binds to a CD4 receptor protein on the surface of these cells and enters the cell using this protein and a co-receptor protein; one common co-receptor is CCR5. The CCR5 protein results from expression of the *CCR5* receptor gene and has a normal function in the immune system as a chemokine receptor. Most people are homozygous for the "normal" *CCR5* allele: *CCR5*+, but some people naturally have a "defective" allele: *CCR5-Δ32. CCR5-Δ32* is named such because of a 32 base pair deletion in its coding region that results in a premature stop codon. Individuals

who are homozygous for *CCR5-Δ32* (genotype: 32/32) lack normal CCR5 function and are resistant to common strains of HIV. Individuals who are heterozygous for *CCR5* (+/32) exhibit HIV resistance that is intermediate to the two homozygous genotypes; HIV infections progress more slowly in these individuals than those with +/+ genotype.

Questions

1. Why is this research important? Describe at least two ways that learning more about the *CCR5* gene and its different alleles could benefit human health.

2. What, specifically, should scientists research about *CCR5?* List as many *CCR5*-related research questions that you can come up with in five minutes. Pick one question and describe in detail how what you have learned so far in this course applies to this question. Use correct vocabulary and concepts from class in your description.

Part II – Predicting Frequency of *CCR5-Δ32*

One interesting early finding about *CCR5* is that the frequency of *CCR5-Δ32* varies among human populations from different parts of the world. Many populations have no or almost no occurrence of *CCR5-Δ32;* the frequency of *CCR5-Δ32* in these populations is 0 or close to 0. A few areas of the world exhibit higher *CCR5-Δ32* frequencies of 0.16 or more, in that 16% of *CCR5* alleles in these populations are *CCR5-Δ32* (Limborska *et al.*, 2002). Some research suggests that the worldwide distribution of *CCR5-Δ32* is not random or due solely to genetic drift (Galvani, 2005). Instead, *CCR5-Δ32* may have reached higher frequencies in some populations due to selection. But selection by what?

Questions

1. If HIV were the selective factor driving differences in the worldwide frequency of *CCR5-Δ32*, where in the world would you expect the frequency of *CCR5-Δ32* to be the highest? Where would you expect it to be the lowest?

2. The map below shows parts of Europe, Asia and Africa. Predict the frequencies of *CCR5-Δ32* across these continents (if HIV drove selection for this allele) by coloring or shading the map below. For example, use darker shading for areas that you predict would have a higher frequency of *CCR5-Δ32*. Include a figure legend showing how your shading corresponds to *CCR5-Δ32* allele frequency.

Explain why you shaded the figure this way:

Part III –Experimental Results

Novembre *et al.* (2005) compiled data from several studies to construct a geographic frequency map of the *CCR5-Δ32* allele (Figure 2). Additional relevant results from this and other studies include:

- The frequency of *CCR5-Δ32* is 0 (or close to it) in Aboriginal populations from the rest of Africa and the rest of the world not shown on the map (Galvani, 2005).
- *CCR5-Δ32* likely originated once from a mutation event (Galvani, 2005).
- The age of the *CCR5-Δ32* mutation has been estimated to be as recent as 700 years to as old as 5000 years (Galvani, 2005).
- HIV likely jumped from apes to humans in Africa in the 1800s (World Health Organization, 2016).

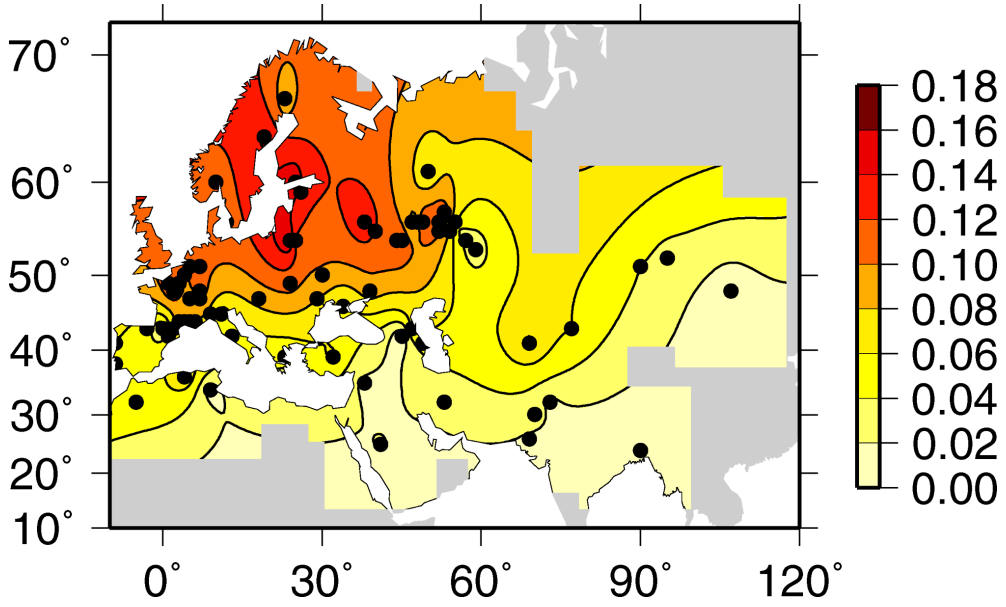

Figure 2. Distribution of *CCR5-Δ32* frequency across the Old World. Latitude and longitude are shown on the axes. The black points indicate sites where *CCR5-Δ32* frequency was directly sampled. Shading and contour lines indicate estimated frequency of the *CCR5-Δ32* allele across Europe and parts of northern Africa and Asia based on selection and dispersion models. From Novembre *et al.* 2005.

Questions

1. Compare and contrast your map predictions from Part II to the experimental results shown in Figure 2.

2. Do the experimental results support that HIV is the selective agent driving evolution of *CCR5-Δ32?* Why or why not?

3. What else might be driving the observed distribution of *CCR5-Δ32?* Explain the reasoning behind your answer in detail. For example, based on the data, what predictions can you make about the selective agent (e.g., what it was, where it occurred, its severity and duration, etc.)?

4. Describe how at least two other (not selection) mechanisms of evolution may have contributed to the distribution of *CCR5-Δ32* shown in Figure 2.

Part IV – Why the Variation?

Why is *CCR5-Δ32* so common in northern Europeans but rare elsewhere in the world? One hypothesis is that the *CCR5-Δ32* allele provides resistance to the bubonic plague (caused by the bacteria, *Yersinia pestis),* and that several plague outbreaks in Europe in the 1300s and 1600s—killing up to 30% of the European population in a few years—strongly favored individuals with at least one allele of *CCR5-Δ32*. More recent modeling data supports that smallpox may have been an important selective agent for *CCR5-Δ32*. Smallpox continually caused significant mortality in Europe for the last 700 years (until it was eradicated in 1980). Unlike plague, smallpox primarily affects pre-reproductive individuals (children) and it is a virus that, like HIV, uses the CCR5 co-receptor to infect cells. Another, not mutually exclusive, hypothesis is that the *CCR5-Δ32* mutation originated in the Vikings and was distributed more widely during Viking invasions (gene flow).

Question

1. If *CCR5* affects resistance to HIV, the plague, and smallpox, what other disorders might it affect? Be specific: what do the known diseases that *CCR5* affects have in common? How are they different? Are there diseases that *CCR5* is unlikely to affect? Which ones and why?

Part V – West Nile and HWE

West Nile virus is an emerging disease that has rapidly spread across North America in recent years, infecting birds, humans and horses. It can cause severe flu-like symptoms and death from brain inflammation. Glass *et al.* (2006) sequenced *CCR5* genotypes in several hundred West Nile virus patients in Arizona and Colorado and compared them to a control group of patients who did not have West Nile (Table 1).

Table 1. Number of patients in each group with each *CCR5* genotype.

	Genotype		
	CCR5+/ +	*CCR5+/CCR5Δ32*	*CCR5Δ32/32*
Patients with West Nile	316	62	17
Patients without West Nile	125	19	1

Hardy-Weinberg equilibrium (HWE) can be used to test if the *CCR5-Δ32* allele affects a person's susceptibility to West Nile similarly to what has been observed for HIV. In the questions below, you will test if the CCR5 gene is in HWE in patients with West Nile and, separately, in patients without West Nile.

Questions

1. Explain what testing for HWE can tell us about disease resistance. Keep in mind the following as you write your explanation: What is the HWE model and what are its assumptions? What does it tell us when a gene in a population is (or isn't) in HWE? How does this relate to genetic resistance?

2. Referring to Table 1, calculate the frequency of the *CCR5-Δ32* allele in each of these patient groups.

3. Is *CCR5* in HWE for either patient population? (If the results aren't clear, use a chi-squared test to check your answer. Chi-square test statistic: $x^2 = \Sigma\ [(O - E)^2/E]$, critical value: 3.814.)

4. Does the *CCR5* gene affect a person's susceptibility to West Nile? How so? How do these results compare to the relationship between *CCR5-Δ32* and HIV susceptibility?

5. In Part I, you first thought about the applications of research studying *CCR5*. What potential concerns are there to treating HIV by altering *CCR5* function?

Part VI – Application to Human Health Therapies

Each group member should choose one of the following articles to read from *Nature News*. Read your article and be prepared to present it to the group and discuss it with the class by the next class period.

- Callaway, E. 08 April 2016. Second Chinese team reports gene editing in human embryos. <http://www.nature.com/news/second-chinese-team-reports-gene-editing-in-human-embryos-1.19718>
- Reardon, S. 05 March 2014. Gene-editing method tackles HIV in first clinical test. <http://www.nature.com/news/gene-editing-method-tackles-hiv-in-first-clinical-test-1.14813>
- Ledford, H. 11 February 2009. Stem cell transplant wipes out HIV. <http://www.nature.com/news/2009/090211/full/news.2009.93.html> doi:10.1038/news.2009.93
- Pearson, H. 30 June 2008. Designer protein tackles HIV. <http://www.nature.com/news/2008/080630/full/news.2008.924.html> doi:10.1038/news.2008.924

References

Galvani, A.P., and J. Novembre. 2005. The evolutionary history of the *CCR5-Δ32* HIV-resistance mutation. *Microbes and Infection* 7: 302–309.

Glass, W.G., D.H. McDermott, J.K. Lim, S. Lekhong, S.F. Yu, W.A. Frank, ... and P.M. Murphy. 2006. CCR5 deficiency increases risk of symptomatic West Nile virus infection. *The Journal of Experimental Medicine* 203, 35–40.

Limborska, S.A., O.P. Balanovsky, E.V. Balanovskaya, P.A. Slominsky, M.I. Schadrina, L.A. Livshits, ... and V.A. Spitsyn. 2002. Analysis of *CCR5Δ32* geographic distribution and its correlation with some climatic and geographic factors. *Human Heredity* 53: 49–54.

Novembre, J., A.P. Galvani, and M. Slatkin. 2005. The geographic spread of the *CCR5-Δ32* HIV-resistance allele. *PLOS Biology* 3: e339.

Stephens, J.C., D.E. Reich, D.B. Goldstein, H.D. Shin, M.W. Smith, M. Carrington, ... and B. Gerrard. 1998. Dating the origin of the *CCR5-Δ32* AIDS-resistance allele by the coalescence of haplotypes. *The American Journal of Human Genetics* 62(6): 1507–1515.

World Health Organization. 2016. HIV/AIDS [website]. Retrieved from <http://www.who.int/hiv/en/>.