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Genomics: Discovering Associations with HIV/AIDS

Human immunodeficiency virus, most commonly known today as HIV, is a global pandemic that has led to the deaths of millions of individuals since its debut in the early 1980s. Although there are actually two types of HIV, creatively designated as “HIV-1” and “HIV-2”, the term “HIV” in the United States refers to HIV-1 unless otherwise specified; this custom was used for this paper as well. In 1999, a team of researchers discovered that HIV originated in the native lands of west equatorial Africa in a subspecies of chimpanzee that live there. This original virus, SIV (simian immunodeficiency virus), could have been exposed to humans via hunters unprotected from infected blood (Giraldo-Vela et al., 2008).

Studies of those primates, chimpanzees and rhesus macaques alike, have shown that they are able to control their viral loads and replication, granting them the name of elite controllers (Giraldo-Vela et al., 2008). Similarly, a small percentage of humans throughout the past three decades have been able to seemingly halt disease progression over an extended period of time; they are known as long-term non-progressors (LTNP). On the other side of the spectrum is a small percentage of HIV positive individuals known as rapid progressors (RP) who “rapidly progress” to AIDS when without the benefit of medication. In a small group of HIV negative sex workers in Kenya lies another subset of HIV/AIDS progression: highly exposed persistently seronegative (HEPS). Despite multiple “unprotected sexual exposures to HIV-1 per year”, they have managed to resist HIV infection (Kaul et al., 2001). These varying disease progressions and discovered associations suggest that HIV/AIDS susceptibility and progression are influenced to some degree by an individual’s genetic make-up. Because of that, genomics have been used extensively in the recent decade to expose new associations and further our understanding of this disease.

CCR5 and the Berlin Patient

CCR5, C-C chemokine receptor type 5, is a protein expressed on the surface of CD4-bearing T lymphocytes and a major coreceptor for HIV cell entry (with the other major coreceptor being CXCR4, the CXC chemokine receptor) (Hütter et al., 2009). Mutations in CCR5, such as rs333 (also known as $\Delta 32$), in which thirty-two base pairs are deleted from the coding region of the gene, lead to the production of a truncated protein, therefore not expressing the receptor on the cell surface. In this way, homozygosity of the mutation directly prevents HIV from entering the body's immune cells. This mutation was originally thought to have appeared about 1000 years ago and risen to its relatively high frequency due to strong positive selection through selective agents such as the bubonic plague or smallpox. Somewhat recent findings however suggest that the mutation emerged about 5000 years ago in line with neutral evolution (Sabeti et al., 2005).

Yet no matter when the mutation arose, its clear implications for positive selection have allowed it to increase to a frequency in which one percent of European populations are homozygous for this mutation. This relatively high frequency of appearance however is only present in European Caucasians and is absent "among African, Native American, and East Asian populations, suggesting that the $\Delta 32$ mutation occurred after the separation of the ancestral founders of these populations" (Sabeti et al., 2005). At the genetic level, individuals who are homozygous for this mutation are practically immune to HIV infection; even those who are heterozygous for this mutation have an increased immunity against the virus when compared to those without the mutation since a reduced number of CCR5 molecules are functioning.

In 2007, a forty-year-old man, Timothy Ray Brown, was diagnosed with acute myeloid leukemia. He became a patient of Gero Hütter and other physicians at Charite Hospital in Berlin. Diagnosed with HIV ten years previous, he had been on HAART treatment for four years with no signs or symptoms of AIDS having been observed. The first initial treatment of the leukemia with chemotherapy induced renal failure, leading to the discontinuation of HAART in order to proceed with leukemia treatment, resuming with its completion before a viral steady state could be reached. When the leukemia relapsed ten months later, Brown "underwent allogeneic stem-

cell transplantation with CD34+ peripheral-blood stem cells from an HLA-identical donor who had been screened for homozygosity for the CCR5 $\Delta 32$ allele” (Hütter et al., 2009). He received a second transplant from the same donor over a year later when the acute myeloid leukemia relapsed. HAART was discontinued after the second transplant, and there was no sign of active, replicating HIV in the patient (Hütter et al., 2009).

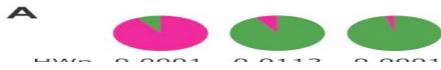
Although there were previous experiments prior to 2007 to control HIV infection with stem cell transplantation, no regard to the donor’s CCR5 $\Delta 32$ status was considered and the efforts failed. However, Timothy Brown, “the Berlin patient”, has not presented any sign of HIV (no detection in peripheral blood, bone marrow, or rectal mucosa) since his second transplant due to the complete alteration of his monocytes from heterozygous to homozygous CCR5 $\Delta 32$ (Hütter et al., 2009). This finding illustrated the depth of CCR5 involvement in HIV susceptibility and acquisition and motivated the development of new treatment ideas for HIV.

DARC Association Studies

Although the CCR5 $\Delta 32$ discovery was revolutionary in terms of HIV acquisition studies, it is limited to European Caucasians since the mutation does not occur in any other populations. Because HIV has infected the most individuals in sub-Saharan Africa, multiple studies have attempted to find a particular gene or set of genes that could potentially explain the high rate of acquisition in Africa.

In 2008, a study on DARC, duffy antigen receptor for chemokines, expanded the potential roles of the protein. A receptor found on red blood cells, it is known to influence plasma levels of several chemokines, including those found in HIV-1 suppression like CCL5/RANTES and is also a red blood cell receptor for *Plasmodium vivax* and *Plasmodium knowlesi* (He et al., 2008). On UniProt, the gene ontology of DARC lists its biological processes as defense response, inflammatory response, and the regulation of chemokine production (*UniProt*). The DARC -46C/C genotype results in a DARC-negative phenotype, meaning that the red blood cells lack the receptor. This genotype occurs due to a SNP (T/C, rs2814778) at the -46 position in the promoter region of DARC. Consequentially, individuals with this genotype are highly resistant to

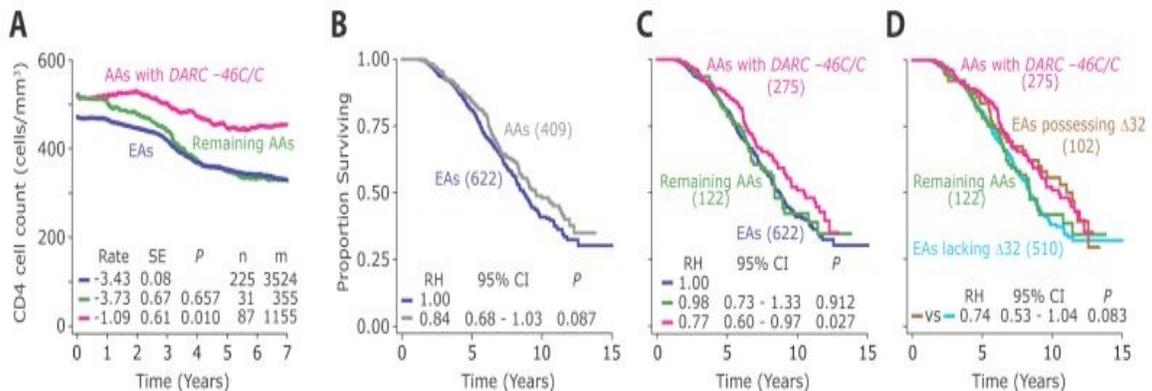
malaria, giving them a biological advantage over those with DARC, especially within an environment well suited for malaria. Due to this selective advantage, the -46C/C genotype is almost universal in sub-Saharan black Africans (He et al., 2008). DARC has been shown to also intervene in the binding of HIV to red blood cells and to target cells due to its regulation of HIV suppressing chemokines.



Consequently, while an individual without DARC on red blood cells is resistant to malaria, the antigen's absence from erythrocytes also creates the possible risk of having increased susceptibility to HIV. This could be due to the fact that the absence of DARC causes a decrease in chemokine levels, thus reducing a potential shield for binding HIV to red blood cells and HIV target cells. However, despite the increased possibility of acquisition, those without DARC happened to have a slower disease progression, gaining a survival advantage comparable to CCR5 32 heterozygosity found in European American populations (He et al., 2008). This retarded progression is thought to be associated with a duller immune response after infection due to the decreased number of proinflammatory HIV-suppressive chemokines. Therefore, relatively, DARC positive individuals have an accelerated rate of progression, although DARC positive phenotypes are much more rare in sub-Saharan Africans and without the increased risk of HIV acquisition. Another scenario could be that HIV is able to bind to DARC after infection, thus making it much more infectious and stable than free-viruses, leading to a faster rate of disease progression (He et al., 2008).

Besides the direct impact that DARC (or the lack of DARC specifically) may have on HIV acquisition and disease progression, Africans, or those descended from African ancestry, historically have lower white blood cell counts than individuals of European ancestry; this phenomenon is known as ethnic leukopenia (< 4000 cells/mm³), typically considered to be a benign phenotype. "Admixture mapping and other genetic studies in large nonimmunosuppressed cohorts of European Americans and African Americans" have shown that DARC -46C/C is the main genetic factor associated with ethnic based leukopenia in Africans

(Kulkarni et al., 2009). However, in the early stages of HIV infection, leukopenia is common amongst all populations, regardless of ethnicity. Despite this, individuals in a HIV-positive cohort lacking DARC (African Americans) still maintained a survival advantage over HIV-positive European Americans. While European Americans with low white blood cell counts had more rapid disease courses than individuals with high white blood cell counts from the same population, African Americans, no matter the white blood cell count, progressed at a relatively similar rate (although those with high white blood cell status tended to have a slightly longer disease course) (Kulkarni et al., 2009).



A risk to association studies like DARC is that there could be other genotypes that influence HIV susceptibility and progression that have yet to be discovered. There is extensive linkage disequilibrium around the -46C/C locus, thereby preventing the definitive conclusion that the effects of HIV susceptibility and disease progression observed in Africans is attributed to the -46C/C genotype since they could be caused by another genotype, polymorphism, or set of polymorphisms linked to rs2814778 (-46C/C mutation) (Kulkarni et al., 2009). In addition, there is a possibility that DARC is not a cause for ethnic leukopenia amongst Africans, although the -46C/C mutation does display a counter-intuitive advantage to the population like leukopenia. A lower white blood cell count, while signaling a weaker immune response, decreases inflammatory response to viral infections and consequentially cell entry. Therefore, although not definitive, there appears to be a delicate interaction between the survival advantage granted from DARC -46C/C and leukopenia supported from previous associations between the DARC-negative state and malaria.

Genome-Wide Association Studies

In 2008, the NIH created a policy for the sharing of data regarding genome-wide association studies. Those studies are defined as association studies in which the concentration of genetic patterns and the linkage disequilibrium are used to illustrate “a large portion of the common variation in the human genome in the population under study” (*Genome-Wide Association Studies*). The use of various phenotypes in GWAS has the potential to be significant help in determining what genetic factors are associated with certain HIV acquisition responses. For example, a GWAS of AIDS through the study of a nonprogressive HIV positive cohort has found that the HCP5 gene in the HLA region on chromosome 6 may have a major role in HIV infection, as shown through its linkage disequilibrium to several other genes in the HLA locus (Manen et al., 2011).

Genomic practices and association studies led to the discovery of the CCR5 $\Delta 32$ mutation and its benefits on the small percentage of affected European Caucasians. Those same practices have led to the completion of countless studies, like the one on DARC, investigating associations between genotype, phenotype, and disease. These studies, although relatively new, may just give mankind the advantage it needs against an enemy as formidable as HIV. The discovery of new SNPs and genes and the production of better diagnostic technologies and cost-effective solutions through GWAS has the potential, in time, to lead to better antiviral therapies, improved patient care, and a solution in combating this viral opponent.

Works Cited

Genome-Wide Association Studies. National Institutes of Health, n.d. Web.

<<http://gwas.nih.gov/01faq2.html>>.

Giraldo-Vela, Juan P., Richard Rudersdorf, Chungwon Chung, Ying Qi, Lyle T. Wallace, Benjamin Bimber, Gretta J. Borchardt, Debra L. Fisk, Chrystal E. Glidden, John T. Loffredo, Shari M. Piaskowski, Jessica R. Furlott, Juan P. Morales-Martinez, Nancy A. Wilson, William M. Rehrauer, Jeffrey D. Lifson, Marry Carrington, and David I. Watkins. "The Major Histocompatibility Complex Class II Alleles Mamu-DRB1*1003 and -DRB1*0306 Are Enriched in a Cohort of Simian Immunodeficiency Virus-Infected Rhesus Macaque Elite Controllers." *Journal of Virology* 82.2 (2008): 859-70. Web. <<http://jvi.asm.org/content/82/2/859.full>>.

He, W., S. Neil, H. Kulkarni, E. Wright, B. Agan, V. Marconi, M. Dolan, R. Weiss, and S. Ahuja. "Duffy Antigen Receptor for Chemokines Mediates Trans-Infection of HIV-1 from Red Blood Cells to Target Cells and Affects HIV-AIDS Susceptibility." *Cell Host & Microbe* 4.1 (2008): 52-62. Web. <<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2562426/>>.

He, Weijing, Vincent C. Marconi, John Castiblanco, Hemant Kulkarni, Robert A. Clark, Matthew J. Dolan, Robin A. Weiss, and Sunil K. Ahuja. "Response: Association of Duffy Antigen Genotypes with HIV-AIDS Susceptibility." *Cell Host & Microbe* 5.5 (2009): 418-19. Web. <<http://www.sciencedirect.com/science/article/pii/S1931312809001486>>.

"HIV/AIDS Basics." *Centers for Disease Control and Prevention*. Centers for Disease Control and Prevention, 6 Nov. 2006. Web. 05 Dec. 2012. <<http://www.cdc.gov/hiv/resources/qa/definitions.htm>>.

Hütter, Gero, Daniel Nowak, Maximilian Mossner, Susanne Ganepola, Arne Müßig, Kristina Allers, Thomas Schneider, Jörg Hofmann, Claudia Kücherer, Olga Blau, Igor W. Blau, Wolf K. Hofmann, and Eckhard Thiel. "Long-Term Control of HIV ByDelta32/Delta32 Stem-Cell Transplantation." *New England Journal of Medicine* 360.7 (2009): 692-98. Web. <<http://www.nejm.org/doi/full/10.1056/NEJMoa0802905#t=articleTop>>.

- Kaul, Rupert, Sarah L. Rowland-Jones, Joshua Kimani, Keith Fowke, Tao Dong, Peter Kiama, John Rutherford, Ephantus Njagi, Francis Mwangi, Tim Rostron, James Onyango, Juliaas Oyugi, Kelly S. MacDonald, Job J. Bwayo, and Francis A. Plummer. "New Insights into HIV-1 Specific Cytotoxic T-lymphocyte Responses in Exposed, Persistently Seronegative Kenyan Sex Workers." *Immunology Letters* 79.1-2 (2001): 3-13. Web. <<http://www.sciencedirect.com/science/article/pii/S0165247801002607>>.
- Kulkarni, Hemant, Vincent C. Marconi, Weijing He, Michael L. Landrum, and Jason F. Okulicz. "The Duffy-null State Is Associated with a Survival Advantage in Leukopenic HIV-infected Persons of African Ancestry." *Journal of the American Society of Hematology* 114.13 (2009): 2783-792. *The Duffy-null State Is Associated with a Survival Advantage in Leukopenic HIV-infected Persons of African Ancestry*. Web. 05 Dec. 2012. <<http://bloodjournal.hematologylibrary.org/content/114/13/2783.full>>.
- Limou, Sophie, Sigrid Le Clerc, Cédric Coulonges, Wassila Carpentier, Christian Dina, Olivier Delaneau, Taoufik Labib, Lieng Taing, Rob Sladek, Christiane Deveau, Rojo Ratsimandresy, Matthieu Montes, Jean Louis Spadoni, Jean Daniel Lelièvre, Yves Lévy, Amu Therwath, François Schächter, Fumihiko Matsuda, Ivo Gut, Philippe Froguel, Jean François Delfraissy, Serge Hercberg, and Jean François Zagury. "Genomewide Association Study of an AIDS Nonprogression Cohort Emphasizes the Role Played By Genes (ANRS Genomewide Association Study 02)." *The Journal of Infectious Diseases* 199.3 (2009): 419-26. Web. <<http://jid.oxfordjournals.org/content/199/3/419.long>>.
- Manen, Daniëlle Van, Oliver Delaneau, Neeltje A. Kootstra, Brigitte D. Boeser-Nunnink, Sophie Limou, Sebastiaan M. Bol, Judith A. Burger, Aeilko H. Zwinderman, Perry D. Moerland, Ruben Van't Slot, Jean-Francois Zagury, Angelique B. Van't Wout, and Hanneke Schuitemaker. "Genome-Wide Association Scan in HIV-1-Infected Individuals Identifying Variants Influencing Disease Course." *PLoS One* 6.7 (2011): n. pag. 21 July 2011. Web. <<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3141012/>>.

Nibbs, Robert J.B. "HIV: Getting to the Heart of DARCness." *Blood* 114.13 (2009): 2570-571.

Web. <<http://bloodjournal.hematologylibrary.org/content/114/13/2570.full>>.

Petrovski, Slave, Jacques Fellay, Kevin V. Shianna, Nicole Carpenetti, Johnstone Kumwenda, Gift Kamanga, Deborah D. Kamwendo, Noman L. Letvin, Andrew J. McMichael, Barton F. Haynes, Myron S. Cohen, and David B. Goldstein. "COMMON HUMAN GENETIC VARIANTS AND HIV-1 SUSCEPTIBILITY: A GENOME-WIDE SURVEY IN A HOMOGENEOUS AFRICAN POPULATION." *AIDS* 25.4 (2011): 513-18. Web.

<<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3150594/>>.

Sabeti, Pardis C., Emily Walsh, Steve F. Schaffner, Patrick Varilly, Ben Fry, Holli B. Hutcheson, Mike Cullen, Tarjei S. Mikkelsen, Jessica Roy, Nick Patterson, Richard Cooper, David Reich, David Altshuler, Stephen O'Brien, and Eric S. Lander. "The Case for Selection at CCR5- 3 2." *PLoS Biology* 3.11 (2005): E378. Web.

<<http://www.plosbiology.org/article/info%3Adoi%2F10.1371%2Fjournal.pbio.0030378>>.

UniProt. N.p., 3 Oct. 2012. Web. <<http://www.uniprot.org/uniprot/Q16570>>.