Of Microbes and Men

Introduction

In the beginning, there were microbes. Some of these microbes caused disease in the human species. The infections caused by these pathogenic bacteria, protozoan, and viruses have, over time, brought changes to the genome itself as human hosts have had to evolve in order to survive. The human body's immune system is constantly evolving against various pathogens and must adapt to defend against these outside invaders. Other microbes have not caused disease in the human species but have instead coevolved alongside us and bestowed beneficial mutations that have altered the genome and human evolution overall. This symbiosis between humans and microbes is known as the microbiome. In both cases, parasitic and symbiotic, the human genome has had to evolve along with bacteria, parasites, and viruses to ensure the survival of the human species. Changes brought about in the human genome, specifically genes within the immune system, by both pathogenic and salubrious microbes will be discussed.

Genetic variation in modern populations has been significantly molded by the evolutionary 'arms race' between the human species and pathogenic microorganisms. It has been demonstrated that genes within the immune system have recently been positively selected for in recent human history. In their research article, Lachance and Tishkoff discuss the fact that genes that have recently been positively selected for are none other than genes that play a role in the immune system (1). As humans have migrated across the globe, they have encountered new environments teeming with pathogenic organisms. They have also domesticated animals that have the potential to be reservoirs for various viruses and bacteria. Zoonotic events have led to epidemics that have changed the course of not only human history but also to the human genome (2). Pathogens that cause lower rates of reproductive probability within a population (whether by reduced health in the individual or early death) drive selection to select for genetic variants that offer the population resistance to the effects of the pathogenic infection. These signatures of selection that can be found within the human genome are dependent on three criteria: how long the pathogen has been circulating within a population, the geographical spread of the pathogen, and the pathogen's virulence (3). Recent positive selection within the human genome (5,000-10,000 years) are characterized by: unusually high frequency of newly derived variants, unusually large allele frequency differences between populations, and unusually extended linkage disequilibrium caused by a single allele rapidly increasing in frequency (3).

Bubonic Plague's Effect on the Human Genome

One particular example of how a microbe can alter the human genome is none other than the bubonic plague. Bubonic plague, also called the black plague, ravaged the human species from 1348-1350 in one of the most devastating epidemics known to human history. The plague is caused by a bacterium called *Yersinia pestis*. Symptoms of the disease include: flu-like symptoms such as fever, headache, chills, and body aches. The lymph nodes also swell and

become painful and tender during the course of infection. The bacterium was spread by rats in the cities during this particular epidemic. In one study, it was demonstrated how the bubonic plague left its mark upon the human genome. In the study, the genomes of the Rroma, a population that migrated to Europe from India (also known as gypsies), European Romanians, and residents of Northwestern India were compared. There were genetic changes in both the populations of the Rroma and the European Romanians that were not seen in the genomes of the Indians. These changes were comprised of approximately 20 genes. Of these 20 genes, there were genes for inflammation and skin pigmentation but most importantly there were genes that are involved with immune response. There were three immune system genes located on chromosome four that were altered when comparing the three populations- they were more closely related in the Rroma and European Romanian population than in the Indian population. The immune system genes coded for toll-like receptors. Toll-like receptors are proteins that have the ability to latch onto foreign bacterial entities and then initiate a defensive response against them. To test the theory whether these genes were due to the effects of the plague the researchers exposed them to *Yersinia pestis* to see if a response would be elicited. They found that immune response depended on the production of those certain toll-like receptors found in the genes on chromosome four. This verified that the genes had indeed been positively selected for in the Rroma and European Romanian populations due to exposure to bubonic plague (4).

Smallpox's Effect on the Human Genome

Another recent addition to the human genome is the *CCR5-Δ32* deletion allele. This allele has been found at high frequency in European populations. Interestingly, it confers resistance against the human immunodeficiency virus (HIV) by not allowing the virus to gain entry into $CD4^+$ T cells and macrophages. Individuals with this allele do not have receptors for HIV on their cells making it impossible for the virus to cause infection. However, it is understood that it was not this particular virus that gave rise to the CCR5-*Δ*32 deletion allele- this allele originated too early for HIV to be the causal agent for this evolution within the genome (5). This variant is not found in populations of Africans, American Indians, and East Asians further supporting the hypothesis that it evolved before the rise of HIV. If HIV did not cause this change in the genome what did? Researchers set out to discover the answer by carrying out coalescence of haplotypes (6). This analysis revealed that the allele originated some 700 years ago- clearly HIV could not be the cause. Another study took it a step further and tried to determine what pathogen had caused this allele to be selected for initially. The researchers narrowed it down to bubonic plague and smallpox. After sampling genomes and entering the data into various models, the researchers came to the conclusion that smallpox was the most likely cause of the deletion. This conclusion was based on several facts- one being that the CCR5-*Δ*32 deletion allele could not have reached current frequencies if bubonic plague was responsible. The plague would not have created enough selective pressure to drive the allele to the current rate of frequency since the plague was an epidemic for only about 400 years. On the other hand, smallpox has been circulating for more than 2,000 years and would have provided sufficient selective pressure to cause the allele to reach the frequencies it is currently at in the human genome. Furthermore, the geographic distribution of the CCR5-*Δ*32 deletion allele also indicates that smallpox is the most likely cause of the mutation (5).

Smallpox has also impacted the genomes of Native Americans- a population that is across the ocean from European populations where it has altered that population's genome. When Europeans came to North America to colonize its wild lands they brought with them deadly diseases such as smallpox and measles that Native Americans had never been exposed to thus they had no immunity to these illnesses. Research has shown that the immune systems of Native Americans adapted to this change of pathogens within their environment. DNA samples from 25 deceased individuals belonging to the Tsimshian population were analyzed for specific genes that were related to immune response. Analysis of the DNA revealed that ancient Native Americans carried a variant of a gene known as HLA-DQA1. This gene codes for proteins that can distinguish healthy cells from bacteria and viruses- leading to better immunity. The HLA-DQA1 gene was found in almost 100% of ancient individuals but it was found in only 36% of modern Native American genomes. Researchers have speculated that a possible cause for this discrepancy is the fact that ancient Native Americans were well equipped immunologically to resist infection from native pathogens but when the Europeans arrived in North America and brought new, never-before encountered pathogens the HLA-DQA1 gene was not sufficient in offering immunity thus it was no longer as heavily selected for in the population (7).

Cholera's Effect on the Human Genome

Cholera is a deadly disease caused by the bacterium *Vibrio cholera*. This bacterium produces toxins that binds to the cells lining the small intestine where its disrupts cellular processes to cause severe diarrhea. The disease has a 50% mortality rate when left untreated as those who are infected die from loss of electrolytes and shock. In one study, individuals of a Bangladeshi population underwent a genome-wide scan for positive selection. The scan revealed approximately 305 selected regions within the population. Three types of genes were statistically overrepresented within the Bangladeshi population. One type of gene that was discovered to be under positive selection code for potassium channels that release chloride ions into the intestines. The second type of gene that was overrepresented codes for proteins that manage the NF- kB protein. This protein is responsible for controlling inflammation- prime immune response during infection. The third type of gene plays a role in kick starting the inflammasome. The inflammasome is a protein aggregation that detects pathogens and then initiates the pathway of inflammation in response to their presence. Selection for these three classes of genes indicates that the human immune system is indeed evolving to become more resistant to *Vibrio cholera* (8).

Malaria's Effect on the Human Genome

Another disease that has had a significant impact on the human genome is malaria. Malaria is caused by the protozoan plasmodium. The parasite infects red blood cells and causes symptoms such as chills and fever. This disease kills more than one million children each year. Malaria has been deemed one of the strongest forces for selection in the human genome in recent history (9). The population of sub-Saharan Africans have a nearly complete resistance to *Plasmodium vivax* infection when compared to other human populations that are susceptible to this species of malaria parasite. This population's resistance is conferred by a single nucleotide polymorphism (SNP) in the *FY* gene which results in the Duffy blood group- negative phenotype. Infection caused by *Plasmodium vivax* is common in South America and Asia but it is rare in

sub-Saharan Africa. This rarity of infection is due to that mutation in the FY gene rather than environment because sub-Saharan Africa has the perfect climate for the transmission of this parasite (9). Another ethnic group that has high resistance to malaria when compared to other populations within the same area are the Tharu people. It is thought that their resistance is due to a high frequency of α thalassemia within the Tharu population. Haemogolbin beta (HBB) has three different coding SNPs: hemoglobin S (HbS), hemoglobin C (HbC), and hemoglobin E (HbE). Each of these versions of HBB bequeath resistance against malaria and are found in high frequencies in different populations. The HbS allele is found in parts of the Middle East as well as sub-Saharan Africa. This allele is a double-edged sword. Individuals that are homozygous for this allele will have molecular defects within their red blood cells due to the disease sickle cell anemia- this often leads to an early death. On the other hand, individuals that are heterozygous for the allele will not have sickle cell anemia, but they will still be resistant to malaria. HbC is found in populations in many parts of West Africa. It has less frequency than HbS. Individuals that are homozygous with the HbC allele suffer from a mild form of hemolytic anemia but they are protected from malaria. Heterozygous individuals do not have significant reductions in their hemoglobin levels and they are conferred resistance against malaria as well. The HbE allele is found in Southeast Asia. It seems that the mutation is recent in the human genome despite its rapid rise in allele frequency. Homozygous individuals typically have symptomless anemia and it is assumed that they have resistance against severe malaria. Heterozygous individuals are quite resistant to infection by the species *Plasmodium falciparum* (9). Another example of change within the human genome that has been induced by malaria is the rise of HLA-Bw53, a human leucocyte class I antigen. This antigen is rarely found in other racial groups but it is predominant in West African populations. HLA-Bw53 is associated with protection from severe malaria (10).

Autoimmune Disorders and Parasites

At times when a person encounters certain environmental factors such as peanuts or pollen, the immune system sometimes elicits an inappropriate immune response towards these environmental antigens. Rather than recognizing that the environmental antigen in question is not harmful the immune system views it as something destructive and initiates a response that presents itself as an allergic reaction. Antibodies called immunoglobulin E (IgE) are produced and they travel to cells that release chemicals that cause the reaction. It has recently been proposed that IgE mediated immune responses evolved in humans to offer protection against metazoan parasites rather than to cause allergies. To validate this hypothesis, researchers compared the properties of environmental allergens to the properties of metazoan parasite antigens that are targeted by IgE in infected populations. Researchers took 2,712 protein molecules that are known to be IgE antigens and compared them to a dataset of proteins found in parasitic arthropods and helminths. Of the proteins compared, 2,445 of the parasite proteins were significantly similar in structure and sequence to proteins that are produced during an allergic reaction. These findings suggest that the 'off-target' effects of the IgE mediated immune system in allergies has evolved to act as a protection against parasitic infections (11). This is a prime example of how the human genome has changed due to pathogen influence.

The Microbiome's Influence on Human Genome Evolution

Pathogenic microorganisms are not the only contributors to change in or regulation of the human genome. Microorganisms that have a symbiotic relationship with human hosts are finally being recognized for their contribution to human genetics. Microbes that live naturally within the human body and make up the microbiome are constantly altering how genes are expressed. It has been demonstrated that microbes within the human gut have the ability to drive gene expression in the host through alteration of the epigenome. The epigenome is defined as the chemical information that regulates which genes will be active and which genes will be inactive (12). It has recently been discovered that bacteria within the human gut play a role in the evolution of the adaptive immune system. Research has demonstrated that the absence of microbiota within the gut results in many immunity deficiencies. There is a lack of production of toll-like receptors- major players in the body's defense system against invading pathogens. The number and cytotoxicity of intra-epithelial lymphocytes (a specialized group of T cells) are significantly reduced as well as the amount of $CD4^+$ T cells. The effects are not limited to the immunity defenses in the intestines but have far reaching consequences that effect the immune system systemically throughout the body. There are reduced numbers of $CD4^+$ T cells within the spleen as well as diminished systemic antibody levels. Thus, the microbes within the gut not only affect the immune health of the intestines but of the entire immune system. It seems that microbiota and host mechanisms have evolved collaboratively to protect the body from harmful pathogens (13).

Conclusion

The human genome has been influenced by invading, pathogenic microorganisms as well as by microbes that reside within the body naturally and have formed a symbiotic relationship with their human hosts. The human immune system is in a constant arms race against pathogenic microbes and must continually evolve and adapt to ensure the reproductivity and survival of the species. It has been demonstrated by numerous research that pathogens are one of the strongest selective forces on the human genome. As microorganisms have increased in virulence and infectivity the human immune system has had to evolve in order to ensure the survival of the human species. These adaptations have been incorporated into the human genome and can be seen in modern populations still today. Without the influence of microbes, the human genome would not be what it is currently. Suffice it to say, microbes both pathogenic and salubrious have indelibly left their mark on the human genome. They have made us who we, as a species, are today.

References:

1. Lachance, Joseph, and Sarah A. Tishkoff. "Population Genomics of Human Adaptation." *Annu. Rev. Ecol. Evol. Syst. Annual Review of Ecology, Evolution, and Systematics* 44.1 (2013): 123-43. Web.

2. Fumagalli M, Sironi M, Pozzoli U, Ferrer-Admetlla A, Pattini L, et al. (2011) Signatures of Environmental Genetic Adaptation Pinpoint Pathogens as the Main Selective Pressure through Human Evolution. PLoS Genet 7(11): e1002355. doi:10.1371/journal.pgen.1002355

3. Karlsson, Elinor K., Dominic P. Kwiatkowski, and Pardis C. Sabeti. "Natural Selection and Infectious Disease in Human Populations." *Nature Reviews. Genetics*. U.S. National Library of Medicine, 2014. Web. 26 Nov. 2016.

4. Laayounia, et al. *Proceedings of the National Academy of Sciences*. N.p., n.d. Web. 21 Nov. 2016.

5. Galvani, Alison P., and Montgomery Slatkin. "Evaluating Plague and Smallpox as Historical Selective Pressures for the *CCR5-Δ32* HIV-resistance Allele." *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, 2003. Web. 21 Nov. 2016.

6. Stephens, J. C., et al. "Dating the Origin of the CCR5-Delta32 AIDS-resistance Allele by the Coalescence of Haplotypes." *American Journal of Human Genetics*. U.S. National Library of Medicine, 1998. Web. 22 Nov. 2016.

7. *Nature.com*. Nature Publishing Group, n.d. Web. 26 Nov. 2016.

8. Elinor K. Karlsson, et al., Science Translational Medicine03 Jul 2013: 192ra86. "Natural Selection in a Bangladeshi Population from the Cholera-Endemic Ganges River Delta." *Natural Selection in a Bangladeshi Population from the Cholera-Endemic Ganges River Delta | Science Translational Medicine*. N.p., n.d. Web. 26 Nov. 2016.

9. Kwiatkowski, Dominic P. "How Malaria Has Affected the Human Genome and What Human Genetics Can Teach Us about Malaria." *American Journal of Human Genetics*. The American Society of Human Genetics, 2005. Web. 25 Nov. 2016.

10. *Nature.com*. Nature Publishing Group, n.d. Web. 25 Nov. 2016.

11. Tyagi, Nidhi, Edward J. Farnell, Colin M. Fitzsimmons, Stephanie Ryan, Edridah Tukahebwa, Rick M. Maizels, David W. Dunne, Janet M. Thornton, and Nicholas Furnham. "Comparisons of Allergenic and Metazoan Parasite Proteins: Allergy the Price

of Immunity." *PLoS Computational Biology*. Public Library of Science, 2015. Web. 26 Nov. 2016.

12. University of Wisconsin-Madison. "Gut's microbial community shown to influence host gene expression." ScienceDaily. ScienceDaily, 23 November 2016. <www.sciencedaily.com/releases/2016/11/161123124256.htm>.

13. Lee, Yun Kyung, and Sarkis K. Mazmanian. "Has the Microbiota Played a Critical Role in the Evolution of the Adaptive Immune System?" *Science (New York, N.Y.)*. U.S. National Library of Medicine, 2010. Web. 26 Nov. 2016.