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Instances of Human Genetic Resistance Against Pathogens

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ABSTRACT

This paper will review research on genetic mutations in humans that grant resistance against pathogens. It will describe the characteristics of these genetic traits, which pathogens they grant resistance to, and the mechanism by which these traits provide resistance. It will then discuss the detrimental aspects of these traits, if any, and the potential applications of the genetic resistance mechanisms towards populations without the trait. Finally, it will cover further research on the genetic trait or pathogen that should be conducted. The following traits will be discussed within this review: the Δ32 mutation in the CCR5 gene and HIV resistance, the erythrocyte silent mutation in the Duffy antigen promoter and malaria resistance, and the sickle cell mutation in the hemoglobin gene and malaria resistance. The research conducted on these genetic traits has allowed us to understand more of the pathophysiology of the disease they resist against.

Introduction

Throughout much of humans' time on earth, disease caused by pathogens has been a common cause of death. For this reason, pathogens have exerted selective pressure on the genome of many organisms. Various mutations present in certain human populations have been suggested to be prevalent in those populations due to the resistance they provide against disease. By understanding which mutations provide resistance to a disease, one can potentially protect those without the mutation from the disease. For example, drugs have been created that prevent certain strains of HIV from infecting new cells based on the knowledge that a mutation in certain individuals grants them HIV resistance.

Three mutations will be covered in this review, each of which provides some form of resistance to a pathogen. These mutations are the Δ32 mutation in the CCR5 gene, the erythrocyte silent mutation in the Duffy antigen promoter, and the HbS mutation in the hemoglobin gene. This review will describe the characteristics of these genetic traits, which pathogens they grant resistance to, and the mechanism by which these traits provide resistance. It will also discuss the detrimental aspects of these traits, if any, and the potential applications of their disease resistance mechanisms. Through understanding these genetic traits, we can also gain a better understanding of how the diseases they protect against can be treated.

Δ32

HIV, CCR5, and Δ32

Human immunodeficiency virus (HIV) is a pathogen responsible for hundreds of thousands of deaths each year [1]. HIV is transmitted from person to person through bodily fluids and infects immune cells such as monocytes, macrophages, and T-cells [2]. When infecting these cells, HIV may use two different proteins to enter the cell, depending on its tropism; R5-tropic HIV infects cells through the CC chemokine receptor 5 (CCR5) protein, and X4-tropic HIV infects cells through the C-X-C chemokine receptor 4 (CXCR4) protein. The majority of HIV is exclusively R5-tropic

during primary infection [3], but as infection progresses mutation may cause the HIV to become X4-tropic or both R5 and X4 tropic.

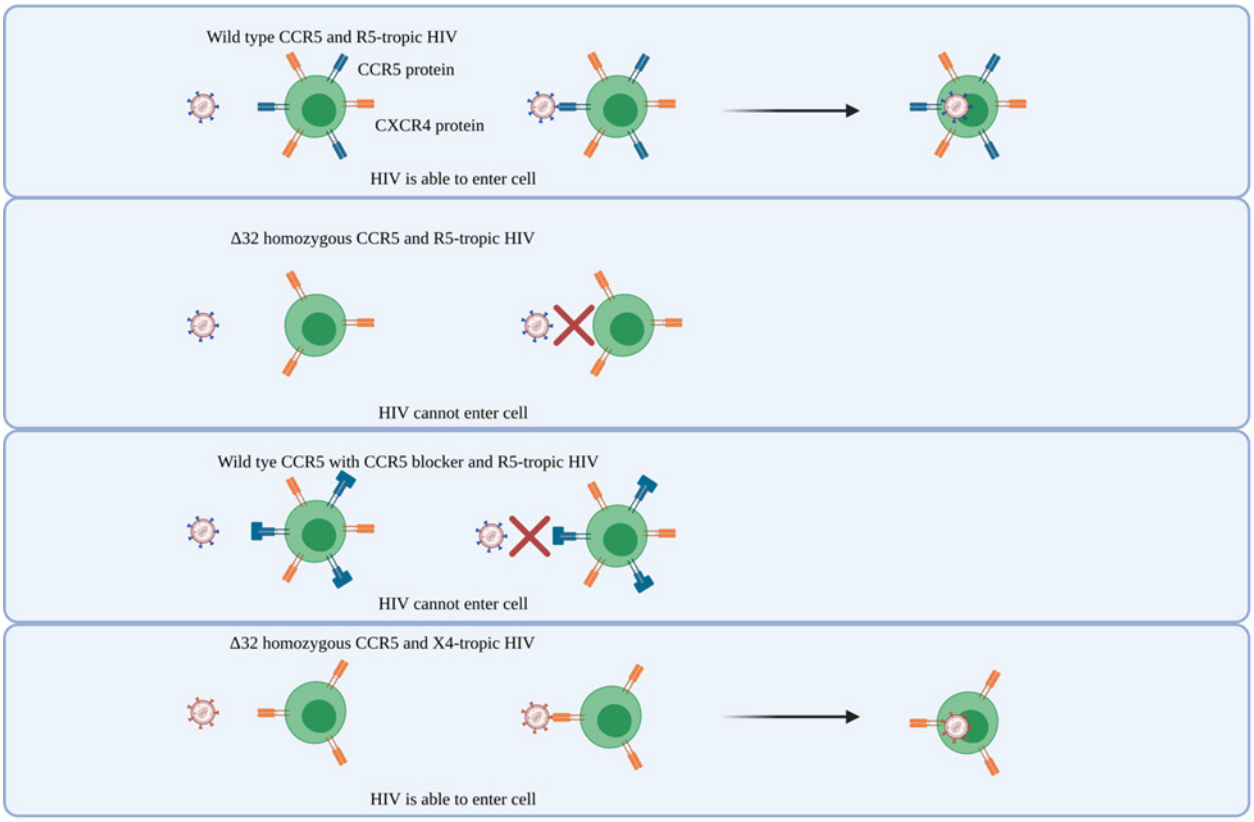

Figure 1. A diagram comparing the vulnerability of the cells of different individuals to HIV invasion. Going from top to bottom: (1) WT CCR5 on host cells and R5-tropic HIV enables HIV to infect the host cells. (2) Δ32 mutation CCR5 on host cells and R5-topic HIV means that HIV cannot infect the host cells. (3) WT CCR5 host cells and R5-tropic HIV in the presence of a CCR5 blocker means that HIV cannot infect the host cells. (4) Δ32 mutation CCR5 on host cells and X4-tropic HIV enables HIV to infect the host cells. Figure created using BioRender.com.

CCR5, the receptor used by over 90% of HIV during primary infection, is a chemokine receptor responsible for directing immune cells to inflamed sites during inflammatory response [4], and is expressed by immune cells, such as T cells and macrophages. Some individuals have a mutation in the CCR5 gene, called Δ32, that truncates their CCR5 protein. Individuals homozygous for the Δ32 mutation only express the truncated, nonfunctional CCR5 proteins, and are resistant to HIV [5] (Figure 1). Individuals heterozygous for the mutation still express some functional CCR5 proteins, albeit at a reduced rate to homozygous wild type individuals, and are not resistant to HIV. Despite the lack of a functional CCR5 protein, Δ32 homozygous individuals do not suffer obvious deleterious effects from their mutation.

Mechanism of resistance

Because R5-tropic HIV requires CCR5 as a gateway to enter cells, it cannot enter cells that do not have the full length CCR5 proteins on their surface. Because the Δ32 mutation causes an improper formation of the CCR5 protein that prevents it from appearing on the surface of their immune cells, individuals homozygous for the Δ32 mutation are resistant to R5-tropic HIV. While individuals heterozygous for Δ32 do not have the same resistance to HIV as they

still have some CCR5 proteins on the surface of their immune cells, they may have a slower onset of symptoms than homozygous wild-type individuals [6].

Detrimental aspects of Δ32

The full extent of the consequences of a nonfunctional CCR5 protein are not presently understood. It is widely believed that the CCR5 mutation has little effect on an individual's health. However, there is evidence to suggest that lacking a functioning CCR5 protein could have some negative effects. Gene knockouts of the CCR5 gene in mice showed that CCR5- mice had a much lower rate of survival when infected with *C. neoformans*, a fungal pathogen, than CCR5+ mice [7]. Another gene knockout study showed that the $CD4^+$ and $CD8^+$ T cells of CCR5- mice produced less interferon gamma, a cytokine responsible for the control of certain immune responses, compared to CCR5+ mice [8]. Additionally, studies of Δ32 homozygous umbilical cord blood units found that the total nucleated cell and CD34+ cell (a type of hematopoietic stem cell) counts of the CBUs was lower than that of heterozygous or wild type homozygous CBUs [6], which could indicate that Δ32 homozygous individuals produce less nucleated and CD34+ cells.

Use of Δ32 to treat HIV

The understanding that a nonfunctional CCR5 protein protects against HIV has led to the advent of anti-HIV procedures and drugs based around altering the CCR5 protein in patients.

HIV has been cured by hematopoietic stem cell transplants using bone marrow from individuals homozygous for the Δ32 mutation [9]. Because hematopoietic stem cell transplants lead to the replacement of the cells HIV infects with cells similar to those of the donor, HSCTs from Δ32 homozygous donors cause the receiver to become resistant to HIV [10]. However, HSCTs require regimens of chemotherapy and radiation before transplantation that destroy bone marrow and immune cells in the patient [11], and therefore pose a great risk to the patient. The HSCTs that cured patients of HIV were performed primarily to treat cancers, not to cure HIV.

New anti-HIV treatments have been created in the form of drugs that bind to the CCR5 protein, preventing HIV from infecting individuals through the CCR5 protein [5]. These CCR5 blockers can be used effectively in conjunction with other drugs as a treatment for HIV that prevents the virus from infecting new cells. However, this treatment cannot currently be used to cure HIV, because some of the cells that the virus infects do not begin immediately producing new copies of HIV, instead creating reservoirs of dormant HIV infected cells [12] that allow for the reestablishment of symptomatic infection once the use of CCR5 blockers on the patient is discontinued. This makes elimination through CCR5 blockers alone impossible, though certain CCR5 blockers may be used in conjunction with other drugs to eliminate these reservoirs [13]. Theoretically, CCR5 blockers could be used in a prophylactic strategy against HIV, but they have the potential for side effects [14] and cannot protect against X4-tropic HIV, so other preventative strategies will likely be more effective. It is also unlikely that CCR5 blockers could be used to effectively prevent the establishment of permanent infection after a person is exposed to HIV, as the virus begins creating its reservoirs in the first stage of infection [15].

Genetic editing is another means by which the CCR5 protein could be altered as a means to protect patients from HIV. By altering an individual's CCR5 gene to prevent the CCR5 protein from appearing on the surface of their immune cells, that individual would be resistant to R5-tropic HIV. In fact, the first known genetic editing of human embryos, announced in November of 2018 by Biophysicist Jiankui He, was done to edit the CCR5 gene to protect the embryos from HIV (see the potential outcomes of the editing in Figure 2). Dr. He's team genetically edited the CCR5 gene in two twins, Lulu and Nana [16]. Though neither twin had 32 base pairs of their CCR5 gene removed, as in the Δ32 mutation, both received edits intended to mimic the protective effects of Δ32 by making their CCR5 protein nonfunctional. Lulu received edits to both copies of her CCR5 gene in order to make her CCR5 protein non-functional. While this could make her HIV resistant, it is possible that not all of Lulu's cells were edited, and some may still be able to be infected by HIV. Nana only had one of her CCR5 genes edited in an in-frame deletion [17], and therefore should still be susceptible to HIV. In addition to potentially not having full resistance to HIV, Lulu may face complications as a result of lacking the CCR5 protein [16]. She may lack the necessary alternative pathways in the chemokine

system that the ancestors of Δ32 carriers evolved to manage their lack of CCR5. This may lead to similar side effects to those seen in individuals using CCR5 blockers, such as poor bone development and remodeling. Considering the unknown impact of altering the CCR5 gene, along with the potential for CRISPR's tendency to cause unintended mutations in the edited genome [18], and the ethical concerns surrounding genetic editing, it can be concluded that more work must be done in the field before we can effectively and humanely protect patients against HIV through genetic editing.

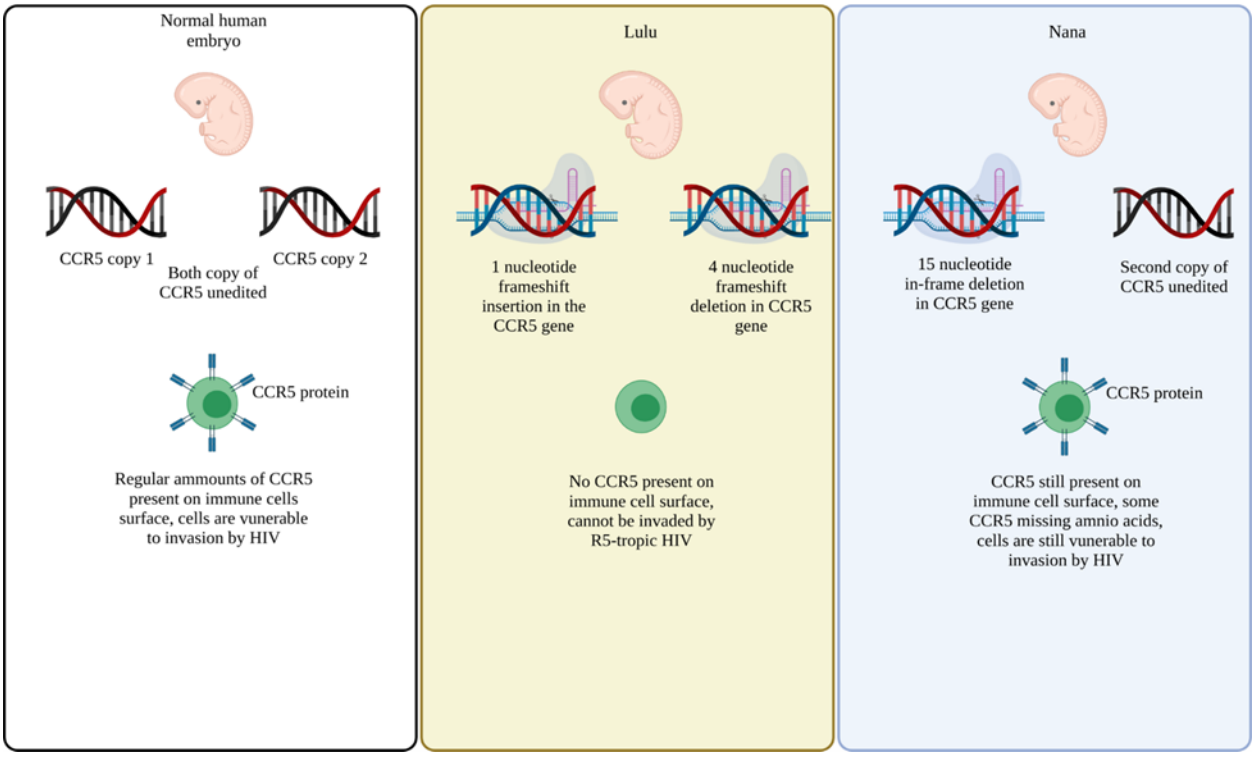

Figure 2 Diagram of all the edits in Lulu and Nana and their effects on Lulu and Nana's CCR5 expression, compared to a wild-type CCR5 individual. Going from left to right: (1) Normal WT human embryo with respect to CCR5 and thus has vulnerability to HIV. (2) Lulu's edited genome wherein both copies of the CCR5 have frameshift deletions. Therefore there should be no CCR5 present on immune cell surfaces and thus no HIV vulnerability. (3) Nana's edited genome wherein 1 copy is unedited and the other has an in-frame deletion. Therefore there is still CCR5 present on immune cell surfaces and thus Nana is still vulnerable to HIV. Note that this diagram assumes that the edits affected all cells vulnerable to R5-tropic HIV. Figure created using BioRender.com.

It is important to note that when using strategies based around altering the CCR5 protein to treat HIV, the same strategies will not be effective against X4-tropic HIV, which does not require the CCR5 protein to infect cells, so testing for HIV's tropism should be conducted before treatment.

Δ32 and other pathogens

Although HIV has only emerged recently as a human pathogen, Δ32 is quite prevalent within European populations. Comparing the prevalence of the Δ32 allele to how long HIV has been a human infecting pathogen shows that HIV has not exerted pressure on the human genome for long enough to fully explain the prevalence of the Δ32 allele. This

indicates that another pressure may also selects for HIV, and has led some researchers to suggest that Δ32 also provides resistance to a pathogen other than HIV [19].

One proposed pathogen the Δ32 mutation could provide resistance to is *Y. pestis*, the bacteria that causes bubonic plague. Stephens et al. suggests that that *Y. pestis* could be the selective agent responsible for the prevalence of the Δ32 mutation, based on when the black death, a plague that is thought to be caused by *Y. pestis*, occurred and how long the Δ32 allele must have been selected for to reach its current prevalence [20]. However, there is debate as to whether this is the case. Cohn and Weaver argue that the bubonic plague was not the selective agent responsible for the current prevalence of the Δ32 mutation [21]. They point towards studies of mice infected with *Y. pestis* that found no significant difference in mortality between wild type CCR5 mice and mice that had their CCR5 gene knocked out [9] [10]. This seems to agree with their hypothesis that the Δ32 allele provides no resistance to the bubonic plague. Another piece of evidence Cohn and Weaver present is maps of black death mortality from 1346–53 compared to maps of present day Δ32 frequencies that show areas most impacted by the black death have the lowest presence of the Δ32 allele today, and vice versa [21]. They argue that this indicates the black death did not exert enough pressure on areas with high present day Δ32 prevalence, and therefore is not responsible for the prevalence. Cohn and Weaver's counterargument against Stephen et al.'s hypothesis is fairly convincing, as it presents direct evidence showing that lacking the CCR5 receptor does not seem to affect vulnerability to *Y. pestis*, and presents evidence that can be argued to counter the claim that *Y. pestis* was a significant selective pressure for the Δ32 allele.

Another suggested pathogen that Δ32 could provide resistance to is smallpox. Galvani and Slatkin use a model to suggest that while the black death and other subsequent plagues did not affect Europe for long enough to account for the prevalence of Δ32 within European populations today, smallpox was active enough in Europe to cause the prevalence of the allele [22]. However, Cohn and Weaver also argue against this hypothesis, claiming it does not account for the fact that the Δ32 allele is only prevalent in Europe, while smallpox impacted other areas of the world to an equal, if not greater, extent [21]. Cohn and Weaver's argument against the Galvani and Slatkin's hypothesis is not as convincing as their argument against Stephen et al.'s hypothesis, as it does not present evidence to disprove Galvani and Slatkin's model showing that smallpox was active in Europe long enough to provide sufficient pressure for the Δ32 allele, and provides no evidence contrary to the claim that lacking the CCR5 protein protects against smallpox, as they did with *Y. pestis*. However, further evidence for Galvani and Slatkin's hypothesis must be presented for it to be considered as a theory.

Further research

More work must be done to determine what selective pressure increased the prevalence of the Δ32 mutation before HIV emerged. Furthermore, the potentially detrimental aspects of Δ32 suggested by the evidence of altered immune response should be studied further, and if individuals with the Δ32 mutation are more vulnerable to certain diseases, prophylactic measures should be created to safeguard them from harm.

One valuable aspect of mutations that provide resistance against pathogens is that they allow us to learn more about those pathogens. The fact that the Δ32 mutation provides resistance to R5-tropic HIV and that CCR5 blockers prevent R5-tropic HIV infection from progressing should provide a selective advantage to X4-tropic HIV. But there are other factors that may affect the viability of X4-tropic HIV, such as how shifts from R5 to X4 tropism of HIV in an infected person are correlated with a decrease in the number of $CD4^+$ T cells [23], which often coincides with an advancement of infection. This poses the question: Is X4-tropic HIV selected for? To determine this, a new study should begin genetically sequencing populations of infected individuals over time to determine how the rate of R5-tropic, X4-tropic, and dual (both R5 and X4)-tropic HIV cases changes over time. It is possible that selection is based on how long infection progression has occurred for, and therefore the time since initial infection should be considered as a potential confounding variable. To control for this, it may be reasonable to only sample early HIV infections to control for tropism changes. But this again could cause issues if one specific tropism is more easily detected, during early infection, and therefore disproportionately sampled. It would also be advised to consider the location of testing,

to determine if the selected tropism is related to factors of the local area, such as climate, human promiscuity, or use of CCR5 blockers. If the sequencing study determines the X4-tropism is selected for, it may be best to avoid investing in CCR5-based treatments for HIV, as long as the X4-tropism continues to be selected for. However, if the R5-tropism is selected for, further investment in CCR5-based treatments for HIV should be done as long as the R5-tropism continues becoming more common.

Duffy antigen polymorphisms

Malaria, Plasmodium vivax, and Plasmodium knowlesi

Plasmodium is a genus of protozoa and the cause of malaria [24]. Plasmodium can infect many different classes of animals, including mammals, birds, reptiles, and insects. In humans, plasmodium infection occurs when a mosquito infected by plasmodium consumes blood from a human. After being inoculated into the human, the plasmodium first infects liver cells. The parasite matures in the liver cells before causing the cells to rupture, releasing the parasite into the bloodstream. The parasite then begins infecting red blood cells (RBCs,) and reproducing, causing symptomatic infection [28]. During blood stage infection, malarial parasites can infect mosquitoes when they consume blood from the infected human. Additionally, some species of plasmodium can enter a hypnozoite stage while in the liver, where they remain dormant and can reemerge after long periods of time to restart symptomatic infection in the host. Malaria causes hundreds of thousands of deaths per year, and children are particularly at risk from being killed by malaria [34]. This should make it a significant selective pressure in its endemic areas.

Plasmodium vivax is a species of plasmodium. *P. vivax* is the most geographically widespread cause of malaria in humans [25]. Additionally, *P. vivax* accounted for an estimated 7% of 229 million malaria cases in 2000 [26]. Though it is not the most deadly species of Plasmodium, *P. vivax* infections present their own challenges due to the fact that *P. vivax* is one of the plasmodium species able to enter the hypnozoite stage, where they remain dormant in the liver and reemerge to reinfect blood at a later time [27]. This not only makes *P. vivax* malaria a recurring event in infected patients, but also allows the parasite to avoid some effects of certain antimalarials [28], making infection more difficult to eliminate. During the erythrocytic stage of human infection, *P. vivax* uses the Duffy antigen to enter RBCs.

Plasmodium knowlesi is a species of plasmodium that, until recently, was only thought to infect humans on rare occasions. It mainly infects long tailed and pig tailed macaques [29]. Like *P. vivax*, *P. knowlesi* uses the Duffy antigen to infect RBCs during the erythrocyte stage of its life cycle [30].

The Duffy antigen

The Duffy antigen is a chemokine receptor expressed on the surface of cells, including RBCs, as well as the endothelium and several other organs throughout the body [31]. There are two codominant alleles for the Duffy antigen, FY*A and FY*B (henceforth A and B,) which each allow for the expression of Fy^a and Fy^b polymorphisms of the Duffy antigen, respectively. An individual can display one, both or neither of these polymorphisms. In individuals homozygous for the A allele, only the Fy^a polymorphism of the Duffy antigen is expressed. Likewise, individuals homozygous for the B allele only express Fy^b Duffy antigens. In individuals with a genotype of A/B, both forms of the antigen are expressed.

Some individuals have a mutation in the promoter for the Duffy antigen that causes the Duffy antigen to not be expressed on RBCs. This mutation is referred to as the erythrocyte silent polymorphism, and individuals with this mutation will have a genotype of A^{ES} or B^{ES} for at least one of their Duffy antigen alleles. An individual whose genotype is A/B^{ES} will express only the Fy^a Duffy antigen on RBCs, and only at about half the rate of an A/A individual [32]. An individual who only has alleles for the enterocyte silent expression of the Duffy antigen will not express the Duffy antigen on their RBCs. These individuals are referred to as Duffy negative, and are resistant to infection by *P. vivax* and *P. knowlesi.*

Mechanism of resistance

Because both P. vivax and P. knowlesi use the Duffy antigen to enter RBCs, RBCs that lack the Duffy antigen cannot be infected by those plasmodium species through normal means. Other species of malarial parasites are still able to infect Duffy negative RBCs, as they utilize different receptors to enter RBCs [30]. Additionally, both P. vivax and P. knowlesi are still able to infect liver cells, even without the Duffy antigen, as they do not use the Duffy antigen to infect liver cells. However, the infection will be asymptomatic if the parasites remain in the liver and do not enter the bloodstream [33]. It should be noted that there have been reports of blood stage malaria caused by P. vivax in Duffy negative individuals [34].

Use of the Duffy antigen to treat malaria

Because *P. vivax* and *P. knowlesi* generally require the Duffy antigen to enter cells, it has been suggested that Duffy antigen blockers be used to treat malaria cases caused by those species of plasmodium, similar to how CCR5 blockers have been suggested to treat HIV [35]. This method would not be effective for eliminating *P. vivax* in the liver, and infection would progress once the Duffy antigen blockers are no longer administered, but the blocker would stop the parasite from causing malaria symptoms [36]. However, a potential danger of using a Duffy blocker is that it may bind to Duffy antigens all over the body, not just those of RBCs. This could cause negative side effects not seen in people with the erythrocyte silent polymorphisms, since they only lack the Duffy antigen on their RBCs. Another potential method is using a gene editing tool, such as CRISPR, to alter the Duffy antigen promoter so it is not expressed on RBCs, mimicking the erythrocyte silent polymorphism of the Duffy antigen. Again, this would protect against malaria by preventing parasites from invading RBCs, but in this case the protective effect would be permanent. Additionally, so long as only the Duffy antigen promoter is edited, this method will not have the off target effects that a blocker might. However, this would not account for any evolutionary adaptations Duffy erythrocyte silent individuals have to manage their lack of Duffy antigen on their RBCs.

Infection of Duffy negative individuals by P. vivax

Despite the protective factor granted to Duffy negative individuals against *P. vivax*, there have been cases of individuals who are Duffy negative with malaria caused by *P. vivax*. Research by Golassa et al. displays multiple reports of Duffy negative individuals infected by *P. vivax* and has suggested that the parasite may be able to infect Duffy negative RBCs through an unknown alternate mechanism [34]. The researchers suggested that *P. vivax*'s new method of invasion could be through *P. vivax* reticulocyte binding proteins (PvRBPs), a family of proteins used to adhere to the surface of reticulocytes, young RBCs. In Duffy positive infection, *P. vivax* uses a PvRBP to bind to transferrin receptor 1, a protein found on reticulocytes, before invading the cell through the Duffy antigen. Invasion of Duffy negative RBCs could also be related to the proteins *P. vivax* merozoite surface protein-1 paralog and *P. vivax* glycosylphosphatidylinositol-anchored micronemal antigen (PvMSP1P and PvGAMA,) both of which facilitate the attachment of *P. vivax* to RBCs and can bind to Duffy negative RBCs. To test what proteins are responsible for *P. vivax*'s ability to invade Duffy negative RBCs, one could perform gene knockouts on PvRBPs, PvGAMA, and PvMSP1P in *P. vivax* that is shown to be able to infect Duffy negative RBCs before the knockouts, and expose the post-knockout *P. vivax* to Duffy negative RBCs to determine if one, a combination of two or more, or none of these proteins are necessary for *P. vivax*'s invasion of Duffy negative RBCs. However, another possibility that should be considered is that the Duffy antigen is still expressed on RBCs of supposedly Duffy negative individuals infected with *P. vivax*. Though the erythrocyte silent polymorphism usually prevents individuals from expressing the Duffy antigen on their RBCs, it could be the case that other genetic variations can allow for the Duffy antigen to still be expressed on RBCs, in which case invasion by *P. vivax* through its normal methods would be possible. Tests for the presence of the Duffy antigen on RBCs of supposedly Duffy negative malaria cases should be performed along with genotyping. Regardless, the fact that individuals homozygous for erythrocyte silent variations of the Duffy antigen can still be vulnerable to *P. vivax* infection may create issues for basing prophylactic strategies around preventing its invasion of the Duffy antigen,

and the apparent vulnerability of Duffy negative individuals to *P. vivax* should be taken into account when creating strategies to control its spread.

Detrimental aspects of Duffy negative

Although individuals without the Duffy antigen present on their RBCs do not seem to suffer any ill effects from lacking the antigen, there could also be more subtle consequences of lacking the Duffy antigen on RBCs. Some researchers have shown gene knockouts in mice that prevented the expression of the Duffy antigen on both RBCs and vascular endothelial cells found increased growth of prostate tumors as evidence for the dangerous aspect of lacking the Duffy antigen. However, counterevidence has been presented that appears to refute this; most humans only lack the Duffy antigen on RBCs, while still having the antigen present on their vascular endothelial cells, and a study of prostate cancer patients did not find a link between being Duffy negative and developing or having worse outcomes for prostate cancer [37].

*Resistance to P. vivax through the FY*A allele*

There is evidence that, along with the erythrocyte silent polymorphism, the Fy^a polymorphism of the Duffy antigen may have a protective effect against *P. vivax*, suggested by a study conducted by King et al. [32]. The study attempted to determine if an individual's vulnerability to *P. vivax* infection was related to their genotype for the Duffy antigen. The study followed 400 individuals for malaria and found that the order of vulnerability to *P. vivax* infection for each genotype, from least to most vulnerable, was A/B^{ES}, A/A, A/B, B/B^{ES}, then B/B. Since A homozygotes express the Duffy antigen at approximately the same rate as B homozygotes, the difference in vulnerability to infection cannot be attributed only to the level of Duffy expression in each individual. The protective factor of the A allele could be explained by in vitro testing showing that RBCs expressing the FyA antigen have less binding activity with *P. vivax* Duffy binding protein (PvDBP, a protein used by *P. vivax* in the invasion of human RBCs) compared with RBCs expressing FyB antigens. Furthermore, antibodies naturally produced during *P. vivax* infection that prevent the binding of PvDBP to the Duffy antigen were found to inhibit binding of PvDBP more effectively in A homozygous RBCs than in B homozygous RBCs, though this was tested using higher concentrations of the antibody than are found naturally in the body.

Further research

Reports of *P. vivax* infected Duffy negative individuals must be investigated further to determine if *P. vivax* is truly able to infect people without the Duffy antigen. If *P. vivax* has developed an alternate mechanism of infection not using the Duffy antigen, this mechanism must be understood in order to figure out how to protect infected individuals, and Duffy negative individuals must be considered at least somewhat vulnerable to infection by *P. vivax*. It could be investigated if *P. knowlesi* has also developed alternate pathways to infect Duffy negative individuals, but since *P. knowlesi* infections in humans are rare, its ability to infect Duffy negative individuals is less of a concern.

The reports of *P. vivax* infections in Duffy negative individuals are troubling. If these reports are correct, and the ability of *P. vivax* to infect Duffy negative individuals becomes prevalent, it will mean that many people once considered safe from *P. vivax* will become vulnerable. However, reports show that even though some *P. vivax* is able to infect Duffy negative individuals, Duffy negative individuals generally have less severe infections of *P. vivax* compared to Duffy positive individuals [34]. This calls into question how vulnerable Duffy negative individuals are to *P. vivax* infection. It will be henceforth assumed that certain *P. vivax* strains are capable of infecting RBCs without use of the Duffy antigen, as opposed to any alternate hypothesis for why apparently Duffy negative individuals are infected with *P. vivax*. These strains of *P. vivax* able to infect Duffy negative individuals will be referred to as DNC (Duffy Negative Competent) *P. vivax*. To determine how threatening strains of DNC *P. vivax* are, new studies will be needed to determine the extent to which Duffy negative individuals are now vulnerable to *P. vivax* infection. For this reason,

an experiment to determine *P. vivax*'s effectiveness in infecting Duffy negative individuals should be developed. Cases of Duffy negative individuals infected with *P. vivax* must be found and samples of the DNC *P. vivax* must be taken. This *P. vivax* should be tested to determine its ability to infect Duffy negative and Duffy positive RBCs, and compared to a strain of *P. vivax* unable to infect Duffy negative RBCs to determine if the DNC *P. vivax* is able to infect Duffy negative RBCs efficiently, and furthermore, to see if its infectivity of Duffy positive RBCs has been compromised by its ability to infect Duffy negative RBCs. Additionally, different strains of *P. vivax* able to infect Duffy negative RBCs should be genetically sequenced and compared to determine if they share a common ancestor or the ability to infect Duffy negative RBCs has evolved independently.

Sickle Cell Trait

Plasmodium Falciparum

Plasmodium falciparum is the most common cause of malaria in humans, responsible for hundreds of millions of infections each year [38]. Additionally, *P. falciparum* is the most lethal form of malarial parasite in humans [39]. Some individuals have a genetic trait, called sickle cell trait, that makes them resistant to *P. falciparum*.

Sickle cell disease and sickle cell trait

Sickle cell disease is a homozygous genetic disease caused by a mutation in the hemoglobin gene. Individuals with sickle cell disease, who have a hemoglobin genotype of SS, have their RBCs deformed into a sickle shape. These sickle cells cause symptoms that include anemia and pain caused by the sickle cells blocking circulation [40]. However, individuals who are heterozygous for the sickle cell allele, who have a hemoglobin genotype of AS, do not experience sickle cell symptoms, and have resistance to *P. falciparum* [41]. Individuals with a hemoglobin genotype of AS are considered to have sickle cell trait.

Mechanism of resistance

Though there is evidence that being heterozygous for the sickle cell trait provides protection against *P. falciparum*, it is still unclear exactly how sickle cell trait provides this resistance. Many mechanisms have been proposed as the factor that gives individuals with sickle cell trait resistance to *P. falciparum*, and it is possible that there are multiple features of sickle cell trait responsible for its protective nature (See Figure 3 below for an overview of these potential features).

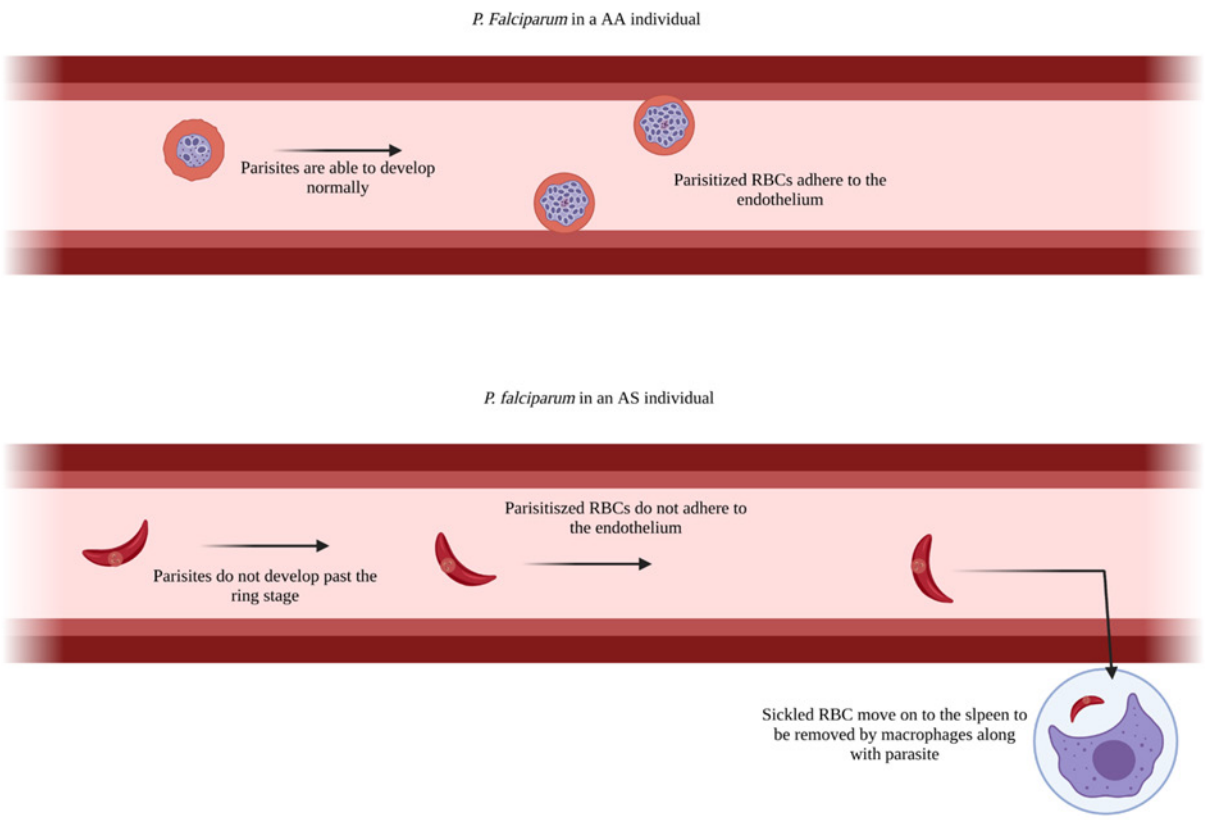

Figure 3. Diagram showing how an individual with AS hemoglobin may be affected differently by P. falciparum compared to an individual with AA hemoglobin. Going from top to bottom: (1) Parasites are able to develop normally in an AA individual and adhere to the endothelium. (2) Parasites cannot develop normally in an AS individual and thus do not adhere to the endothelium resulting in fewer symptoms. The infected RBCs are also removed by macrophages. Note that this diagram assumes all of the potential factors for AS individuals' resistance to P. falciparum. Figure created using BioRender.com.

One proposed reason for the resistance of AS individuals to malaria is the selective sickling of cells infected by *P. falciparum* parasites [42]. When a RBC of an AS individual is invaded by plasmodium parasites, changes in the cell caused by the parasite make it more prone to sickling. These sickled cells are then removed along with their parasites by macrophages in the spleen.

Another potential mechanism for AS individual's malaria resistance is the lack of growth of *P. falciparum* when in low oxygen environments inside AS RBCs [43]. Experiments by Archer et al., intended to determine how oxygen levels affect parasite growth in AS RBCs, suggested that in low oxygen AS RBCs inoculated with *P. falciparum*, only a small percentage of *P. falciparum* parasites had hemozoin, a byproduct of the parasite's hemoglobin digestion, whereas in high oxygen AS RBCs and low oxygen wild type homozygous (AA) RBCs, nearly all *P. falciparum* parasites had hemozoin. This indicated that *P. falciparum* parasites in AS RBCs struggle to break down hemoglobin under low oxygen conditions. Further testing done to determine if the sickling of AS RBCs in low oxygen conditions was responsible for the growth arrest was done by exposing low oxygen AS RBCs to an anti-sickling agent showed that parasite DNA replication (another measure of parasite growth) was able to progress to levels similar to parasites in AA RBCs also exposed to the anti-sickling agent. This seems to indicate that the sickling of AS RBCs in low oxygen environments is responsible for the growth arrest of *P. falciparum* parasites. The study is unclear about whether this sickling also protects SS individuals from *P. falciparum* infection.

A third possible mechanism by which sickle cell protects against *P. falciparum* is by preventing RBCs infected by the parasite from adhering to the endothelial cells that line blood vessels. Research by Lansche et al. to determine the difference in adhesion between AA parasitized RBCs and AS parasitized RBCs has shown that RBCs containing sickle hemoglobin adhere less to the endothelium. This could be a mechanism of resistance granted by sickle cell trait, as it may prevent complications of malarial infection. The study proposes that because their shape is different from normal parasitized RBCs, parasitized sickle cells have reduced adhesion to endothelial cells. This may prevent the dangerous aspects of malaria, such as vaso-occlusion, that arise when parasitized RBCs adhere to endothelial cells.

Lack of resistance to P. falciparum from sickle cell disease

Despite the protective benefits sickle cell trait seems to provide against *P. falciparum*, sickle cell disease does not seem to have the same protective effects. In fact, though SS individuals appear to be as vulnerable to malaria infection as AA individuals, they are much more likely to be killed by said infection than AA individuals [42]. This is because the symptoms SS individuals experience as a result of their disease, such as anemia, are often compounded by malarial infection. Furthermore, sickle cell disease may compromise an individual's ability to fight off malaria by damaging the spleen. It is possible that some of the resistance mechanisms present in AS individuals are not present in SS individuals. For example, the phagocytosis of infected sickle RBCs may be hindered by the compromised spleens of SS individuals. However, other resistance mechanisms, such as reduced adhesion of parasitized sickle RBCs, should still be present and need to be accounted for in the understanding of sickle cell disease individual's vulnerability to malaria. Testing with SS RBCs inoculated with *P. falciparum* to observe the presence or absence of protective factors granted by sickle cell trait should be conducted and case studies of *P. falciparum* infected SS individuals must be observed to gain a further understanding of why individuals with sickle cell disease do not seem to be protected from *P. falciparum*. For example, the study conducted by Lansche et al. [44] could be replicated using HbSS RBCs to determine if they also have reduced adhesion to the endothelium.

Detrimental aspects of sickle cell

As discussed previously, individuals with only one copy of the sickle cell gene generally do not suffer any detrimental effects, though they may experience cell sickling under certain conditions that allow for it, such as in a low oxygen environment [45]. However, individuals with sickle cell disease often experience a variety of health problems because of their disease, such as anemia, vaso-occlusion, coronary artery disease, and inflammation [40]. These factors cause severe complications and individuals with sickle cell disease have a lowered life expectancy if not given medical prophylaxis [46].

Potential applications

Genetic editing could be used as a protective strategy against *P. falciparum* by changing wild type hemoglobin homozygotes to heterozygotes for sickle cell. So long as the gene edits are made locally in hematopoietic stem cells so that the germline cells of these patients are not edited, there would be no risk of the child of two genetically edited patients receiving sickle cell alleles (Figure 4). However, the scale of this genetic editing project may make it less viable than alternative preventative measures.

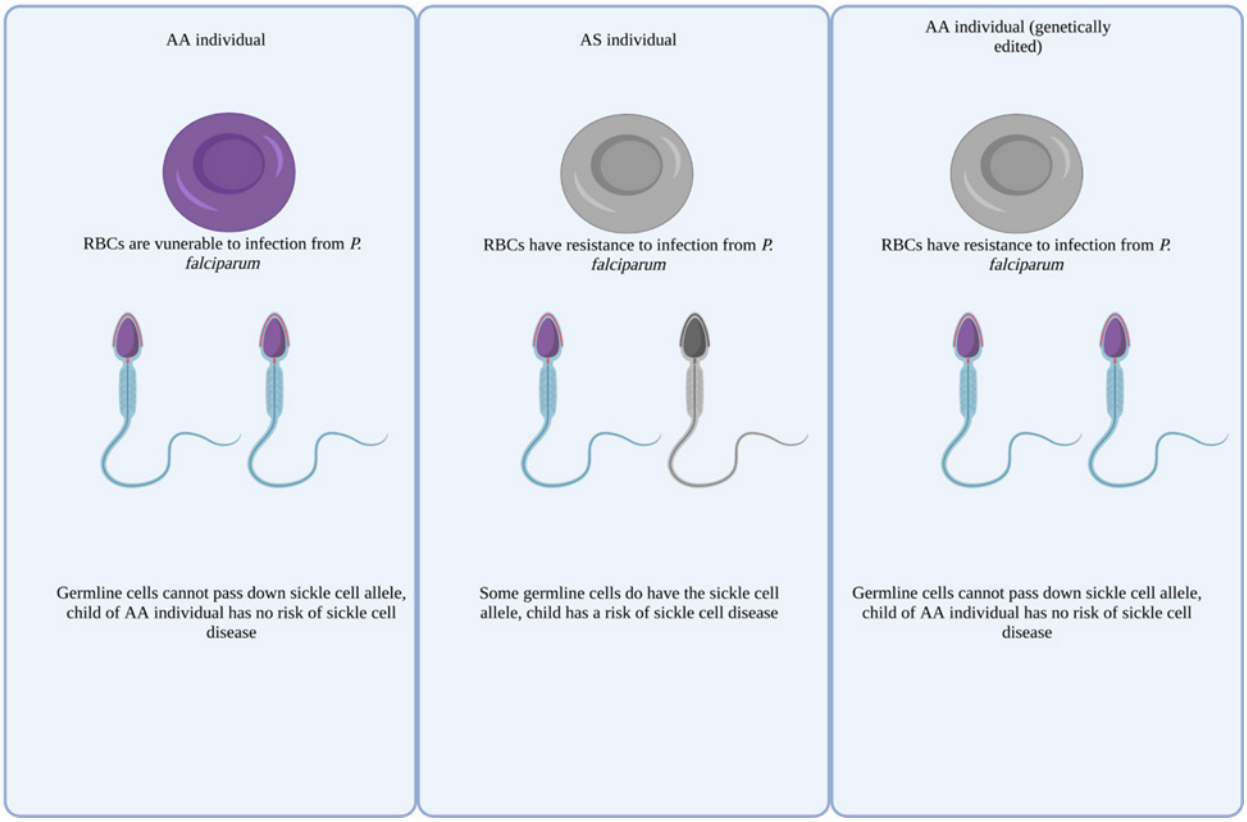

Figure 4 Diagram comparing individuals with and without sickle cell trait, and how an individual with the hemoglobin gene edited in certain cells could have both resistance to P. falciparum and would have no chance of offspring with sickle cell disease. Going from left to right: (1) An AA individual is vulnerable to infection but, because they do not have the sickle cell trait, there is no chance that offspring would have the sickle cell disease. (2) An AS individual is not vulnerable to infection but, because they do have the sickle cell trait, there is a chance that offspring would have the sickle cell disease. (3) An AA individual whose RBCs edited such that they are now AS is not vulnerable to infection but, because their germ cells do not have the sickle cell trait, there is no chance that offspring would have the sickle cell disease. Figure created using BioRender.com.

Further understanding of the safeguarding factors of sickle cell trait could be utilized in the development **of** antimalarials. For example, a drug could be used to halt hemoglobin digestion to prevent parasite growth. Some already existing antimalarials do target plasmodium's hemoglobin digestion [47]. For example, chloroquine. Though chloroquine does not prevent the digestion of hemoglobin, it prevents the formation of hemozoin after hemoglobin digestion, leaving behind a toxic byproduct of the digestion, called heme, that destroys the parasite. Another possibility is a drug that reduces the lethality of infection, such as a drug that prevents the adhesion of parasitized RBCs to endothelial cells which could be used to avoid complications such as the sequestration crises that occur due to the adhesion of the parasitized RBCs.

Further research

There is a clear need for a better understanding of the mechanisms that allow for the resistance of sickle cell trait individuals against *P. falciparum*, and more research must be done to determine if SS individuals also have resistance to *P. falciparum.*

Another area of interest for further research of sickle cell is whether it provides resistance to species of plasmodium other than *P. falciparum*. When determining this, an important focus would be *P. vivax*; as the second most common cause of malaria, knowledge of mechanisms that protect against *P. vivax* are valuable. In addition, *P. vivax* is present in many areas where the sickle cell allele is prevalent in the population, meaning that infection of AS individuals is likely a common occurrence. It is possible that some of the traits of sickle cell that confer resistance to *P. falciparum* also confer resistance to *P. vivax*. For example, *P. vivax* does digest hemoglobin [48], and therefore may have its hemoglobin digestion interfered with in sickle hemoglobin RBCs. However, some factors of *P. vivax* infection may be unaffected by sickle cell trait. For instance, whereas *P. falciparum* creates knobs on RBCs to adhere to the endothelium, *P. vivax* does not form knobs to adhere to the endothelium [49], which may mean that the different shape of a sickle RBC may not prevent its adherence to the endothelium when infected by *P. vivax*. In order to determine if sickle cell trait does in fact provide resistance to *P. vivax*, a study could follow individuals with the sickle cell trait in *P. vivax* endemic areas and compare their vulnerability to infection with individuals without the trait. Ideally, this would be done in an area where the Duffy antigen is present on the local population's RBCs, as an area with high prevalence of the Duffy antigen erythrocyte silent polymorphism and the *P. vivax* resistance conferred by this trait could throw off the study's results. Though there are no places where the Duffy antigen erythrocyte silent polymorphism is absent and both the sickle cell allele and *P. vivax* are present, certain parts of India have a relatively high frequency of *P. vivax* [50] and the sickle cell allele [51] while also having a low frequency of the Duffy antigen erythrocyte silent polymorphism [52]. This makes India an ideal location for the study. In terms of the time span over which the study would take place, a period of interest is the individual's childhood years, when they are most vulnerable to death by malaria [39], so perhaps the study could follow individuals from age 0-10 for *P. vivax* infection. Clinical outcome of any *P. vivax* infection and parasitemia in sickle trait individuals would be recorded and compared to those of the control group to determine if sickle cell has a protective effect against *P. vivax*. Additional factors, such as rate of phagocytosis of parasitized RBCs, hemozoin levels in parasites, and rate of adhesion, may be recorded to see if sickle trait influences these factors. In the case that sickle cell does provide some protection against *P. vivax*, experiments done to elucidate the mechanisms by which *P. falciparum* is resisted in sickle cell individuals could be repeated using *P. vivax*, to determine which of the mechanisms by which sickle cell protects against *P. falciparum* also provides protection against *P. vivax*, if any.

Conclusion

Multiple mutations in the human genome have provided humans with resistance to various pathogens. Understanding these mutations help bolster our comprehension of evolution and the selective pressures that allow for specific mutations to become prevalent. For practical purposes, knowledge of these mutations helps us understand the pathophysiology of the diseases they help resist and can be used as a basis for treatment of those diseases.

Several other potential candidates for protective mutations have been identified, such as tay-sachs [53] and cystic fibrosis [54]. Many of these mutations have detrimental effects, and yet still exist in populations at relatively high rates, which could be explained by the resistance they provide against pathogens. If the selective pressure a pathogen exerts on a genome is great enough, it could allow for the prevalence of traits that are otherwise detrimental, so long as those traits provide resistance to the pathogen. By confirming whether these traits are protective or not and elucidating the mechanisms by which these traits provide resistance, the prevalence of them within the human gene pool could be explained.

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