Genetic Polymorphisms in LRP1, a Newly Identified Receptor for Emerging Bunyaviruses

by

Bayley Fields

Bachelor of Arts, Slippery Rock University, 2020

Submitted to the Graduate Faculty of the

Department of Infectious Diseases and Microbiology

School of Public Health in partial fulfillment

of the requirements for the degree of

Master of Public Health

University of Pittsburgh

2023

UNIVERSITY OF PITTSBURGH

SCHOOL OF PUBLIC HEALTH

This essay is submitted

by

Bayley Fields

on

April 19, 2023

and approved by

Essay Advisor: Dr. Amy L. Hartman, Ph.D., Associate Professor, Infectious Diseases and Microbiology, University of Pittsburgh, School of Public Health

Essay Reader: Dr. Jeremy Martinson, D. Phil, Assistant Professor, Infectious Diseases and Microbiology and Human Genetics, University of Pittsburgh, School of Public Health

Essay Reader: Robert Binder, Ph.D., Professor and Director, Department of Immunology, University of Pittsburgh, School of Medicine

Genetic Polymorphisms in LRP1, a Newly Identified Receptor for Emerging Bunyaviruses

Bayley Fields, MPH

University of Pittsburgh, 2023

Abstract

Bunyaviruses, such as Rift Valley Fever (RVFV), are becoming increasingly important in public health. RVFV is being prioritized by the World Health Organization (WHO) due to its potential to become an epidemic. RVFV is transmitted by mosquitos and has the potential to spread to new areas due to climate change. There is no treatment for RVFV since many aspects of the disease are still unknown. Recently, a surface receptor known as low-density lipoprotein receptor-related protein 1 (LRP1) was identified as a factor in RVFV cellular uptake. This finding could be important for progression and may be a potential target for drug therapies or vaccines. Polymorphisms in the human LRP1 gene have been documented and are associated with adverse outcomes such as cholesterol and cardiovascular diseases. We hypothesize that polymorphisms in LRP1 may affect protein expression, function, and/or structure, which could then affect the susceptibility of cells to infection by RVFV. We identified nine polymorphisms of interest: rs138854007, rs1799986, rs1800127, rs1800137, rs34577247, rs1800194, rs12814239, and rs7397167. RVFV is an infectious disease that can lead to serious health outcomes in infected people. As climate change results in disease-carrying mosquitos spreading to other areas, more people will be exposed. Without effective treatment or vaccine, this could seriously impact human health. LRP1 could be significant in RVFV disease progression; therefore, it could be a valuable avenue for treatment or vaccine development. Polymorphisms influencing LRP1 function could be significant in understanding susceptibility to RVFV infection and clinical disease progression.

Table of Contents

List of Tables

List of Figures

Preface

I want to express my sincere gratitude to the following individuals for their invaluable contributions to this work:

First and foremost, I would like to thank Dr. Amy Hartman for her guidance and mentorship throughout my research on LRP1; I have greatly enjoyed learning more about this topic and challenging myself. I would also like to thank the Hartman Lab for being so welcoming and helping me when needed; I have enjoyed getting to know the team.

Additionally, I extend my thanks to Dr. Robert Binder for reviewing my essay and providing insightful feedback.

I would also like to thank Dr. Jeremy Martinson for his assistance with the essay and his unwavering support since we met during the 2017 Summer Edge program, where I first discovered my passion for Public Health. His guidance and encouragement have been invaluable to me throughout my academic journey.

I am deeply grateful to my family, who have always supported my educational goals and pushed me to challenge myself.

Furthermore, I would like to thank my fiancé, Cordell, for his patience, unwavering support, and constant encouragement. Throughout this program, he has been a source of strength and inspiration.

Last but not least, I want to acknowledge my late cat, Chester, for his unconditional love and companionship. His presence in my life was a constant source of comfort and motivation, and I will always cherish the memories we shared.

Thank you all for your support and encouragement.

1.0 Introduction

Bunyaviruses, belonging to the order *Bunyavirales*, contain hundreds of viruses and can infect many plants, animals, and humans (Boshra, 2022; Elliott, 2009; Kwasnik et al., 2021). Viruses belonging to this family are enveloped, mostly tri-segmented, and single-stranded with negative or ambi-sense coding (Boshra, 2022; Elliott, 2009). Typically, viruses in this family are transmitted through arthropods, commonly mosquitos, or rodents (Boshra, 2022; Elliott, 2009). Bunyaviruses are known to cause febrile and hemorrhagic diseases, which can be severe (Boshra, 2022). There is also a concern for viral reassortment due to the segmented genome, which could lead to more severe virus strains (Schwarz et al., 2022). One such bunyavirus, Rift Valley Fever Virus (RVFV), has been of significant public health concern due to the severity of the disease it causes and its recent spread. Recently, a new host entry factor, low-density lipoprotein-related protein 1 (LRP1), has been identified to mediate cellular infection by RVFV. LRP1 is a member of the low-density lipoprotein receptor family, and known mutations in the protein have been linked to cardiovascular disease (Ganaie et al., 2021). Human polymorphisms in LRP1 could impact RVFV uptake and, therefore, disease progression. Identifying polymorphisms could help with understanding the disease and preventing severe outcomes.

1.1 Rift Valley Fever Virus Background

RVFV is a zoonotic disease transmitted by mosquitos in most parts of Africa, even becoming endemic in certain areas (Linthicum et al., 2016). A member of the *Phenuiviridae* family

in the *Phlebovirus* genus, RVFV is related to hantaviruses and Crimean-Congo hemorrhagic fever (CCHF) virus (Ganaie et al., 2021). RVFV is a negative sense RNA virus; other notable negative sense RNA viruses include rabies, influenzas, and Ebola (Borkenhagen et al., 2019; Fisher et al., 2018; Munoz-Fontela & McElroy, 2017)

RVFV is transmitted by *Aedes* mosquitos, specifically *Ae.mcintoshi,* and *Ae.vexans,* which flourish in floodwater and can transmit the virus to their offspring (Ikegami & Makino, 2011). The RVFV transmission cycle involves vertical virus transmission from female mosquitos to their eggs, which can survive for years (CDC, 2020b). Heavy rainfall leads to the hatching of eggs and increases in the mosquito population, leading to virus transmission to animals and humans (CDC, 2020b). Animal outbreaks also increase virus transmission to humans (CDC, 2020b). Livestock, such as cattle, sheep, and goats, are most affected by the virus and can have severe disease outcomes such as abortion, fetal malformation, febrile illness, and acute hepatitis (Ikegami & Makino, 2011). The disease is more severe in younger animals, such as lambs and goats, with mortality rates between 70% and 100%; this is particularly true in pregnant livestock, where abortion rates are nearly 100% when infected (Gray et al., 2012; WOAH, 2022). Sheep and calves also have high mortality rates, between 20% and 70% (WOAH, 2022). These high mortality rates illustrate the importance of RVFV; in areas where the disease is endemic, there is substantial economic loss and increased stress in the community.

RVFV can be transmitted to humans through mosquitos or contact with infected animals' blood, body fluids, or tissues; this contact can occur during slaughter/butchering, veterinary care, or consuming raw or undercooked animal products (CDC, 2020b). There is no evidence of person-to-person transmission; most transmission is zoonotic (CDC, 2020b). In about 50% of people, the disease presents as a mild self-limiting febrile illness, making the condition challenging to

diagnose (Ikegami & Makino, 2011; Kwasnik et al., 2021). However, RVFV can become severe and potentially deadly, with some people developing symptoms such as encephalitis, semi-permanent vision loss, hemorrhagic fever, and thrombosis (Ikegami & Makino, 2011). Overall, the case fatality rate in humans is between 0.5% and 2%, but in some instances, the mortality rate has been up to 28% (Javelle et al., 2020). There is no treatment or vaccine for RVFV since many aspects of the disease, specifically, host factors contributing to cellular infection, remain unknown (Ganaie et al., 2021). Therefore, both the National Institutes of Health (NIH) and the World Health Organization (WHO) consider RVFV a priority pathogen (Bopp et al., 2021).

1.1.1 History

RVFV was first identified in 1934 by veterinarians in a research laboratory in Kenya; however, a similar report of the disease in 1913 was found in sheep in the Rift Valley (Davies, 2010). Kenya continued to have livestock outbreaks and noted the increased disease pattern during heavy rainfall (Davies, 2010). In 1951, humans in South Africa became ill after handling infected animals, and the disease was found in livestock in East and South Africa (Davies, 2010). The largest epidemic was in 1977 in Egypt, where livestock and humans were impacted, with 10,000 to 20,000 human cases and 600 deaths in humans (Davies, 2010; Gray et al., 2012). Afterward, the disease was seen in West Africa with high human mortality, which was early evidence that the disease was spreading (Davies, 2010). The first instance of RVFV outside of Africa occurred in 2000 in Saudi Arabia and has remained in that area periodically (Davies, 2010). There have been substantial outbreaks in Africa in 2006 and from 2009 to the present, proving that RVFV is a problem throughout the continent (CDC, 2020a). There was also an outbreak in Mayotte, France, from 2018-2019, where 142 human cases were confirmed (Youssouf et al., 2020). Mayotte is an

island near RVFV endemic areas with a similar climate and heavy tourism, making it a likely location for the spread of RVFV (Youssouf et al., 2020). Although this outbreak was likely due to exposure to infected animals or bodily fluids, there was also evidence of increased illegal imports of animals from endemic areas, potentially contributing to the outbreak (Youssouf et al., 2020). This shows that RVFV is not going away and is becoming more prevalent and spreading to more countries.

1.1.2 Prevention Methods

RVFV is prevalent in forests and grasslands with high rainfall (Davies, 2010). Although historically, rainfall data was used to warn livestock owners of a risk of an outbreak, currently, various technologies help provide an accurate prediction of when rainfall will be heavy and thus lead to more disease (Consultative Group for, 2010; Davies, 2010). Historical data and soil type identification allow experts to predict where disease-carrying mosquitoes will be found (Davies, 2010). These methods enable emergency preparedness interventions to be implemented about two to three months before infected mosquitoes are seen (Davies, 2010). One of these interventions includes livestock vaccinations using an attenuated live virus vaccine which helps prevent significant economic losses (Consultative Group for, 2010; Davies, 2010). Some issues associated with vaccination are that it can induce abortions in livestock, and it can also be risky if the disease is already being transmitted since needles are sometimes not changed between animals, causing more transmission between animals (Davies, 2010). However, practicing safe needle and vaccination techniques can prevent such spread, but it may not be feasible in low-income areas. An added challenge is the short shelf life of vaccines, which is about four years; due to the infrequency of outbreaks (anywhere from four to twenty years), many veterinary clinics cannot

afford to maintain adequate stock of the vaccine, therefore, when an outbreak does occur, vaccines are not always readily available (Consultative Group for, 2010). This problem is also seen with manufacturers who cannot maintain vaccine supplies but also take several months to produce and distribute the vaccine (Consultative Group for, 2010). Another intervention is mosquito control measures, including larvicides at breeding sites, increased public awareness, and preparation of medical institutions (Davies, 2010). While proper surveillance and response could contain the disease, these resources are unavailable in many areas where the virus is endemic. Additionally, the lack of laboratory testing of suspected cases further complicates reporting in endemic areas where resources are unavailable for routine testing (Grossi-Soyster & LaBeaud, 2020). Furthermore, stigmas and economic loss from livestock trade restrictions in areas with outbreaks may contribute to the lack of reporting (Grossi-Soyster & LaBeaud, 2020). Therefore, unless significant changes in public health infrastructure exist, RVFV will continue to be a problem and potentially spread to new countries.

1.2 RVFV Pathogenesis

Many cells can be infected by RVFV, including neurons, immune cells, and epithelial cells, which allows the virus to attack many areas of the body (Ganaie et al., 2021). During the early stage of infection, the virus is met with innate immune cells, such as macrophages and dendritic cells (DCs). RVFV can replicate with macrophages, and its NSs protein from the S segment of the gene appears to inhibit the secretion of specific antiviral proteins essential in inducing proinflammatory responses (Terasaki & Makino, 2015). Previous studies have shown that the virus can infect immature DCs through C-type lectin DC-SIGN, although this is poorly understood, and

other host factors could be required for viral entry (Ganaie et al., 2021; Leger et al., 2016; Lozach et al., 2011; Phoenix et al., 2016; Terasaki & Makino, 2015). A study by Gommet et al. indicates that macrophages and DCs increase RVFV pathogenicity, as mice deficient in both cell types had prolonged survival and slower viral replication and infection (Gommet et al., 2011; Terasaki & Makino, 2015).

The middle or hepatic phase of infection occurs after the virus enters the bloodstream and replicates within many organs (Terasaki & Makino, 2015). RVFV preferentially targets the liver, where it replicates extensively and subsequently causes damage (Terasaki & Makino, 2015). Infection in the liver results in hepatic necrosis followed by uncontrolled inflammation and cytotoxicity, ultimately leading to severe liver damage (Terasaki & Makino, 2015). Animal research has shown that infected livers are enlarged, congested, and have black discoloration or lesions upon death (Gray et al., 2012; Kwasnik et al., 2021). It has been demonstrated in animals that liver necrosis is a significant cause of mortality, and it is thought to be the same for humans (Gray et al., 2012).

Late-stage infection involves brain infection, specifically neurons, resulting in encephalitis and other neurologic disorders. This stage of the disease typically has a higher mortality rate of about 50% and is more common when the virus is transmitted via aerosol (Madani et al., 2003). While brain infection by RVFV is still poorly understood, some aspects are becoming more known. The adaptive immune response appears necessary to prevent severe late-stage outcomes; this response involves CD4 and CD8 T cells and antibodies (Harmon et al., 2018). During late-stage infection, immune cells in the brain called microglia respond using mitochondrial antiviral signaling (MAVs) protein to secrete cytokines and kill infected host cells (Hum et al., 2022).

Without a proper immune response in the brain, excessive inflammation leads to severe neurological outcomes such as encephalitis.

Depending on various factors, there appear to be three infection patterns in animals and possibly humans. The first pattern is a severe acute infection with a large abundance of virus in the bloodstream resulting in a quick death (Kwasnik et al., 2021). The second pattern is the self-limiting febrile illness that can be mild or asymptomatic with lower amounts of virus in the blood, with increased survival (Kwasnik et al., 2021). Finally, the third pattern involves fever and an abundance of virus in the bloodstream, which then spreads to other organs, such as the liver and the brain; this causes many complications and death (Kwasnik et al., 2021).

1.3 Cellular Infection

RVFV is an enveloped virus containing three RNA segments: large (L), medium (M), and small (S) (**Figure 1**) (Terasaki & Makino, 2015). The L segment encodes the RNA-dependent RNA polymerase, which is essential for RNA replication (Terasaki & Makino, 2015). The M segment encodes the glycoprotein precursor (GPC) and two accessory proteins; GPC is eventually cleaved into Gn and Gc (Ganaie et al., 2021). Gn forms glycoprotein spikes, and Gc is a fusion protein, Gn and Gc are found on the virus surface and aid in virus fusion and entry into the host cells (Ganaie et al., 2021; Leger et al., 2016). Finally, the S segment encodes for nucleocapsid protein N and protein NSs (Ganaie et al., 2021).

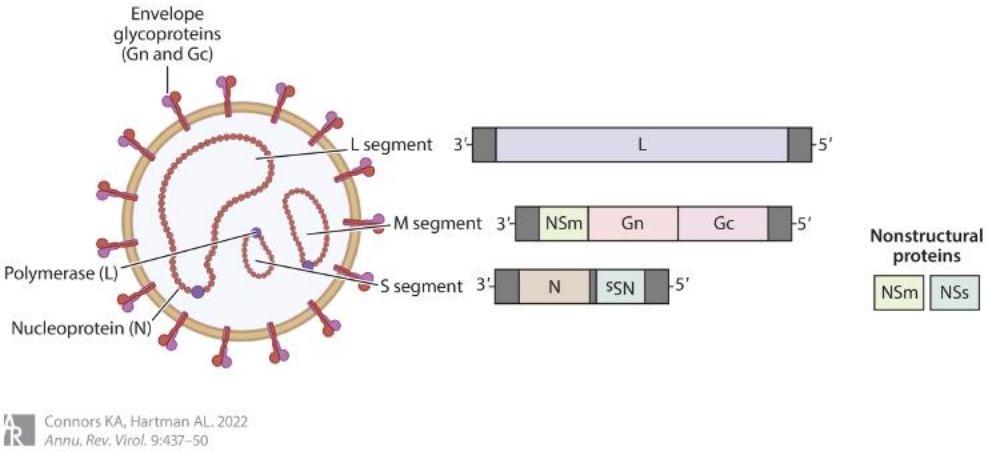

Figure 1. RVFV Genome and Structure (Connors & Hartman, 2022).

The virus enters host cells by first using glycoproteins Gn and Gc to attach to the cell; then, the virus uses receptor-mediated endocytosis for entry. Next, nucleocapsids are released into the host cell cytoplasm using a pH-mediated fusion of viral and endosomal membranes (Hartman, 2017; Kwasnik et al., 2021). Next, the virus replicates its genome within the host cell's cytosol, which helps the virus remain undetected by the cell while utilizing cellular resources; however, it can lead to inflammation (Leger et al., 2016). After replicating, the virus particles are assembled and buds from the Golgi apparatus, where they are released from the cell to infect others (Hartman, 2017).

One mode of cellular attachment is DC-specific intercellular adhesion molecule 3-grabbing nonintegrin (DC-SIGN), a calcium-dependent (C-type) lectin found on the surface of dermal DCs and some macrophages, responsible for antigen capture and presentation (Lozach et al., 2011). DC-SIGN binds to high mannose N-glycans found on RVFV Gn and Gc, leading to cellular attachment (Lozach et al., 2011; Phoenix et al., 2016). Furthermore, RVFV was found to utilize DC-SIGN to infect immature DCs and other cells expressing the lectin, with lectin expression

levels directly related to infection rates (Lozach et al., 2011; Phoenix et al., 2016). However, due to RVFV's broad tropism, DC-SIGN cannot be the only receptor that mediates the virus entry (de Boer et al., 2012). Another potential receptor for cellular attachment is liver/lymph node-specific intercellular adhesion molecule 3-grabbing nonintegrin (L-SIGN), a C-type lectin similar to DC-SIGN, found on the surface of human liver sinusoidal endothelial cells (LSECS) (Leger et al., 2016). L-SIGN captures antigens by binding to high mannose N-glycans on the virus, which can then lead to cellular entry (Leger et al., 2016). It was found that RVFV can utilize L-SIGN to infect host cells; however, while more research is needed, it could explain why the liver is a target for RVFV (Leger et al., 2016). However, it was found that Gn glycosylation, the attachment method used by DC-SIGN and L-SIGN, is not necessary for RVFV entry (Ganaie et al., 2021). Therefore, there must be other factors contributing to viral entry.

Heparan sulfate is a glycosaminoglycan (GAG) abundant on most cell types and has been known to be an attachment factor for many viruses (de Boer et al., 2012). When comparing RVFV infection in cell lines with heparan sulfate and without, infection is significantly reduced in cell lines without heparan sulfate, but it is not eliminated, suggesting another attachment factor is present (de Boer et al., 2012). This was further supported by Riblett et al., who found that cell lines with deficiencies in GAG synthesis were resistant to RVFV infection. They concluded that heparan sulfate enhances infection but is not necessary (Riblett et al., 2016). In addition, Ganaie et al. identified Exostosin-2, a protein involved with heparan sulfate biosynthesis, as a host factor for RVFV but later found that deletion of the gene encoding for the protein did not impact viral infection, further concluding that heparan sulfate is not necessary for infection (Ganaie et al., 2021).

1.4 Low-Density Lipoprotein Receptor-Related Protein 1 (LRP1)

Recently, LRP1 has been identified as a host factor for RVFV infection and is present in many animal species, including those susceptible to RVFV infection. In addition, chaperone proteins RAP and Grp94 were also identified as factors for cellular entry by RVFV (Ganaie et al., 2021). Cells without the chaperone proteins had decreased expression of LRP1 and, subsequentially, less binding by RVFV; similarly, cells lacking LRP1 also had less binding (Ganaie et al., 2021). In addition, it has been shown that glycoprotein Gn on RVFV binds to LRP1 at CL_{IV} and $CL_{II,}$ resulting in cellular infection (**Figure 2**) (Ganaie et al., 2021). LRP1 comprises two chains, a 515-kDa extracellular alpha chain which is non-covalently attached to an 85-kDa intracellular beta chain (Ganaie et al., 2021; Potere et al., 2019). The alpha chain consists of four clusters of complement-like repeats (CL_1-C_{IV}, green in **Figure 2**) that act as ligand binding sites, along with epidermal growth factor repeats (EGF, light green in **Figure 2**) and β-propeller (YWTD, dark green in **Figure 2**) domains (Ganaie et al., 2021; Potere et al., 2019). The beta chain comprises a tetra amino acid YxxL motif, two NPxY motifs, and tyrosine residues (Potere et al., 2019). The NPxY motifs act as docking sites for signaling adaptor proteins. The tyrosine residues are used for phosphorylation; both are important for proper cell signaling, and their dysfunction can lead to disease (Herz & Strickland, 2001; Potere et al., 2019).

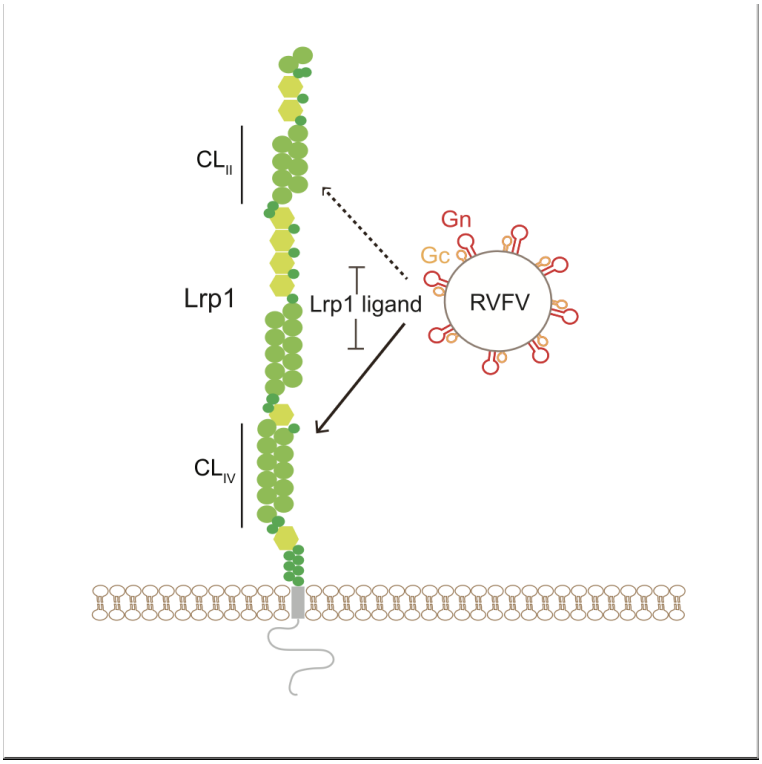

Figure 2. LRP1 Structure (Ganaie et al., 2021).

LRP1 belongs to the low-density lipoprotein receptor (LDLR) family, which is involved in cholesterol regulation (Goldstein & Brown, 2009). Cholesterol is carried by low-density lipoproteins (LDLs) through the bloodstream to other cells (Goldstein & Brown, 2009). They bind to LDLRs and are internalized into the cell through receptor-mediated endocytosis and metabolized (Goldstein & Brown, 2009). LDLRs dissociate from ligands once they encounter lower endosome pH and are recycled back to the cell surface (Goldstein & Brown, 2009). LDLR expression depends on feedback regulation; when the cell has increased cholesterol levels, it reduces the production of LDLRs and vice versa (Goldstein & Brown, 2009). Therefore, defects in LDLRs lead to increased LDL levels which can have many adverse health implications, such as heart attacks (Goldstein & Brown, 2009).

LRP1 plays many roles and is classified as a multifunctional transmembrane receptor (Potere et al., 2019; Schwarz et al., 2022). LRP1 is a large protein and binds to over 100 different ligands of various types (Mantuano et al., 2022). It is most known for lipoprotein endocytosis but also acts as a scavenger, scaffold, and regulatory protein (Potere et al., 2019). Therefore, LRP1 functions in "ligand endocytosis, cell signaling, lipoprotein metabolism, blood-brain barrier maintenance, and angiogenesis" (Schwarz et al., 2022). LRP1 is ubiquitous but, interestingly, is expressed highly in the liver and brain, where severe outcomes of RVFV occur (Potere et al., 2019). LRP1 is involved in cancers, Alzheimer's disease, and cardiovascular diseases; in addition, it can cause harm to the nervous system; therefore, LRP1 is involved in numerous diseases (Gonias & Campana, 2014; Potere et al., 2019).

RAP, a chaperone for LRP1, helps regulate the protein by preventing ligand interactions while the protein is cycled to the cell surface (Bu & Schwartz, 1998; Ganaie et al., 2021). Like LRP1, RAP is ubiquitous, with high expression in the kidney and brain (Bu & Schwartz, 1998). This chaperone primarily resides in the ER and Golgi compartments and can block the binding of all ligands to LRP1 (Bu & Schwartz, 1998). LRP1 has multiple RAP binding sites that overlap with other ligand-binding sites, which RAP competitively binds to; in fact, RAP is the only ligand identified that can bind to CL_I and CL_{III} (Bu & Schwartz, 1998; Herz & Strickland, 2001; Horn et al., 1997). RAP binds to LRP1 early in the secretory pathway, reducing binding activity and thus preventing LRP1 from binding ligands too early in the pathway; once the RAP-LRP1 complexes reach low pH in the medial-Golgi compartments, RAP dissociates, and LRP1 can bind to ligands and continue in the pathway (Bu & Schwartz, 1998). It has also been recorded that RAP interacts with LRP1 in the ER to ensure proper folding of LRP1, which is essential for protein function (Bu

& Rennke, 1996). Therefore, RAP is critical in LRP1 function, and a reduction in RAP expression is correlated with a reduction in RVFV infection (Ganaie et al., 2021).

Proprotein convertase subtilisin kexin type 9 (Pcsk9) is a sterol-regulated gene, meaning that Pcsk9 is activated during low sterol levels and suppressed during high sterol levels, in order to regulate sterol levels, mainly cholesterol (Poirier et al., 2015). It is primarily expressed in the liver, similar to LRP1, and somewhat expressed in the kidney and intestines (Poirier et al., 2015). When bound to LRP1, Pcsk9 directs LRP1 to endocytic compartments for degradation instead of recycling to the cell surface; therefore, Pcsk9 negatively regulates LRP1 expression (Ganaie et al., 2021; Poirier et al., 2015). An ER resident chaperone, Grp94, binds to Pcks9 to prevent its release from the cell, which prevents LRP1 degradation (Ganaie et al., 2021; Poirier et al., 2015). When Grp94 expression is decreased and Pcsk9 expression is increased, LRP1 degradation is increased, leading to less expression (Poirier et al., 2015). Similarly, less Pcsk9 expression leads to increased LRP1 expression and RVFV infection (Ganaie et al., 2021).

RVFV would not be the first pathogen to use LRP1 as a host cell entry factor. For example, human rhinoviruses account for most common colds; a minor-group human rhinovirus was found to utilize the LDL receptor family, including LRP1, as a factor for cellular infection (Herz & Strickland, 2001; Hofer et al., 1994). In addition, the rhinovirus can use LRP1 as a docking receptor for endocytosis and, subsequently, host cell infection (Herz & Strickland, 2001; Hofer et al., 1994). Similarly, it was found that LRP1 can exclusively internalize Pseudomonas exotoxin A, produced by the bacteria *Pseudomonas aeruginosa*, which can have deadly consequences for host cells (Herz & Strickland, 2001; Kounnas et al., 1992). LRP1 also plays a role in HIV activation by binding to the HIV-Tat protein, which then mediates its uptake into host neurons, potentially accelerating severe outcomes relating to HIV infection (Herz & Strickland, 2001; Liu

et al., 2000). Interestingly, it was found that dengue virus reduces LRP1 expression in order to increase cholesterol levels and facilitate virus binding (Tree et al., 2019). Therefore, using LRP1 by pathogens is possible and may not be unique to RVFV. In addition, LDLRs have been found to be associated with the binding and entry of hepatitis C virus and vesicular stomatitis virus, further illustrating the role of this protein family in infectious diseases (Molina et al., 2007; Nikolic et al., 2018).

1.5 Genetics Background

Genetics plays a significant role in determining an individual's susceptibility to infectious diseases. An individual's genetics can influence the immune response to a pathogen and can determine the ability to recognize and eliminate a pathogen (Sorensen et al., 1988). Furthermore, certain genetic variations can either increase or decrease the risk of developing an infection or even the severity of the disease (Chapman & Hill, 2012). Additionally, genetics can influence an individual's response to vaccines and treatments (Kwok et al., 2021). Therefore, understanding the genetic basis of infectious diseases is crucial for developing effective prevention and treatment strategies.

Single nucleotide polymorphisms (SNPs) are one genetic variation that can influence an individual's susceptibility to infectious diseases. SNPs are the most common variation or polymorphism, which involves switching one nucleotide for another (Brookes, 1999; Shastry, 2009). An individual often has many SNPs in their genome since SNPs typically occur one in every 1,000 base pairs (Brookes, 1999; Shastry, 2009). It has been found that "50% of SNPs occur in noncoding regions, 25% lead to missense mutations, and the remaining 25% are silent

mutations" (Shastry, 2009). SNPs can impact a gene by changing gene expression by impacting the promoter region, decreasing gene stability by impacting messenger RNA (mRNA), and impacting translational activity (Shastry, 2009).

LRP1 is a large protein containing over 4,500 amino acids (Shinohara et al., 2017). SNPs in LRP1 that change the amino acid in a coding region (missense mutation) or impact the promoter region could affect LRP1 structure, expression, or stability. We hypothesize that SNPs in LRP1 may change how RVFV can bind to LRP1 or the amount of LRP1 on the cell surface to mediate viral entry. It was found that ligands can bind with equal affinity to CLII and CLIV, leading to the theory that LRP1 has a functional duplication. A functional duplication occurs when a portion of a protein is duplicated within the same sequence, causing identical regions. This can increase binding affinity or be utilized as a backup in case of mutations such as an SNP. Herz et al. performed a deletion analysis which found that ligands interact with multiple ligand-binding repeats, which could span two or more clusters (Herz & Strickland, 2001). LRP1 has 31 ligand-binding-type repeats with various features that each ligand utilizes uniquely for high-affinity binding (Herz & Strickland, 2001).

1.6 Public Health Significance

As discussed in the previous sections, RVFV is a grave concern and a public health priority. One of the major concerns associated with RVFV is the lack of treatment and vaccination for human use. Since many aspects of the disease need to be better understood, it is difficult for potential treatments or vaccines to be developed. Currently, there are vaccines for animal use that effectively prevent and control infection (Faburay et al., 2017). One such vaccine, MP-12, is

commonly used in animals and has proven safe for humans; however, the antibody response has yet to be well defined and has halted its use in humans (Pittman et al., 2016). Providing MP-12 or a similar vaccine may benefit at-risk groups such as veterinarians, butchers, farmers, and slaughterhouse workers (Faburay et al., 2017). Another strategy to reduce the risk of RVFV outbreaks is the routine vaccination of livestock in endemic areas (Faburay et al., 2017). Since the primary mode for transmission to humans is via contact with infected animals, this strategy would significantly reduce the number of RVFV cases in humans and potentially prevent large human outbreaks (Faburay et al., 2017). Currently, it is recommended for farmers to vaccinate their livestock if an outbreak is predicted using various tracking systems; however, there needs to be a policy enforcing this strategy (Davies, 2010; Faburay et al., 2017). With a policy implementing routine vaccinations for livestock, there would be a lower risk of transmission if an outbreak occurs without proper prediction, which may be the case in the future with climate change. However, areas endemic to RVFV often need more resources to enforce a vaccination policy or help pay for the costs of a vaccine (Faburay et al., 2017). Therefore, a potential animal vaccination would need to be low-cost, and public health infrastructure in endemic areas would need to be more robust. With the current state of public health infrastructure in RVFV endemic areas, the virus will likely continue to spread to new places as it is poorly contained. In that case, vaccines for use in humans are highly critical since there is no treatment, and human cases can be quite severe. If an RVFV pandemic occurs, there may be mass panic due to the potential severity of the disease and lack of treatment or vaccine.

RVFV outbreaks become more likely as climate change worsens, especially in new areas. Since RVFV outbreaks seem to be linked to climate due to mosquitoes and heavy rainfall, the spread of the virus as the climate warms is probable. This idea is further supported by RVFV

spread from the Rift Valley to most of Africa and recently to neighboring areas such as Egypt, Madagascar, the Comoros Islands, and the Arabian Peninsula (Linthicum et al., 2016). In addition, the geographical range of RVFV-competent mosquitoes has been expanding, and more continents, including the Americas, are at risk (Linthicum et al., 2016). Similarly, susceptible animal hosts such as livestock are seen in most parts of the world, further facilitating the spread of this virus (Linthicum et al., 2016). International travel and trade also play a role as infected people or animals can be brought to new places and introduce the virus to a naïve population. Therefore, it is of great concern to the global livestock trade since asymptomatic animal cases are relatively common (Linthicum et al., 2016). Also, mosquitoes and their eggs can be transported to new areas and spread from there. Once the virus is introduced to a new location, it can become endemic if RVFV-competent mosquitoes become infected, especially as over 30 different species of mosquitoes can be infected (Rolin et al., 2013).

In endemic areas, RVFV has more implications than disease burden; it also impacts communities' economies, society, and food security. During outbreaks, families that rely on livestock for food and economic benefit suffer the most loss due to herd size significantly decreasing due to the high mortality and abortion storms in livestock (Peyre et al., 2015). Those who rely on livestock for transportation or work struggle to maintain their livelihood (Peyre et al., 2015). Bans on livestock trading due to outbreaks can impact a whole nation’s economy, which harms countless people (Peyre et al., 2015). In addition, less livestock hurts farmers and butchers, who may have to find new income sources (Peyre et al., 2015). Finally, the resulting food insecurity can cause other health problems unrelated to the virus. The full socio-economic impact of RVFV in endemic areas is under-research. It, therefore, is an area of focus in the future, although

many believe the burden is relatively high due to the instability outbreaks cause (Peyre et al., 2015).

RVFV has significant potential to be utilized as a weapon of bioterrorism and introduced to naïve areas in that capacity. Many national authorities have recognized RVFV as a bioterrorism threat to humans and animals (Mandell & Flick, 2010; Rolin et al., 2013). RVFV was developed as a bioterrorism weapon by the United States in the biological weapons program, proving that the disease can be used in this manner (Rolin et al., 2013). The impacts of RVFV as a bioterrorism weapon would be costly, especially since severe outcomes are more likely when the virus is in its aerosolized form, which would have lasting effects on human health (Madani et al., 2003). In the event of a bioterrorist attack, there would be extensive public panic at the possibility of a hemorrhagic fever spreading (Mandell & Flick, 2010). Since there is no vaccine or treatment for this disease, human suffering could be substantial. Losses could also occur due to disease spread in livestock impacting food supply and trade. The loss in human and animal health would negatively impact the economy, causing even more stress. Additionally, once RVFV is introduced, it has a high potential for becoming endemic, causing long-lasting negative impacts.

Polymorphisms in LRP1 may play a role in disease progression or severity. For example, it has been demonstrated that cells lacking LRP1, RAP, or Grp94 had less binding of RVFV and thus had fewer instances of disease (Ganaie et al., 2021). Polymorphisms that change LRP1 expression may significantly influence an individual's susceptibility to RVFV. Furthermore, understanding these polymorphisms and their impact could inform treatment or vaccine development.

2.0 Methods

The literature search was conducted using keywords such as “LRP1”, “LDL-receptor,” “polymorphisms,” “SNPs,” “mutations,” “cardiovascular disease,” and “human cholesterol diseases.” Literature was found through PubMed, the National Library of Medicine, Wiley Online Library, and ScienceDirect. Literature had to be peer-reviewed to be included and published after 2000. 13 publications met these criteria, and of these, nine publications were included in this paper. We chose polymorphisms reported in the literature that likely impacted LRP1 structure and expression; as a result, most of the polymorphisms included occur in exons except for one polymorphism in the promoter region.

We also identified exons corresponding to the cluster regions of the protein to identify polymorphisms that alter the amino acid sequence in the cluster regions that could impact RVFV binding (Lee et al., 2017). After identifying the cluster exon locations, we utilized the University of California-Santa Cruz (UCSC) Genomics Institute Genome Browser to identify the common SNPs with an allele frequency greater than 1% in the exons (Kent WJ, 2002). We used the standard definition of common SNPs to identify the polymorphisms that may result in a selective advantage or disadvantage at the population level.

3.0 Results

A literature search was performed to identify polymorphisms in LRP1 that have a known impact on disease, focusing on cholesterol-related diseases. Once a SNP is identified, it is assigned a reference SNP (rs) number used to refer to that specific SNP. However, often SNP common names are used in literature, which usually include the location of the SNP and the resulting amino acid change. Since SNP databases do not typically include common names, it can be challenging to find a SNP in the literature in the database.

One notable polymorphism was first discovered by Schulz et al., referred to as c.1-25 C>G (rs138854007), and found in the promoter region of the gene (Schulz et al., 2002). The polymorphism leads to a new GC-box, a conserved sequence of nucleotides recognized by Sp1 and Sp3 proteins that regulate gene expression (Schulz et al., 2002). It was found that those with the polymorphism had higher mRNA expression levels, which could lead to higher LRP1 protein levels (Aledo et al., 2014; Schulz et al., 2002). Therefore, it is believed that this polymorphism impacts LRP1 transcription. Furthermore, this polymorphism has been indicated to cause an increase in coronary atherosclerosis (Aledo et al., 2014; Schulz et al., 2002). Concerning RVFV, the polymorphism leads to higher LRP1 expression, potentially increasing RVFV binding and host entry. This would make the host more susceptible to RVFV infection and potentially more severe disease outcomes.

Another important polymorphism, 677 C>T (rs1799986), is a silent polymorphism, meaning that it does not change the amino acid sequence of the gene (Jusic, 2018). However, this polymorphism impacts the splicing efficiency of exon three, which can impact the mRNA length, potentially impacting LRP1 function (Jusic, 2018). If LRP1 is not functioning properly, it may not

bind and endocytose ligands properly, potentially resulting in decreased RVFV cellular infection. This polymorphism is relatively common in the human population, showing up in about 22% of the population (Vučinić et al., 2017). It has been associated with numerous disease states, including metabolic syndrome, cardiovascular disease, and Alzheimer's disease (Aledo et al., 2012; Jusic, 2018; Vučinić et al., 2017). It should be noted that this polymorphism has been referred to as both 677 C>T in most cases and occasionally 667 C>T but appears to be the same polymorphism in all cases.

Polymorphism 663 C>T (rs1800127) impacts exon six and is associated with an increased risk of coronary heart disease and a potentially higher risk of recurrent venous thromboembolism (Pocathikorn et al., 2003; Vormittag et al., 2007). While the full impact of the polymorphism on the LRP1 gene has not been described, it most likely impacts ligand binding. The paper by Vormittag et al. describes how LRP1 is responsible for the uptake and degradation of clotting factor (F) VIII (Vormittag et al., 2007). When LRP1 is not functioning correctly or not expressed, FVIII levels increase, leading to a higher risk of venous thromboembolism (Vormittag et al., 2007). This polymorphism leads to an increase in FVIII levels, meaning that LRP1 is not effectively taking in and degrading FVIII, potentially due to an inability of FVIII to bind to LRP1 (Vormittag et al., 2007). This is important because the polymorphism prevents the binding of a ligand to LRP1; in the context of RVFV, this could lead to a decrease in cellular infection of the virus due to an inability of ligand binding.

Another polymorphism that impacts FVIII levels is rs1800137, located in exon 8, which causes a frameshift in exon nine and thus leads to premature termination codons, which often cause mRNA to be degraded or become unstable (Lee et al., 2017). Those without the polymorphism have higher mRNA expression levels than those with the polymorphism (Lee et al., 2017). The

polymorphism is found in about 8% of the Chinese population; due to the lack of studies in other populations, it is unclear how common this polymorphism is in the general population (Lee et al., 2017). Those with the polymorphism may have a decreased risk of cellular infection by RVFV due to the lower mRNA expression levels leading to a decrease in LRP1 on cell surfaces.

D2080N (rs34577247) is another polymorphism that impacts FVIII levels; located in exon 39, it changes the amino acid sequence (Morange et al., 2005). This change in the amino acid sequence could impact LRP1 structure and, consequently, ligand binding (Morange et al., 2005). Regarding FVIII levels, the D2080N polymorphism decreases FVIII levels, indicating that the polymorphism leads to increased LRP1 binding (Morange et al., 2005). Therefore, this polymorphism may increase cellular infection by RVFV due to increased ligand binding through an unknown mechanism.

Additionally, we identified polymorphisms in the exons corresponding to the cluster regions of the protein, seen in **Table 1** below. The polymorphisms were identified using the UCSC Genome Browser; we listed the common polymorphisms with an allele frequency greater than 1% in the population (Kent WJ, 2002). By this definition, 35 exonic polymorphisms in the whole LRP1 gene are considered common (Kent WJ, 2002). While these polymorphisms were identified in the Genome Browser, little is known about how they impact disease states or the protein.

Table 1. Known Common SNPS in LRP1 Cluster Regions.

Location	Known Common SNPs	Description
Cluster 1 (exon 2)	None	N/A
Cluster 2 (exons 18-25)	exon 22: rs79365493 [C/T] exon 22: rs1800194 [C/A/T] exon 23: rs12814239 [C/A/T]	synonymous change [Asn->Asn], likely benign nonsense mutation nonsense mutation
Cluster 3 (exons 52-61)	exon 54: rs1800154 [C/T] exon 54: rs7397167 [A/C/T] exon 56: rs1800156 [C/T] exon 56: rs7308698 [T/A/C/G] exon 61: rs1140648 [G/A/C],	synonymous change [Cys->Cys], likely benign missense variants [Gln->Pro and Gln->Leu] synonymous change [Asp->Asp], likely benign synonymous change [Thr->Thr], likely benign synonymous change [Thr->Thr], likely benign
Cluster 4 (exons 71-81)	None	N/A

Of these identified common polymorphisms in the cluster regions, three are particularly interesting: rs1800194, rs12814239, and rs7397167. The two nonsense mutations in CL_{II}, rs1800194 and rs12814239, result in premature stop codons that stop translation prematurely, often resulting in a non-functional protein. This could lead to a decrease in RVFV infection due to the decrease in functional LRP1 proteins. The polymorphisms are also notable since it is known that RVFV binds to $CL_{II.}$ Therefore, these polymorphisms could have a direct impact on RVFV binding. Finally, the polymorphism, rs7397167, in CL_{III} is notable because it could alter the 3-D structure of the protein through its amino acid substitution to proline (Krieger et al., 2005). This change in LRP1 structure could impact RVFV binding, either positively or negatively, regardless of where in the gene the polymorphism is.

Table 2. Summary of Notable Polymorphisms in LRP1.

Polymorphism	Impact	Disease Association
rs138854007	LRP1 expression	Coronary atherosclerosis
rs1800137	LRP1 expression	FVIII levels
rs1799986	LRP1 function	Numerous disease states, including metabolic syndrome, cardiovascular disease, and Alzheimer's disease.
rs1800194	LRP1 function	Unknown
rs12814239	LRP1 function	Unknown
rs1800127	LRP1 structure	FVIII levels
rs34577247	LRP1 structure	FVIII levels
rs7397167	LRP1 structure	Unknown

4.0 Discussion

LRP1 is a recently identified receptor for RVFV cellular infection and is a rather sizeable multifunctional protein (Ganaie et al., 2021). The mechanisms for RVFV cellular infection are still poorly understood, although it is known that RVFV binds to cluster regions II and IV (Ganaie et al., 2021). Identifying polymorphisms in LRP1 may give insight into how host cells are infected and individual susceptibility. There are many polymorphisms in LRP1; however, we have identified eight that potentially impact RVFV infection. These eight polymorphisms are as follows: c.1-25 C>G (rs138854007), 677 C>T (rs1799986), 663 C>T (rs1800127), rs1800137, D2080N (rs34577247), rs1800194, rs12814239, and rs7397167. The polymorphisms were identified through a literature search and the UCSC Genome Browser (Kent WJ, 2002). The polymorphisms could impact LRP1 expression, functionality, or structure, significantly affecting RVFV binding.

The amount of LRP1 expressed on the surface of host cells directly affects RVFV binding; if there is less LRP1 to bind to, there is less opportunity for cellular infection and vice versa. Two polymorphisms were identified that likely impact LRP1 expression: rs138854007 and rs1800137. The first polymorphism, rs138854007, occurs in the gene's promoter region and directly impacts transcription, leading to a higher expression of LRP1 (Aledo et al., 2014; Schulz et al., 2002). The polymorphism has been associated with coronary atherosclerosis (Aledo et al., 2014; Schulz et al., 2002). We hypothesize that this polymorphism would cause an increase in host cell infection by RVFV due to the increased expression of LRP1. Conversely, the polymorphism rs1800137 causes a decrease in LRP1 expression and is associated with increased FVIII levels (Lee et al., 2017). This is due to the polymorphism causing a frameshift mutation in exon nine, leading to premature termination codons, causing mRNA to be degraded or become unstable (Lee et al., 2017). We

hypothesize that this polymorphism would cause a decrease in host cell infection by RVFV due to the decreased expression of LRP1.

LRP1 function is essential for cellular uptake and processing, which RVFV utilizes for cellular infection. If the gene is not functioning properly, RVFV infection will be directly impacted, either positively or negatively. Three polymorphisms potentially affecting LRP1 function were identified: rs1799986, rs1800194, and rs12814239. The first polymorphism, rs1799986, is a silent polymorphism impacting the splicing efficiency of exon three (Jusic, 2018). This affects mRNA length and can affect LRP1 function (Jusic, 2018). This common polymorphism impacts about 22% of the population and is associated with numerous diseases (Aledo et al., 2012; Jusic, 2018; Vučinić et al., 2017). Little is known about the other polymorphisms, rs1800194 and rs12814239, identified in $CL_{II,}$ resulting in nonsense mutations. The polymorphisms cause premature stop codons, which often generate a non-function protein. We hypothesize that the three polymorphisms would decrease RVFV cellular infection due to decreased LRP1 functionality.

LRP1 structure determines if ligands can bind to the protein and the affinity to which they can bind. Depending on the structure, RVFV may be able to bind easily and with more affinity or vice versa. We found three polymorphisms that could impact the LRP1 structure: rs1800127, rs34577247, and rs7397167. While the first polymorphism, rs1800127, is not entirely understood, it is known to impact exon six and cause an increase in FVIII levels (Vormittag et al., 2007). Since LRP1 is essential in the clearance of FVIII, it is believed that the polymorphism changes the structure of LRP1 to prevent ligand binding (Vormittag et al., 2007). Therefore, we hypothesize that this polymorphism would decrease RVFV cellular infection due to the change in LRP1 structure decreasing ligand binding. Conversely, the polymorphism rs34577247, located in exon

39, changes the amino acid sequence and impacts the LRP1 structure, and results in a decrease in FVIII levels (Morange et al., 2005). Therefore, we hypothesize that this polymorphism would increase RVFV cellular infection due to increased ligand binding. Interestingly, both previously mentioned polymorphisms (rs1800127 and rs34577247) likely affect FVIII ligand binding, which binds to the same cluster regions as RVFV, suggesting that RVFV binding may be impacted similarly. While the exact mechanism of FVIII and LRP1 binding is still unknown, it has been found that a combination of binding techniques is utilized during the interaction, including bivalent binding and non-canonical binding (Chun et al., 2022). One primary binding technique of interest is bivalent binding, in which two binding sites on the receptor bind to two parts of the ligand to increase the affinity (Chun et al., 2022). With this binding technique, it has been found that tryptophan residues in certain regions of LRP1 are critical in interactions with RAP and FVIII; as such, substituting this residue results in decreased binding for both ligands (Chun et al., 2022). Therefore, polymorphisms in LRP1 that result in an amino acid change from tryptophan could impact ligand binding, as seen with RAP and FVIII, and could be of interest.

Finally, the polymorphism, rs7397167, could alter LRP1 structure due to its amino acid substitution to proline. It is unknown if this would positively or negatively impact RVFV binding. However, we utilized a platform, AlphaFold, to predict the 3D structure of LRP1 with the amino acid substitution to proline. AlphaFold is an AI-based platform that uses a protein's amino acid sequence to predict 3D structure; while it is the most accurate platform available, its reliability is still unknown (Jumper et al., 2021; Pak et al., 2023). Due to the size of LRP1, we had to input a smaller protein region to visualize. Since the polymorphism of interest occurs in CL_{III} (exons 52-61), we selected exons 50-65 to visualize; therefore, we do not know how the rest of LRP1 responds to the polymorphism. **Figures 3 and 4**, seen below, show the difference between the wild

type (non-mutated) and mutated 3D predicted structures. While the exact impacts of this polymorphism are still unknown, there is a clear difference between the structures. Even though RVFV does not bind to CL_{III} of LRP1, this drastic change in structure could impact ligand binding.

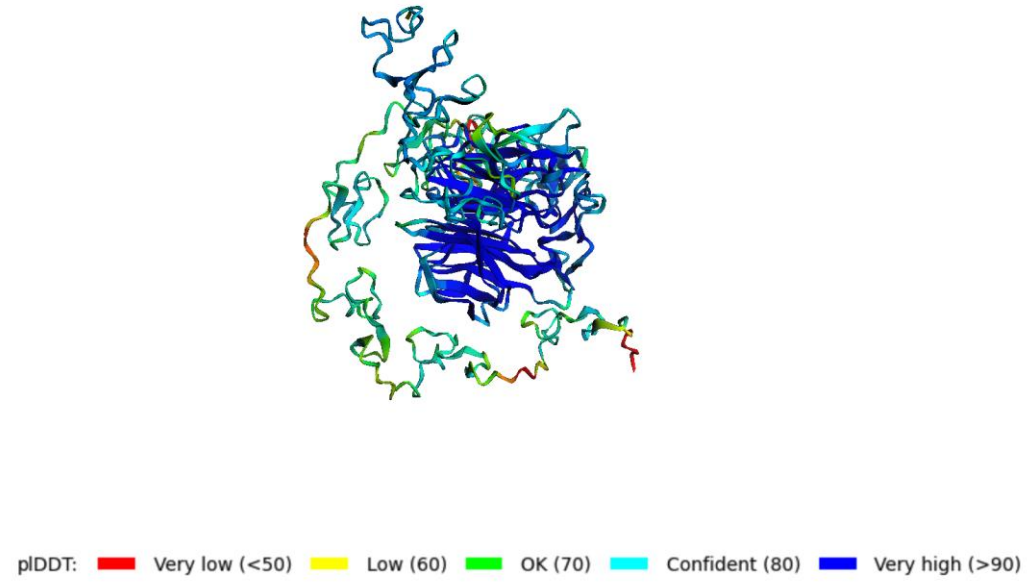

Figure 3. AlphaFold LRP1 Wild Type Structure (exons 50-65).

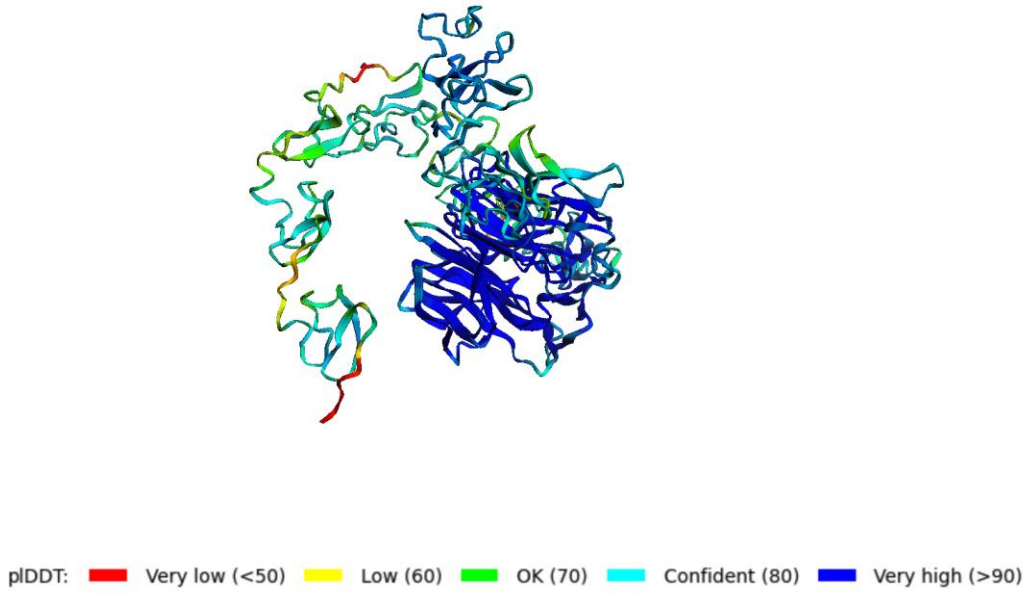

Figure 4. AlphaFold LRP1 Proline Substitution Structure (exons 50-65).

4.1 Limitations

There are several limitations to the paper. First, we focused on polymorphisms that likely impact LRP1 structure and expression, preferring polymorphisms that alter the amino acid sequence. As a result, polymorphisms occurring in introns were not included. However, SNPs occurring in introns can also impact gene expression and could be an essential consideration. This paper serves as a starting point for identifying significant polymorphisms in LRP1 and RVFV; many potentially important polymorphisms were not included.

Also, it is believed that LRP1 has functional duplications due to its ligands' ability to bind to multiple clusters with equal affinity (Herz & Strickland, 2001). Ligands interact with multiple ligand-binding repeats on LRP1 (Herz & Strickland, 2001). Therefore, it is possible that a

polymorphism that impacts one cluster of the protein would not change overall RVFV binding since the virus could bind to another region. It is unknown if functional duplications with the protein change the impact of polymorphisms, but it is possible.

Additionally, while many polymorphisms have been identified in LRP1, little is known about them. Most literature identifies an association of a polymorphism with clinical disease outcomes, but more needs to be done to study the underlying reasons mechanistically. Therefore, it is difficult to understand other ways a polymorphism can impact the protein. More research is needed to identify and characterize human polymorphisms in LRP1 and determine their potential effects on the protein.

4.2 Future Directions

This paper highlights notable polymorphisms in the LRP1 gene that could impact RVFV infection. Targeted research on these polymorphisms should be conducted to identify their specific impact on RVFV infection. A potential first step could be to model the LRP1 protein 3D structure using prediction software such as AlphaFold to identify how specific polymorphisms could change the structure of LRP1. Afterward, a promising approach would be to employ site-directed mutagenesis techniques to identify the effects of each polymorphism on LRP1 and RVFV binding. Additionally, dual-luciferase assay offers a reliable means of measuring gene expression levels associated with these polymorphisms and their potential impact on protein expression. In-depth research into these polymorphisms could offer valuable insights into the mechanisms underlying RVFV cellular entry and infection, eventually leading to novel vaccines and treatments.

Due to the scope of this paper, polymorphisms in introns were not addressed but could still significantly impact LRP1 and RVFV binding. Therefore, research similar to this paper should be conducted to identify key polymorphisms occurring in introns and their potential impact. Afterward, similar approaches using AlphaFold, site-directed mutagenesis, and dual-luciferase assays could be employed to learn more about the polymorphisms.

Further research should also be conducted on polymorphisms in RAP and GRP94, proteins associated with LRP1 that are important in its expression and function (Ganaie et al., 2021). Polymorphisms in these proteins could impact LRP1 severely, resulting in a change in RVFV cellular infection. Therefore, it is crucial to understand how polymorphisms in related proteins play a role in overall infection by RVFV.

5.0 Conclusion

Several notable polymorphisms within the LRP1 gene may impact RVFV infection. These include: rs138854007, rs1799986, rs1800127, rs1800137, rs34577247, rs1800194, rs12814239, and rs7397167. The effect of these polymorphisms could alter gene expression, function, or structure, which has significant implications for RVFV cellular infection. Further research is required to determine the exact extent to which each polymorphism impacts RVFV infection. By understanding the impact of these polymorphisms on disease susceptibility, this research could shed light on mechanisms underlying RVFV infection, potentially paving the way for the development of treatments and vaccines.

Bibliography

A Aledo, R., Alonso, R., Mata, P., Llorente-Cortes, V., Padro, T., & Badimon, L. (2012). LRP1 gene polymorphisms are associated with premature risk of cardiovascular disease in patients with familial hypercholesterolemia. *Rev Esp Cardiol (Engl Ed), 65*(9), 807-812. https://doi.org/10.1016/j.recesp.2012.03.013

Aledo, R., Costales, P., Ciudad, C., Noe, V., Llorente-Cortes, V., & Badimon, L. (2014). Molecular and functional characterization of LRP1 promoter polymorphism c.1-25 C>G (rs138854007). *Atherosclerosis*, *233*(1), 178-185. https://doi.org/10.1016/j.atherosclerosis.2013.12.014

Bopp, N. E., Fernandez, D., & Aguilar, P. V. (2021). Closing the Rift: Discovery of a novel virus receptor. *Cell*, *184*(20), 5084-5086. https://doi.org/10.1016/j.cell.2021.09.004

Borkenhagen, L. K., Salman, M. D., Ma, M. J., & Gray, G. C. (2019). Animal influenza virus infections in humans: A commentary. *Int J Infect Dis*, *88*, 113-119. https://doi.org/10.1016/j.ijid.2019.08.002

Boshra, H. (2022). An Overview of the Infectious Cycle of Bunyaviruses. *Viruses*, *14*(10). https://doi.org/10.3390/v14102139

Brookes, A. J. (1999). The essence of SNPs. *Gene*, *234*(2), 177-186. https://doi.org/10.1016/s0378-1119(99)00219-x

Bu, G., & Rennke, S. (1996). Receptor-associated protein is a folding chaperone for low density lipoprotein receptor-related protein. *J Biol Chem*, *271*(36), 22218-22224. https://doi.org/10.1074/jbc.271.36.22218

Bu, G., & Schwartz, A. L. (1998). RAP, a novel type of ER chaperone. *Trends Cell Biol*, *8*(7), 272-276. https://doi.org/10.1016/s0962-8924(98)01283-5

CDC. (2020a). *Outbreak Summaries*. Centers for Disease Control and Prevention. Retrieved February 21 from https://www.cdc.gov/vhf/rvf/outbreaks/summaries.html

CDC. (2020b). *Transmission*. Centers for Disease Control and Prevention. Retrieved February 21 from https://www.cdc.gov/vhf/rvf/transmission/index.html

Chapman, S. J., & Hill, A. V. (2012). Human genetic susceptibility to infectious disease. *Nat Rev Genet*, *13*(3), 175-188. https://doi.org/10.1038/nrg3114

Chun, H., Kurasawa, J. H., Olivares, P., Marakasova, E. S., Shestopal, S. A., Hassink, G. U., Karnaukhova, E., Migliorini, M., Obi, J. O., Smith, A. K., Wintrode, P. L., Durai, P., Park, K., Deredge, D., Strickland, D. K., & Sarafanov, A. G. (2022). Characterization of

interaction between blood coagulation factor VIII and LRP1 suggests dynamic binding by alternating complex contacts. *J Thromb Haemost*, *20*(10), 2255-2269. https://doi.org/10.1111/jth.15817

Connors, K. A., & Hartman, A. L. (2022). Advances in Understanding Neuropathogenesis of Rift Valley Fever Virus. *Annu Rev Virol*, *9*(1), 437-450. https://doi.org/10.1146/annurev-virology-091919-065806

Consultative Group for, R. V. F. D. S. (2010). Decision-support tool for prevention and control of Rift Valley fever epizootics in the Greater Horn of Africa. *Am J Trop Med Hyg*, *83*(2 Suppl), 75-85. https://doi.org/10.4269/ajtmh.2010.83s2a03

Davies, F. G. (2010). The historical and recent impact of Rift Valley fever in Africa. *Am J Trop Med Hyg*, *83*(2 Suppl), 73-74. https://doi.org/10.4269/ajtmh.2010.83s2a02

de Boer, S. M., Kortekaas, J., de Haan, C. A., Rottier, P. J., Moormann, R. J., & Bosch, B. J. (2012). Heparan sulfate facilitates Rift Valley fever virus entry into the cell. *J Virol*, *86*(24), 13767-13771. https://doi.org/10.1128/jvi.01364-12

Elliott, R. M. (2009). Bunyaviruses and climate change. *Clin Microbiol Infect*, *15*(6), 510-517. https://doi.org/10.1111/j.1469-0691.2009.02849.x

Faburay, B., LaBeaud, A. D., McVey, D. S., Wilson, W. C., & Richt, J. A. (2017). Current Status of Rift Valley Fever Vaccine Development. *Vaccines (Basel)*, *5*(3). https://doi.org/10.3390/vaccines5030029

Fisher, C. R., Streicker, D. G., & Schnell, M. J. (2018). The spread and evolution of rabies virus: conquering new frontiers. *Nat Rev Microbiol*, *16*(4), 241-255. https://doi.org/10.1038/nrmicro.2018.11

Ganaie, S. S., Schwarz, M. M., McMillen, C. M., Price, D. A., Feng, A. X., Albe, J. R., Wang, W., Miersch, S., Orvedahl, A., Cole, A. R., Sentmanat, M. F., Mishra, N., Boyles, D. A., Koenig, Z. T., Kujawa, M. R., Demers, M. A., Hoehl, R. M., Moyle, A. B., Wagner, N. D., . . . Hartman, A. L. (2021). Lrp1 is a host entry factor for Rift Valley fever virus. *Cell*, *184*(20), 5163-5178 e5124. https://doi.org/10.1016/j.cell.2021.09.001

Goldstein, J. L., & Brown, M. S. (2009). The LDL receptor. *Arterioscler Thromb Vasc Biol*, *29*(4), 431-438. https://doi.org/10.1161/ATVBAHA.108.179564

Gommet, C., Billecocq, A., Jouvion, G., Hasan, M., Zaverucha do Valle, T., Guillemot, L., Blanchet, C., van Rooijen, N., Montagutelli, X., Bouloy, M., & Panthier, J. J. (2011). Tissue tropism and target cells of NSs-deleted rift valley fever virus in live immunodeficient mice. *PLoS Negl Trop Dis*, *5*(12), e1421. https://doi.org/10.1371/journal.pntd.0001421

Gonias, S. L., & Campana, W. M. (2014). LDL receptor-related protein-1: a regulator of inflammation in atherosclerosis, cancer, and injury to the nervous system. *Am J Pathol*, *184*(1), 18-27. https://doi.org/10.1016/j.ajpath.2013.08.029

Gray, K. K., Worthy, M. N., Juelich, T. L., Agar, S. L., Poussard, A., Ragland, D., Freiberg, A. N., & Holbrook, M. R. (2012). Chemotactic and inflammatory responses in the liver and brain are associated with pathogenesis of Rift Valley fever virus infection in the mouse. *PLoS Negl Trop Dis*, *6*(2), e1529. https://doi.org/10.1371/journal.pntd.0001529

Grossi-Soyster, E. N., & LaBeaud, A. D. (2020). Rift Valley Fever: Important Considerations for Risk Mitigation and Future Outbreaks. *Trop Med Infect Dis*, *5*(2). https://doi.org/10.3390/tropicalmed5020089

Halushka, M. K., Fan, J. B., Bentley, K., Hsie, L., Shen, N., Weder, A., Cooper, R., Lipshutz, R., & Chakravarti, A. (1999). Patterns of single-nucleotide polymorphisms in candidate genes for blood-pressure homeostasis. *Nat Genet*, *22*(3), 239-247. https://doi.org/10.1038/10297

Harmon, J. R., Spengler, J. R., Coleman-McCray, J. D., Nichol, S. T., Spiropoulou, C. F., & McElroy, A. K. (2018). CD4 T Cells, CD8 T Cells, and Monocytes Coordinate To Prevent Rift Valley Fever Virus Encephalitis. *J Virol*, *92*(24). https://doi.org/10.1128/JVI.01270-18

Hartman, A. (2017). Rift Valley Fever. *Clin Lab Med*, *37*(2), 285-301. https://doi.org/10.1016/j.cll.2017.01.004

Herz, J., & Strickland, D. K. (2001). LRP: a multifunctional scavenger and signaling receptor. *J Clin Invest*, *108*(6), 779-784. https://doi.org/10.1172/JCI13992

Hofer, F., Gruenberger, M., Kowalski, H., Machat, H., Huettinger, M., Kuechler, E., & Blaas, D. (1994). Members of the low density lipoprotein receptor family mediate cell entry of a minor-group common cold virus. *Proc Natl Acad Sci U S A*, *91*(5), 1839-1842. https://doi.org/10.1073/pnas.91.5.1839

Horn, I. R., van den Berg, B. M., van der Meijden, P. Z., Pannekoek, H., & van Zonneveld, A. J. (1997). Molecular analysis of ligand binding to the second cluster of complement-type repeats of the low density lipoprotein receptor-related protein. Evidence for an allosteric component in receptor-associated protein-mediated inhibition of ligand binding. *J Biol Chem*, *272*(21), 13608-13613. https://doi.org/10.1074/jbc.272.21.13608

Hum, N. R., Bourguet, F. A., Sebastian, A., Lam, D., Phillips, A. M., Sanchez, K. R., Rasley, A., Loots, G. G., & Weilhammer, D. R. (2022). MAVS mediates a protective immune response in the brain to Rift Valley fever virus. *PLoS Pathog*, *18*(5), e1010231. https://doi.org/10.1371/journal.ppat.1010231

Ikegami, T., & Makino, S. (2011). The pathogenesis of Rift Valley fever. *Viruses*, *3*(5), 493-519. https://doi.org/10.3390/v3050493

Javelle, E., Lesueur, A., Pommier de Santi, V., de Laval, F., Lefebvre, T., Holweck, G., Durand, G. A., Leparc-Goffart, I., Texier, G., & Simon, F. (2020). The challenging management of Rift Valley Fever in humans: literature review of the clinical disease and algorithm proposal. *Ann Clin Microbiol Antimicrob*, *19*(1), 4. https://doi.org/10.1186/s12941-020-0346-5

Jumper, J., Evans, R., Pritzel, A., Green, T., Figurnov, M., Ronneberger, O., Tunyasuvunakool, K., Bates, R., Zidek, A., Potapenko, A., Bridgland, A., Meyer, C., Kohl, S. A. A., Ballard, A. J., Cowie, A., Romera-Paredes, B., Nikolov, S., Jain, R., Adler, J., . . . Hassabis, D. (2021). Highly accurate protein structure prediction with AlphaFold. *Nature*, *596*(7873), 583-589. https://doi.org/10.1038/s41586-021-03819-2

Jusic, A. A., Aldijana; Hadžiavdic, V.; Junuzović, I.; Kurtcehajic, Dzenita. (2018). Identification of 677 C > T Polymorphism of LRP1 gene by PCR-RFLP method. *TEM Journal*, *7*, 803-807.

Kent WJ, S. C., Furey TS, Roskin KM, Pringle TH, Zahler AM, Haussler D. (2002). *The human genome browser at UCSC.*

Kounnas, M. Z., Morris, R. E., Thompson, M. R., FitzGerald, D. J., Strickland, D. K., & Saelinger, C. B. (1992). The alpha 2-macroglobulin receptor/low density lipoprotein receptor-related protein binds and internalizes Pseudomonas exotoxin A. *J Biol Chem*, *267*(18), 12420-12423.

Krieger, F., Moglich, A., & Kiefhaber, T. (2005). Effect of proline and glycine residues on dynamics and barriers of loop formation in polypeptide chains. *J Am Chem Soc*, *127*(10), 3346-3352. https://doi.org/10.1021/ja042798i

Kwasnik, M., Rozek, W., & Rola, J. (2021). Rift Valley Fever - a Growing Threat To Humans and Animals. *J Vet Res*, *65*(1), 7-14. https://doi.org/10.2478/jvetres-2021-0009

Kwok, A. J., Mentzer, A., & Knight, J. C. (2021). Host genetics and infectious disease: new tools, insights and translational opportunities. *Nat Rev Genet*, *22*(3), 137-153. https://doi.org/10.1038/s41576-020-00297-6

Lee, J. D., Hsiao, K. M., Chang, P. J., Chen, C. C., Kuo, Y. W., Huang, Y. C., Hsu, H. L., Lin, Y. H., Wu, C. Y., Huang, Y. C., Lee, M., Hsu, C. Y., Pan, Y. T., Kuo, C. Y., & Lin, C. H. (2017). A common polymorphism decreases LRP1 mRNA stability and is associated with increased plasma factor VIII levels. *Biochim Biophys Acta Mol Basis Dis*, *1863*(6), 1690-1698. https://doi.org/10.1016/j.bbadis.2017.04.015

Leger, P., Tetard, M., Youness, B., Cordes, N., Rouxel, R. N., Flamand, M., & Lozach, P. Y. (2016). Differential Use of the C-Type Lectins L-SIGN and DC-SIGN for Phlebovirus Endocytosis. *Traffic*, *17*(6), 639-656. https://doi.org/10.1111/tra.12393

Linthicum, K. J., Britch, S. C., & Anyamba, A. (2016). Rift Valley Fever: An Emerging Mosquito-Borne Disease. *Annu Rev Entomol*, *61*, 395-415. https://doi.org/10.1146/annurev-ento-010715-023819

Liu, Y., Jones, M., Hingtgen, C. M., Bu, G., Laribee, N., Tanzi, R. E., Moir, R. D., Nath, A., & He, J. J. (2000). Uptake of HIV-1 tat protein mediated by low-density lipoprotein receptor-related protein disrupts the neuronal metabolic balance of the receptor ligands. *Nat Med*, *6*(12), 1380-1387. https://doi.org/10.1038/82199

Lozach, P. Y., Kuhbacher, A., Meier, R., Mancini, R., Bitto, D., Bouloy, M., & Helenius, A. (2011). DC-SIGN as a receptor for phleboviruses. *Cell Host Microbe*, *10*(1), 75-88. https://doi.org/10.1016/j.chom.2011.06.007

Madani, T. A., Al-Mazrou, Y. Y., Al-Jeffri, M. H., Mishkhas, A. A., Al-Rabeah, A. M., Turkistani, A. M., Al-Sayed, M. O., Abodahish, A. A., Khan, A. S., Ksiazek, T. G., & Shobokshi, O. (2003). Rift Valley fever epidemic in Saudi Arabia: epidemiological, clinical, and laboratory characteristics. *Clin Infect Dis*, *37*(8), 1084-1092. https://doi.org/10.1086/378747

Mandell, R. B., & Flick, R. (2010). Rift Valley fever virus: an unrecognized emerging threat? *Hum Vaccin*, *6*(7), 597-601. https://doi.org/10.4161/hv.6.7.11761

Mantuano, E., Azmoon, P., Banki, M. A., Gunner, C. B., & Gonias, S. L. (2022). The LRP1/CD91 ligands, tissue-type plasminogen activator, α(2)-macroglobulin, and soluble cellular prion protein have distinct co-receptor requirements for activation of cell-signaling. *Sci Rep*, *12*(1), 17594. https://doi.org/10.1038/s41598-022-22498-1

Molina, S., Castet, V., Fournier-Wirth, C., Pichard-Garcia, L., Avner, R., Harats, D., Roitelman, J., Barbaras, R., Graber, P., Ghersa, P., Smolarsky, M., Funaro, A., Malavasi, F., Larrey, D., Coste, J., Fabre, J. M., Sa-Cunha, A., & Maurel, P. (2007). The low-density lipoprotein receptor plays a role in the infection of primary human hepatocytes by hepatitis C virus. *J Hepatol*, *46*(3), 411-419. https://doi.org/10.1016/j.jhep.2006.09.024

Morange, P. E., Tregouet, D. A., Frere, C., Saut, N., Pellegrina, L., Alessi, M. C., Visvikis, S., Tiret, L., & Juhan-Vague, I. (2005). Biological and genetic factors influencing plasma factor VIII levels in a healthy family population: results from the Stanislas cohort. *Br J Haematol*, *128*(1), 91-99. https://doi.org/10.1111/j.1365-2141.2004.05275.x

Munoz-Fontela, C., & McElroy, A. K. (2017). Ebola Virus Disease in Humans: Pathophysiology and Immunity. *Curr Top Microbiol Immunol*, *411*, 141-169. https://doi.org/10.1007/82_2017_11

Nikolic, J., Belot, L., Raux, H., Legrand, P., Gaudin, Y., & A, A. A. (2018). Structural basis for the recognition of LDL-receptor family members by VSV glycoprotein. *Nat Commun*, *9*(1), 1029. https://doi.org/10.1038/s41467-018-03432-4

Pak, M. A., Markhieva, K. A., Novikova, M. S., Petrov, D. S., Vorobyev, I. S., Maksimova, E. S., Kondrashov, F. A., & Ivankov, D. N. (2023). Using AlphaFold to predict the impact of single mutations on protein stability and function. *PLoS One*, *18*(3), e0282689. https://doi.org/10.1371/journal.pone.0282689

Peyre, M., Chevalier, V., Abdo-Salem, S., Velthuis, A., Antoine-Moussiaux, N., Thiry, E., & Roger, F. (2015). A Systematic Scoping Study of the Socio-Economic Impact of Rift Valley Fever: Research Gaps and Needs. *Zoonoses Public Health*, *62*(5), 309-325. https://doi.org/10.1111/zph.12153

Phoenix, I., Nishiyama, S., Lokugamage, N., Hill, T. E., Huante, M. B., Slack, O. A., Carpio, V. H., Freiberg, A. N., & Ikegami, T. (2016). N-Glycans on the Rift Valley Fever Virus Envelope Glycoproteins Gn and Gc Redundantly Support Viral Infection via DC-SIGN. *Viruses*, *8*(5). https://doi.org/10.3390/v8050149

Pittman, P. R., Norris, S. L., Brown, E. S., Ranadive, M. V., Schibly, B. A., Bettinger, G. E., Lokugamage, N., Korman, L., Morrill, J. C., & Peters, C. J. (2016). Rift Valley fever MP-12 vaccine Phase 2 clinical trial: Safety, immunogenicity, and genetic characterization of virus isolates. *Vaccine*, *34*(4), 523-530. https://doi.org/10.1016/j.vaccine.2015.11.078

Pocathikorn, A., Granath, B., Thiry, E., Van Leuven, F., Taylor, R., & Mamotte, C. (2003). Influence of exonic polymorphisms in the gene for LDL receptor-related protein (LRP) on risk of coronary artery disease. *Atherosclerosis*, *168*(1), 115-121. https://doi.org/10.1016/s0021-9150(03)00087-x

Poirier, S., Mamarbachi, M., Chen, W. T., Lee, A. S., & Mayer, G. (2015). GRP94 Regulates Circulating Cholesterol Levels through Blockade of PCSK9-Induced LDLR Degradation. *Cell Rep*, *13*(10), 2064-2071. https://doi.org/10.1016/j.celrep.2015.11.006

Potere, N., Del Buono, M. G., Mauro, A. G., Abbate, A., & Toldo, S. (2019). Low Density Lipoprotein Receptor-Related Protein-1 in Cardiac Inflammation and Infarct Healing. *Front Cardiovasc Med*, *6*, 51. https://doi.org/10.3389/fcvm.2019.00051

Riblett, A. M., Blomen, V. A., Jae, L. T., Altamura, L. A., Doms, R. W., Brummelkamp, T. R., & Wojcechowskyj, J. A. (2016). A Haploid Genetic Screen Identifies Heparan Sulfate Proteoglycans Supporting Rift Valley Fever Virus Infection. *J Virol*, *90*(3), 1414-1423. https://doi.org/10.1128/JVI.02055-15

Rolin, A. I., Berrang-Ford, L., & Kulkarni, M. A. (2013). The risk of Rift Valley fever virus introduction and establishment in the United States and European Union. *Emerg Microbes Infect*, *2*(12), e81. https://doi.org/10.1038/emi.2013.81

Schulz, S., Schagdarsurengin, U., Greiser, P., Birkenmeier, G., Müller-Werdan, U., Hagemann, M., Riemann, D., Werdan, K., & Gläser, C. (2002). The LDL receptor-related protein (LRP1/A2MR) and coronary atherosclerosis--novel genomic variants and functional consequences. *Hum Mutat*, *20*(5), 404. https://doi.org/10.1002/humu.9070

Schwarz, M. M., Price, D. A., Ganaie, S. S., Feng, A., Mishra, N., Hoehl, R. M., Fatma, F., Stubbs, S. H., Whelan, S. P. J., Cui, X., Egawa, T., Leung, D. W., Amarasinghe, G. K., & Hartman, A. L. (2022). Oropouche orthobunyavirus infection is mediated by the cellular host factor Lrp1. *Proc Natl Acad Sci U S A*, *119*(33), e2204706119. https://doi.org/10.1073/pnas.2204706119

Shastry, B. S. (2009). SNPs: impact on gene function and phenotype. *Methods Mol Biol*, *578*, 3-22. https://doi.org/10.1007/978-1-60327-411-1_1

Shinohara, M., Tachibana, M., Kanekiyo, T., & Bu, G. (2017). Role of LRP1 in the pathogenesis of Alzheimer's disease: evidence from clinical and preclinical studies. *J Lipid Res*, *58*(7), 1267-1281. https://doi.org/10.1194/jlr.R075796

Sorensen, T. I., Nielsen, G. G., Andersen, P. K., & Teasdale, T. W. (1988). Genetic and environmental influences on premature death in adult adoptees. *N Engl J Med*, *318*(12), 727-732. https://doi.org/10.1056/NEJM198803243181202

Terasaki, K., & Makino, S. (2015). Interplay between the Virus and Host in Rift Valley Fever Pathogenesis. *J Innate Immun*, *7*(5), 450-458. https://doi.org/10.1159/000373924

Tree, M. O., Londono-Renteria, B., Troupin, A., Clark, K. M., Colpitts, T. M., & Conway, M. J. (2019). Dengue virus reduces expression of low-density lipoprotein receptor-related protein 1 to facilitate replication in Aedes aegypti. *Sci Rep*, *9*(1), 6352. https://doi.org/10.1038/s41598-019-42803-9

Vormittag, R., Bencur, P., Ay, C., Tengler, T., Vukovich, T., Quehenberger, P., Mannhalter, C., & Pabinger, I. (2007). Low-density lipoprotein receptor-related protein 1 polymorphism 663 C > T affects clotting factor VIII activity and increases the risk of venous thromboembolism. *J Thromb Haemost*, *5*(3), 497-502. https://doi.org/10.1111/j.1538-7836.2007.02337.x

Vučinić, N., Stokić, E., Djan, I., Obreht, D., Veličković, N., Stankov, K., & Djan, M. (2017). The LRP1 Gene Polymorphism is associated with Increased Risk of Metabolic Syndrome Prevalence in the Serbian Population. *Balkan J Med Genet*, *20*(1), 51-58. https://doi.org/10.1515/bjmg-2017-0004

WOAH. (2022). *Rift Valley Fever*. World Organization for Animal Health. Retrieved February 21 from https://www.woah.org/en/disease/rift-valley-fever/

Youssouf, H., Subiros, M., Dennetiere, G., Collet, L., Dommergues, L., Pauvert, A., Rabarison, P., Vauloup-Fellous, C., Le Godais, G., Jaffar-Bandjee, M. C., Jean, M., Paty, M. C., Noel, H., Oliver, S., Filleul, L., & Larsen, C. (2020). Rift Valley Fever Outbreak, Mayotte, France, 2018-2019. *Emerg Infect Dis*, *26*(4), 769-772. https://doi.org/10.3201/eid2604.191147