

## The Rh Factor in Humans: Beyond Blood Compatibility; Examining Associations with *Helicobacter pylori*, Norovirus, Cholera, Malaria, Cardiovascular Disease, and COVID-19

Nicholas A Kerna<sup>1,2\*</sup>, Uzoamaka Nwokorie<sup>3</sup>, Abdullah Hafid<sup>4</sup>, Kevin D Pruitt<sup>5</sup>, Oluwatosin A Atolagbe<sup>6</sup>, Fernand Jean-Baptiste<sup>7</sup>, Shain Waugh<sup>8</sup>, Joseph Anderson II<sup>9</sup> and ND Victor Carsrud<sup>10</sup>

<sup>1</sup>SMC–Medical Research, Thailand

<sup>2</sup>First InterHealth Group, Thailand

<sup>3</sup>University of Washington, USA

<sup>4</sup>Academy of Integrative Health and Medicine (AIHM), USA

<sup>5</sup>Kemet Medical Consultants, USA

<sup>6</sup>Zorvis Inc., USA

<sup>7</sup>Department of Biological Sciences, Florida Atlantic University, USA

<sup>8</sup>Fettle Path, USA

<sup>9</sup>International Institute of Original Medicine, USA

<sup>10</sup>Lakeline Wellness Center, USA

**\*Corresponding Author:** Nicholas A Kerna, (mailing address) POB47 Phatphong, Suriwongse Road, Bangkok, Thailand 10500.

Contact: medpublab+drkerna@gmail.com.

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### Abstract

The term “Rhesus factor” was put forth by Karl Landsteiner and Alexander S. Wiener in 1937. The serum of rabbits and guinea pigs agglutinated 85% of the human samples following the immunization with RBCs of *Macacus rhesus* monkeys. If the Rhesus factor is present in blood cells, the blood is termed Rh-positive. In the absence of Rhesus factor, the blood is termed Rh-negative. Rh grouping is significant in assessing blood compatibility, performing a crucial role in Rh-negative women exposed to Rh-positive blood through transfusion or pregnancy. The Rh-negative blood type is distinctly human, originating from a mutation in the RHD gene. Depending on the source, about 85% of the United States’ population is Rh-positive, while 15% is Rh-negative. However, this ratio varies from country to country; globally, the ratio is estimated at 92% Rh-positive with the remaining Rh-negative. Technological advances have resulted in a better understanding of Rh antigens’ molecular basis; approximately 170 alleles of the Rh gene RHD have been identified. It is projected that the percentage of Rh-negative people should drop as the population growth rate in Europe is low, and Europeans contribute significantly to the Rh-negative population. The most familiar and established connection regarding Rh is in blood compatibility. Nevertheless, investigations are ongoing into similarities between ABO grouping and the Rh factor regarding susceptibility or predilection towards specific psychological disorders, physical conditions and diseases, and infections. One aim of future research is in developing a universal blood type, making donor blood more readily available and blood transfusions and im-mixing harmless.

**Keywords:** Allele; Antigen; Gene Mutation; COVID-19; Polymorphism; Rhesus Box; Schizophrenia

### Abbreviations

cDNA: Complementary DNA; COVID-19: Coronavirus Disease 2019; CVD: Cardiovascular Disease; HDN: Hemolytic Disease of the Newborn; IVIG: Intravenous Immunoglobulin Therapy; PCR: Polymerase Chain Reaction; RBC: Red Blood Cell; RHAG: Rhesus Antigen; RHCE: Rh Blood Group CcEe Antigens; RhoGAM: Rho(D) immune globulin

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## Introduction

### Historical perspective

The designation “Rh” was derived from the “Rhesus factor”. This abridgement was put forth by Karl Landsteiner and Alexander S. Wiener in 1937. These researchers noted that the serum of rabbits and guinea pigs agglutinated 85% of the human samples following immunization with red blood cells (RBCs) of *Macacus Rhesus* monkeys [1]. If the Rh factor is present on RBCs, the blood is termed Rh-positive. Otherwise, the blood is termed Rh-negative. This discovery was and still is a significant finding as it determines blood compatibility of two individuals.

Initially, the researchers thought that the Rh factor was found in both humans and animals [1]. However, it was discovered in monkeys and found to be homologous to the ammonium transfer (AMT) protein. Hence, the presence or absence of the factor plays no role in the evolutionary chain. However, the term offered by Landsteiner and Wiener remains in use.

Rh grouping is significant in determining blood compatibility, playing a crucial role in Rh-negative women exposed to Rh-positive blood through transfusion or pregnancy. This grouping leads to vital fetal blood typing applications, which involves typing fetuses for D and K, C, c, or E antigens to assess a newborn’s risk of hemolytic diseases.

Levine., *et al.* (1939) described an unusual case of intra-group agglutination [2]. They reported a severe transfusion-related reaction in a woman who received blood from her husband following the delivery of a stillborn child with erythroblastosis fetalis. It was found that the patient’s serum agglutinated the RBCs in the husband’s blood (and about 80% of the Caucasian ABO compatible donor’s blood). This syndrome is referred to as hemolytic disease of the newborn (HDN) due to the maternal immune system’s sensitization to the D antigen. HDN arising because of D antigen’s incompatibility is common among Caucasians, having the highest prevalence of the D-negative phenotype (15–17%) [3,4].

This particular type of HDN is rare among people with different ethnic backgrounds. The incidence of HDN changed after the development of the Rh immunoglobulin. By the 1960s, HDN arising from anti-D was preventable [3,4]. Following the ABO blood grouping system, the Rh blood grouping system is a polymorphic and clinically-significant blood grouping system, comprising forty-nine distinct antigens [2–6].

Technological advances, such as cloning complementary DNA (cDNA) and genetic sequencing, led to the investigation and subsequent understanding of the Rh antigens’ molecular basis. Following the ABO system, the Rh system is a complex blood grouping system. In addition to D antigens, prevalent Rh antigens include allele C or c and E or e. Gene cloning has facilitated research into the Rh system. It is now known that the Rh locus’ arrangement and configuration promotes the genetic conversion and production of hybrid proteins (additional Rh antigens). Genetic testing identifies the predilection for recessive D cells other than RBCs, such as kidney, liver, brain, and skin cells [5,7–9].

Comparative genomics and structure-function studies have revealed Rh proteins’ presence in various tissues. Research is ongoing regarding the relationship between Rh proteins and mechanisms of transport. The majority of the blood group proteins have some known functions. During purification of the human Rhesus protein, American researcher Peter Agre identified a water transporter protein, for which he received the Noble Prize in 2003 [4].

## Discussion

### Groupings, nomenclatures, and designations

The Rh-negative blood type is distinctly human, originating from a mutation in the RHD gene. The antigens are designated as D, C, c, E, and e. The Rh blood group system is attributed to two genes, RHD and RhCE, located on chromosome 1 [4,7–9]. The RHD gene is dominant; hence, the D antigen's expression depends on whether an RHD gene has been inherited from one or both parents. A person is considered D positive whenever the RHD gene is present, even though it may have only been inherited from one parent. Conversely, a person will be D-negative if no RHD gene is inherited.

The Rh genes are denoted with capital letters with or without italics: RHD, RHCE, and RhAG; the non-RBC Rh genes are denoted as RhBG and RhCG. The different alleles of the genes are distinguished based on the parental gene. For example, in the different alleles of RHCE genes, they are denoted as RHce, RHcE, or RHCe based on the antigens coded by them [4,7–9]. The proteins are denoted as RHD. The Rh blood group CcEe antigens can be denoted as Rhce, RhCe, and RhcE based on the specific antigens carried by them (RhAG, RhBG, or RhCG); the latter two being Rh proteins found on extraerythrocytic hemoglobin and exo-erythrocytic tissues.

### Molecular basis of Rh alleles

Of all the Rh genes, the RhCE gene was the first to be discovered in 1990. The RHD gene was discovered in 1992; the complete deletion of this gene led to the European D-negative phenotype. Since the detection of the Rh gene RHD, approximately 170 alleles have been found. Recently, DNB, the most common of the partial D alleles in European ethnicity individuals, was identified [7,10]. In 2002, using data from the Human Genome Project and the Mammal Genome Project, a better understanding of the two Rh genes on chromosome 1 was gained [11].

(Note: Rather than develop another graphic representing the function of the Rh antigen complex or the molecular basis of the Rh system, the reader is referred to a plethora of such, which can be readily viewed by a simple online search, using search terms such as “structure of the Rh antigen”).

### Gene transformation pertinent to Rh

The RHD gene in humans developed from the duplication of the ancestral Rh gene during the evolution process that led to the deletion of RHD. Hence, in many modern humans, the RHD gene is entirely lacking. Across the world, this particular haplotype is the most common source of the D-negative phenotype. The Rh alleles are typically grouped per their molecular structures. Ammonia or carbon dioxide transport is considered a function of the Rh-RHAG complex [4].

There is a point mutation in most groups (single nucleotide polymorphisms; SNPs) that might lead to different types of mutations, such as missense, nonsense, and frameshift. In some cases, RHDCE-D hybrid alleles are produced through gene conversion [4].

### Molecular basis of the Rhesus phenotype

Only slight differences exist between the two Rh proteins, RHD and RHCE; they differ by 36 out of a total of 417 amino acids. Each of the proteins has extracellular and intracellular segments. There are twelve intracellular and six extracellular loops. Both the amino and the carboxyl terminals are located within the erythrocyte [6,10].

## **D-negative phenotype**

The most clinically-significant difference between the Rhesus-positive and Rhesus-negative erythrocytes is the presence or absence of the RHD protein on their surfaces. It is not usual for human cells, including erythrocytes, to lack these types of cell proteins. Thus, this particular genetic characteristic results in a robust immune response to the RHD protein.

As the ancestral Rh gene was duplicated during evolution, two DNA segments were formed (termed as “Rhesus box”) [7]. Deletion of RHD led to unequal crossover occurring as the two DNA segments in the Rhesus box are homologous. The hybrid Rhesus box is seen in Europeans with an RHD negative haplotype. The molecular differences among various types of Rhesus boxes are utilized in various genetic testing types [6].

## **D antigen variants**

Besides the lack of RHD protein, the D-negative phenotype occurs due to several RHD protein changes responsible for the difference in phenotype. Based on the phenotype and corresponding molecular structure, the RHD alleles are categorized as partial D, weak D, and DEL [8].

### **Partial D**

In this variation, the RHD protein passes through the erythrocyte membrane numerous times, leaving only a portion of the surface’s protein structure. Thus, if an amino acid is added or substituted—at that part of the RHD located on the erythrocyte’s outer surface—single epitopes of the D antigen can be lost, or sometimes, a completely new antigen is produced [6–8].

RHD negative, mentioned earlier, is the most common variation of RHD found in the European population. In the RHD gene structure, homologous exons of the RHCE gene can be inserted, leading to the formation of a hybrid Rhesus allele and corresponding hybrid proteins. These genetic changes result in different categories of D (III, IV, V, and VI).

### **Weak D**

In this phenotype, the amino substitution is located within the erythrocyte membrane or in the erythrocyte’s cytoplasm [6,7,11]. Hence, if incorporating the RHD protein in the erythrocyte membranes is blocked, there results weak quantitative expression of the D antigen. However, there are no qualitative changes, and thus, no anti-D immunization. This phenotype is most frequently seen in the European population.

The Rh antigens on the surface of an Rh-positive cell allow its attachment to specific antibodies where a reaction incites RBCs’ sensitization. When there is a positive complement fixation reaction (i.e. the patient’s serum contains the antibody against Rh antigen), there is no lysis of RBCs since no complement remains. On the contrary, an adverse reaction occurs when the patient’s serum contains antibodies against Rh antigens—complement remains and the sensitized RBCs are lysed [4].

The Rh genes are 97% identical; they are located next to each other on chromosome 1. The D/d polymorphism most commonly arises from a deletion of the entire RHD gene [7–11]. The C/c polymorphism arises from four SNPs that cause four amino acid changes, one of which (S103P) determines the C or c antigen specificity. No population is either entirely Rh-positive or Rh-negative.

### **DEL**

DEL (previously termed as Del) is a specific (and the weakest) form of weak D expression—only observed during elution. During the process of elution, antibodies are separated from erythrocytes and present in the elute. The underlying molecular changes associated

with DEL are comparatively more severe than the weak D phenotype, leading to considerable (if not complete) resistance to incorporating the D antigen in the erythrocyte membrane. This DEL allele is rare among Europeans; however, 30% of D-negative people in Asia are carriers of the DEL allele RHD [6,7,10].

### **C/c and E/e antigens**

Other variations of the Rh antigen occur due to alterations in the RHCE protein. C, c, E, and e antigens are produced due to changes in specific amino acids at five sites. The term 'antithetical' describes antigens when a protein is present only in one of them, caused by protein polymorphism [7–11].

In many cases, there are two variants of a protein differing by only one amino acid, such as Rhesus antigen E and e. RHCE alleles, with the amino acid proline at position 226, lead to the production of E antigens. RhCE alleles with alanine at the same position allow the production of e antigens. Similar findings are seen in RhCE alleles expressing C and c antigens. However, the antigen pairs C/c and E/e are not antithetical as their expressions occur due to the substitution of amino acids at various locations. The frequency of occurrence of these four possible combinations is varied and hierarchical; the expressions among Europeans being Ce > ce > cE > CE, presenting as haplotypes [11].

### **Clinical applications**

Similar to most clinical investigations, genetic tests are ordered and performed in appropriate circumstances [6,8,10]. Molecular technologies for the identification of the Rh allele are employed under specific clinical situations. Frequently utilized molecular methods include polymerase chain reaction (PCR) to amplify the target gene, followed by identification through electrophoresis, sequencing, and hybridization [5,6,10].

### **Anti-D patients**

Few patients have this type of RHD allele. A majority of the patients express partial D, and in rare cases, weak D. Among these alleles, category DVI is the most consequential variation. Monoclonal anti-D antibodies are recommended for typing, which do not interact with category DVI [11–13].

This previous recommendation was included in the German chemotherapy guidelines of 1996 and other international standards. Individuals with the DVI allele are typed as false-negative to prevent D-positive blood transfusion and immunization against D [12].

In contrast to partial D, no anti-D alloimmunization has been documented for weak D alleles (type 1, 2, and 3) [14,15]. From a clinical perspective, this data is quite helpful as most of the D alleles—around 90% of the German population—are weak D alleles (type 1, 2, and 3). Such patients can receive D-positive blood with no risk of anti-D alloimmunization, conserving an estimated 5% of all D-negative blood products [14,15].

### **Pregnancy and anti-D prophylaxis; squandered resources**

Pregnant women who type weak D (type 1, 2, or 3) can also receive D-positive blood products without anti-D prophylaxis. Each year, studies show that a simple genetic test to identify the pregnant carriers of weak D (type 1, 2, or 3) supports that 5% of all the D-negative pregnant women can avoid the repeated administration of anti-D (about 3,500 pregnant women in Germany). This testing can also avoid possible side effects of prophylaxis and the cost of testing [16,17]. However, other pregnant women with a rare type of weak D alleles require anti-D prophylaxis.

To reiterate, identifying a D-negative fetus in a D-negative or weak D mother excludes anti-D prophylaxis (making anti-D prophylaxis unnecessary in approximately 40% of all cases otherwise receiving it). Fetal D-positivity can be determined through fetal DNA circulating in the peripheral maternal blood [16]. Fetal D antigens can be directly detected through amniocentesis or by sampling the trophoblasts [17].

### **Anti-D antibodies and childbearing**

Suppose the father of the fetus is heterozygous for RHD deletion, and the mother is D-negative. There is a 50% chance of the fetus being D-negative, eliminating any hematological risks in this scenario. However, if the father is homozygous for RHD deletion (and the mother remains D-negative), the chance of the fetus carrying the D antigen is 100%, carrying a high risk for hematological diseases.

For many years, the heterozygous or homozygous status of RHD was impossible to detect using serological methods and being inconclusive. Now, it is possible to identify the status using genetic sampling techniques [18].

### **Application of blood typing in various diseases**

In cases where standard serological tests fail, genetic tests are conclusive in blood group typing of patients with autoimmune or alloimmune anemias [4,7]. Despite the persistence of transfusion of leucocytes in some cases, they do not interfere with genetic tests.

Children with maternal-fetal Rh incompatibility are at increased relative risk for developing schizophrenia [7]. Rh incompatibility can be prevented with Rho(D) immune globulin (RhoGAM). Therefore, prevention remains the best treatment.

Rh incompatibility occurs most frequently when an Rh-negative mother is exposed to Rh-positive fetal RBCs secondary to hemorrhage during pregnancy (from spontaneous or induced abortion, trauma, invasive obstetric procedures, or standard delivery). It can also occur when an Rh-negative female receives an Rh-positive blood transfusion. Infants with mild Rh incompatibility are treated with phototherapy using bilirubin lights and specific intravenous immunoglobulin (IVIG) therapy. In some cases, an exchange transfusion of blood may be needed, decreasing the blood's bilirubin level.

### **Blood donors**

D-negative blood donors (weak D or DEL) can be identified by genetic testing of the RHD gene and, thus, should only be administered to D positive patients [18]. Without proper diagnosis, D-negative recipients will still be immunized by the D antigen contained in the blood (of patients with weak D or DEL) [19–21]. Moreover, mistyped D-negative donors with D-/D+ chimeras can also be identified correctly. Appropriate identification of D-positive blood is confirmed only through genetic testing [6,10,19]. Anti-D immunization is of significant clinical importance for girls and women of reproductive age as a D positive fetus in a D-negative mother results in a high risk of Rhesus hemolytic disease in the newborn [22].

### **Population data**

Worldwide data suggests that the percentage of Rh-positive and Rh-negative people is 94% and 6%, respectively [6,10]. However, the percentage of Rh-negative people will drop further as the population growth rate in Europe is low, and Europeans mainly contribute to the Rh-negative population.

### **Rh blood group and disease and infection predilection and susceptibility**

There is evidence of an association between protection from specific infectious diseases and the inheritance and expression of the ABO blood group and Lewis antigens regarding *Helicobacter pylori*, norovirus, cholera, malaria, and cardiovascular disease [23–25]. At

this time, no such connection has been established between Rh-positive or Rh-negative people; however, investigations into possible connections continue.

As mentioned previously, highly immunogenic Rh antigens result in transfusion reactions and HDN.

### **Rh grouping and COVID-19**

A few recent studies have explored a correlation between Rh blood grouping and coronavirus disease (COVID-19) [26–28]. While some studies reported no relationship between the Rh blood group and the risk or severity of COVID-19, some studies hinted at a possible correlation.

In a demographic study conducted in Sudan, researchers determined that Rh-positive individuals could be at a higher risk of COVID-19 infection than Rh-negative individuals [28]. However, as knowledge about the disease is evolving and research on this topic is in the earliest stages, further research is warranted.

### **Diseases associated with Rh grouping**

#### **Hemolytic disease of the newborn**

This alloimmune condition occurs in a fetus at or around birth—if the mother is Rh-negative and the father is Rh-positive. Anti-Rh antibodies pass from the placental circulation and attack antigens of fetal RBCs. Consequently, reticulocytosis and anemia develop in the fetus. The severity of the disease can be mild to severe. In severe cases, fetal death occurs. Treatment options include phototherapy, IVIG, and exchange transfusion [29–31].

#### **Transfusion reactions due to Rh incompatibility**

If Rh-positive blood is transfused into an Rh-negative individual, the first episode of transfusion results in the development of anti-Rh antigens; in a subsequent episode of Rh-positive blood transfusion in the same patient, it might elicit an incompatibility reaction (similar to ABO). Frequent symptoms include hemolysis, jaundice, and hemoglobinuria. If a woman with Rh-negative blood becomes sensitized by Rh-positive blood via an Rh-positive baby or previous transfusion of Rh-positive blood, there is an increased risk of hemolytic disease in the newborn in the next pregnancy due to Rh incompatibility [29–31].

### **Conclusion**

The Rh blood group is the most consequential blood grouping system after the ABO system. Of the forty-nine blood group antigens of the Rh system, six antigens, namely C, D, E, c, d, and e, are critical. With the advent of newer genetic tools and a better understanding of the Rh system, Rh incompatibility can be prevented and predicted in most cases. A majority of the world's population is Rh-positive. Unlike the ABO system, clinical evidence of any correlation between a person's Rh status (positive or negative) and conditions, such as *Helicobacter pylori*, norovirus, cholera, malaria, and cardiovascular disease, are not yet established. The Rh system's clinical significance lies in Rh incompatibility reactions, such as HDN and transfusion reactions. If universal blood typing can be developed through agents, such as enzymes, distinct blood groups could be converted to Type O—the universal blood type—thereby making donor blood more readily available and overcoming blood shortages. This enzymatic conversion to Type O is currently being pursued with Type A blood only as it is more common than Type B. Later, perhaps, the Rh factor will be addressed accordingly.

## Conflict of Interest Statement

The authors declare that this paper was written in the absence of any commercial or financial relationship that could be construed as a potential conflict of interest.

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