HHS Public Access

Author manuscript

Transfusion. Author manuscript; available in PMC 2017 July 01.

Published in final edited form as:

Transfusion. 2016 July ; 56(7): 1699–1706. doi:10.1111/trf.13622.

Blood group A and Rh(D)-negativity are associated with symptomatic West Nile virus infection

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Abstract

Background—West Nile virus (WNV) infection is mostly asymptomatic but 20% of subjects report WNV fever and 1% of patients experience neurological diseases with higher rates in elderly and immunosuppressed persons. With no treatment and no vaccine to prevent the development of symptomatic infections, it is essential to understand prognostic factors influencing symptomatic disease outcome. Host genetic background has been linked to the development of WNV neuroinvasive disease. The present study investigates the association between the ABO and Rh(D) blood group status and WNV disease outcome.

Study Design and Methods—The distribution of blood groups was investigated within a cohort of 374 WNV+ blood donors including 244 asymptomatic (AS) and 130 symptomatic (S) WNV+ blood donors. Logistic regression analyses were used to examine associations between A, B, O and Rh(D) blood groups and WNV clinical disease outcome.

Results—Symptomatic WNV+ donors exhibited increased frequencies of blood group A (S 47.6% AS 36.8%, *P*=0.04, OR [95%CI] 1.56 [1.01–2.40]) and Rh(D)-negative individuals (S 21.5% AS 13.1%, *P*=0.03, OR [95%CI] 1.82 [1.04–3.18]).

Conclusion—The findings suggest a genetic susceptibility placing blood group A and Rh(D)-negative individuals at risk for the development of symptomatic disease outcome after WNV infection.

Keywords

ABO Rh(D) Blood groups; West Nile Virus; symptomatic disease outcome; Red Blood Cells antigens

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Conflict of interest: The authors have reported no conflict of interest.

Introduction

West Nile Virus (WNV) is a mosquito-borne flavivirus that was introduced to the US in 1999 and is now the leading cause of arboviral encephalitis in the US.[1] Over the past 17 years, WNV has been responsible for more than 42,000 reported cases, of which 19,160 presented with neuroinvasive disease, 22,434 with West Nile (WN) fever, and 1,7957 had a fatal outcome.[2] Because WNV infection is asymptomatic in 80% of cases,[1] these numbers underrepresent the overall frequency of infection. A recent study projected that over 3 million persons have been infected with WNV in the US from 1999 to 2010, resulting in about 780,000 illnesses.[3, 4]

To date there is no treatment and no vaccine to prevent the development of symptomatic infections in humans.[5] Therefore it is essential to understand the reasons for symptomatic outcome and prognostic factors influencing severe disease outcome to better manage patients care. Underlying risk factors associated with severe WNV disease are older age, hypertension,[6] immunosuppression,[7] and genetic factors. Indeed host genetic background such as mutations or deletions in the CCR5,[8] IRF-3 and OAS1 genes,[9] and host HLA class I alleles, such as the HLA-A*68 and C*08 alleles,[10] have been linked to the development of WNV neuroinvasive disease. Research has begun to clarify the relationship between immune protection and disease,[11] however the correlates of protective immunity in humans remain uncharacterized. It is, therefore, determinant to characterize aspects of the host-virus interaction to identify the host and viral parameters influencing WNV disease outcome.

Studies have suggested that WNV could bind to red blood cells (RBCs)[12] and WNV RNA was found to persist longer in whole blood than in plasma.[13, 14] The mechanisms underlying compartmentalization of WNV in the RBC fraction of the blood are unclear and the consequences on pathogenesis are unknown. RBCs are nonnucleated biconcave discs that contain hemoglobin and have no cellular machinery to synthesize *de novo* virions, it is therefore unlikely that WNV would be able to replicate in RBCs. However, reticulocytes constitute 1% of the circulating RBCs and could support WNV replication. With approximately five million RBCs per cubic millimeter in adults and the ability to access vascularized tissues through the smallest capillaries, RBCs represent potent vehicles for pathogens to be carried around the body.

RBCs harbor glycosylated antigens that include the ABO blood group antigens. Those result from the expression of inherited specific genes which code for glycosyltransferases whose sequential action builds the H, A, or B antigens. RBCs also carry other type of antigens such as the rhesus (Rh) antigens. Those are non-glycosylated hydrophobic transmembrane proteins that are integral proteins of the RBC membrane and assembled into a multimeric complex with other glycoproteins such as the Rh50 glycoprotein, [15] CD47, glycophorin B, Duffy, and LW.[16]

Various studies have reported the association between specific blood groups and susceptibility to particular diseases or manifestations of disease caused by bacterial, parasitic, and viral infections such as SARS CoV,[17] *V. cholerae*,[18–20] *P.falciparum*,[21–24] *H.pylori and gastric ulcers or cancers*,[25–28] norovirus,[29–32] rotavirus, dengue virus,[33] and

hepatitis infections.[34–36] The present study investigates the association between ABO and Rh(D) blood groups and WNV disease outcome in a cohort of 374 WNV+ blood donors.

Materials and methods

Study population

A total of 374 WNV+ subjects were enrolled in this study through the Blood Systems Research Institute (BSRI) between 2004 and 2011. Demographics such as age and gender were collected for all donors (Table 1). Race data were missing for 24 donors and available for 350 donors (298 Caucasian, 2 Black, 1 Asian, 40 Hispanic, and 9 donors from other race groups). All were enrolled by BSRI from the United Blood Services network after they tested positive for WNV RNA by routine donation screening (index). Infection was confirmed using follow-up samples showing seroconversion to anti-WNV IgM. Blood typing was performed by the blood bank at the time of donation. All of these donors were enrolled after signing an informed consent approved by the UCSF Committee on Human Research. Symptom questionnaires covering 12 possible WNV-related symptoms (fever, headache, eye pain, body aches, new skin rash, swollen lymph nodes, nausea or vomiting, muscle weakness, confusion, disorientation, memory problems, or other symptom) were administered at study enrollment and two weeks later. As previously described,[37] a cutoff of four symptoms was used to categorized blood donors as asymptomatic (AS, number of reported symptoms < 4, n = 244) or symptomatic (S, number of reported symptoms ≥ 4, n=130) as previously used for pathogenesis studies[10, 14, 38, 39]. The average age was 47.4 years for the WNV+ cohort, 48.2 years for asymptomatic, and 46 for symptomatic WNV+ donors (P AS vs. S = 0.15) (Table 1).

Statistical analysis

The statistical package SAS was used for data analyses. Chi-square test of independence and Fisher Exact test were used to compare the demographic characteristics between asymptomatic and symptomatic WNV+ blood donors and to compare the distribution of blood groups within WNV+ blood donors and US blood donors.[40]

Multivariate logistic regression analyses were used to examine associations between A, B, O and WNV clinical disease outcome, adjusting for race and Rh(D) blood group status. Multivariate logistic regression analyses were used to examine associations between Rh(D) blood group status and WNV clinical disease outcome adjusting for race. For statistical presentation, instead of using a reference group, each group is compared to all other donors (A versus non-A, O versus non-O, Rh(D)-negative versus Rh(D)-positive, etc.). Statistical significance was determined at $P < 0.05$. False discovery rates (FDR) were calculated by PROC MULTITEST (SAS 9.3). FDR-controlling procedures are designed to control the probability of Type I errors in analyses with multiple cmparisons. FDR<0.1 would support the signficance of P-values while FDR>0.1 would indicate that caution needs to be applied when analyzing the signficance of the findings.

Results

WNV+ blood donors have the same blood type distribution as the general blood donor population

Demographics and blood type data were collected for the 374 WNV+ blood donors included in this analysis (Table 1). This cohort consisted of 79.7% White non-Hispanic Caucasians, 10.7% Hispanics, 0.5% Blacks, 0.3% Asians, and 2.4% others with race data missing for 6.4% of the subjects. The cohort had 56.7% of males and 43.3% of females. The mean age was 47.4 years.

The frequencies of subjects with O, A, B, AB, RhD-positive, and RhD-negative blood types were calculated for the whole cohort. The blood type distribution within the WNV+ cohort was the same as within the general blood donor population in the US,[40] with a majority of O (45.5%), then A (40.6%), then B (10.2%), a minority of AB (3.7%), and 84% of Rh(D)-positive versus 16% of Rh(D)-negative subjects (Table 2).

A and Rh(D)-negative blood groups are associated with symptomatic WNV disease outcome

Symptom questionnaires were analyzed as previously described,[10, 37, 39] and revealed that 130 of the 374 WNV+ blood donors included in this cohort developed four or more symptoms during the three week period around index donation and could be categorized as symptomatic (S) while 244 subjects remained asymptomatic (AS). No significant difference was found in the demographic characteristics of asymptomatic and symptomatic WNV+ blood donors included in this analysis (Table 1). Asymptomatic and symptomatic WNV+ blood donors in this study did not have significant difference of mean age (48.2 and 46 years, respectively, $P = 0.15$).

The frequencies of O, A, B, AB, Rh(D)-positive, and Rh(D)-negative blood groups were calculated within 244 asymptomatic and 130 symptomatic WNV+ blood donors. The distribution of blood groups was compared between asymptomatic and symptomatic WNV+ blood donors and a higher frequency of blood group A subjects was found within symptomatic than within asymptomatic WNV+ blood donors (47.6% in S vs. 36.8% in AS, $P = 0.04$) (Table 2). The odds ratio of symptomatic WNV disease outcome among blood donors with blood group A was significantly higher than within blood donors with blood group O (OR [95%CI] = 1.56 [1.01, 2.40]). A higher frequency of Rh(D)-negative subjects was found within symptomatic WNV+ blood donors (21.5% in S vs. 13.1% in AS, $P = 0.03$). The odds ratio of symptomatic WNV disease outcome among Rh(D)-negative blood donors was significantly higher than within Rh(D)-positive blood donors (OR[95%CI] = 1.82 [1.04, 3.18]) (Table 2, unadjusted *P*-values obtained using the Chi-square test of independence).

Previous studies have reported the highest percentages of Rh(D)-negative subjects are present in the white non-Hispanic race group.[40] A multivariate logistic regression analysis was used to adjust for race, the association between blood group A and symptomatic WNV disease outcome persisted after adjustment for both Rh(D) status and race (P S vs. AS = 0.02, OR [95%CI] = 1.70 [1.08, 2.66]) and the association between Rh(D)-negativity and

symptomatic WNV disease outcome persisted after adjustment for race (*P* S vs. AS = 0.02, OR [95%CI] = 1.96 [1.1, 3.48]). Therefore A and Rh(D)-negative blood groups are independently associated with symptomatic WNV disease outcome and this association persisted after controlling for race (Table 2, adjusted *P*-values obtained by multivariate logistic regression adjusting for race).

Therefore, blood group A and Rh(D)-negativity could represent potential risk factors associated with symptomatic WNV clinical outcome.

Within Rh(D)-positive WNV+ blood donors, the frequency of blood group A individuals within symptomatic WNV+ blood donors was higher but not significantly higher than within asymptomatic WNV+ blood donors (*P* = 0.33) though blood group A was significantly associated with symptomatic WNV disease outcome after adjustment for Rh(D) and race status (*P* = 0.02, OR [95%CI] = 1.70 [1.08, 2.66]). Within Rh(D)-positive WNV+ blood donors, the frequency of blood group B individuals within symptomatic WNV+ blood donors was significantly lower than within asymptomatic WNV+ blood donors (*P* = 0.002, OR[95%CI] = 0.27 [0.11,0.65]) suggesting a potential association between B/Rh(D)-positive (B+) blood groups though blood group B was not significantly associated with asymptomatic WNV disease outcome after adjustment for Rh(D) status (*P* = 0.32).

Within Rh(D)-negative WNV+ blood donors, the frequency of blood group A individuals within symptomatic WNV+ blood donors was significantly higher than within asymptomatic WNV+ blood donors (*P* = 0.03) and the odds of developing symptomatic disease for A/Rh(D)-negative (A−) (OR[95%CI] =2.33 [1.04,5.20]) was even higher than for blood group A (OR[95%CI] =1.56 [1.01, 2.40]) or Rh(D)-negative individuals (OR[95%CI] = 1.82 [1.04,3.18]) suggesting a cumulative effect when both risk factors were combined. Within Rh(D)-negative WNV+ blood donors, the frequency of blood group B individuals within symptomatic WNV+ blood donors was higher than within asymptomatic WNV+ blood donors (*P* = 0.04 OR[95%CI] = 3.88 [0.95, 15.8]) suggesting the protective role of blood group B is lower than the risk associated with Rh(D)-negative blood group.

The frequency of blood group A subjects is higher in the symptomatic WNV+ blood donor population than in the healthy US blood donor population

When the WNV+ blood donor population was compared to the US blood donor population, the frequency of Rh(D)-negative subjects was significantly higher within symptomatic WNV + blood donors than within the US blood donor population (21.5% for S vs. 14.6% for US blood donors, *P* = 0.03, OR[95%CI] = 1.61 [1.06–2.44]) (Table 3, upper part) but the difference was not statistically significant when the analysis was restricted to white non-Hispanic individuals (22.7% for S vs. 17.3% for US blood donors, *P* = 0.13) (Table 3, lower part) suggesting that race may be a confounder factor. Indeed, there was a higher frequency of white non-Hispanic individuals within the symptomatic WNV+ population than within the US blood donor population (84.6% within S WNV+ donors vs. 71.7% within US blood donors, *P* = 0.0007).

However, there was an over-representation of blood group A subjects within symptomatic WNV+ blood donors compared to the US blood donor population (47.6% within S WNV+

donors vs. 37.1% within US blood donors, $P = 0.01$, OR[95%CI] = 1.55 [1.10–2.18]) (Table 3, upper part) and this difference persisted when the analysis was restricted to white non-Hispanic individuals (49.1% within S WNV+ donors vs. 39.7% within US blood donors, $P = 0.05$, OR[95%CI] = 1.46 [1.01–2.13]) (Table 3, lower part). The frequency of blood group A individuals was also higher within symptomatic WNV+ Rh(D)-positive blood donors than within the US Rh(D)-positive blood donor population (47.1% within S WNV+ donors vs. 36.9% within US blood donors, $P = 0.03$, OR[95%CI] = 1.52 [1.03–2.24]) (Table 3, upper part). Therefore, blood group A individuals are more common within symptomatic WNV+ donors than within the healthy US blood donor population.

Discussion

While race, gender, and age were not associated with WNV disease outcome in this cohort, blood group A was found at higher frequency in symptomatic than in asymptomatic WNV+ donors. Rh(D)-negativity was also more frequent in symptomatic than in asymptomatic WNV+ donors. The data suggest an association between blood groups and WNV disease outcome, with blood group A and Rh(D)-negativity being risk factors for the development of symptomatic WNV disease outcome. When the distribution of blood groups was compared within the WNV+ donor population and within the US blood donor population using the numbers previously reported by Garratty et al., an increased frequency of blood group A individuals was found within symptomatic WNV+ donors than within the US blood donor population[40].

While the comparison of blood group distribution within WNV+ groups experiencing different disease outcomes (asymptomatic versus symptomatic) is straightforward, the comparison of blood group distribution within WNV+ donor population and US blood donor population is interesting but limited. Indeed, comparing blood group distribution within WNV+ blood donor population enrolled by Blood Systems Inc. from 2004 to 2011 to blood group distribution as reported by Garratty et al. within US blood donors enrolled at five other blood centers between 1991 and 2000[40] may be biased by donor stratification resulting from collection based on blood group need that would potentially change over different period of time and geographic areas. Therefore, the comparison between blood group distribution within the WNV+ donor population and within the US blood donor population is worth reporting but the key findings are more related to the differences of blood group distribution identified within symptomatic versus asymptomatic WNV+ donors which would not be impacted by blood group recruitment biase.

In a previous study, higher WNV viral loads were observed over the three months post-index in blood group A WNV+ subjects than in blood group O WNV+ subjects.[14] This was not the case for Rh(D)-negative donors, suggesting the mechanisms that underlie the association between blood group A and WNV disease outcome are different from the ones underlying the association between blood group Rh(D)-negative and WNV disease outcome. From an evolutionary stand point, it is however interesting to note that the frequency of Rh(D)-negative individuals is higher in white non-Hispanic, than in African, and Asian populations.[40] Therefore the world distribution of Rh(D)-negative humans is concentrated in geographic areas where WNV outbreaks have been documented for the increased severity of

symptoms, as reported in the 90's around the Mediterranean's, and since 1999 in North America. WNV was first isolated in 1937 in Uganda from a women with fever and studies have documented the widespread distribution of WNV in Africa through seroprevalence studies conducted in the 50's and revealing a high frequency of young adults immunized during childhood.[42] The symptoms associated with WNV infection seemed more severe after WNV introduction in North America. Whether this is due to virus and human co-evolution and immunization earlier during childhood in endemic areas, or underreporting of more severe symptoms is unclear but it is worth to note that the frequency of Rh(D)-negative subjects is the highest in Causian populations of Europe and North America and the lowest in the Asian population where WNV infection remains unnoticed.

Numerous studies have been published to date on the association between blood groups and diseases.[43, 44] However, most studies are based on statistical analyses and some are limited by sample size, racial heterogeneity, methodological approaches.[45] Therefore it is important to reproduce the findings in different cohorts. Noteworthy, similar results were recently presented by Politis et al.[46] Indeed, an association was reported between increased frequencies of blood group A and Rh(D)-negative individuals within 132 patients previously infected with lineage 2 WNV in Greece compared to healthy blood donors from the same WNV affected areas. This is supporting the findings of the present study.

The body of literature reporting an association between blood groups and diseases is growing. Some studies have clearly established the association between blood group O and reduced risk of severe malaria, with the theory that Plasmodium falciparum could have exerted a selective pressure on the human population in favor of individuals with blood group O in malaria-endemic regions.[22] The mechanisms underlying the association between blood groups and malaria outcome are unclear but reports have suggested that blood group A antigen could serve as a co-receptor for Plasmodium falciparum,[21] potentially explaining the association between blood group A and severe malaria.[22] It was also recently reported that human macrophages have a higher avidity for Plasmodium falciparum-infected O erythrocytes than for Plasmodium falciparum-infected A or B erythrocytes and therefore subjects with blood group O might have accelerated parasite clearance compared to others.[24] In the present study, similar mechanisms could underlie the association between blood group A and symptomatic WNV disease. Indeed, it was shown that WNV interacts with LBP and αVβ3 integrin at the cell surface of host cells through its E glycoprotein,[47, 48] and blood group A antigen could serve as a co-receptor for WNV. This would explain the findings that subjects with blood group A demonstrated higher viral loads in whole blood than subjects with other blood groups,[14] however, this would not explain why the same assoication is not observed for subjects with blood group AB, whose erythrocytes express approximately half of the number of A sites than A erythrocytes[49] and this would not explain the association with Rh(D)-negativity. An alternative hypothesis would be that other glycosylated structures expressed differentially at the surface of erythrocytes from blood group A and Rh(D)-negative donors would facilitate WNV binding through glycan-glycan or lectin-glycan interactions in a Velcro-like interaction or would serve as receptors or co-receptors. While the potential mechanisms underlying such associations have been previously hypothesized and discussed,[14] further investigation is needed to clearly understand the association between blood group and WNV pathogenesis.

The present study suggests an association between blood groups and WNV pathogenesis with A and Rh(D)-negative blood groups being potential new risk factors for the development of symptoms after WNV infection. This study adds to our previous report of WNV RNA persisting at higher levels in whole blood from blood group A WNV+ individuals. The findings reported in the present study are based on the statistical analysis of epidemiologic data collected from a cohort of individuals infected with lineage 1 WNV and are strengthened by the findings obtained on a different cohort of individuals infected with lineage 2 WNV.[46] Therefore the present findings support the interest in further pursuing the investigation of the mechanisms underlying the association between blood group A and Rh(D)-negativity and symptomatic WNV disease outcome.

Acknowledgments

We thank Daniel Hindes for the management of the West Nile virus symptom questionnaires.

Sources of support: this research was supported by grant R01 C1000214-01 from the Centers for Disease Control and Prevention and grant RC2HL101 from the National Heart Lung and Blood Institute.

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TABLE 1

Demographics of the WNV+ blood donor population

Demographics		AS (n=244)		S (n=130)		Total (n=374)		P-value	OR	Lower CL	Upper CL
		n	%	n	%	n	%				
Race	Caucasians	188	**77.1**	110	**84.6**	298	**79.7**	0.16	1.59	0.82	3.06
	Blacks	1	**0.4**	1	**0.8**	2	**0.5**	0.67	1.83	0.11	29.50
	Asians	1	**0.4**	0	**0.0**	1	**0.3**	-	-	-	-
	Hispanics	31	**12.7**	9	**6.9**	40	**10.7**	0.07	0.49	0.23	1.07
	Others	5	**2.1**	4	**3.1**	9	**2.4**	0.23	1.47	0.39	5.59
	Missing	18	**7.4**	6	**4.6**	24	**6.4**	-	-	-	-
Gender	Male	143	**58.6**	69	**53.0**	212	**56.7**		1.00		
	Female	101	**41.4**	61	**47.0**	162	**43.3**	0.30	1.25	0.82	1.92
Mean Age		48.2		46.0		47.4		0.15			

TABLE 2

Comparison of blood group distribution within asymptomatic and symptomatic WNV+ blood donors

Blood groups	WNV+						S versus AS by Chi-square/ Fisher Exact tests					S versus AS by multivariate logistic regression					
	AS (n=244)		S (n=130)		Total (n=374)												
	n	%	n	%	n	%	P-value	OR	Lower CL	Upper CL	FDR	P-value	OR	Lower CL	Upper CL	FDR	Adjusted for
O	114	**46.8**	56	**43.0**	170	**45.5**	0.50	0.86	0.56	1.32	0.50	0.42	0.83	0.53	1.30	0.42	Rh(D) Race
A	90	**36.8**	62	**47.6**	152	**40.6**	***0.04***	***1.56***	***1.01***	***2.40***	***0.06***	***0.02***	***1.70***	***1.08***	***2.66***	***0.06***	Rh(D) Race
B	27	**11.0**	11	**8.4**	38	**10.2**	0.42	0.74	0.35	1.55	0.50	0.32	0.67	0.31	1.46	0.40	Rh(D) Race
AB	13	**5.4**	1	**0.8**	14	**3.7**	0.02	0.13	0.01	1.06	0.06	0.05	0.13	0.02	1.04	0.09	Rh(D) Race
Rh(D)+	212	**86.9**	102	**78.5**	314	**84.0**	0.03	0.55	0.31	0.96	0.06	0.02	0.51	0.29	0.91	0.06	Race
Rh(D)−	32	**13.1**	28	**21.5**	60	**16.0**	***0.03***	***1.82***	***1.04***	***3.18***	***0.06***	***0.02***	***1.96***	***1.10***	***3.48***	***0.06***	Race
O+	97	**45.8**	48	**47.1**	145	**46.2**	0.60	0.88	0.57	1.37	0.60	0.45	0.84	0.53	1.32	0.60	Race
A+	78	**36.8**	48	**47.1**	126	**40.1**	0.33	1.24	0.79	1.94	0.50	0.25	1.31	0.83	2.07	0.51	Race
B+	25	**11.8**	6	**5.9**	31	**9.9**	***0.002***	***0.27***	***0.11***	***0.65***	***0.01***	***0.05***	***0.37***	***0.14***	***0.99***	***0.19***	Race
AB+	12	**5.7**	0	**0.0**	12	**3.8**	-	-	-	-	-	-	-	-	-	-	-
O−	17	**53.1**	8	**28.6**	25	**41.7**	0.76	0.87	0.36	2.08	0.76	0.82	0.90	0.37	2.18	0.82	Race
A−	12	**37.5**	14	**50.0**	26	**43.3**	***0.03***	***2.33***	***1.04***	***5.20***	***0.08***	***0.02***	***2.69***	***1.16***	***6.26***	***0.09***	Race
B−	2	**6.3**	5	**17.9**	7	**11.7**	0.04	3.88	0.95	15.80	0.08	0.07	4.70	0.90	24.65	0.13	Race
AB−	1	**3.1**	1	**3.6**	2	**3.3**	0.65	1.88	0.11	30.36	0.76	0.70	1.74	0.11	28.13	0.82	Race

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Table 3

Comparison of blood group distribution between asymptomatic or symptomatic WNV+ blood donors and US blood donors

Blood Groups		WNV+ blood donors				US blood donors[a]		WNV+ S versus US				
		AS (n=244)		S (n=130)		(n=3,086,215)						
		n	%	n	%	n	%	P-value	OR	Lower CL	Upper CL	FDR
All races	O	114	**46.8**	56	**43.0**	1438176	**46.6**	0.42	0.87	0.61	1.23	0.42
	A	90	**36.8**	62	**47.6**	1144986	**37.1**	***0.01***	***1.55***	***1.10***	***2.18***	***0.05***
	B	27	**11.0**	11	**8.4**	376518	**12.2**	0.20	0.67	0.36	1.23	0.24
	AB	13	**5.4**	1	**0.8**	126535	**4.1**	0.09	0.18	0.03	1.30	0.13
	Rh (D)+	212	**86.9**	102	**78.5**	2635628	**85.4**	***0.03***	***0.62***	***0.41***	***0.95***	***0.05***
	Rh(D)−	32	**13.1**	28	**21.5**	450587	**14.6**	***0.03***	***1.61***	***1.06***	***2.44***	***0.05***
	O+	97	**45.8**	48	**47.1**	1228314	**46.6**	0.93	1.02	0.69	1.50	0.97
	A+	78	**36.8**	48	**47.1**	972158	**36.9**	***0.03***	***1.52***	***1.03***	***2.24***	***0.10***
	B+	25	**11.8**	6	**5.9**	327139	**12.4**	0.05	0.44	0.19	1.01	0.10
	AB+	12	**5.7**	0	**0.0**	108018	**4.1**	0.97	N/A	N/A	N/A	0.97
	O−	17	**53.1**	8	**28.6**	212949	**47.3**	0.06	0.45	0.20	1.03	0.23
	A−	12	**37.5**	14	**50.0**	172828	**38.4**	0.20	1.62	0.77	3.41	0.32
	B−	2	**6.3**	5	**17.9**	49379	**11.0**	0.24	1.78	0.68	4.68	0.32
	AB−	1	**3.1**	1	**3.6**	18517	**4.1**	0.89	0.87	0.12	6.41	0.89
Blood Groups		**WNV+ blood donors**				**US blood donors[a]**		**WNV+ S versus US**				
		AS (n=188)		**S (n=110)**		**(n=2,215,623)**						
		n	%	n	%	n	%	**P-value**	**OR**	**Lower CL**	**Upper CL**	**FDR**
White non-Hispanic	O	87	**46.3**	47	**42.7**	1001462	**45.2**	0.60	0.90	0.62	1.32	0.60
	A	69	**36.7**	54	**49.1**	879602	**39.7**	***0.05***	***1.46***	***1.01***	***2.13***	***0.20***
	B	22	**11.7**	8	**7.3**	241503	**10.9**	0.22	0.64	0.31	1.32	0.27
	AB	10	**5.3**	1	**0.9**	90841	**4.1**	0.13	0.21	0.03	1.54	0.20
	Rh(D)+	162	**86.2**	85	**77.3**	1832320	**82.7**	0.13	0.71	0.46	1.11	0.20
	Rh(D)−	26	**13.8**	25	**22.7**	383303	**17.3**	0.13	1.41	0.90	2.20	0.20

[a] As reported by Garratty et al.[40]