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Short communication

Human CCR5Δ32 (rs333) polymorphism has no influence on severity and mortality of influenza A(H1N1)pdm09 infection in Brazilian patients from the post pandemic period

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ABSTRACT

Background: Influenza is an acute and highly contagious viral respiratory infection that causes significant morbidity and mortality. The identification of host genetic factors associated with susceptibility and severity of influenza virus infection is of paramount importance. Previous studies evaluating the potential involvement of the CCR5Δ32 polymorphism (rs333), a 32 base pair deletion in C−C motif chemokine receptor 5 (CCR5) gene, in severity and mortality of influenza A(H1N1)pdm09 infected individuals have been reported, but their results are quite conflicting.

Objectives: The aim of this study was the evaluation of the CCR5Δ32 frequency in individuals with mild, severe and fatal influenza A(H1N1)pdm09 infection and its putative association with clinical and epidemiologic data.

Patients/Methods: A total of 432 individuals were included in this study and classified according to their clinical status, into the following groups: influenza like illness (ILI) (n = 153); severe acute respiratory infection (SARI) (n = 173) and fatal (n = 106) cases. The samples were collected in the post pandemic period, from 2012 to 2018. Individuals were further stratified according to their clinical and epidemiological data. The CCR5Δ32 variant was genotyped by PCR amplification and a subset of samples was further submitted to Sanger sequencing.

Results: The different clinical groups (ILI, SARI and fatal) presented similar distribution of wt/wt and wt/Δ32 genotypes and CCR5Δ32 allele frequencies. Genotype Δ32/Δ32 was not detected in our study. Additionally, no association between wt/wt and wt/Δ32 genotypes and dyspnea, a clinical factor for influenza complications was found. Similarly, no significant differences in the distribution of wt/wt and wt/Δ32 genotypes and CCR5Δ32 variant allele frequencies were observed in samples from the different Brazilian geographical regions.

Conclusions: The CCR5Δ32 variant does not influence the susceptibility to influenza A(H1N1)pdm09 severe disease or mortality in individuals from Brazil.

1. Introduction

Influenza is an acute and contagious viral respiratory infection that affects 5–10% of adults and 20–30% of children (World Health Organization, 2012), reaching 290,000–650,000 deaths annually worldwide (Iuliano et al., 2018). Risk groups for severe disease include pregnant women, children < 5 years, the elderly and individuals with underlying health conditions (World Health Organization, 2012). Influenza viruses are classified into four types (A, B, C and D). Currently, only influenza A(H1N1)pdm09 and A(H3N2) subtypes, as well as influenza B lineages (Victoria and Yamagata), are endemic in the human population (World Health Organization, 2017). It should be noted that influenza A(H1N1)pdm09 infection is more related to infections in children and adults < 65 years adults (Barr and Cheng, 2018; Van Kerkhove et al., 2013), when compared to influenza A(H3N2) (Barr and Cheng, 2018).

Human inter-individuality in influenza infection outcome is resultant of host and viral genetic components. Viral genetic determinants

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of virulence and host immune response have been widely considered, while host determinants remain poorly understood (Ciancanelli et al., 2016; Horby et al., 2012).

The C−C motif chemokine receptor 5 (CCR5) is a member of the chemokine receptor subclass of the G-Protein-Coupled receptor superfamily protein (Oppermann, 2004). CCR5 is a functional receptor for several inflammatory CC-chemokines expressed in lymphoid organs such as the thymus and spleen, as well as in peripheral blood leukocytes, including macrophages, NK and T cells (Lee et al., 2009). A 32 bp deletion in the C−C motif chemokine receptor 5 (CCR5) gene (CCR5Δ32) at its third exon results in a truncated protein, not bearing the third intracellular loop and carboxy-terminal cytoplasmic domains, involved in G-protein coupling (Zhao et al., 1998). This deletion leads to decreased expression and dysfunction of CCR5 (Liu et al., 1996; Samson et al., 1996). CCR5Δ32 is not associated with any specific phenotype during homeostasis, although it has been associated with susceptibility to some infectious and inflammatory diseases, specially HIV infection, in which individuals caring the CCR5Δ32 allele are more resistant to infection (Baltus et al., 2016; de Souza et al., 2015; Liu et al., 1996; Pokorny et al., 2005; Samson et al., 1996; Scheibel et al., 2008; Telini et al., 2014). In contrast, CCR5Δ32 is related to susceptibility to West Nile virus (WNV) (Lim et al., 2008) and tickborne encephalitis virus (TBEV) (Kindberg et al., 2008) infection.

Studies on CCR5Δ32 and severity of influenza A(H1N1)pdm09 infection have been conflicting and inconclusive. Some studies in specific cohorts have implied the putative relationship of CCR5Δ32 allele and susceptibility for severe influenza cases (Falcon et al., 2015; Keynan et al., 2010; Rodriguez et al., 2013), while others have failed to detect any association (Maestri et al., 2015a; Sironi et al., 2014). In the present study, we evaluated the CCR5Δ32 polymorphism (rs333) in 432 individuals with mild, severe and fatal influenza A(H1N1)pdm09 infection and its putative association with clinical, epidemiological and geographical data.

2. Materials and methods

2.1. Samples and data collection

In Brazil, the Influenza Surveillance System (ISS) is present in all Brazilian States and there are 3 National Influenza Centers (NIC). Fiocruz NIC receives a subset of samples from 9 out of the 27 States. In the 2012–2018 period, approximately 25,000 influenza A(H1N1) pdm09 cases were detected in the Brazilian ISS. From them, 1804 were received at Fiocruz NIC. This study inclusion criteria was availability of an accurate patient record for the classification of influenza disease into influenza-like illness (ILI) and severe acute respiratory infection (SARI). Thus, 432 cases were included. The clinical samples are nasopharyngeal swabs or aspirates and *post mortem* specimens. ILI cases were defined as presenting fever and cough or sore throat and one of the following symptoms: headache, myalgia or arthralgia. In children < 2 years, ILI cases were defined as presenting fever and cough, coryza and nasal obstruction. SARI cases were defined as requiring hospitalization and presenting dyspnea or one of the following signs: peripheral capillary oxygen saturation < 95%, respiratory distress or acute respiratory insufficiency. Samples were aliquoted and frozen at −80 °C before testing. This study was approved by the Fiocruz-IOC Ethics Committee under the number 68118417.6.0000.5248.

2.2. Influenza diagnosis

Viral RNA was extracted using commercial kit (QIAmp Viral RNA Mini kit, Qiagen, Germany), according to the manufacturer's instructions. For influenza detection and subtyping, RNA was tested in a one-step real time RT-PCR assay with primers and probes specific for influenza (CDC, USA), according to provider's instructions.

2.3. Genotyping

DNA was extracted from clinical samples using the PureLink™ Genomic DNA Mini Kit (Invitrogen, USA) according to manufacturer's instructions. The CCR5Δ32 variant (rs333 locus) was amplified by PCR using the following primers: F – 5' CTCCCAGGAATCATCTTTACCA 3′ and R – 5' TTTTAGGATTCCCGAGTAGCA 3′ (Maestri et al., 2015a). Products were visualized in 3% agarose gels stained with SYBR Safe 1 × (Thermo Fisher Scientific, USA) under a Gel Doc™ EZ Gel Documentation System (Bio-Rad, USA). The electrophoresis genotype patterns consisted of (i) a single 178 bp product (wt/wt), or (ii) a double 178 and 146 bp product (wt/Δ32). The unique 146 bp product pattern (Δ32/Δ32) was not detected in our samples. In addition, we performed Sanger sequencing in a subset of samples ($n = 273$) to confirm CCR5 classification. Noteworthy, the electrophoresis and Sanger sequencing genotype patterns were totally concordant.

2.4. Genetic and statistical analysis

Genotype strata were classified as Δ32 allele non-carriers (wt/wt) and Δ32 allele carriers (wt/Δ32). The Hardy-Weinberg equilibrium (HWE), allele frequency and genotype distribution were assessed by using the web program (http://www.oege.org/software/hwe-mr-calc.shtml) (Rodriguez et al., 2009), and deviation from HWE was assessed by Chi-square test. Further statistical analyses were performed using SPSS for Windows, version 19.0 software (IBM Inc., USA). Contingence table statistics (Chi-square or Fisher's exact test for dichotomous variables and Krustall-Wallis test for median) were employed to explore either eventual differences in demographic features, signs and symptoms, as putative associations between CCR5Δ32 genotype and influenza clinical outcomes (ILI, SARI and fatal cases). Results were considered statistically significant when $p < .05$.

3. Results

A total of 432 influenza A(H1N1)pdm09 positive patients were included in this study, whose samples were collected in the post pandemic period, from 2012 to 2018. Of note, influenza A(H1N1)pdm09 was the most prevalent virus during Brazilian 2012, 2013, 2016 and 2018 seasons, and had an overall prevalence of > 50% from 2012 to 2018 (data from Brazilian ISS, available at http://portalms.saude.gov.br/saude-de-a-z/gripe/situacao-epidemiologica-dados).
Demographical, clinical and epidemiological characteristics are summarized in Table 1. The majority of samples accounted for patients from Southern and Southeastern regions of Brazil (48.1% and 31.5%, respectively). The remaining 20.4% of samples were from the Northeastern region (Table 1). From the 432 patients, 50.7% were male and the median age was 41 years. ILI cases were significantly younger than SARI and fatal subjects (Table 1) ($p < .001$). Moreover, the presence of any comorbidity was significantly higher among those who reported SARI or fatal cases, when compared to ILI patients (Table 1). Among SARI and fatal cases, 57.2% (99/173) and 27.4% (29/106) reported respiratory distress, and 37.0% (64/173) and 22.6% (24/106) reported oxygen saturation < 95%, respectively. However, this information was not systematically collected among influenza patients during the studied period.

In our population, CCR5Δ32 allele frequencies were in compliance with HWE, even after stratification into clinical groups ILI, SARI and fatal ($p > .05$). Putative association between CCR5Δ32 genotype and clinical and epidemiological data are shown in Tables 2 and 3. Analysis of wt/wt and wt/Δ32 genotypes and CCR5Δ32 alleles frequency in clinical groups revealed no significant difference ($p = .31$ and $p = .33$, respectively) (Table 2). Moreover, CCR5Δ32 genotypes (wt/wt and wt/Δ32) were not associated with dyspnea, a clinical sign of influenza severity (Table 3).

Furthermore, as previous analysis reported that population from the

Table 1
Demographical, clinical and epidemiological characteristics of influenza A(H1N1)pdm09 patients according to clinical severity.

Characteristics	Total	ILI	SARI	Fatal
Number of samples	432	153	173	106
Demographic features				
Gender (male, n%)	219 (50.7)	83 (54.2)	77 (44.5)	59 (55.7)
Age (years)				
Median	41	31*	46	47
IQR	32	31	31	26
Geographic region				
South	208 (48.1)	89 (58.2)	53 (29.0)	66 (62.3)
Southeast	136 (31.5)	26 (17.0)	84 (48.5)	26 (24.5)
Northeast	88 (20.4)	38 (24.8)	36 (20.8)	14 (13.2)
Signs and symptoms				
Cough	325/340 (95.6)	102/107 (95.3)	171/173 (98.8)	52/60 (86.79)
Sore Throat	125/262 (47.7)	60/98 (61.2)	55/129 (42.6)	10/35 (28.6)
Coryza	35/209 (16.7)	27/85 (31.8)	5/93 (5.4)	3/31 (9.7)
Dyspnea	206/309 (66.7)	NA	147/167 (88.0)	59/62 (95.2)
Comorbidities	114/221 (51.6)	14/56 (25.0)*	78/127 (61.4)	22/38 (57.9)

ILI – influenza like illness; SARI – severe acute respiratory infection; n = number of cases with the presented characteristic; N = number of cases with the available information; NA – non-applicable. Statistical significance was considered when *P*-value < .05 and was assessed by Chi-square test for dichotomous variables Krustall-Wallis test for median. *$p < .001$.

Table 2
CCR5Δ32 genotype distribution and allele frequency in influenza A(H1N1) pdm09 patients analyzed in the study, and according to their classification based on the clinical status of disease.

Genotype	ILI	SARI	Fatal	*p*-value
wt/wt	141	152	98	0.31
wt/Δ32	12	21	8	
Δ32/Δ32	0	0	0	
Δ32 allele frequency	0.04	0.06	0.04	0.33

ILI – influenza like illness; SARI – severe acute respiratory infection.

Table 3
CCR5Δ32 genotype distribution, according to presence of dyspnea and Brazilian geographical regions.

Variable	wt/wt	wt/Δ32	*p*-value	Δ32 allele frequency
Dispnea – n/N (%)	185/278 (66.5%)	21/31 (67.7%)	0.89	–
Geographic region				
South	184 (88.5%)	24 (11.5%)	0.25	0.06
Southeast	124 (91.2%)	12 (8.8%)		0.04
Northeast	83 (94.3%)	5 (5.7%)		0.03

n = number of cases with the presented characteristic; N = number of cases with the available information.

Brazilian South and Southeastern regions presented a higher CCR5Δ32 allele frequency than those found in the North and Northeastern regions (Silva-Carvalho et al., 2016), the respective distribution was explored in our study population. Similar CCR5Δ32 allele frequencies were found in the assessed Brazilian geographical regions: 6.0% in South, 4.0% in the Southeast and 3.0% in the Northeast ($p = .25$) (Table 3).

4. Discussion

The role of CCR5Δ32 allele in infectious diseases has been investigated since its association with protection to HIV infection (Liu et al., 1996; Samson et al., 1996). During this infection, CCR5 functions as a co-receptor for the virus entry in macrophages and CCR5Δ32 homozygotes are clearly less susceptible to this infection (Dean et al., 1996; Ruiz-Mateos et al., 2018). Therefore, many studies have been conducted to examine CCR5Δ32 relationship with infection diseases. In WNV and TBEV flaviviruses infections, CCR5 responses are related to leukocytes trafficking to the brain and viral clearance (Glass et al., 2006), and CCR5Δ32 homozygotes are more susceptible to their infection and/or severity (Glass et al., 2006; Kindberg et al., 2008; Mickienė et al., 2014). For hepatitis infections, in which CCR5 signaling is important for the immune response against this virus (Ahlenstiel et al., 2004; Coenen and Nattermann, 2010), CCR5Δ32 has no influence in hepatitis viruses infection susceptibility (Ellwanger et al., 2018; Glas et al., 2003; Khorramdelazad et al., 2013; Ruiz-Ferrer et al., 2004). In influenza infections, the functional role of CCR5 in human immune and inflammatory responses has been related to NK cells recruitment to lung airways and epithelium (Carlin et al., 2018), as well as localization of memory CD8 T cells to the epithelium (Kohlmeier et al., 2008). Also, studies with influenza A infected Ccr5−/− mice showed higher accumulation of macrophages in the lungs and increased mortality (Dawson et al., 2000). Previous reports on CCR5Δ32 and influenza A(H1N1)pdm09 infection severity have been conflicting so far.

In the present study, we report the CCR5Δ32 allele frequency in Brazilian individuals infected with influenza A(H1N1)pdm09 viruses, from 2012 to 2018. Individuals are from nine Brazilian States, which have a population of approximately 90 million people. The majority of the samples were collected from Southern and Southeastern Brazilian regions, which report most of Brazilian influenza A(H1N1)pdm09 cases (Brazilian MoH, 2017). Also, we observed a relationship between age and severity of disease, as SARI and fatal cases presented higher median ages. This finding is consistent with the literature in showing that elderly people usually have more influenza complications, hospitalization or death, because they have more comorbidities and are more prone to have secondary bacterial infections (Brazilian MoH, 2017; New South Wales, 2017).

In this report, CCR5Δ32 allele was not associated with disease clinical strata (ILI, SARI and fatal) or signs of disease severity, as in a previous study involving Brazilian influenza A(H1N1)pdm09 infected subjects, in which no relationship between CCR5Δ32 and hospitalization was reported (Maestri et al., 2015a). Importantly, this previous Brazilian study was performed in samples collected in the pandemic period, from Brazil's North and Northeast geographical regions, whereas we investigated samples from the post pandemic period and mostly from Southern and Southeastern regions. Despite differences in analyses and stratification criteria, both studies found complementary outcomes. Also in line with our findings, no relationship between CCR5Δ32 and disease severity was reported in patients infected during the pandemic period in the Southern Europe, mainly Italians (Sironi et al., 2014). As the frequency of CCR5Δ32 heterozygotes in severe and fatal influenza cases was similar to heterozygotes frequency in mild cases, these data suggest that an intermediate amount of CCR5, as only one gene copy is disrupted, could be sufficient for response mechanisms in influenza infection.

As opposed to our findings, analysis from a small cohort of Canadian patients with severe influenza A(H1N1)pdm09 infection showed higher frequency of CCR5Δ32 heterozygotes (55.5%), when compared to Caucasian overall population (10–15%) (Keynan et al., 2010). Besides, a report detected CCR5Δ32 homozygosity in a Spanish fatal influenza A(H1N1)pdm09 case (Rodriguez et al., 2013). Also, a study with Spanish influenza A(H1N1)pdm09 infected patients showed CCR5Δ32 association with mortality (Falcon et al., 2015). Noteworthy, these studies are limited by adopting different criteria for disease severity definition and, except for the former, by sample size.CCR5Δ32 allelic overall frequency in our cohort (5.0%) is in accordance with a systematic review and meta-analysis with samples throughout the country, which showed an allelic frequency of 4.0% in Brazil (Silva-Carvalho

et al., 2016). However, differently from the slightly higher CCR5Δ32 allelic frequency reported by them in South and Southeast, when compared to North and Northeast, no regional patterns could be perceived in our samples. CCR5Δ32 distribution can depend on the region and genetic ancestry. Higher allele frequencies have been detected in European (Hütter et al., 2015; Martinson et al., 1997; Su et al., 2000) than in African and Asian countries (Rahimi et al., 2014; Salem et al., 2009; Su et al., 2000). In the past, Brazilian South and Southeast regions sheltered immigrants mainly from Europe. In contrast, in Brazil's Northeast, especially in Bahia state, Africa played a stronger influence in genetic population patterns (Alves-Silva et al., 2000; Kehdy et al., 2015). However more recent migratory patterns, in which Northern and Northeastern populations migrated towards South and Southeast regions (Alves-Silva et al., 2000), may have contributed to the dilution of CCR5Δ32 allele frequency among Brazilian populations.

In conclusion, in our study with Brazilian individuals, CCR5Δ32 allele did not influence the susceptibility to influenza severe disease or mortality. Also, our work presents the largest number of studied influenza A(H1N1)pdm09 cases and CCR5Δ32 status and represents a country, Brazil, with a lot of genetic ancestry miscegenation. Nevertheless, comprehensive multicentric studies, including larger sample size, are necessary to fully address this issue. As prioritized by the WHO (World Health Organization (WHO), 2017), the identification of host genetic factors on influenza infection is of great importance for a better understanding of the biological host predispositions and the mechanisms associated with the development of severe influenza. Some interesting candidates have been identified in studies performed with Brazilian population, such as polymorphisms in phosphatidyl inositol 3 Kinase-gamma (PI3Kγ) (Garcia et al., 2018) and ST3 beta-galactosidase alpha–2,3-sialyltransferase 1 (ST3GAL1) genes (Maestri et al., 2015b).

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References

Ahlenstiel, G., Woitas, R.P., Rockstroh, J., Spengler, U., 2004. CC-chemokine receptor 5 (CCR5) in hepatitis C – at the crossroads of the antiviral immune response? J. Antimicrob. Chemother. 53, 895–898. https://doi.org/10.1093/jac/dkh239.

Alves-Silva, J., da Silva Santos, M., Guimarães, P.E., Ferreira, A.C., Bandelt, H.J., Pena, S.D., Prado, V.F., 2000. The ancestry of Brazilian mtDNA lineages. Am. J. Hum. Genet. 67, 444–461. https://doi.org/10.1086/303004.

Baltus, T.H.L., Kallaur, A.P., Lozovoy, M.A.B., Morimoto, H.K., Delongui, F., Alfieri, D.F., Iriyoda, T.M.V., Dichi, I., Simão, A.N.C., Reiche, E.M.V., 2016. CCR5Δ32 (rs333) polymorphism is associated with the susceptibility to systemic lupus erythematosus in female Brazilian patients. Rheumatol. Int. 36, 7–15. https://doi.org/10.1007/s00296-015-3308-z.

Barr, I.G., Cheng, A.C., 2018. Difficulties of predicting the timing, size and severity of influenza seasons. Respirology 23, 562–563. https://doi.org/10.1111/resp.13310.

Brazilian MoH, 2017. Informe Epidemiológico Secretaria de Vigilância em Saúde – Ministério da Saúde Influenza: Monitoramento até a Semana Epidemiológica 50 de 2017. Available at. http://portalarquivos2.saude.gov.br/images/pdf/2017/dezembro/27/Informe-Epidemiologico-Influenza-2017-SE-50.pdf.

Carlin, L.E., Hemann, E.A., Zacharias, Z.R., Heusel, J.W., Whitmire, J.K., Mckinstry, K.K., Legge, K.L., 2018. Natural killer cell recruitment to the lung during influenza a virus infection is dependent on CXCR3, CCR5, and virus exposure dose. Front. Immunol. 9, 1–14. https://doi.org/10.3389/fimmu.2018.00781.

Ciancanelli, M.J., Abel, L., Zhang, S.-Y., Casanova, J.-L., 2016. Host genetics of severe influenza: from mouse Mx1 to human IRF7. Curr. Opin. Immunol. 38, 109–120. https://doi.org/10.1016/j.coi.2015.12.002.

Coenen, M., Nattermann, J., 2010. The role of CCR5 in HCV infection. Eur. J. Med. Res. 15, 97–101. https://doi.org/10.1186/2047-783X-15-3-97.

Dawson, T.C., Beck, M.A., Kuziel, W.A., Henderson, F., Maeda, N., 2000. Contrasting effects of CCR5 and CCR2 deficiency in the pulmonary inflammatory response to influenza a virus. Am. J. Pathol. 156, 1951–1959. https://doi.org/10.1016/S0002-9440(10)65068-7.

Dean, M., Carrington, M., Winkler, C., Huttley, G.A., Smith, M.W., Allikmets, R., Goedert, J.J., Buchbinder, S.P., Vittinghoff, E., Gomperts, E., Donfield, S., Vlahov, D., Kaslow, R., Saah, A., Rinaldo, C., Detels, R., O'Brien, S.J., 1996. Genetic restriction of HIV-1 infection and progression to AIDS by a deletion allele of the CKR5 structural gene. Hemophilia Growth and Development Study, Multicenter AIDS Cohort Study, Multicenter Hemophilia Cohort Study, San Francisco City Cohort, ALIVE Study. Science 273, 1856–1862.

Ellwanger, J.H., Leal, B.K., Valverde-Villegas, J.M., Simon, D., Marangon, C.G., Mattevi, V.S., Lazzaretti, R.K., Sprinz, E., Kuhmmer, R., Chies, J.A.B., 2018. CCR5Δ32 in HCV infection, HCV/HIV co-infection, and HCV-related diseases. Infect. Genet. Evol. 59, 163–166. https://doi.org/10.1016/j.meegid.2018.02.002.

Falcon, A., Cuevas, M.T., Rodriguez-Frandsen, A., Reyes, N., Pozo, F., Moreno, S., Ledesma, J., Martínez-Alarcón, J., Nieto, A., Casas, I., 2015. CCR5 deficiency predisposes to fatal outcome in influenza virus infection. J. Gen. Virol 96, 2074–2078. https://doi.org/10.1099/vir.0.000165.

Garcia, C.C., Tavares, L.P., Dias, A.C.F., Kehdy, F., Alvarado-Arnez, L.E., Queiroz-Junior, C.M., Lima, B.H., Matos, A.R., Soriani, F.M., Moraes, M.O., Siqueira, M.M., Machado, A.M.V., Russo, R.C., Teixeira, M.M., 2018. Phosphatidyl Inositol 3 kinase-gamma balances antiviral and inflammatory responses during influenza a H1N1 Infection: from murine model to genetic association in patients. Front. Immunol. 9, 975. https://doi.org/10.3389/fimmu.2018.00975.

Glas, J., Török, H.P., Simperl, C., König, A., Martin, K., Schmidt, F., Schaefer, M., Schiemann, U., Folwaczny, C., 2003. The Delta 32 mutation of the chemokine-receptor 5 gene neither is correlated with chronic hepatitis C nor does it predict response to therapy with interferon-alpha and ribavirin. Clin. Immunol. 108, 46–50.

Glass, W.G., McDermott, D.H., Lim, J.K., Lekhong, S., Yu, S.F., Frank, W.A., Pape, J., Cheshier, R.C., Murphy, P.M., 2006. CCR5 deficiency increases risk of symptomatic West Nile virus infection. J. Exp. Med. 203, 35–40. https://doi.org/10.1084/jem.20051970.

Horby, P., Nguyen, N.Y., Dunstan, S.J., Baillie, J.K., 2012. The role of host genetics in susceptibility to influenza: a systematic review. PLoS One 7, e33180. https://doi.org/10.1371/journal.pone.0033180.

Hütter, G., Blüthgen, C., Elvers-Hornung, S., Klüter, H., Bugert, P., 2015. Distribution of the CCR5-delta32 deletion in Southwest Germany. Anthropol. Anz. 72, 303–309. https://doi.org/10.1127/anthranz/2015/0479.

Iuliano, A.D., Roguski, K.M., Chang, H.H., Muscatello, D.J., Palekar, R., Tempia, S., Cohen, C., Gran, J.M., Schanzer, D., Cowling, B.J., Wu, P., Kyncl, J., Ang, L.W., Park, M., Redlberger-Fritz, M., Yu, H., Espenhain, L., Krishnan, A., Emukule, G., van Asten, L., Pereira da Silva, S., Aungkulanon, S., Buchholz, U., Widdowson, M.-A., Bresee, J.S., Global Seasonal Influenza-Associated Mortality Collaborator Network, E,Cheng, P.-Y., Dawood, F., Foppa, I., Olsen, S., Haber, M., Jeffers, C., MacIntyre, C.R., Newall, A.T., Wood, J.G., Kundi, M., Popow-Kraupp, T., Ahmed, M., Rahman, M., Marinho, F., Proschle, C.V.S., Mallegas, N.V., Luzhao, F., Sa, L., Barbosa-Ramírez, J., Sanchez, D.M., Gomez, L.A., Vargas, X.B., Herrera, aBetsy A., Llanés, M.J., Fischer, T.K., Krause, T.G., Mølbak, K., Nielsen, J., Trebbien, R., Bruno, A., Ojeda, J., Ramos, H., Heiden, M. an der, Signor, L. del C.C., Serrano, C.E., Bhardwaj, R., Chadha, M., Narayan, V., Kosen, S., Bromberg, M., Glatman-Freedman, A., Kaufman, Z., Arima, Y., Oishi, K., Chaves, S., Nyawanda, B., Al-Jarallah, R.A., Kuri-Morales, P.A., Matus, C.R., Corona, M.E.J., Burmaa, A., Darmaa, O., Obtel, M., Cherkaoui, I., Wijngaard, C.C. van den, Hoek, W. van der, Baker, M., Bandaranayake, D., Bissielo, A., Huang, S., Lopez, L., Newbern, C., Flem, E., Grøneng, G.M., Hauge, S., Cosío, F.G. de, Moltó, Y. de, Castillo, L.M., Cabello, M.A., Horoch, M. von, Osis, J.M., Machado, A., Nunes, B., Rodrigues, A.P., Rodrigues, E., Calomfirescu, C., Lupulescu, E., Popescu, R., Popovici, O., Bogdanovic, D., Kostic, M., Lazarevic, K., Milosevic, Z., Tiodorovic, B., Chen, M., Cutter, J., Lee, V., Lin, R., Ma, S., Cohen, A.L., Treurnicht, F., Kim, W.J., Delgado-Sanz, C., Ontañón, S. de mateo, Larrauri, A., León, I.L., Vallejo, F., Born, R., Junker, C., Koch, D., Chuang, J.-H., Huang, W.-T., Kuo, H.-W., Tsai, Y.-C., Bundhamcharoen, K., Chittaganpitch, M., Green, H.K., Pebody, R., Goñi, N., Chiparelli, H., Brammer, L., Mustaquim, D., 2018. Estimates of global seasonal influenza-associated respiratory mortality: a modelling study. Lancet (London, England) 391, 1285–1300. https://doi.org/10.1016/S0140-6736(17)33293-2.

Kehdy, F.S.G., Gouveia, M.H., Machado, M., Magalhães, W.C.S., Horimoto, A.R., Horta, B.L., Moreira, R.G., Leal, T.P., Scliar, M.O., Soares-Souza, G.B., Rodrigues-Soares, F., Araújo, G.S., Zamudio, R., Sant Anna, H.P., Santos, H.C., Duarte, N.E., Fiaccone, R.L., Figueiredo, C.A., Silva, T.M., Costa, G.N.O., Beleza, S., Berg, D.E., Cabrera, L., Debortoli, G., Duarte, D., Ghirotto, S., Gilman, R.H., Gonçalves, V.F., Marrero, A.R., Muniz, Y.C., Weissensteiner, H., Yeager, M., Rodrigues, L.C., Barreto, M.L., Lima-Costa, M.F., Pereira, A.C., Rodrigues, M.R., Tarazona-Santos, E., Brazilian EPIGEN Project Consortium, T.B.E.P, 2015. Origin and dynamics of admixture in Brazilians and its effect on the pattern of deleterious mutations. Proc. Natl. Acad. Sci. U. S. A. 112, 8696–8701. https://doi.org/10.1073/pnas.1504447112.

Keynan, Y., Juno, J., Meyers, A., Ball, T.B., Kumar, A., Rubinstein, E., Fowke, K.R., 2010. Chemokine receptor 5 Δ32 allele in patients with severe pandemic (H1N1) 2009. Emerg. Infect. Dis. 16, 1621–1622. https://doi.org/10.3201/eid1610.100108.

Khorramdelazad, H., Hakimizadeh, E., Hassanshahi, G., Rezayati, M., Sendi, H., Arababadi, M.K., 2013. CCR5 Δ 32 mutation is not prevalent in Iranians with chronic HBV infection. J. Med. Virol. 85, 964–968. https://doi.org/10.1002/jmv.23510.

Kindberg, E., Mickienė, A., Ax, C., Åkerlind, B., Vene, S., Lindquist, L., Lundkvist, Å., Svensson, L., 2008. A deletion in the chemokine receptor 5 *(CCR5)* gene is associated with tickborne encephalitis. J. Infect. Dis. 197, 266–269. https://doi.org/10.1086/524709.

Kohlmeier, J.E., Miller, S.C., Smith, J., Lu, B., Gerard, C., Cookenham, T., Roberts, A.D., Woodland, D.L., 2008. The chemokine receptor CCR5 plays a key role in the early memory CD8+ T cell response to respiratory virus infections. Immunity 29, 101–113. https://doi.org/10.1016/j.immuni.2008.05.011.

Lee, B., Doranz, B.J., Ratajczak, M.Z., Doms, R.W., 2009. An intricate web: chemokine receptors, HIV-1 and hematopoiesis. Stem Cells. https://doi.org/10.1002/stem.160079.

Lim, J.K.K., Louie, C.Y.Y., Glaser, C., Jean, C., Johnson, B., Johnson, H., McDermott, D.H.H., Murphy, P.M.M., 2008. Genetic deficiency of chemokine receptor CCR5 is a strong risk factor for symptomatic West Nile virus infection: a meta-analysis of 4 cohorts in the US epidemic. J. Infect. Dis. 197, 262–265. https://doi.org/10.1086/524691.

Liu, R., Paxton, W.A., Choe, S., Ceradini, D., Martin, S.R., Horuk, R., MacDonald, M.E., Stuhlmann, H., Koup, R.A., Landau, N.R., 1996. Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. Cell 86, 367–377. https://doi.org/10.1016/S0092-8674(00)80110-5.

Maestri, A., dos Santos, M.C., Ribeiro-Rodrigues, E.M., de Mello, W.A., Sousa, R.C.M., Dos Santos, S.E., Sortica, V.A., 2015a. The CCR5Δ32 (rs333) polymorphism is not a predisposing factor for severe pandemic influenza in the Brazilian admixed population. BMC Res. Notes 8, 1–5. https://doi.org/10.1186/s13104-015-1299-1.

Maestri, A., Sortica, V.A., Tovo-Rodrigues, L., Santos, M.C., Barbagelata, L., Moraes, M.R., Alencar de Mello, W., Gusmao, L., Sousa, R.C., Emanuel Batista Dos Santos, S., 2015b. Siaalpha2-3Galbeta1-receptor genetic variants are associated with influenza A(H1N1)pdm09 Severity. PLoS One 10, e0139681. https://doi.org/10.1371/journal.pone.0139681.

Martinson, J.J., Chapman, N.H., Rees, D.C., Liu, Y.-T., Clegg, J.B., 1997. Global distribution of the CCR5 gene 32-basepair deletion. Nat. Genet. 16, 100–103. https://doi.org/10.1038/ng0597-100.

Mickienė, A., Pakalnienė, J., Nordgren, J., Carlsson, B., Hagbom, M., Svensson, L., Lindquist, L., 2014. Polymorphisms in chemokine receptor 5 and Toll-like receptor 3 genes are risk factors for clinical tick-borne encephalitis in the Lithuanian population. PLoS One 9, e106798. https://doi.org/10.1371/journal.pone.0106798.

New South Wales, 2017. Influenza Monthly Epidemiology Report. Available at. https://www.health.nsw.gov.au/Infectious/Influenza/Publications/2017/december-flu-report.pdf.

Oppermann, M., 2004. Chemokine receptor CCR5: insights into structure, function, and regulation. Cell. Signal. 16, 1201–1210. https://doi.org/10.1016/J.CELLSIG.2004.04.007.

Pokorny, V., McQueen, F., Yeoman, S., Merriman, M., Merriman, A., Harrison, A., Highton, J., McLean, L., 2005. Evidence for negative association of the chemokine receptor CCR5 d32 polymorphism with rheumatoid arthritis. Ann. Rheum. Dis. 64, 487–490. https://doi.org/10.1136/ard.2004.023333.

Rahimi, H., Farajollahi, M.M., Hosseini, A., 2014. Distribution of the mutated delta 32 allele of CCR5 co-receptor gene in Iranian population. Med. J. Islam Repub. Iran. 28, 140.

Rodriguez, S., Gaunt, T.R., Day, I.N.M., 2009. Hardy-Weinberg equilibrium testing of biological ascertainment for Mendelian randomization studies. Am. J. Epidemiol. 169, 505–514. https://doi.org/10.1093/aje/kwn359.

Rodriguez, A., Falcon, A., Cuevas, M.T., Pozo, F., Guerra, S., García-Barreno, B., Martinez-Orellana, P., Pérez-Breña, P., Montoya, M., Melero, J.A., Pizarro, M., Ortin, J., Casas, I., Nieto, A., 2013. Characterization in vitro and in vivo of a pandemic H1N1 influenza virus from a fatal case. PLoS One 8. https://doi.org/10.1371/journal.pone.0053515.

Ruiz-Ferrer, M., Barroso, N., Antiñolo, G., Aguilar-Reina, J., 2004. Analysis of CCR5-Delta 32 and CCR2-V64I polymorphisms in a cohort of Spanish HCV patients using real-time polymerase chain reaction and fluorescence resonance energy transfer technologies. J. Viral Hepat. 11, 319–323. https://doi.org/10.1111/j.1365-2893.2004.00510.x.

Ruiz-Mateos, E., Tarancon-Diez, L., Alvarez-Rios, A.I., Dominguez-Molina, B., Genebat, M., Pulido, I., Abad, M.A., Muñoz-Fernandez, M.A., Leal, M., 2018. Association of heterozygous CCR5Δ32 deletion with survival in HIV-infection: a cohort study. Antivir. Res. 150, 15–19. https://doi.org/10.1016/j.antiviral.2017.12.002.

Salem, A.H., Farid, E., Fadel, R., Abu-Hijleh, M., Almawi, W., Han, K., Batzer, M.A., 2009. Distribution of four HIV type 1-resistance polymorphisms (*CCR5 -Δ 32*, *CCR5-m303*, *CCR2-64I,* and *SDF1-3′A*) in the Bahraini population. AIDS Res. Hum. Retrovir. 25, 973–977. https://doi.org/10.1089/aid.2009.0066.

Samson, M., Libert, F., Doranz, B.J., Rucker, J., Liesnard, C., Farber, C.-M., Saragosti, S., Lapouméroulie, C., Cognaux, J., Forceille, C., Muyldermans, G., Verhofstede, C., Burtonboy, G., Georges, M., Imai, T., Rana, S., Yi, Y., Smyth, R.J., Collman, R.G., Doms, R.W., Vassart, G., Parmentier, M., 1996. Resistance to HIV-1 infection in Caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene. Nature 382, 722–725. https://doi.org/10.1038/382722a0.

Scheibel, I., Veit, T., Neves, A.G., Souza, L., Prezzi, S., Machado, S., Kohem, C., Icarelli, M., Xavier, R., Brenol, J.C., Chies, J.A.B., 2008. Differential CCR5Δ32 allelic frequencies in juvenile idiopathic arthritis subtypes: evidence for different regulatory roles of CCR5 in rheumatological diseases. Scand. J. Rheumatol. 37, 13–17. https://doi.org/10.1080/03009740701631935.

Silva-Carvalho, W.H.V., de Moura, R.R., Coelho, A.V.C., Crovella, S., Guimarães, R.L., 2016. Frequency of the CCR5-delta32 allele in Brazilian populations: a systematic literature review and meta-analysis. Infect. Genet. Evol. 43, 101–107. https://doi.org/10.1016/j.meegid.2016.05.024.

Sironi, M., Cagliani, R., Pontremoli, C., Rossi, M., Migliorino, G., Clerici, M., Gori, A., 2014. The CCR5Δ32 allele is not a major predisposing factor for severe H1N1pdm09 infection. BMC Res. Notes 7, 32–35. https://doi.org/10.1186/1756-0500-7-504.

de Souza, M., Do, S.Q.A., de Souza, C.A., Cunha, L.M.P., de Souza, A.Q.A., de Morais, M.S., Rabenhorst, S.H.B., 2015. A new look at osteomyelitis development–focus on CCR5delta32. Study in patients from Northeast Brazil. Infect. Genet. Evol 31, 61–63. https://doi.org/10.1016/j.meegid.2015.01.012.

Su, B., Sun, G., Lu, D., Xiao, J., Chakraborty, R., Deka, R., Jin, L., 2000. Distribution of three HIV-1 resistance-conferring polymorphisms (SDF1-3′A, CCR2-64I, and CCR5-Δ32) in global populations. Eur. J. Hum. Genet. 8, 975–979.

Telini, B., Veit, T.D., Chies, J.A.B., Vianna, P., 2014. The CCR5Δ32 polymorphism as a pre-eclampsia susceptibility marker: an evaluation in Brazilian women. Arch. Gynecol. Obstet. 290, 1–3. https://doi.org/10.1007/s00404-014-3246-0.

Van Kerkhove, M.D., Hirve, S., Koukounari, A., Mounts, A.W., H1N1pdm serology working group, for the H. serology working, 2013. Estimating age-specific cumulative incidence for the 2009 influenza pandemic: a meta-analysis of A(H1N1)pdm09 serological studies from 19 countries. Influenza Other Respi. Viruses 7, 872–886. https://doi.org/10.1111/irv.12074.

World Health Organization, 2012. Vaccines against influenza WHo position paper – November 2012. Wkly Epidemiol. Rec. 87, 461–476. https://doi.org/10.1016/j.actatropica.2012.04.013.

World Health Organization, 2017. Review of global influenza activity, October 2016–October 2017. Wkly Epidemiol. Rec. 92, 761–780.

World Health Organization (WHO), 2017. WHO public health research agenda for influenza: 2017 update.

Zhao, J., Ma, L., Wu, Y.L., Wang, P., Hu, W., Pei, G., 1998. Chemokine receptor CCR5 functionally couples to inhibitory G proteins and undergoes desensitization. J. Cell. Biochem. 71, 36–45. https://doi.org/10.1002/(SICI)1097-4644(19981001)71:1<36::AID-JCB4>3.0.CO;2-2.