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The *CCR5* $\Delta 32$ (rs333) polymorphism is not a predisposing factor for severe pandemic influenza in the Brazilian admixed population

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Abstract

Background: Recent studies have tried to identify host genetic variants that could explain severe cases and deaths in infection with Influenza A(H1N1)pdm09, especially among children and young adults. *CCR5* is a chemokine receptor expressed on T cells, macrophages and dendritic cells, which is an important mediator of leukocyte chemotaxis during the immune response. A deletion mutation ($\Delta 32$) in this gene interferes with the response of immune cells, impairing viral clearance. We evaluated the *CCR5* $\Delta 32$ polymorphism (rs333) in individuals of the Brazilian admixed population with a diagnosis of Influenza A(H1N1)pdm09 infection.

Methods: A total of 330 subjects with a diagnosis of Influenza A(H1N1)pdm09, evaluated at health services in the northern and northeastern regions of Brazil between June 2009 and August 2010, were genotyped for the $\Delta 32$ deletion (rs333). The cases were classified according to the progression of infection into a group of hospitalized patients (n = 156) and a group of non-hospitalized patients (n = 174).

Results: No significant differences in the allele or genotype frequencies of the *CCR5* $\Delta 32$ polymorphism were observed between non-hospitalized and hospitalized patients (p = 0.289 and p = 0.431, respectively).

Conclusion: The $\Delta 32$ deletion in the *CCR5* gene is not associated with an unfavorable outcome in patients infected with Influenza A(H1N1)pdm09 in the Brazilian admixed population.

Keywords: A(H1N1)pdm09 infection, Influenza, *CCR5* $\Delta 32$

Background

On April 21, 2009 [1], the Centers for Disease Control and Prevention (CDC) announced two flu cases in children from California, USA, caused by a new influenza strain originated by the quadruple reassortment between other already circulating influenza viruses [2]. The new viral subtype spread around the world, a fact that culminated in the announcement by the World Health Organization (WHO) on June 11, 2009, of the first flu pandemic in the 21st century [3]. The lethality of the new viral strain did not add to the flu-related death statistics;

however, the large number of severe cases and deaths among children and young adults called the attention of the scientific community [4].

Recent studies have tried to identify host genetic variants that could explain severe cases of the disease [5]. On the basis of the cycle of viral replication of the pandemic strain in human cells, genetic variants that could influence viral clearance [6] have been identified. One genetic variant is a 32-bp deletion in the *CCR5* gene ($\Delta 32$) [7].

CCR5 is a chemokine receptor expressed on T cells, macrophages and dendritic cells, which is an important mediator of leukocyte chemotaxis during the response to chemokines. The interaction of this receptor with its ligands results in the homing of different immune cells to the sites of viral infection on the mucosal surface. Studies have shown that the $\Delta 32$ deletion in the *CCR5* gene

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interferes with the response of immune cells through CCL3, CCL4 and CCL5, impairing viral clearance [8–10]. In a population of 20 Canadian patients who developed severe forms of infection with the pandemic flu virus, the mutation at position 32 of the CCR5 gene (rs333) was detected in five patients and was associated with an unfavorable clinical evolution [7]. However, the same mutation evaluated in 29 Italian patients also infected with the pandemic strain was not associated with poor clinical outcome [11]. In the present study, the *CCR5*Δ32 polymorphism (rs333) was investigated in individuals from a Brazilian admixed population with a diagnosis of Influenza A(H1N1)pdm09 infection.

Methods

Population

Between June 2009 and August 2010, the Virology Section of the Evandro Chagas Institute (Seção de Virologia do Instituto Evandro Chagas—SEVIR/IEC) received 5,427 nasal swab or nasopharyngeal aspirate samples from subjects with a clinical suspicion of flu-like illness who sought health services in the states of the northern and northeastern regions of Brazil. Of these, 1,524 samples were positive for the pandemic strain and 330 samples with a diagnosis of Influenza A(H1N1)pdm09 were randomly included in the study. All patients enrolled in the study provided their written informed consent. Underage participants (younger than 18 years $n = 115$) had the informed consents signed by parents to participate in the study. The study was approved by the Ethics Committee of the Center of Tropical Medicine, Federal University of Pará (Núcleo de Medicina Tropical, Universidade Federal do Pará).

Laboratory diagnosis

Diagnostic confirmation was performed at the Laboratory of Respiratory Viruses, SEVIR/IEC, Ananindeua, Pará, using the SuperScript III™ One-Step qRT-PCR System with Platinum Taq® (Invitrogen Life Technologies, Carlsbad, CA, USA) according to the protocol recommended by the CDC [12].

Genotyping of *CCR5*Δ32 (rs333)

Genomic DNA was extracted from the leukocyte aggregate found in the nasal aspirate or nasopharyngeal swab using the QIAamp DNA Mini Kit (Qiagen, Valencia, CA, USA) according to manufacturer instructions. All DNA samples were genotyped by PCR. The primers (forward: CTCCCAGGAATCATCTTTACCA and reverse: TTTT-TAGGATTCCTCGAGTAGCA) were designed using the Primer3 software (<http://www.genome.wi.mit.edu/cgi-bin/primer/primer3>) and tested with the AutoDimer software.

PCR was carried out in a final volume of 12.5 μL containing PCR buffer 1 with 3 mM of MgCl₂, 125 mM of each dNTP, 2 U Platinum AmpliTaq DNA polymerase (Invitrogen Life Technologies, Carlsbad, CA, USA) and 10–20 ng genomic DNA. The PCR conditions were: 11 min at 95°C; 10 cycles of 1 min at 94°C, 1 min at 60°C and 2 min at 70°C; 17 cycles of 1 min at 90°C, 1 min at 60°C and 2 min at 70°C, and a final extension of 60 min at 60°C. For capillary electrophoresis, 1 mL of the PCR product was added to 8.5 mL Hi-Di formamide (Applied Biosystems, Foster City, CA, USA) and 0.5 mL GeneScan 500 LIZ Size Standard (Applied Biosystems, Foster City, CA, USA). The amplicons were separated in an ABI Prism 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) and analyzed using the v3.2 GeneMapper ID software (Applied Biosystems, Foster City, CA, USA).

Population substructure

The proportions of African, European and Amerindian genetic ancestry of the infected patients were estimated using a panel of 48 ancestry-informative markers as described previously [13].

Statistical analysis

Allele and genotype frequencies were estimated by direct counting. Deviation from Hardy–Weinberg equilibrium was verified by Chi squared analysis. Differences in quantitative and qualitative characteristics between the groups of hospitalized and non-hospitalized patients were evaluated using the Student *t* test, Chi squared test and Fisher's exact test. Differences in the proportions of genetic ancestry between groups were determined using the Wilcoxon–Mann–Whitney test. Fisher's exact test was used to analyze differences in the allele and genotype frequencies of the deletion studied between the groups of patients. Logistic regression was used to assess the effect of the *CCR5* polymorphism on the severity of Influenza A(H1N1)pdm09 infection. European and African genetic ancestry and comorbidities (dichotomous variable, presence or not of comorbidity) were included in the model as confounders, as they presented differences between patients groups. Statistical analysis was performed using the SPSS 18.0 software, adopting a level of significance of $p < 0.05$.

Results

The 330 patients included in the study exhibited the classical clinical symptoms of the disease and were divided according to the progression of infection into two groups: patients that presented severe acute respiratory syndrome (SARS) and were hospitalized ($n = 156$) following Brazilian Ministry of Health protocol [14] and

patients with mild symptoms non-hospitalized (n = 174) (Table 1). There was a predominance of women among the patients studied (61.5%) and the mean patient age

Table 1 Epidemiological and clinical characteristics of hospitalized and non-hospitalized patients infected with Influenza A(H1N1)pdm09

| Characteristics | All patients | Non-hospitalized | Hospitalized | p value |
|----------------------------------|--------------|------------------|--------------|---------|
| N | 330 | 174 | 156 | |
| Female sex | 203 (61.5) | 102 (58.6) | 101 (64.7) | 0.254 |
| Age (years) | 24.7 ± 15.3 | 23.8 ± 14.2 | 25.8 ± 16.5 | 0.270 |
| Pregnant | 47 (31.8) | 19 (26.0) | 28 (37.3) | 0.140 |
| Smoking | 18 (5.5) | 6 (3.4) | 12 (7.7) | 0.090 |
| Signs and symptoms | | | | |
| Fever | 320 (97.0) | 167 (96.0) | 153 (98.1) | 0.267 |
| Cough | 311 (94.2) | 161 (92.5) | 150 (96.2) | 0.158 |
| Shortness of breath | 245 (74.2) | 101 (58.0) | 144 (92.3) | <0.001 |
| Muscle aches | 212 (64.2) | 117 (67.2) | 95 (60.9) | 0.230 |
| Rhinorrhea | 197 (59.7) | 113 (64.9) | 84 (53.8) | 0.040 |
| Sore throat | 192 (58.2) | 107 (61.5) | 85 (54.5) | 0.198 |
| Chills | 138 (41.8) | 72 (41.4) | 66 (42.3) | 0.864 |
| Joint pain | 118 (35.8) | 64 (36.8) | 54 (34.6) | 0.682 |
| Headache | 63 (19.1) | 40 (23) | 23 (14.7) | 0.057 |
| Diarrhea | 55 (16.7) | 24 (13.8) | 31 (19.9) | 0.139 |
| Conjunctivitis | 21 (6.4) | 15 (8.6) | 6 (3.8) | 0.076 |
| Abnormal chest radiograph | 81 (24.5) | 0 (0) | 81 (51.9) | <0.001 |
| Without comorbidities | 220 (66.7) | 128 (73.6) | 92 (59.0) | 0.005 |
| With comorbidities | | | | |
| Chronic lung disorder | 65 (19.6) | 29 (16.7) | 36 (23.1) | 0.144 |
| Chronic cardiovascular condition | 22 (6.6) | 10 (5.7) | 12 (7.7) | 0.470 |
| Metabolic disorder | 12 (3.6) | 1 (0.6) | 11 (7.1) | 0.002 |
| Immunosuppression | 7 (2.1) | 0 (0) | 7 (4.5) | 0.005 |
| Obesity | 8 (2.4) | 1 (0.6) | 7 (4.5) | 0.021 |
| Hemoglobinopathy | 3 (0.9) | 1 (0.6) | 2 (1.3) | 0.490 |
| Chronic kidney disease | 4 (1.2) | 1 (0.6) | 3 (1.9) | 0.260 |
| Death | 87 (26.3) | 0 (0) | 87 (55.7) | <0.001 |
| Genetic ancestry | | | | |
| Native American | 0.264 ± 0.18 | 0.250 ± 0.19 | 0.280 ± 19 | 0.147 |
| European | 0.574 ± 0.20 | 0.600 ± 0.20 | 0.544 ± 19 | 0.006 |
| African | 0.162 ± 0.11 | 0.150 ± 0.10 | 0.176 ± 11 | 0.010 |

Age and genetic ancestry were reported as the mean ± SD. All other variables are reported as number (%).

was 24.7 years (1–80 years). Forty-seven of the women were pregnant and 28 were hospitalized. Radiologic alterations were found in 51.9% of hospitalized patients. Most hospitalized patients had no comorbidities (59%). However, patients with comorbidities (metabolic disorders, immunosuppression and obesity) were significantly more frequent in the group of hospitalized patients. Deaths occurred in 55.7% of hospitalized patients. The population studied exhibited a mean European genetic contribution of 57.4%, a mean Native American contribution of 26.4% and a mean African contribution of 16.2% (Table 1) in agreement with previously data from Brazilian populations [13, 15]. In non-hospitalized patients the mean European contribution was 60% ranging from 4.6 to 91.9%, the mean Native American contribution was 25% ranging from 3.2 to 93.2%, and the mean African contribution was 15% ranging 2.2–61%. In hospitalized patients, genetic contribution was European 54.4% ranging from 5.9 to 93%, Native American 28% ranging from 3.5 to 90.6%, and African 17.6% ranging 2.4–67.6% (Additional files 1, 2). European and African genetic ancestry showed statistical differences between groups (p = 0.006 and p = 0.010, respectively).

There were no significant differences in the allele or genotype frequencies of the *CCR5Δ32* polymorphism between non-hospitalized and hospitalized patients (p = 0.289 and 0.431, respectively) (Table 2). A logistic regression analysis was performed to assess the effect of *CCR5Δ32* polymorphism on infection severity controlling for European and African ancestry and the presence of comorbidities to avoid confounding effects. No association between patients carrying the Δ32 allele and severity was found (Table 3).

Discussion

Since the demonstration of the protective role of a 32-bp deletion in the *CCR5* gene in two individuals exposed to, but not infected with HIV [16], studies on the protective or regulatory role of this deletion have multiplied [17].

Table 2 Genotype and allele distribution of CCR5Δ32 in non-hospitalized and hospitalized patients infected with Influenza A(H1N1)pdm09

| | Non-hospitalized | Hospitalized | p value |
|----------|------------------|--------------|---------|
| Genotype | | | |
| Wt/Wt | 160 (92.0) | 148 (94.9) | 0.431 |
| Wt/Δ32 | 13 (7.5) | 8 (5.1) | |
| Δ32/Δ32 | 1 (0.6) | 0 (0) | |
| Allele | | | |
| Wt | 333 (0.96) | 304 (0.97) | 0.289 |
| Δ32 | 15 (0.04) | 8 (0.03) | |

Table 3 *CCR5*Δ32 effect on A(H1N1)pdm09 infection severity

| Genotypes | B | OR | CI 95% | P value |
|---------------------------|--------|-------|------------|---------|
| Wt/Wt ^a | | | | |
| Δ32 carriers ^b | −0.582 | 0.559 | 0.22; 1.26 | 0.219 |

Covariates in Regression model: European and African ancestry and comorbidities.

^a Reference genotype.

^b Wt/Wt + Wt/Δ32.

The protective role of this polymorphism in HIV infection was extensively studied and utilized as model for the development of new therapeutics for AIDS [18].

However, the presence of the deletion can be a determinant factor of morbidity and mortality in the case of other infectious diseases. In a meta-analysis of four cohorts evaluating the deletion in patients with West Nile fever, genetic deficiency of the *CCR5* gene was a strong risk factor of symptomatic arbovirus infection [19]. In another study comparing 129 patients with a diagnosis of tickborne encephalitis virus infection and 79 subjects with aseptic meningitis negative for tickborne encephalitis and 134 healthy controls, a higher frequency of the homozygous Δ32/Δ32 genotype was observed among patients with tickborne encephalitis, particularly among severe cases of the disease [20].

In the present study, no difference in the Δ32 deletion of the *CCR5* gene was observed between the groups of individuals infected with Influenza A(H1N1)pdm09 in a Brazilian admixed population, who presented the classical clinical symptoms [21]. Thus, no correlation could be established between the presence of the mutation and a more severe outcome of the disease. The present results agree with those reported in a recent study involving an Italian population [11], but disagree with the findings obtained for a Canadian population [7].

The Brazilian population was formed by extensive admixture of Native American, European and African populations, nevertheless European genetic ancestry is predominantly in Brazil [13, 15]. In the population studied the mean European contribution was around 57% and the *CCR5*Δ32 allele frequency was 3.6%, similar to previously described for Brazilian populations [22]. Despite ethnic differences with the populations previously analyzed, in the present study thirteen heterozygous and one homozygous for the Δ32 deletion were found in patients with mild symptoms and eight heterozygous for this deletion were found in hospitalized group showing no evidence of this allele effect on severity.

Previous researches adopted different criteria for severe patients [7, 11], which could contribute for conflicting

results among studies, but despite those differences, we emphasize the large size of the sample studied here compared to previously published studies, supporting the hypothesis that the Δ32 mutation is not a predisposing factor for severe Influenza A(H1N1)pdm09 infection.

Conclusion

Although studies have reported an association between mutation Δ32 in the *CCR5* gene and a more severe evolution of some infectious diseases, our findings demonstrate that the same cannot be confirmed for infection with Influenza A(H1N1)pdm09 in the Brazilian admixed population.

Additional files

Additional file 1: Genetic ancestry admixture of patients infected with Influenza A(H1N1)pdm09 sorted by African ancestry. Each individual ancestry is depicted as a column, whereas color represents the proportion of ancestry estimated for that individual (African = blue; European = brown; Native American = green). (A) Non-hospitalized patients and (B) Hospitalized patients.

Additional file 2: Genetic ancestry admixture of patients infected with Influenza A(H1N1)pdm09 sorted by European ancestry. Each individual ancestry is depicted as a column, whereas color represents the proportion of ancestry estimated for that individual (African = blue; European = brown; Native American = green). (A) Non-hospitalized patients and (B) Hospitalized patients.

Abbreviations

CDC: Centers for Disease Control and Prevention; IEC: Evandro Chagas Institute; SEVIR: Virology Section; WHO: World Health Organization.

Authors' contributions

AMN designed the study concepts, conducted the study and prepared the manuscript. VAS performed the data analysis and interpretation and drafted the manuscript. MCS, EMR and WAS were involved in sample and data collection, and final approval of the manuscript. SEB and RCM designed the study concepts, drafting the manuscript and final approval of the manuscript. All authors read and approved the final manuscript.

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Compliance with ethical guidelines

Competing interests

The authors declare that they have no competing interests.

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References

- Centers for Diseases Control and Prevention (2009) Swine influenza A (H1N1) infection in two children-Southern California, March–April 2009. *MMWR Morb Mortal Wkly Rep* 58(15):400–402
- Neumann G, Noda T, Kawaoka Y (2009) Emergence and pandemic potential of swine-origin H1N1 influenza virus. *Nature* 459(7249):931–939
- World Health Organization (2009) Influenza A(H1N1) in the Americas, vol 21. In: Pan American Health Organization (ed) World Health Organization, p 2
- Fineberg HV (2014) Pandemic preparedness and response—lessons from the H1N1 influenza of 2009. *N Engl J Med* 370(14):1335–1342
- Keynan Y, Malik S, Fowke KR (2013) The role of polymorphisms in host immune genes in determining the severity of respiratory illness caused by pandemic H1N1 influenza. *Public Health Genomics* 16(1–2):9–16
- Zhang L, Katz JM, Gwinn M, Dowling NF, Khoury MJ (2009) Systems-based candidate genes for human response to influenza infection. *Infect Genet Evol* 9(6):1148–1157
- Keynan Y, Juno J, Meyers A, Ball TB, Kumar A, Rubinstein E et al (2010) Chemokine receptor 5 Δ 32 allele in patients with severe pandemic (H1N1) 2009. *Emerg Infect Dis* 16(10):2
- Sanchooli J, Sanadgol N, Kazemi Arababadi M, Kennedy D (2014) CCR5 plays important roles in hepatitis B infection. *Viral Immunol* 27(1):2–6
- Samson M, Labbe O, Mollereau C, Vassart G, Parmentier M (1996) Molecular cloning and functional expression of a new human CC-chemokine receptor gene. *Biochemistry* 35:6
- Ghorban K, Dadmanesh M, Hassanshahi G, Momeni M, Zare-Bidaki M, Arababadi MK et al (2012) Is the CCR5 Δ 32 mutation associated with immune system-related diseases? *Inflammation* 36(3):633–642
- Sironi M, Cagliani R, Pontremoli C, Rossi M, Migliorino G, Clerici M et al (2014) The CCR5 Δ 32 allele is not a major predisposing factor for severe H1N1 pdm09 infection. *BMC Research Notes* 7(1):504
- Centers for Diseases Control and Prevention (2009) CDC protocol of realtime RTPCR for influenza A(H1N1). In: Centers for Disease Control and Prevention, Atlanta, p 7
- Santos NPC, Ribeiro-Rodrigues EM, Ribeiro-dos-Santos ÁKC, Pereira R, Gusmão L, Amorim A et al (2010) Assessing individual interethnic admixture and population substructure using a 48-insertion-deletion (INSEL) ancestry-informative marker (AIM) panel. *Hum Mutat* 31(2):184–190
- Ministério da Saúde do Brasil (2009) Brasil: Protocolo de manejo clínico e vigilância epidemiológica da influenza. In: SVS, Brasília, p 32
- Saloum de Neves Manta F, Pereira R, Vianna R, Rodolfo Beutenmüller de Araújo A, Leite Góes Gitai D, Aparecida da Silva D et al (2013) Revisiting the genetic ancestry of Brazilians using autosomal AIM-Indels. *PLoS One* 8(9):e75145
- Liu R, Paxton WA, Choe S, Ceradini D, Martin SR, Horuk R et al (1996) Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. *Cell* 86:11
- Klein Robyn S (2008) A moving target: the multiple roles of CCR5 in infectious diseases. *J Infect Dis* 197(2):183–186
- O'Brien SJ, Hendrickson SL (2013) Host genomic influences on HIV/AIDS. *Genome Biol* 14(1):201–213
- Lim Jean K, Louie Christine Y, Glaser C, Jean C, Johnson B, Johnson H et al (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(2):262–265
- Kindberg E, Mickienė A, Ax C, Åkerlind B, Vene S, Lindquist L et al (2008) A deletion in the chemokine receptor 5(CCR5) gene is associated with tickborne encephalitis. *J Infect Dis* 197(2):266–269
- Bautista E, Chotpitayasunondh T, Gao Z, Harper SA, Shaw M, Uyeki TM et al (2010) Clinical aspects of pandemic 2009 influenza A (H1N1) virus infection. *N Engl J Med* 362(18):1708–1719
- Vargas AE, Marrero AR, Salzano FM, Bortolini MC, Chies JA (2006) Frequency of CCR5delta32 in Brazilian populations. *Braz J Med Biol Res* 39(3):321–325

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