Hospitalization Risk Due to Respiratory Illness Associated with Genetic Variation at IFITM3 in Patients with Influenza A(H1N1)pdm09 Infection: A Case-Control Study

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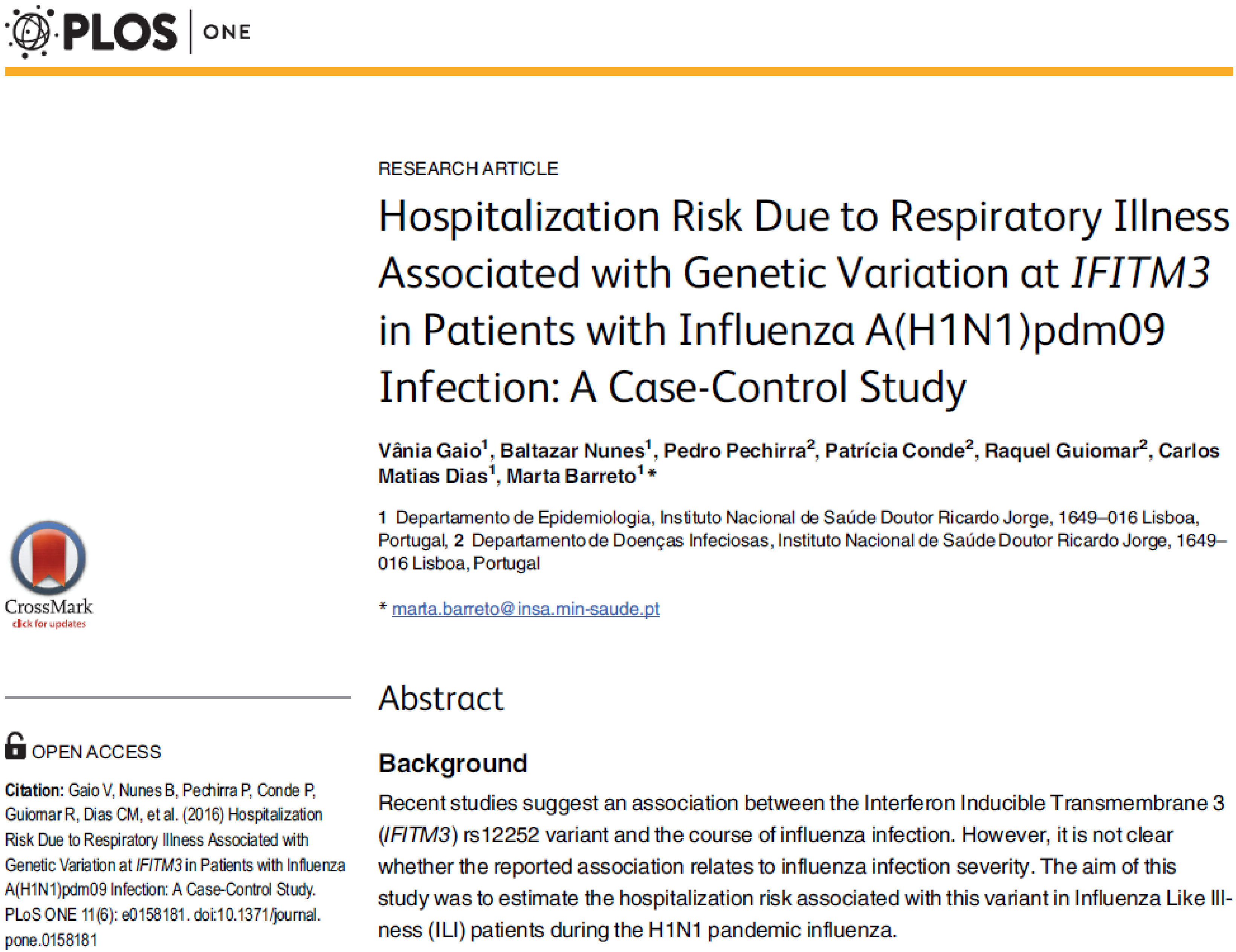
Lisboa, 4 de junho de 2019

Seminários Ricardo Jorge



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RESEARCH ARTICLE

Hospitalization Risk Due to Respiratory Illness Associated with Genetic Variation at *IFITM3* in Patients with Influenza A(H1N1)pdm09 Infection: A Case-Control Study

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Abstract

Background

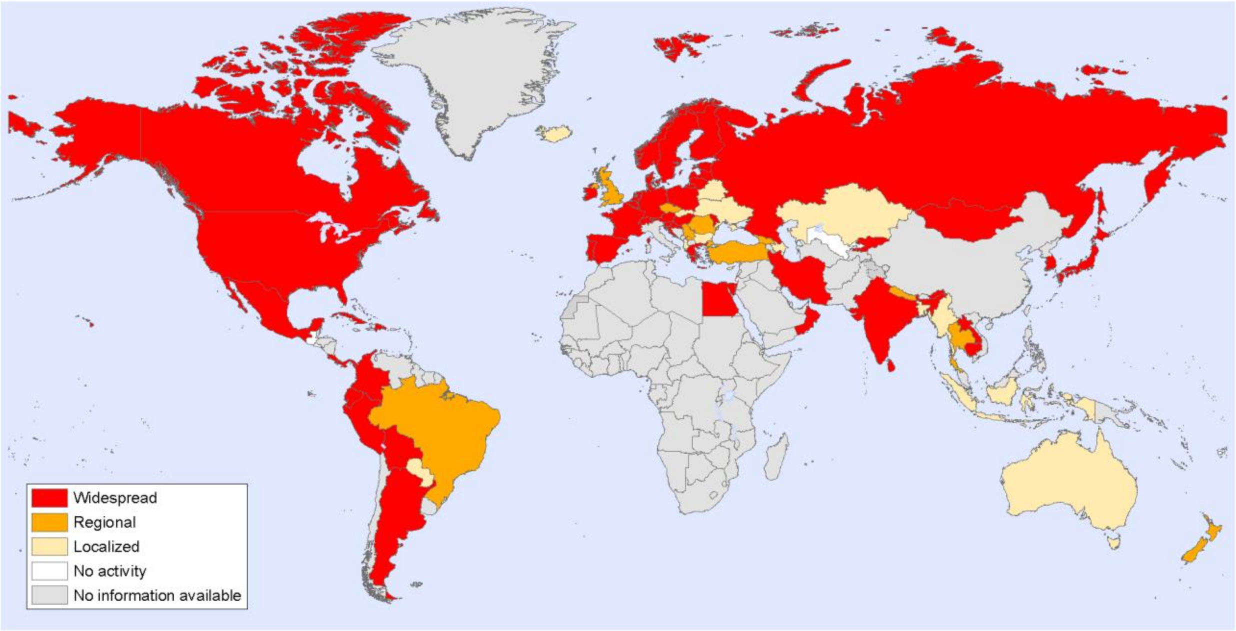
Recent studies suggest an association between the Interferon Inducible Transmembrane 3 (*IFITM3*) rs12252 variant and the course of influenza infection. However, it is not clear whether the reported association relates to influenza infection severity. The aim of this study was to estimate the hospitalization risk associated with this variant in Influenza Like Illness (ILI) patients during the H1N1 pandemic influenza.

1.Background

Geographic spread of influenza activity

(Geographic spread reflects the number and distribution of regions within a country reporting influenza activity.)

Status as of Week 48
23 Nov - 29 Nov 2009



The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement.

Map produced: 22 December 2009, 14:10 GMT

Data Source: World Health Organization
Map Production: Public Health Information and Geographic Information Systems (GIS)
World Health Organization

World Health Organization

Influenza A(H1N1)pdm09:

- 15,000 deaths in less than a year;
- Severe cases in healthy young people (90% of all deaths occurred in those younger than 65 years).

1. Background

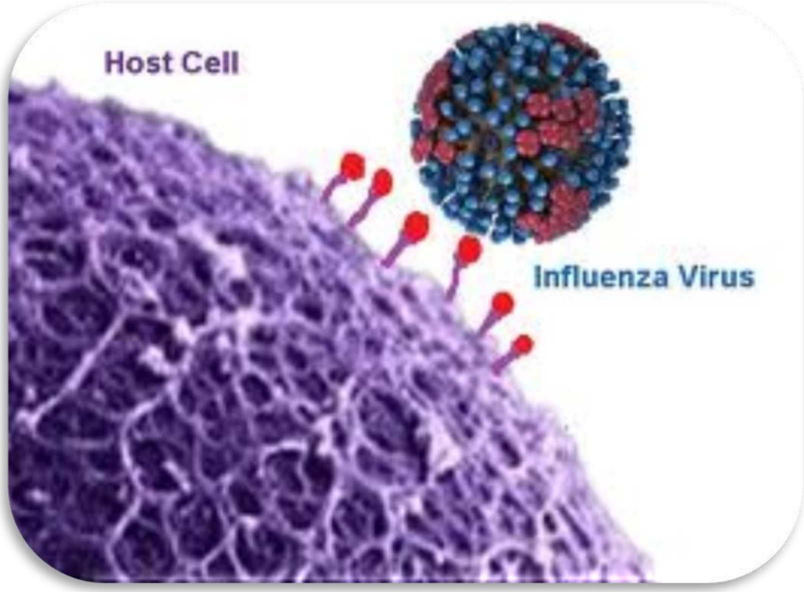
Portugal

According to the Portuguese Influenza Surveillance Program (NISP):

- 64% of the laboratory-confirmed cases for the influenza A(H1N1)pdm09 virus were detected in the age group of 5–14 years old;
- A total of 1436 hospitalizations were reported;
- the estimated mortality rate was 1.17 per 100000 inhabitants, corresponding to 124 laboratory confirmed influenza deaths.

Why do some individuals resist to infection or recover quickly, whereas others experience severe disease associated with the infection?

- ➢ Virus pathogenicity } Intensive research
- ➢ Host genetic susceptibility } **?**



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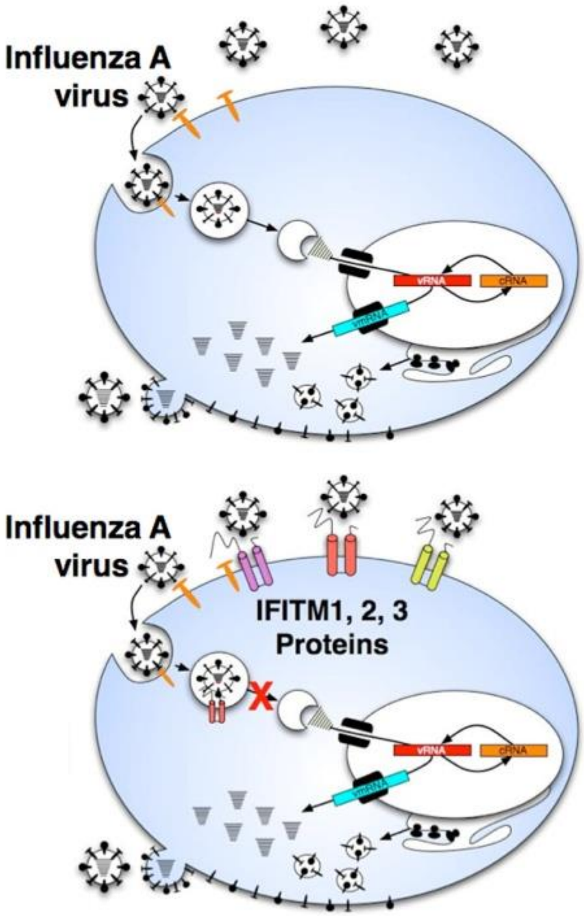
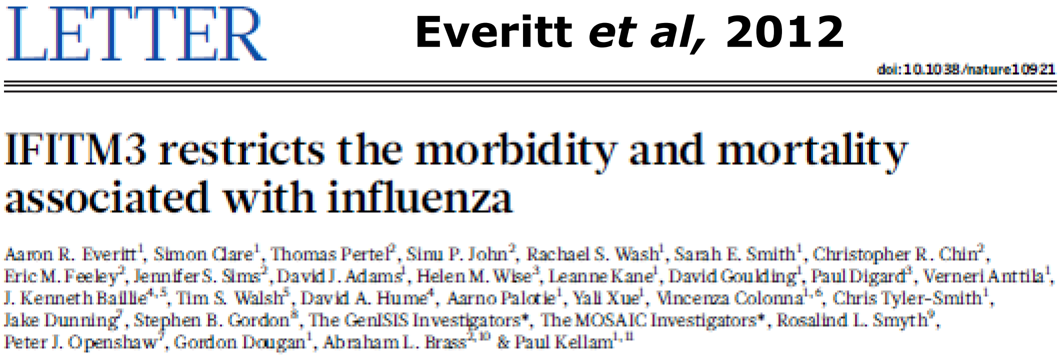
WHO (2009):
identified studies of the host genetic factors’ role on susceptibility
to severe influenza as a priority.

1. Background



Everitt *et al,* 2012



LETTER

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IFITM3 restricts the morbidity and mortality associated with influenza

Aaron R. Everitt[1], Simon Clare[1], Thomas Pertel[2], Simu P. John[2], Rachael S. Wash[1], Sarah E. Smith[1], Christopher R. Chin[2], Eric M. Feeley[2], Jennifer S. Sims[2], David J. Adams[1], Helen M. Wise[3], Leanne Kane[1], David Goulding[1], Paul Digard[3], Verneri Anttila[1], J. Kenneth Baillie[4,5], Tim S. Walsh[5], David A. Hume[4], Aarno Palotie[1], Yali Xue[1], Vincenza Colonna[1,6], Chris Tyler-Smith[1], Jake Dunning[7], Stephen B. Gordon[8], The GenISIS Investigators*, The MOSAIC Investigators*, Rosalind L. Smyth[9], Peter J. Openshaw[7], Gordon Dougan[1], Abraham L. Brass[2,10] & Paul Kellam[1,11]

- *IFITM3* alters the course of influenza virus infection (animal models);

- They found a higher frequency of a minor *IFITM3* allele (SNP rs12252-C) in hospitalized subjects with Influenza infection in comparison with the European 1000 genomes subjects.

2.Objetive

To estimate the association between the IFITM3 rs12252 variant (C allele) and the risk of hospitalization due to respiratory illness in Portuguese patients with influenza A(H1N1)pdm09 infection

3. Methods

3.1. Study design and participants

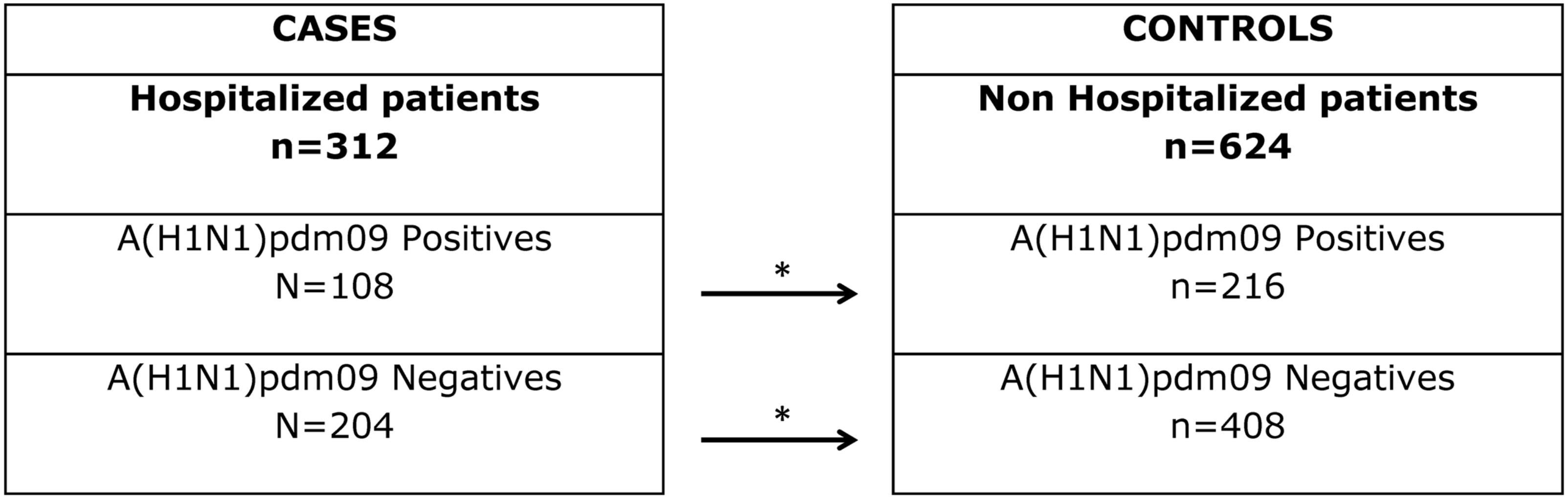
- Case-control genetic association study;
- Comparison of the allele frequency between hospitalized influenza-like illness (ILI) patients (cases) vs non hospitalized ILI patients (controls);
- Nasopharyngeal/oropharyngeal swabs received at INSA for diagnostic purposes from the Portuguese Laboratory Network for the Diagnosis of Influenza Infection (between 09/2009 and 02/2010)

Exclusion criteria:

1. Patients over 65 years old;
2. Immunodepressed or transplanted patients;
3. Patients with chronic diseases (diabetes, lung, kidney, cardiovascular, liver, neurological, immunologic and oncologic diseases);
4. Pregnant women;
5. Notification data before the mitigation phase (01-09-2009);
6. Patients for whom the time lapse between the symptoms onset and the sample collection was over 7 days;
7. Samples with unavailable information about hospitalization;
8. Samples with unavailable laboratorial result for A(H1N1)pdm09 virus;
9. Samples stored outside of the INSA.

3. Methods

Cases and controls selection (1 case: 2 paired controls)



CASES		**CONTROLS**
Hospitalized patients **n=312**		**Non Hospitalized patients** **n=624**
A(H1N1)pdm09 Positives N=108	$\xrightarrow{*}$	A(H1N1)pdm09 Positives n=216
A(H1N1)pdm09 Negatives N=204	$\xrightarrow{*}$	A(H1N1)pdm09 Negatives n=408

* Matched selection of 2 Non Hospitalized patients

- **Hospitalization**: measure of disease severity (defined as a hospital admission due to ILI complications with a concomitant hospital stay for more than 24 hours).

Genotyping:

- DNA extraction: automatic system (MagNA Pure LC—Roche);
- Genotyping: RFLP (restriction fragment length polymorphism).

Statistical analysis:

- R program;
- Stratified analysis by ILI A(H1N1)pdm09 positive and negative patients;
- Conditional Logistic Regression for matched Pairs Data including the potential confounding effect of age and gender variables was used to estimate adjusted Odds-Ratio.

4. Results

Table S1- Genotyping success rates in each ILI patients group.

	ILI A(H1N1)pdm09 positive patients		**ILI A(H1N1)pdm09 negative patients**		***p***
	Hospitalized (cases)	Non-hospitalized (controls)	Hospitalized (cases)	Non-hospitalized (controls)	
n	96	212	198	403	-
Genotyped samples (%)	84 (87.5%)	184 (86.8%)	173 (84.4%)	351 (87.1%)	0.998[1]

[1] *p-values* were obtained by the Pearson's chi-squared test.

- ✓ From the 936 patients selected, 909 had sufficient available frozen swabs for DNA extraction;
- ✓ The overall success rate was 87% and it was similar in all the 4 groups of patients (p = 0.998).

4. Results

Table 1. Patients characterization, regarding the age and gender variables.

	ILI A(H1N1)pdm09 positive patients			ILI A(H1N1)pdm09 negative patients		
	Hospitalized (cases)	Non-hospitalized (controls)	*p*	Hospitalized (cases)	Non-hospitalized (controls)	*p*
Age (years)						
Mean ± sd	16.6 ± 17.6	14.0 ± 12.4	0.815[1]	15.7 ± 20.2	13.5 ± 16.1	0.382[1]
Median (range)	10 (0-60)	9 (0-54)		4 (0-64)	6 (0-62)	
Gender						
% of women (95% CI)	40.5 (30.0-51.0)	46.2 (39.0-53.4)	0.382[2]	43.9 (36.5-51.3)	47.3 (42.1-52.5)	0.468[2]

[1] *p-values* were obtained by the Wilcoxon test. [2] *p-values* were obtained by the Pearson's chi-squared test. (CI, Confidence interval;

- ✓ No significant differences were found regarding age and sex.

4. Results

Table 2. *IFITM3* rs12252 genotypic and allelic frequencies its association with hospitalization, assuming a dominant model.

	ILI A(H1N1)pdm09 positive patients				ILI A(H1N1)pdm09 negative patients			
	Hospitalized (cases)	Non-hospitalized (controls)	OR Crude (95% CI)	OR Adjusted (95% CI)	Hospitalized (cases)	Non-hospitalized (controls)	OR Crude (95% CI)	OR Adjusted (95% CI)
n	84	184	-	-	173	351	-	-
*IFITM3*_rs12252								
Genotypes								
TT (%)	73 (86.9)	152 (82.6)	-	-	134 (77.5)	312 (88.9)	-	-
CT (%)	9 (10.7)	32 (17.4)			39 (22.5)	38 (10.8)		
CC (%)	2 (2.4)	0 (0)			0 (0)	1 (0.28)		
Alleles								
C (%)	13 (7.7)	32 (8.7)	-	-	39 (11.3)	40 (5.7)	-	-
T (%)	155 (92.3)	336 (91.3)			207 (92.3)	662 (94.3)		
Dominant model[1]								
CT/CC (%)	11 (13.1)	32 (17.4)			39 (22.5)	39 (11.1)		
vs			0.72	0.73[2]			2.33	2.54[2]
TT (%)	73 (86.9)	152 (82.6)	(0.34-1.50)	(0.33-1.50)	134 (77.5)	312 (88.9)	(1.43-3.79)	(1.54-4.19)

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[1] Other genetic models were not considered due the low frequency of CC genotype and the existence of zero patients with CC genotypes in 2 groups. [2] Adjustment for age and gender by logistic regression.

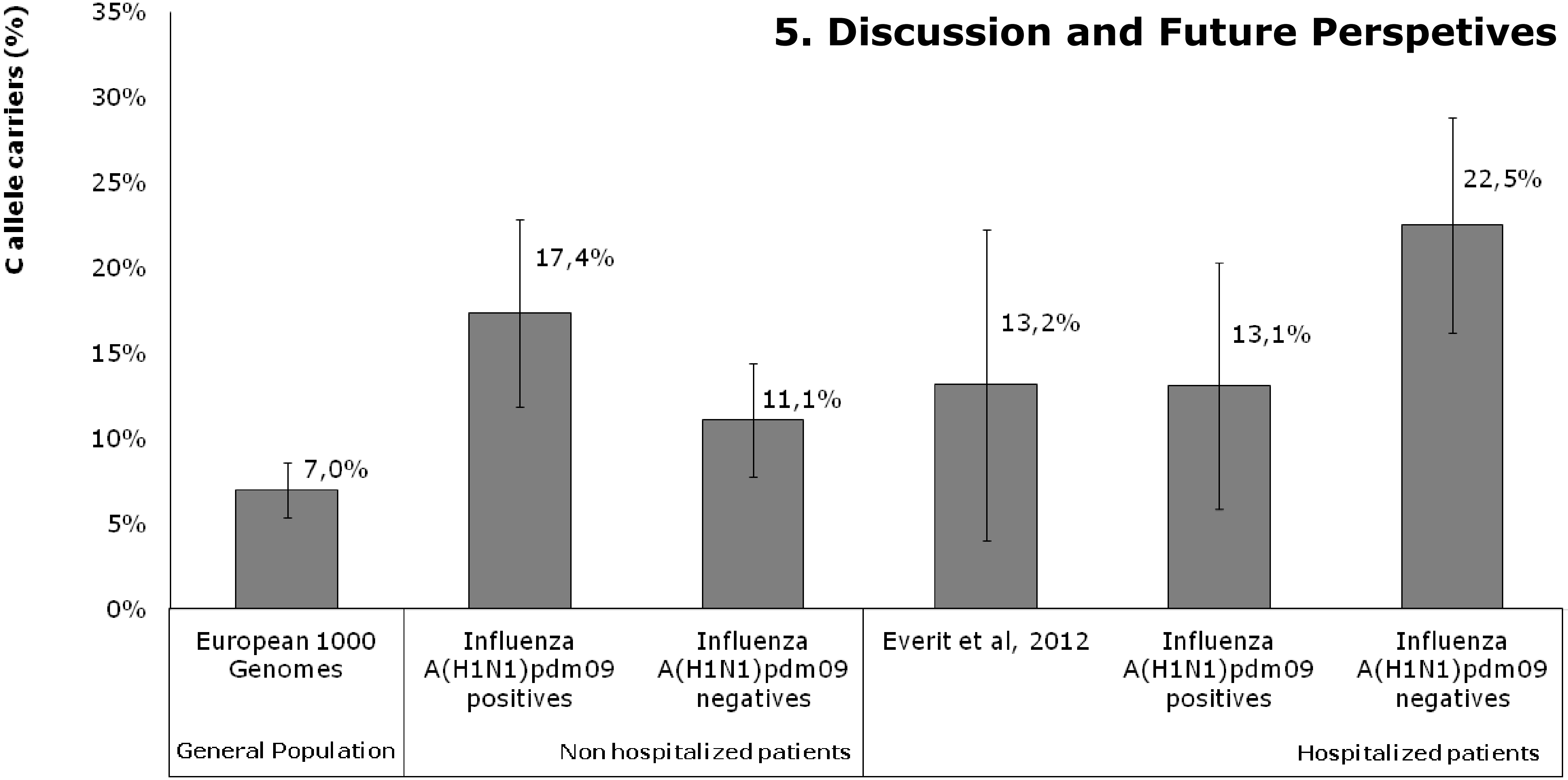
- ✓ In patients negative for A(H1N1)pdm09 virus, the risk of being hospitalized among the CT/CC genotype carriers is significantly higher than the risk of being hospitalized in the TT genotype carriers.

5. Discussion and Future Perspectives

- ✓ IFITM3 rs12252-C is involved in the ILI symptoms severity associated with other respiratory infections (Influenza negative samples could be positive for other respiratory virus);

- ✓ The future detection of other respiratory virus in the analyzed samples would be important to clarify our results.

5. Discussion and Future Perspetives



- Similar % of C allele carriers between Everitt *el al*. 2012 and the Influenza A(H1N1)pdm09 hospitalized positive cases (13.2% vs 13.1%).

- % of C allele carriers in the Portuguese population (INSEF pilot study, n=193) was 7.8%.

Limitations:

- ✓ Use of Hospitalization as a measure of the infection severity (Less severe cases might have been hospitalized due to the initial pandemic alert): this bias was reduced by excluding patients with disease onset reported in the contention phase;
- ✓ Confounding adjustment only for age and sex variables in the logistic regression analysis: the possible confounding bias was reduced by the exclusion criteria used in the patients selection;
- ✓ Sample size limitation due to the low C allele frequency.

5. Discussion and Future Perspetives

- ✓ To perform a future integrative approach to clarify why some healthy individuals resist infection or recover quickly, others experience severe disease associated with the influenza infection.

↓

Considering not only the virus-host genome interactions but also immunity, vaccination, weather conditions and other environmental factors that could help to clarify the present results.

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Muito obrigada!

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