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

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).
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.
1. Background

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

- Virus pathogenicity
- Host genetic susceptibility

Intensive research

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

https://www.flickr.com/photos/ibm_research_zurich/7250979508/
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.

1. Background

LETTER

Everitt et al, 2012

IFITM3 restricts the morbidity and mortality associated with influenza

➢ IFITM3 alters the course of influenza virus infection (animal models);
2. Objective

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)

<table>
<thead>
<tr>
<th>CASES</th>
<th>CONTROLS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hospitalized patients</strong></td>
<td><strong>Non Hospitalized patients</strong></td>
</tr>
<tr>
<td>n=312</td>
<td>n=624</td>
</tr>
<tr>
<td>A(H1N1)pdm09 Positives</td>
<td>A(H1N1)pdm09 Positives</td>
</tr>
<tr>
<td>N=108</td>
<td>n=216</td>
</tr>
<tr>
<td>A(H1N1)pdm09 Negatives</td>
<td>A(H1N1)pdm09 Negatives</td>
</tr>
<tr>
<td>N=204</td>
<td>n=408</td>
</tr>
</tbody>
</table>

* 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).
3. Methods

**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.

<table>
<thead>
<tr>
<th></th>
<th>ILI A(H1N1)pdm09 positive patients</th>
<th>ILI A(H1N1)pdm09 negative patients</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hospitalized (cases)</td>
<td>Non-hospitalized (controls)</td>
<td>Hospitalized (cases)</td>
</tr>
<tr>
<td>n</td>
<td>96</td>
<td>212</td>
<td>198</td>
</tr>
<tr>
<td>Genotyped samples</td>
<td>84</td>
<td>184</td>
<td>173</td>
</tr>
<tr>
<td>(%)</td>
<td>(87.5%)</td>
<td>(86.8%)</td>
<td>(84.4%)</td>
</tr>
</tbody>
</table>

*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.

<table>
<thead>
<tr>
<th></th>
<th>ILI A(H1N1)pdm09 positive patients</th>
<th>ILI A(H1N1)pdm09 negative patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hospitalized (cases)</td>
<td>Hospitalized (cases)</td>
</tr>
<tr>
<td></td>
<td>Non-hospitalized (controls)</td>
<td>Non-hospitalized (controls)</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>Mean ± sd</td>
<td>16.6 ± 17.6</td>
</tr>
<tr>
<td></td>
<td>Median (range)</td>
<td>10 (0-60)</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td>% of women</td>
<td>40.5</td>
</tr>
<tr>
<td></td>
<td>(95% CI)</td>
<td>(30.0-51.0)</td>
</tr>
</tbody>
</table>

\[p-values\] were obtained by the Wilcoxon test. 1 \[p-values\] 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.

<table>
<thead>
<tr>
<th></th>
<th>ILI A(H1N1)pdm09 positive patients</th>
<th>ILI A(H1N1)pdm09 negative patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hospitalized (cases)</td>
<td>Non-hospitalized (controls)</td>
</tr>
<tr>
<td>n</td>
<td>84</td>
<td>184</td>
</tr>
</tbody>
</table>

**IFITM3 rs12252**

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>TT (%)</th>
<th>CT (%)</th>
<th>CC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT (%)</td>
<td>73 (86.9)</td>
<td>152 (82.6)</td>
<td>-</td>
</tr>
<tr>
<td>CT (%)</td>
<td>9 (10.7)</td>
<td>32 (17.4)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>CC (%)</td>
<td>2 (2.4)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Alleles</th>
<th>C (%)</th>
<th>T (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C (%)</td>
<td>13 (7.7)</td>
<td>32 (8.7)</td>
</tr>
<tr>
<td>T (%)</td>
<td>155 (92.3)</td>
<td>336 (91.3)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dominant model¹</th>
<th>CT/CC (%)</th>
<th>TT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>vs</td>
<td>11 (13.1)</td>
<td>73 (86.9)</td>
</tr>
<tr>
<td></td>
<td>32 (17.4)</td>
<td>152 (82.6)</td>
</tr>
<tr>
<td></td>
<td>0.72</td>
<td>(0.34-1.50)</td>
</tr>
<tr>
<td></td>
<td>0.73²</td>
<td>(0.33-1.50)</td>
</tr>
<tr>
<td></td>
<td>39 (22.5)</td>
<td>134 (77.5)</td>
</tr>
<tr>
<td></td>
<td>39 (11.1)</td>
<td>312 (88.9)</td>
</tr>
<tr>
<td></td>
<td>2.33</td>
<td>(1.43-3.79)</td>
</tr>
<tr>
<td></td>
<td>2.54²</td>
<td>(1.54-4.19)</td>
</tr>
</tbody>
</table>

¹ Other genetic models were not considered due to 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.
Similar % of C allele carriers between Everitt et 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 Perspectives

✓ 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.
6. References

Muito obrigada!

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