Association of *Helicobacter pylori* vacA polymorphisms with the risk of gastric precancerous lesions in a Moroccan population

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Abstract

Introduction: *Helicobacter pylori* infection is the major risk factor of atrophic gastritis and intestinal metaplasia. The *vacA* gene is one of the most virulence factors of *H. pylori* and genetic diversity in its s, m, i, and d regions is associated with gastric lesions severity. This study aimed to investigate the association of *vacA* s, m, i, and d regions with the risk of atrophic gastritis and intestinal metaplasia in a Casablanca population.

Methodology: A total of 210 patients suffering from gastric lesions (chronic gastritis, atrophic gastritis, and intestinal metaplasia) were enrolled. The type of lesion was diagnosed by histological examination. Detection of *H. pylori* infection and genotyping of *vacA* regions were carried out by PCR.

Results: The prevalence of *H. pylori* was 95%. The most common *vacA* genotypes were s2 (51.5%), m2 (77%), i2 (60.5%), and d2 (58.5%). *VacA* s1, m1, and i1 genotypes were associated with a high risk of intestinal metaplasia, while the *vacA* d1 genotype increases the risk of atrophic gastritis and intestinal metaplasia. The most common *vacA* combination was s2/m2/i2/d2 (52%), and it was more detected in chronic gastritis. The moderate virulent *vacA* combination (s1/m2/i1/d1) increases the risk of atrophic gastritis, while the most virulent *vacA* combination (s1/m1/i1/d1) increases the risk of intestinal metaplasia.

Conclusions: Genotyping of *vacA* d region might be a reliable marker for the identification of *vacA* virulent strains that represent a high risk of developing precancerous lesions (atrophic gastritis and intestinal metaplasia).

Key words: Atrophic gastritis; *Helicobacter pylori*; intestinal metaplasia; *VacA* gene.

*J Infect Dev Ctries* 2021; 15(8):1124-1132. doi:10.3855/jidc.14435

(Received 02 December 2020 – Accepted 06 January 2021)

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Introduction

*Helicobacter pylori* infection systematically leads to chronic gastritis (CG), which can develop into more severe pathologies such as gastric ulcer, MALT lymphoma, and gastric cancer (GC). GC is a multi-step pathology that develops through a series of lesions known as gastric carcinogenesis and includes: CG, atrophic gastritis (AG), intestinal metaplasia (IM), dysplasia, and GC [1]. The mechanisms by which AG and IM (known as precancerous lesions) develop are linked to a complex interaction between *H. pylori* virulence factors, human genetics, and environmental factors.

*H. pylori* is a genetically heterogeneous bacterium and genetic polymorphisms of its virulence factors affect its pathogenicity [2]. For instance, variations of the vacuolating cytotoxin A (*vacA*) gene have been proposed as a means of identifying virulent strains involved in the occurrence of gastric diseases [3].

The *vacA* gene is one of the most important virulence factors of *H. pylori*. This gene encodes for the multifunctional toxin VacA involved in several deleterious biological activities, such as vacuolization, apoptosis, tight junction disruption, and suppression of T cell activation [4]. The *vacA* gene is present in all *H. pylori* strains and comprises four polymorphic regions: signal (s), middle (m), intermediate (i), and deletion (d) region. Each *vacA* region is divided into two subtypes: s1, s2, m1, m2, i1, i2, d1, and d2 [5–7].

The *vacA* s region encodes for the signal peptide of the VacA protein. The *vacA* s1 genotype encodes for the whole signal peptide while the *vacA* s2 genotype encodes for a short signal peptide which results in a low vacuolating activity [8,9]. The *vacA* m region is
responsible for the binding of the VacA protein to host cells. The **vacA** m1 genotype is more active and binds to a wider range of host cells than the m2 genotype [9]. The **vacA** i and d regions have been recently discovered [6,7]. In the **vacA** i region, the i1 genotype is associated with high vacuolation activity than the i2 genotype [6]. In the **vacA** d region, the d1 genotype is characterized by the absence of a 69 to 81 bp deletion, while the d2 genotype exhibits this deletion [7]. Several **vacA** combinations of these genotypes exist, and each of them is more or less associated with the risk of precancerous lesions and GC development.

GC is one of the most aggressive neoplasms and it is associated with a poor prognosis. Because of its late diagnosis, most Moroccan patients detected are at advanced stages of the disease, which results in a five-year survival rate of less than 15% [10]. Finding a marker for the early diagnosis of patients at high risk of developing this cancer is an important step in reducing its mortality. Our study aimed to investigate the polymorphisms of **vacA** s, m, i, and d regions and their association with gastric precancerous lesions in a Casablanca population, in order to use these regions as predictive markers in the identification and follow-up of patients that present high risks of developing this cancer.

**Methodology**

**Study population**

A total of 210 patients consulting in the gastroenterology service of Ibn Rochd University Hospital Center of Casablanca (Morocco) and suffering from digestive pains were included in this study. From all patients, 6 biopsies (2 from antrum, 2 from fundus, and 2 from lesser curvature) have been sampled. Three biopsies (1 from antrum, 1 from fundus, and 1 from lesser curvature) were used for histological examination and the other three biopsies were used for molecular detection. All participants were informed of their inclusion in the study and agreed to it on a writing form. The study protocol has been performed under the ethical standards of Helsinki and was approved by the ethical committee of the Pasteur Institute of Morocco.

**Histology**

The biopsy samples were transported in 10% formalin and embedded in paraffin. Multiple histological sections were obtained from each biopsy. Biopsy sections were then obtained and stained with hematoxylin-eosin for the detection of gastric lesions. The blades were read by a pathologist.

**PCR for *H. pylori* detection**

Total DNA was extracted from gastric biopsies using a genomic DNA extraction kit (Isolate Genomic DNA Kit, Bioline, Memphis, USA). Using primers described by Lu et al [11], the ureC gene (296 bp) was amplified to detect *H. pylori* infection. The PCR reaction mixture was prepared with 0.5 mM dNTPs, 1.5 mM MgCl₂, 0.5 µM of each primer, 1 U of DNA Polymerase (MyTaq DNA Polymerase, BioLine, Memphis, USA), and 300 ng of DNA in a final volume of 20 µL. PCR thermocycling conditions for *H. pylori* detection were: 1 cycle at 95 °C for 1 minute, 35 cycles at 95 °C for 15 seconds, 55 °C for 30 seconds, 72 °C for 30 seconds, and a final extension cycle at 72 °C for 7 minutes.

**Genotyping of **vacA** regions**

*H. pylori*-positive samples were subjected to PCR for genotyping of **vacA** s, m, i, and d regions. Primers used in this study are listed in Table 1 [5-7,12].

For **vacA** s and m regions, the PCR reactions mixtures were prepared with 0.5 mM dNTPs, 1.5 mM MgCl₂, 0.5 µM of each primer, 1 U of MyTaq DNA Polymerase (MyTaq DNA Polymerase, BioLine, Memphis, USA), and 300 ng of DNA in a final volume of 20 µL.

For **vacA** i and d regions, the PCR reactions mixtures were prepared with 0.75 mM dNTPs, 2.25 mM MgCl₂, 0.4 µM of each primer, 1 U of MyTaq DNA Polymerase (MyTaq DNA Polymerase, BioLine, Memphis, USA), and 300 ng of DNA in a final volume of 20 µL.

### Table 1. Primers used in this study.

| Region amplified | Primer name | Primer sequence | Size (bp) | References |
|------------------|-------------|-----------------|-----------|------------|
| s (s1/s2)        | VAI-F       | ATGGAAATACAACAAACACAC | s1 = 259 | [5]        |
|                  | VAI-R       | CTGCTTGAATGCGCAACAC | s2 = 286 |            |
| m (m1/m2)        | VAG-F       | CAATCTGTCCAATCAACGAG | m1 = 567 | [12]       |
|                  | VAG-R       | GCCGCAAAATATTCCACCAG | m2 = 642 |            |
| i1               | VACF1       | GTGGGGATTGGGGGAAATGCCG | 426      | [6]        |
|                  | C1R         | TTAATTAAAGCCTGTGTAAG |          |            |
| i2               | VACF1       | GTGGGGATTGGGGGAAATGCCG | 432      | [6]        |
|                  | C2R         | GATCAACGCTCTCAGTGG |          |            |
| d (d1/d2)        | VAS-5F      | ACTATATTTGCGCAGACGATTTG | d1 = 367-379 | [7]      |
|                  | VAGF-R      | CTGCCCTGTGGCAGACATGG | d2 = 298 |            |
Memphis, USA), and 300 ng of DNA in a final volume of 20 µL. All PCR thermocycling conditions for genotyping of vacA regions are listed in Table 2.

### Statistical analysis

R software version 3.4.0 was used to conduct statistical analysis. Chi-square and ANOVA tests were performed to assess all associations between gastric lesions, age, gender, and vacA s, m, i, and d regions. The association between vacA d genotypes and vacA s, m, and i combinations were calculated using the Fisher test.

For the association between gastric lesions and vacA combinations, gastric lesions were considered as the dependent variable, and vacA s1/m2/i1/d1 and s1/m1/i1/d1 combinations as the predictor variables. The CG group and vacA s1/m1/i1/d1 combination were taken respectively as the control group and the reference strain. Results were expressed as odds ratio (OR), 95% confidence intervals (95% CI), and p-values.

### Results

#### Population characteristics

The population was constituted of 99 (47%) males and 111 (53%) females. The mean age of the population was 49 ± 16. According to histological examination, 61% of patients were diagnosed with CG, 25% with AG, and 13% with IM.

Gastric lesions severity was increasing with age, but without being statistically meaningful ($p = 0.39$) (Table 3). Concerning gender, the frequency of females and males diagnosed with CG was the same (48 and 52%, respectively). AG was more diagnosed among females, whereas IM was predominant among males (Table 3). Association between gender and gastric lesions severity was statistically significant ($p = 0.04$).

The presence of *H. pylori* was detected in the gastric mucosa of 200 patients (95%): 121 (94%) cases in CG, 51(96%) cases in AG, and 28 (100%) cases in IM.

#### Prevalence of vacA genotypes

All vacA regions were determined for all the 200 *H. pylori*-positive patients. In all vacA regions, a dominance of the inactive form of the vacA genotype (s2, m2, i2, and d2) was observed (Table 4).

**Association between vacA genotypes and gastric precancerous lesions**

The frequency of the vacA s1 genotype was shown to increase with gastric lesions severity: 41% in CG, 57% in AG, and 64% in IM. The vacA m1 genotype was detected with low frequency in CG and AG (18 and 20%, respectively) while it was higher in IM (50%). The frequency of the vacA i1 genotype was found to increase with gastric lesions severity: 31% in CG, 45% in AG, and 64% in IM. Similarly, the frequency of the vacA d1 genotype increased with gastric lesions severity: 31% in CG, 49% in AG, and 71% in IM (Table 4). Distributions of vacA s, m, i, and d genotypes according to gastric lesions severity were statistically significant (Table 4).

According to table 4, the association between vacA s, m, and i regions with the risk of AG was not statistically significant. In contrast, the vacA d region was shown to increase the risk of AG by an OR of 2.1 (95% CI = 1.07 – 4.1, p-value = 0.02).

### Table 2. PCR thermocycling conditions for genotyping of vacA regions used in this study.

| VacA region amplified | PCR thermocycling conditions |
|-----------------------|------------------------------|
| s region              | 1 cycle at 95 °C for 1 min, 35 cycles at 95 °C for 1 min, 56 °C for 50 s, 72 °C for 1 min and a final extension cycle at 72 °C for 7 min |
| m region              | 1 cycle at 95 °C for 1 min, 35 cycles at 95 °C for 1 min, 57 °C for 50 s, 72 °C for 1 min and a final extension cycle at 72 °C for 7 min |
| i region              | 1 cycle at 95 °C for 1 min, 35 cycles at 95 °C for 1 min, 53 °C for 1 min, 72 °C for 1 min and a final extension cycle at 72 °C for 7 min |
| d region              | 1 cycle at 95 °C for 1 min, 35 cycles at 95 °C for 1 min, 59 °C for 50 s, 72 °C for 1 min and a final extension cycle at 72 °C for 7 min |

### Table 3. Distribution of gastric pathologies according to age and gender.

|       | CG n = 129 (%) | AG n = 53 (%) | IM n = 28 (%) | p-value |
|-------|----------------|---------------|---------------|---------|
| Age (mean ± sd) | 48 ± 17        | 49 ± 13       | 53 ± 17       | 0.39 * |
| Gender |                |               |               |         |
| Males  | 62 (48)        | 19 (36)       | 18 (64)       | 0.04 **|
| Females| 67 (52)        | 34 (64)       | 10 (36)       |         |

CG: chronic gastritis; AG: atrophic gastritis; IM: intestinal metaplasia; sd: standard deviation. * ANOVA test; **: Chi-square test.
Table 4. Prevalence and association of vacA genotypes and gastric lesions.

| Prevalence of vacA genotypes | CG n = 121 (%) | AG n = 51 (%) | IM n = 28 (%) | p-value * |
|-----------------------------|----------------|---------------|---------------|-----------|
| s region                    |                |               |               |           |
| s1                          | 97 (48.5)      | 50 (41)       | 29 (57)       | 18 (64)   | 0.03     |
| s2                          | 103 (51.5)     | 71 (59)       | 22 (43)       | 10 (36)   |          |
| ** OR, 95% CI, p-value      | -              | 1.87, 0.96 – 3.62, 0.06 | 2.55, 1.08 – 6, 0.03 |           |
| m region                    |                |               |               |           |
| m1                          | 46 (23)        | 22 (18)       | 10 (20)       | 14 (50)   | 0.001    |
| m2                          | 154 (77)       | 99 (82)       | 41 (80)       | 14 (50)   |          |
| ** OR, 95% CI, p-value      | -              | 1.09, 0.47 – 2.52, 0.83 | 4.5, 1.65 – 9.31, 0.002 |           |
| i region                    |                |               |               |           |
| i1                          | 79 (39.5)      | 38 (31)       | 23 (45)       | 18 (64)   | 0.003    |
| i2                          | 121 (60.5)     | 83 (69)       | 28 (55)       | 10 (29)   |          |
| ** OR, 95% CI, p-value      | -              | 1.79, 0.91 – 3.51, 0.11 | 3.93, 1.65 – 9.31, 0.002 |           |
| d region                    |                |               |               |           |
| d1                          | 83 (41.5)      | 38 (31)       | 25 (49)       | 20 (71)   | < 0.001  |
| d2                          | 117 (58.5)     | 83 (69)       | 26 (51)       | 8 (29)    |          |
| ** OR, 95% CI, p-value      | -              | 2.1, 1.07 – 4.1, 0.02 | 5.46, 2.2 – 13.5, < 0.001 |           |

*All p-values were calculated using the Chi-square test. **Odds ratios were calculated using the twoby2 function and CG as a control group. OR: odds ratio; 95% CI: 95% confidence interval; CG: chronic gastritis; AG: atrophic gastritis; IM: intestinal metaplasia.

Table 5. Distribution of the vacA d genotypes according to vacA s, m, and i combinations.

| VacA d region | s1/m1/i1 | s1/m2/i1 | s1/m2/i2 | s2/m2/i2 | s1/m1/i2 | p-value * |
|--------------|----------|----------|----------|----------|----------|-----------|
| d1           | 44 (55)  | 28 (35)  | 2 (2.5)  | 4 (5)    | 2 (2.5)  | < 0.001   |
| d2           | 0 (0)    | 4 (3.3)  | 12 (10)  | 104 (86.7)| 0 (0)    |           |

*The p-value was calculated using Fisher test.

Table 6. Prevalence and distribution of vacA combinations according to gastric pathologies.

| Prevalence of vacA combinations | CG n = 121 (%) | AG n = 51 (%) | IM n = 28 (%) |
|---------------------------------|----------------|---------------|---------------|
| s2/m2/i2/d2                    | 104 (52)       | 71 (59)       | 25 (49)       |
| s1/m1/i1/d1                    | 44 (22)        | 22 (18)       | 8 (16)        |
| s1/m2/i1/d1                    | 28 (14)        | 12 (10)       | 12 (23)       |
| s1/m2/i2/d2                    | 12 (6)         | 8 (6.6)       | 4 (8)         |
| s2/m2/i2/d1                    | 4 (2)          | 2 (1.6)       | -             |
| s1/m2/i1/d2                    | 4 (2)          | 4 (3)         | -             |
| s1/m1/i2/d1                    | 2 (1)          | -             | 2 (4)         |
| s1/m2/i2/d1                    | 2 (1)          | 2 (1.6)       | -             |

CG: chronic gastritis; AG: atrophic gastritis; IM: intestinal metaplasia.

Table 7. Association between vacA s1/m2/i1/d1 combination and the risk of precancerous lesions.

| s2/m2/i2/d2 n (%) | s1/m2/i1/d1 n (%) | OR * | 95% CI | p-value |
|-------------------|-------------------|------|--------|---------|
| CG                | 71 (86)           | -    | -      | -       |
| AG                | 25 (68)           | 2.84 | 1.13 – 7.13 | 0.02    |
| IM                | 8 (67)            | 2.95 | 0.76 – 11.37 | 0.1     |

*Odds ratios were calculated using the twoby2 function, and CG and vacA s2/m2/i2/d2 combination as a control group and reference strain, respectively. CG: chronic gastritis; AG: atrophic gastritis; IM: intestinal metaplasia; OR: odds ratio; 95% CI: 95% confidence interval.
In the case of IM, the vacA s1, m1, i1, and d1 genotypes were found to increase the risk of IM with an OR of 2.55 (95% CI = 1.08 – 6, p-value = 0.03), 4.5 (95% CI = 1.87 – 10.77, p-value = 0.001), 3.93 (95% CI = 1.65 – 9.31, p-value = 0.003), and 5.46 (95% CI = 2.2 – 13.5, p-value < 0.001), respectively (Table 4).

**Association between vacA d genotypes and vacA combinations**

Our results showed that the frequency of vacA d1 genotype was elevated in the vacA s1/m1/i1 combination (55%), followed by the vacA s1/m2/i1 combination (35%), the vacA s2/m2/i2 combination (5%), the vacA s1/m2/i2 combination (2.5%), and the vacA s1/m1/i2 combination (2.5%). In contrast, the vacA d2 genotype was more detected in the vacA s2/m2/i2 combination (86.7%), followed by the vacA s1/m2/i2 combination (10%), and the vacA s1/m2/i1 combination (3.3%) (Table 5).

**Prevalence of vacA combinations**

Considering all vacA regions together, the vacA combinations observed in this study are listed in Table 6. Our population was characterized by a dominance of the vacA s2/m2/i2/d2 combination (52%), followed by the vacA s1/m1/i1/d1 and s1/m2/i1/d1 combinations (22 and 14%, respectively).

**Distribution of vacA combinations according to gastric lesions**

In CG, the most common vacA combination was s2/m2/i2/d2 (59%), followed by the vacA s1/m1/i1/d1 combination (18%), the vacA s1/m2/i1/d1 combination (10%), the vacA s1/m2/i2/d2 combination (6.6%), the vacA s1/m2/i1/d2 combination (3%), the vacA s2/m2/i2/d1 and s1/m2/i2/d1 combinations (1.6%).

In the case of AG, the most common vacA combination was s2/m2/i2/d2 (49%), followed by the vacA s1/m2/i1/d1 combination (23%), the vacA s1/m1/i1/d1 combination (16%), the vacA s1/m2/i2/d2 combination (8%), and the vacA s1/m1/i2/d1 combination (4%).

In IM, the most common vacA combination was s1/m1/i1/d1 (50%), followed by s2/m2/i2/d2 and s1/m2/i1/d1 combinations (29 and 14%, respectively). The vacA s2/m2/i2/d1 combination was detected in 7%, while the other vacA combinations were totally absent. Table 6 shows that the frequency of the lowest virulent vacA combination, s2/m2/i2/d2, decreases according to gastric lesions severity: 59% in CG, 49% in AG, and 29% in IM. In the case of the vacA s1/m2/i1/d1 combination, considered as a moderate virulent combination, it was more detected in AG (23%) compared to CG and IM (10 and 14%, respectively). In contrast, the frequency of the most virulent vacA combination, s1/m1/i1/d1, increases according to gastric lesions severity: 18% in CG, 16% in AG, and 50% in IM.

**Association between vacA combinations and the risk of gastric precancerous lesions**

By taking CG as a control group and vacA s2/m2/i2/d2 combination as a reference strain, the risks of developing AG and IM following infection with the vacA s1/m1/i1/d1 and s1/m2/i1/d1 combinations were estimated.

According to Table 7, the frequency of vacA s1/m2/i1/d1 combination was higher in AG (32%) compared to CG (14%). In contrast, the frequency of vacA s2/m2/i2/d2 combination was lower in AG (68%) compared to CG (86%). Therefore, the risk of developing AG lesion in patients carrying the vacA s1/m2/i1/d1 strains was higher with a factor of OR = 2.84 (95% CI = 1.13 – 7.13, p-value = 0.02), compared to those carrying the vacA s2/m2/i2/d2 strains.

The frequency of the vacA s1/m2/i1/d1 combination was higher among patients suffering from IM (33%) compared to CG (14%), while the frequency of the vacA s2/m2/i2/d2 combination was lower in IM (67%) compared to CG (86%). However, no association was found between the risk of developing IM lesion and patients carrying the vacA s1/m2/i1/d1 combination (OR = 2.95, 95% CI = 0.76 – 11.37, p-value = 0.1).

According to Table 8, the distribution of vacA s1/m1/i1/d1 and s2/m2/i2/d2 combinations were the same in AG and CG. Thus, no association was found between the vacA s1/m1/i1/d1 combination and the risk of AG (OR = 1.03, 95% CI = 0.4 – 2.61, p-value = 1).

*Odds ratios were calculated using the twiby2 function, and CG and vacA s2/m2/i2/d2 combination as a control group and reference strain, respectively. CG: chronic gastritis; AG: atrophic gastritis; IM: intestinal metaplasia; OR: odds ratio, 95% CI: 95% confidence interval.*

**Table 8. Association between vacA s1/m1/i1/d1 combination and the risk of precancerous lesions.**

|          | s2/m2/i2/d2 | s1/m1/i1/d1 | OR* | 95% CI        | p-value |
|----------|-------------|-------------|-----|--------------|--------|
|          | n (%)       | n (%)       |     |              |        |
| CG       | 71 (76)     | 22 (24)     | -   | -            | -      |
| AG       | 25 (76)     | 8 (24)      | 1.03| 0.4 – 2.61   | 1      |
| IM       | 8 (36)      | 14 (64)     | 5.64| 2.09 – 15.22 | < 0.001|
In IM, the frequency of the vacA s1/m1/i1/d1 combination was higher (64%) compared to CG (24%), while the frequency of the vacA s2/m2/i2/d2 combination was lower in IM (36%) compared to CG (76%). Therefore, a significant association was found between the risk of developing IM lesion in patients carrying the vacA s1/m1/i1/d1 strains (OR = 5.64, 95% CI = 2.09 – 15.22, p-value < 0.001).

**Discussion**

Since the discovery of the vacA s, m, and i regions, numerous studies have investigated their association with the risk of precancerous lesions. However, the recently discovered vacA d region remains poorly studied. In this study, we characterized the polymorphisms of the vacA d region in order to study their association with the development of precancerous lesions.

In vacA s, m, and i regions, the most common genotypes were vacA s2 (51.5%), m2 (77%), and i2 (60.5%). This observation is similar to the epidemiological studies conducted on Moroccan, Tunisian, Egyptian, and Kenyan populations [13–16], but differs from a Senegalese study, where vacA s1, m1, and i1 genotypes were predominant [17].

Concerning the vacA d region, the majority of H. pylori strains were vacA d2 genotype (58.5%). Such finding has been reported by several Iranian studies [18–21], while other studies revealed a dominance of the vacA d1 genotype [22,23]. In Africa, there is no data regarding the prevalence of vacA d region, so further studies are needed to establish an accurate profile of this region.

The distribution of vacA genotypes among gastric lesions showed that the frequency of vacA s1, m1, and i1 genotypes tends to increase in AG compared to CG, but without reaching a statistically significant association (Table 4). In IM, all active forms of the vacA regions (s1, m1, and i1 genotypes) were found to be associated with the development of this lesion. Association between vacA genotypes and the development of gastric lesions varied among epidemiological studies. Some reports found that vacA s1, m1, and i1 genotypes increased the risk of both AG and IM [24,25], while others found that vacA s1, m1, and i1 genotypes were only associated with the risk of IM [14,26,27].

In the case of the vacA d region, the vacA d1 genotype was found to be associated with AG and IM. Ogiwara et al reported a positive association between vacA d1 genotype and the development of AG [7]. In addition, the vacA d1 genotype was found to increase the risk of GC by numerous studies [18–20,23]. However, no study has assessed the association between the vacA d region and IM.

The combination of the vacA s, m, and i genotypes allows the differentiation of the vacuolating activity of the VacA protein between H. pylori strains. It is known that the vacA s1/m1/i1 combinations induce cell vacuolation while the vacA s2/m2/i2 combinations do not. In the case of the vacA s1/m2 combinations, the presence of the i1 genotype is associated with a cellular vacuolation activity, while the presence of the i2 genotype is associated with the absence of the vacuolation activity [3,6,25].

In our population, most of our vacA d1 genotype cases were detected in the active forms of vacA combinations (s1/m1/i1 and s1/m2/i1). This observation is similar to previous studies [7,18,22]. In contrast, the inactive forms of vacA combinations (s1/m2/i2 and s2/m2/i2) were characterized by a predominance of the vacA d2 genotype. Even though the physiological role of the vacA d region remains undiscovered, it seems that the vacA d1 and d2 genotypes are highly associated with the active and inactive forms of vacA combinations, which are respectively characterized by high and low vacuolation activity.

The mosaic combination of the vacA s, m, i, and d regions can lead to several vacA combinations. Our population is characterized by the predominance of the nonvirulent vacA combination, s2/m2/i2/d2, followed by the most virulent vacA combination, s1/m1/i1/d1. In an Algerian study, the vacA s2/m2/i2/d2 was the most common combination [28]. Several African studies (Tunisia, Morocco, and Egypt) have also shown the predominance of the vacA s2/m2 combination in their population [13,15,29]. Moreover, a Moroccan and Kenyan study found a high prevalence of the vacA s2/m2/i2 combination, followed by the vacA s1/m1/i1 combination [14,16]. In contrast, the vacA s1/m1/i1 combination was predominantly detected in a Senegalese study [17].

Our finding demonstrated the association between the vacA d2 genotype with the least virulent vacA combination (s2/m2/i2). In addition, most African H. pylori strains are characterized by the predominance of the vacA s2/m2 and s2/m2/i2 combinations. Based on these observations, we might suggest that the African vacA genetic profile could belong to the s2/m2/i2/d2 combination. However, more studies are needed to confirm this hypothesis.

The results of our study suggest that patients infected with the vacA s1/m1/i1/d1 combination are
more susceptible to develop IM (OR = 5.64, 95% CI = 
2.09 – 15.22, p-value < 0.001) than AG (OR = 1.03, 95% CI = 0.4 – 2.61, p-value = 1). Winter et al showed that the vacA s1/i1 combinations are associated with a high risk of IM compared to the vacA s2/i2 combinations [26]. Moreover, a follow-up study conducted by Gonzalez et al showed that progression toward IM was more frequent in patients infected with the vacA s1/m1 combination than patients infected with the vacA s2/m2 combination [30].

The rapid evolution from a simple CG to more severe lesions is linked to the type of vacA combination. Indeed, H. pylori strains carrying the vacA s1/m1/i1 combination are known to be more virulent than H. pylori strains carrying the vacA s2/m2/i2 combination. It was shown that vacA s2/m2/i2 combinations do not cause vacuolation on epithelial cells, while vacA s1/m1/i1 combinations are characterized by a high degree of vacuolating activity [25]. In addition, the vacA s1/m1/i1 combinations are highly apoptotic and induce more intense inflammation [31]. Moreover, H. pylori strains possessing the vacA s1/m1/i1 combination are more likely to carry the cagA gene, which is another H. pylori virulence factor, and considered as an oncoprotein [32–34]. All these factors can explain the association between the vacA s1/m1/i1/d1 combination and the high risk of IM.

In our study, the moderate virulent vacA s1/m2/i1/d1 combination was associated with the risk of AG (OR = 2.84, 95% CI = 1.13 – 7.13, p-value = 0.02). The vacA s1/m2/i1 combination is known to possess an intermediate vacuolating activity compared to vacA s1/m1/i1 combination [35]. This difference is explained by the variation encountered in the vacA m region, which influences host cell tropism between different vacA strains [36–38]. Indeed, vacA combinations with the m1 genotype can bind to a wider range of host cells than the vacA m2 genotype, which results in a great vacuolating effect [9]. This variation in cell tropism may explain the association between the vacA s1/m2/i1/d1 combination and the risk of AG.

Conclusions
We showed in this study that the vacA s2/m2/i2/d2 combination predominates in our Casablanca population. Compared to other vacA s, m, and i regions, the recently discovered vacA d region seems to be a better marker for the risk of AG and IM. In addition, the active form of the vacA d region was exclusively associated with the most virulent vacA combinations (s1/m1/i1 and s1/m2/i1). Moreover, patients infected with the vacA s1/m2/i1/d1 are more susceptible to develop AG, while those infected with the vacA s1/m1/i1/d1 combination are at high risk of developing IM. Taking together, our results show that the vacA d region appears to be a reliable marker for the identification of virulent vacA strains that are a risk factor for AG and IM development.

Acknowledgements
Dr. Maria Kadi and Dr. Meriem Eljihad from Gastroenterology Department, Ibn Rochd, University Hospital Center, Casablanca Morocco. Dr. Nadia El Gnaoui, Mrs. Maria Serdani, Mrs. Saïda Moutahir, and Dr. Amal Oukkadi from the Laboratory of Histo-Cytopathology, Pasteur Institute of Morocco, Casablanca. Dr. Meriem Khyati from Laboratory of Onco-Virology, Pasteur Institute of Morocco, Casablanca.

Authors’ contributions
Mohamed Reda Jouimyi and Fatima Maachi designed the study. Mohamed Reda Jouimyi carried out the study and wrote the manuscript. Wafaa Badre provided the biopsies. Hakima Benomar performed the histological examination. Ghizlane Bounder, Imane Essaïdi, Hasna Boura, Khalid Zerouali, Halima Lebrazi, and Anass Kettani revised the manuscript. All authors read and approved the final version of the manuscript.

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**Conflict of interests:** No conflict of interests is declared.