**BRIEF COMMUNICATION**

**IGHG3** hinge length variation was associated with the risk of critical disease and death in a Spanish COVID-19 cohort

Rocio López-Martinez, Guillermo M. Albaiceta, Laura Amado-Rodríguez, Juan Gómez, Elias Cuesta-Llavona, Marta García-Clemente, Tamara Hermida-Valverde, Ana I. Enríquez-Rodriguez, Cristina Hernández-González, Jesús Martínez-Borra, Carlos López-Larrea, Helena Gil-Peña, Victoria Alvarez, and Eliecer Coto

*IgG3 would play an important role in the immune adaptive response against SARS-CoV-2, and low plasma levels might increase the risk of COVID-19 severity and mortality. The IgG3 hinge sequence has a variable repeat of a 15 amino acid exod with common 4-repeats (M) and 3-repeats (S). This length **IGHG3** polymorphism might affect the IgG3 effector functions. The short hinge length would reduce the IgG3 flexibility and impairs the neutralization and phagocytosis compared to larger length-isofoms. We genotyped the **IGHG3** length polymorphism in patients with critical COVID-19 (N = 516; 107 death) and 152 moderate-severe but no-critical cases. Carriers of the S allele had an increased risk of critical ICU and mortality (p < 0.001, OR = 2.79, 95% CI = 1.66–4.65). This adverse effect might be explained by a less flexibility and reduced ability to induce phagocytosis or viral neutralization for the short length allele. We concluded that the IgG3 hinge length polymorphism could be a predictor of critical COVID-19 and the risk of death. This study was based on a limited number of patients from a single population, and requires validation in larger cohorts.*

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**INTRODUCTION**

Immunoglobulin G (IgG) mediates functions like virus neutralization, opsonization of the infected cells, and modulation of cytokines production. The latter functions are driven by the binding of the IgG-constant region to the cellular Fc receptors (FCGR). There are four IgG subclasses (IgG1-4) with different structural and functional properties depending on their constant regions. IgG1 and IgG3 are the main immunoglobulins implicated in antiviral responses [1]. IgG subclasses could induce different cytokine production through binding to the FcγR, with IgG1 and IgG3 as the main regulators of type I interferon responses [2].

The constant region of the IgGs is encoded by the **IGHG1-4** genes, which are highly homologous and polymorphic. **IGHG** polymorphisms have been associated with differences in the IgG half-life and effector functions [3]. Subsequently, they might be associated with heterogeneous neutralization-capacity and increased risk for infection and viral disease outcome. IgG3 (encoded by the **IGHG3** gene) is the unique subclass that varies in its hinge length by different copies of a 15 amino acid exon-repeat. The most common **IGHG3** has 4 repeats, and a less common 3-repeats and rare 5-repeats have been reported [3]. Some studies have demonstrated that increased hinge length drives better phagocytosis and neutralization capacities, what is likely a consequence of greater flexibility that facilitates the binding to multiple epitopes [4, 5]. Other studies reported that shorter hinge variants induce better antibody-dependent cellular toxicity (ADCC), what might be explained by a closer proximity between natural-killer and its target cell [6].

Low IgG3 titers have been associated with higher SARS-CoV-2 disease (COVID-19) severity and increased mortality [7, 8]. Different SARS-CoV-2 mRNA vaccines elicited different IgG subclass profiles, potentially conferring differential protection [9, 10]. Anti SARS-CoV-2 IgG monoclonal antibodies would exhibit the best neutralizing capacity [11]. Due to the pivotal role of IgG3 in COVID-19, the **IGHG3** hinge length is a candidate polymorphism to modulate the disease outcome and the risk for critical COVID-19. In this context, variants in the FCGR2A have also been associated with ADCC or phagocytosis and variable responses to viral infections, including SARS-CoV-2 [12, 13]. In this work, we studied the association between the **IGHG3** hinge length and the risk of critical COVID-19.

**METHODS**

This study was approved by the Ethical Research Committee of Asturias and the participants or their next of kin gave their informed consent. All the participants were from the region of Asturias (Northern Spain, total population one million, 25% >65 years). Individuals with non-European ancestry were not included, and none of the participants had been vaccinated against SARS-CoV-2. We studied 516 COVID-19 critical patients who required admission to the Intensive Care Unit (ICU) of Hospital Universitario Central Asturias during the period March-2020 to July-2021. The less-severe group was composed of patients (N = 152) with mild-moderate COVID-19 symptoms who attended the Respiratory Department.
with no need for ICU admission. We also studied 180 individuals from the
general population with the same sex and age distribution as the patients.
These controls were followed during the study period and did not have
COVID-19 symptoms, although the absence of SARS-CoV-2 infection was
not confirmed by serological tests.

The DNA was obtained from whole blood leukocytes and all the
individuals were genotyped for the IGHG3 hinge length (alleles of 3-
repeats, 5, 4-repeats, M, and 5-repeats, L) by amplifying a PCR fragment
with primers 5’ CCCACTTGGTGACACAACTCAC and 5’GCTCAAAACCC
CACCTGGTGACACAAAC. These primers were specific for IGHG3 to avoid
amplification of the other highly homologous IGHG genes. The forward
primer was 5’labelled with the fluorochrome S-FAM to facilitate the
detection of the PCR-fragment length through capillary electrophoresis
(Supplementary Fig. 1).

All the patients’ values (age, sex, cardiovascular comorbidities, IL-6,
D-Dimer, corticosteroid treatment) were obtained from the clinical history
at ICU admission. An age <65 years was considered as the cut-off value for
early onset COVID-19. All the data (including the genotypes) were
annotated in an excel file and the statistical analysis was performed by
logistic regression with the R-free software (www.r-project.org).

The post-hoc power (death vs survival) was calculated based on the
observed S-frequencies and the number of deceased and survivors in the
ICU-patients.

### RESULTS AND DISCUSSION

Demographic characteristics for the no-ICU and ICU patients
(death vs survivors) are summarized in Table 1. Mortality in the ICU
patients was significantly associated with late-onset (≥65 years;
\( p = 3.90 \times 10^{-9} \)), hypertension (\( p = 0.002 \)), and hypercholesterole-
mia (\( p = 0.01 \)). High IL-6 (>70 pg/mL) and D-Dimer (>2000 ng/mL)
at ICU admission were also associated with death (\( p = 0.01 \)). Patients
receiving corticosteroid therapy had a significant reduction
in death (\( p = 0.007 \)). In reference to the genotypes, carriers of the
3-repeats S-allele (5S + 5S genotypes) were significantly more
common in the death patients (\( p < 0.001 \)). After multiple logistic
regression with age (linear generalised model) only IL6 and the
 genotype remained as signi
fically associated with late-onset (>65 years) and the total number of death and
survivors (107 and 409) the post-hoc power of the study at an
alpha level = 0.05 was >95%.

IGHG3 S-carriers were significantly more frequent among the
ICU vs no-ICU patients (\( p = 0.005 \)). The S-allele frequency in the
healthy population was higher than in the no-ICU patients (11.5%
vs. 6.5%) but lower than in the critical COVID-19 (11.5% vs 15%)

### Table 1. Main characteristics of the COVID-19 cases.

|                | ICU N = 516 | Death N = 107 | Survivors N = 409 | p-value death vs survivor |
|----------------|-------------|---------------|-------------------|--------------------------|
| Age mean (IQR) | 64 (18–95)  | 71 (32–95)    | 62 (18–84)        | 1.17 \times 10^{-13}     |
| <65            | 258 (50%)   | 25 (23%)      | 233 (57%)         | 4 \times 10^{-9}         |
| ≥65            | 258 (50%)   | 82 (77%)      | 176 (43%)         |                          |
| Male           | 372 (72%)   | 76 (71%)      | 296 (72%)         | 0.790                    |
| BMI mean (range)| 28 (19–53) | 27 (21–50)    | 28 (19–53)        |                          |
| BMI ≥ 30       | 264 (51%)   | 55 (51%)      | 209 (51%)         | 0.300                    |
| Hypertension   | 286 (56%)   | 74 (69%)      | 212 (52%)         | 0.002                    |
| Hypercholesterolemia | 241 (47%) | 62 (58%)      | 179 (44%)         | 0.009                    |
| Diabetes       | 111 (22%)   | 26 (24%)      | 85 (21%)          | 0.430                    |
| IL-6 pg/mL\(^b\) median (IQR) | 74 (35–126) | 91 (52–130) | 71 (31–124) | 0.05 |
| IL-6 > 70 pg/mL| 229 (67%)   | 59 (88%)      | 170 (61%)         | 0.01                     |
| D-dimer ng/mL\(^c\) Median (IQR) | 1111 (634–2076) | 1507 (954–2590) | 1014 (603–1779) | 0.01 |
| D-dimer> 2000 ng/mL | 97 (26%) | 33 (36%) | 64 (23%) | 0.01 |
| Corticosteroids| 454 (88%)   | 86 (80%)      | 368 (90%)         | 0.006                    |

\(^a\)Data obtained from the electronic clinical history.

\(^b\)Measured in 451 ICU (95 death and 356 survivors).

\(^c\)Measured in 375 ICU patients (92 deceased and 283 survivors).

### Table 2. Statistical p-values, Odds Ratio (OR) and 95% confidence intervals (95% CI) in death vs survivors, univariate and multivariate logistic regression (linear generalized model) with age.

|                | UNIVARIATE |          |          | MULTIVARIATE |          |          |
|----------------|------------|----------|----------|--------------|----------|----------|
|                | p          | OR       | 95% CI   | p            | OR       | 95% CI   |
| Age 65 years   | 4 \times 10^{-9} | 4.34    | 2.70–7.20 | Adjusting variable |
| Male           | 0.78       | 0.94     | 0.59–1.51 | 0.39          | 0.91     | 0.56–1.04 |
| Corticosteroids| 0.006      | 0.44     | 0.25–0.80 | 0.06          | 0.56     | 0.31–5.73 |
| BMI > 30       | 0.301      | 0.75     | 0.44–1.29 | 0.88          | 1.04     | 0.67–1.62 |
| Hypertension   | 0.002      | 2.08     | 1.33–3.31 | 0.06          | 1.57     | 0.98–2.54 |
| Diabetes       | 0.430      | 1.22     | 0.73–2.00 | 0.45          | 0.82     | 0.47–1.37 |
| Hypercholesterol| 0.009     | 1.77     | 1.15–2.73 | 0.33          | 1.26     | 0.79–1.99 |
| IL6 > 70       | 0.014      | 1.79     | 1.13–2.87 | 0.02          | 1.76     | 1.10–2.87 |
| D-dimer >2000  | 0.012      | 1.91     | 1.14–3.18 | 0.05          | 1.71     | 1.01–2.88 |
| IGHG3 S-carriers| <0.001     | 2.79     | 1.66–4.65 | <0.001        | 3.47     | 1.98–6.09 |

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Finally, our work has several limitations. Mainly, it was based on a limited number of patients and from a single population and the results would thus require validation in larger cohorts. We compared critical with less severe cases. SARS-CoV-2 positives but asymptomatic were not studied, and the full disease spectrum was not evaluated.

In conclusion, we found that the \textit{IGHG3} short hinge length was associated with an increased risk of mortality among critical COVID-19 patients. This could be explained by the lower efficiency of the short hinge to induce the IgG3 effector functions compared to longer hinge isoforms. The IgG3 hinge length polymorphism might thus serve as a marker to predict disease severity and the risk of death among COVID-19 patients.

**DATA AVAILABILITY**

To facilitate the revision of the results by other researchers, a file with the patient’s data is available as an excel file upon request to the corresponding author.

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AUTHOR CONTRIBUTIONS
Lead researchers: EC, GMA, JG. Study design: EC, GMA, JG. Patient assessment: GMA, LAR, MGC, THV, AIER, CHG, CLL, JMB, VA, HGP. Genetic study: RLM, EC, ECL, JG, VA. Database: RLM, EC, GMA, LAR, ECL. Data filtering and analysis: RLM, EC. Statistical analysis: RLP, EC. Analysis of results: RLM, EC, GMA. Drafting of the manuscript: EC. Revision of the manuscript: all authors. All the authors contributed to this work by recruiting the patients and performing the genetic and statistical analyses. E.C. takes full responsibility for the accuracy of the data. All the authors approved the submission of this manuscript.

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COMPETING INTERESTS
The authors declare no competing interests.

ADDITIONAL INFORMATION
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Correspondence and requests for materials should be addressed to Eliecer Coto.

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