BRIEF COMMUNICATION

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

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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 exon 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-isoforms. 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 Fcγ-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 IgG3 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,

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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, S, 4-repeats, M, and 5-repeats, L) by amplifying a PCR fragment with primers 5´ CCCCACTTGGTGACACAACTCAC and 5´GCTCAAAACCC CACTTGGTGACACAAC. 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 5-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 hypercholesterolemia ($p = 0.01$). 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 (SS + MS 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 significant predictors of death (Table 2; Supplementary Table 1). Based on the observed S-carrier frequencies (28% vs 12%) 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)	1.17×10^{-13}
<65	258 (50%)	25 (23%)	233 (57%)	4×10^{-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[a]	286 (56%)	74 (69%)	212 (52%)	0.002
Hypercolesterolemia[a]	241 (47%)	62 (58%)	179 (44%)	0.009
Diabetes[a]	111 (22%)	26 (24%)	85 (21%)	0.430
IL-6 pg/mL[b] median (IQR)	74 (35–126)	91 (52–130)	71 (31–124)	
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)	
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	4×10^{-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
Hyperchol	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

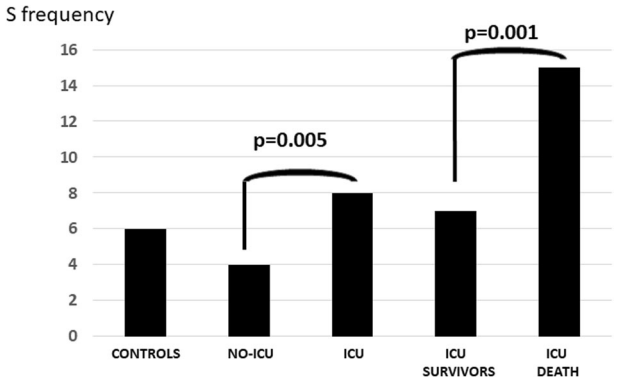

Fig. 1 Frequency of the short IGHG3 hinge (S, 3 repeats) in the study groups. Carriers of the S-allele (MS and SS) were significantly higher in the ICU vs no-ICU, and in the ICU death vs survivors. The raw data are presented as a supplementary table.

(Fig. 1; Supplementary Table 2). This suggested that the 3-repeats *IGHG3* variant was a risk factor for critical COVID-19 death with a significant effect on mortality. The S-allele frequency was higher in the ICU vs no-ICU patients aged <65 and ≥65 years (Supplementery Table 3). The S-allele frequency was also higher among the death independently of the corticosteroid treatment (Supplementary Table 4).

IgG3 and IgG1 are the main IgG subclasses involved in the antiviral response with functions like viral neutralization, opsonization and induction of cytokine production. COVID-19 patients showed high amounts of IgG3 and IgG1 and the blood titers were correlated with the severity of the infection and the production of cytokines [7, 8]. IgG3 and IgG1 can induce cytokine production through the FcγR pathway by metabolic reprogramming of myeloid cells or by monocytes/macrophages infection causing an inflammatory cell death [2, 14]. In addition, the afucosylated structures of the Fc of IgG1 enhanced the production of inflammatory cytokines due to their greater affinity for FcγR [15]. In HIV patients longer hinge length would increase the IgG3 viral neutralization and phagocytosis [4, 5]. Coronaviruses differ from other viruses on their surface by having more separation between their epitopes, which could reduce the neutralization capacity of IgG [16]. This characteristic may imply an even more important role of the IgG hinge length in its effective neutralization. The reduced "flexibility" of the short hinge IgG3 isoform could be associated with lower capacity to drive an antiviral response compared to the longer hinge isoforms, and this could explain the association of the short hinge length with worse COVID-19 and increased mortality. Variants in *IGHG3* have been associated with other infectious diseases such as malaria [17]. The association between *IGHG3* and severe COVID-19 seems plausible given the reported functional effect of the hinge length on IgG3 neutralization capacity and the role of IgG3 in the response to SARS-CoV-2 infection and adverse disease outcome [18–20]. SARS-CoV-2 plasma RNAemia was tightly associated with disease severity and death, and Spike-specific IgG3 was inversely correlated with higher baseline plasma RNAemia, pointing to a direct role for SARS-CoV-2-specific IgG3 humoral immune clearance on viral dissemination, persistence, and disease outcome [21].

To the best of our knowledge, this is the first study that reports the effect of the *IGHG3* hinge length polymorphism in COVID-19. The IgG hinge region is not included in the genome wide association (GWA) studies due to the high homology between the different *IGHG* sequences, which makes difficult the specific amplification of the variants. This genome region was thus not properly covered in the GWAs and would require individualised genotyping approaches to define the association with disease.

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 *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. Statisticalanalysis: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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