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Functional associations between polymorphic regions of the human 3′IgH locus and COVID-19 disease

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ABSTRACT

Purpose: The pandemic diffusion of Coronavirus Disease 2019 (COVID-19) has highlighted significant gender-related differences in disease severity. Despite several hypotheses being proposed, how the genetic background of COVID-19 patients might impact clinical outcomes remains largely unknown.
Methods: We collected blood samples from 192 COVID-19 patients (115 men, 77 women, mean age 67 ± 19 years) admitted between March and June 2020 at two different hospital centers in Italy, and determined the allelic distribution of nine Single Nucleotide Polymorphisms (SNPs), located at the 3′Regulatory Region (3′RR)-1 in the immunoglobulin (Ig) heavy chain locus, including *1 and *2 alleles of polymorphic hs1.2 enhancer region.
Results: In COVID-19 patients, the genotyped SNPs exhibited strong Linkage Disequilibrium and produced 7 specific haplotypes, associated to different degrees of disease severity, including the occurrence of pneumonia. Additionally, the allele *2, which comprises a DNA binding site for the Estrogen receptor alpha (ERα) in the polymorphic enhancer hs1.2 of 3′RR-1, was significantly enriched in women with a less severe disease.
Conclusions: These findings document genetic variants associated to individual clinical severity of COVID-19 disease. Most specifically, a novel genetic protective factor was identified that might explain the sex-related differences in immune response to Sars-COV-2 infection in humans.

1. Introduction

Diffusion of Sars-COV-2 virus was declared a pandemic at the end of 2019, initially in eastern and then in western countries. Previous studies have documented an association with several genes and have identified genetic risk factors responsible for an increased clinical severity of SARS-CoV-2 infection (Severe Covid-19 GWAS Group et al., 2020). Two main genomic regions are significantly linked to severe COVID-19: one locus on chromosome 3, which covers six genes, and another region on chromosome 9 that controls ABO blood groups (Severe Covid-19 GWAS Group et al., 2020).

Epidemiological data has consistently confirmed a higher susceptibility of men relative to women, possibly due to differences in immune response (Mauvais-Jarvis et al., 2020; Takahashi et al., 2020; Bechmann et al., 2022). Indeed, although the host response to infection has been proven to be individual specific, several studies have reported that

Abbreviations: COVID-19, Coronavirus Disease 2019; SNPs, Single Nucleotide Polymorphisms; (3′RR)-1, 3′Regulatory Region; Ig, Immunoglobulin; ERα, Estrogen receptor alpha; ACE2, Angiotensin-converting enzyme 2; TMPRSS2, Transmembrane protease serine 2; heavy (H) chain, Immunoglobulin (Ig); ERE, Estrogen response elements; C, Cytosine; G, Guanine; A, Adenosine; T, Thymine; LD, Linkage Disequilibrium; H, Haplotype.

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mortality rates in men with COVID-19 are higher than among women (Agrawal et al., 2021; Channappanavar et al., 2017; Gadi et al., 2020; Mukherjee and Pahan, 2021). Recent findings suggest that these gender-based differences in COVID-19-related mortality might be linked to a different expression of the angiotensin-converting enzyme 2 (ACE2) receptor and of the transmembrane protease serine 2 (TMPRSS2) enzymes, but also to a different linkage with genes such as SRY and SOX9 (Penna et al., 2020). It is well known that gender predicts a different immune response to infectious diseases (Scepanovic et al., 2018; Billi et al., 2019). In women, the immune system has evolved to shield the embryo during pregnancy and the newborn during breastfeeding. However, a stronger reactivity of the immune system in women can, in turn, explain the higher prevalence of autoimmune diseases (Reardon, 2016).

The pattern of immune response in women has been hypothetically linked to estrogens (Styrt and Sugarman, 1991; Hirokawa et al., 2013; Sund et al., 2022). DNA binding sites of the Estrogen Receptor alpha (ERα) have been reported in the Emu and hs1.2 enhancers, which are important regulatory DNA domains in the immunoglobulin (Ig) heavy (H) chain 3′Regulatory Region (3′RR) (Jones et al., 2016). Moreover, full-genome chromatin immunoprecipitation analysis (ChIP-seq) using DNA from activated mouse B cells and an ERα-specific antibody has identified estrogen response elements (ERE) in heavy chain switch (S) regions (Jones et al., 2016, 2019a). This suggests that estrogens and ER directly modulate class switch recombination (CSR) and antibody expression by binding to the immunoglobulin heavy chain locus. Indeed, the production of immunoglobulins, such as IgM, IgE and IgG1/2, is higher in women than in men (Jones et al., 2019a; Pasternak et al., 2018; Jones et al., 2019b). Also, estrogen levels alter Ig production in response to influenza virus vaccine (Engelmann et al., 2016; Sealy et al., 2019) and infection (Takahashi et al., 2020; Fish, 2008; Jones et al., 2020). Notably, in the hs1.2 enhancers of human IgH locus, EREs are present in a polymorphic region, which is associated with several autoimmune diseases, including dermatitis herpetiformis, psoriatic arthritis and systemic lupus erythematosus (Frezza et al., 2012; Cianci et al., 2008).

Despite this evidence, a definite explanation of the gender-related differences in disease severity among COVID-19 patients is still lacking. Thus, the aim of this study was to investigate whether specific polymorphic regions of the 3′ Regulatory Region-1 of the human immunoglobulin heavy chain locus, situated on the palindrome region between the hs3 and hs1.2 enhancers, could explain a lower risk and a reduced clinical severity among women.

2. Materials and Methods

2.1. Study population and design

The study population included 192 COVID-19 patients consecutively admitted between March and June 2020 to the "Fondazione Policlinico Universitario A. Gemelli", IRCCS, Rome, Italy and of "Fondazione Casa Sollievo della Sofferenza", IRCCS San Giovanni Rotondo, Italy. One hundred fifteen were men, 77 were women, mean age was 67 ± 17.2 years. All patients were of European descent and had a positive RT-PCR test at a nasal and oropharyngeal swab. Socio-demographic information along with clinical and imaging data were derived from electronic medical records. The study has received appropriate institutional ethical approvals by Ethical Committees both at "Casa Sollievo della Sofferenza" Research Ethics Boards (Approval Code: GEN-COVID), and at "Fondazione Policlinico Universitario Agostino Gemelli" (Approval Code: COVID-19-SGR)

2.2. Clinical variables and imaging

Patients were divided into 5 disease severity groups, based on the need of oxygen supplementation and fraction of inspired oxygen (FiO2). Group 0 included patients with only minor symptoms and no need of oxygen. Group 1 included patients on oxygen treatment but with a FiO2 $\leq$ 50%. Group 2 included patients on oxygen supplied through high-flow nasal cannula (HFNC) or receiving not-invasive ventilation. Group 3 included patients assisted with mechanical ventilation or Extra Corporeal Membrane Oxygenation (ECMO) in an intensive care unit. Group 4 included those patients who developed pneumonia and/or died due to COVID-19 during the hospitalization (Table 1). A CT scan was done on all patients to diagnose the presence of interstitial pneumonia.

2.3. Blood samples

All patients signed an informed consent according to the Declaration of Helsinki. Blood samples were collected and stored at the Biobanks of the two Hospitals.

2.4. Genomic DNA extraction from human whole blood samples

DNA purifications and amplifications were performed at the "Casa Sollievo della Sofferenza" Hospital from whole blood samples. Genomic DNA was isolated using the QIAamp DNA Mini and Blood Mini kit (QIAGEN Hilden, Germany) according to the manufacturer's protocol. Aliquots of 200 ul for each of 192 frozen samples were thawed and 20 µl of proteinase K and lysis buffer were added to each sample. Subsequently, tubes were incubated at 56 °C for 10 min and vortex mixed during the incubation period. After purification, DNA concentration was measured for each sample using the NanoDropTM One Microvolume UV–Vis Spectrophotometer (ThermoScientific, Waltham, USA).

2.5. Amplification and sequencing of allelic variants of human IgH 3′ RR1

To investigate the different genetic profile between COVID-19 patients with different clinical severity, we sequenced 9 SNPs in four specific polymorphic regions of the 3′ Regulatory Region-1 (3′RR-1) of the human immunoglobulin heavy chain (IgH) locus, situated on the palindrome region between the hs3 and hs1.2 enhancers (Fig. 1). A 5.4 Kb region of IgH3′EC-1 that includes both hs1.2 and hs3 enhancers, was

Table 1
Demographic distribution and clinical features of considered COVID-19 patients.

	MEN (n = 115)	**WOMEN** (n = 77)	**ALL** (n = 192)
Age (mean ± SD)	64.7 ± 16.3	70.3 ± 18.01	67 ± 17.2
<50 years	23 (20%)	10 (12.9%)	33 (17.2%)
	(38.3 ± 8.64)	(33.8 ± 6.89)	(37.0 ± 8.37)
≥50 years	92 (80%)	67 (87.1%)	159 (82.8%)
	(71.3 ± 9.76)	(75.8 ± 11.66)	(73.2 ± 10.81)
Comorbidities			
0	31 (26.9%)	16 (20.8%)	47 (24.5%)
1	47 (40.9%)	30 (38.9%)	77 (40.1%)
2	17 (14.8%)	21 (27.4%)	38 (19.8%)
3+	20 (17.4%)	10 (12.9%)	30 (15.6%)
Comorbidities			
Hypertension	46 (40%)	29 (37.7%)	75 (39.1%)
NIDDM	16 (13.9%)	7 (9.1%)	23 (12%)
COPD	8 (6.9%)	2 (2.6%)	10 (5.2%)
Cardiac diseases	27 (23.5%)	17 (22.1%)	44 (22.9%)
Obesity	9 (7.8%)	2 (2.6%)	11 (5.7%)
Cancer	19 (16.5%)	18 (23.4%)	37 (19.3%)
Pneumonia	89 (77.4%)	45 (58.4%)	134 (69.8%)
Severity Group			
0	10 (8.7%)	8 (10.4%)	18 (9.4%)
1	47 (40.9%)	35 (45.4%)	82 (42.7%)
2	31 (26.9%)	19 (24.7%)	50 (26%)
3	17 (14.8%)	12 (15.6%)	29 (15.1%)
4	10 (8.7%)	3 (3.9%)	13 (6.8%)

SD: Standard Deviation; NIDDM: Non-Insulin-Dependent Diabetes Mellitus; COPD: Chronic Obstructive Pulmonary Disease; Cardiac diseases (Congestive heart failure, ischemic heart disease); Obesity BMI > 30Kg/m^2.

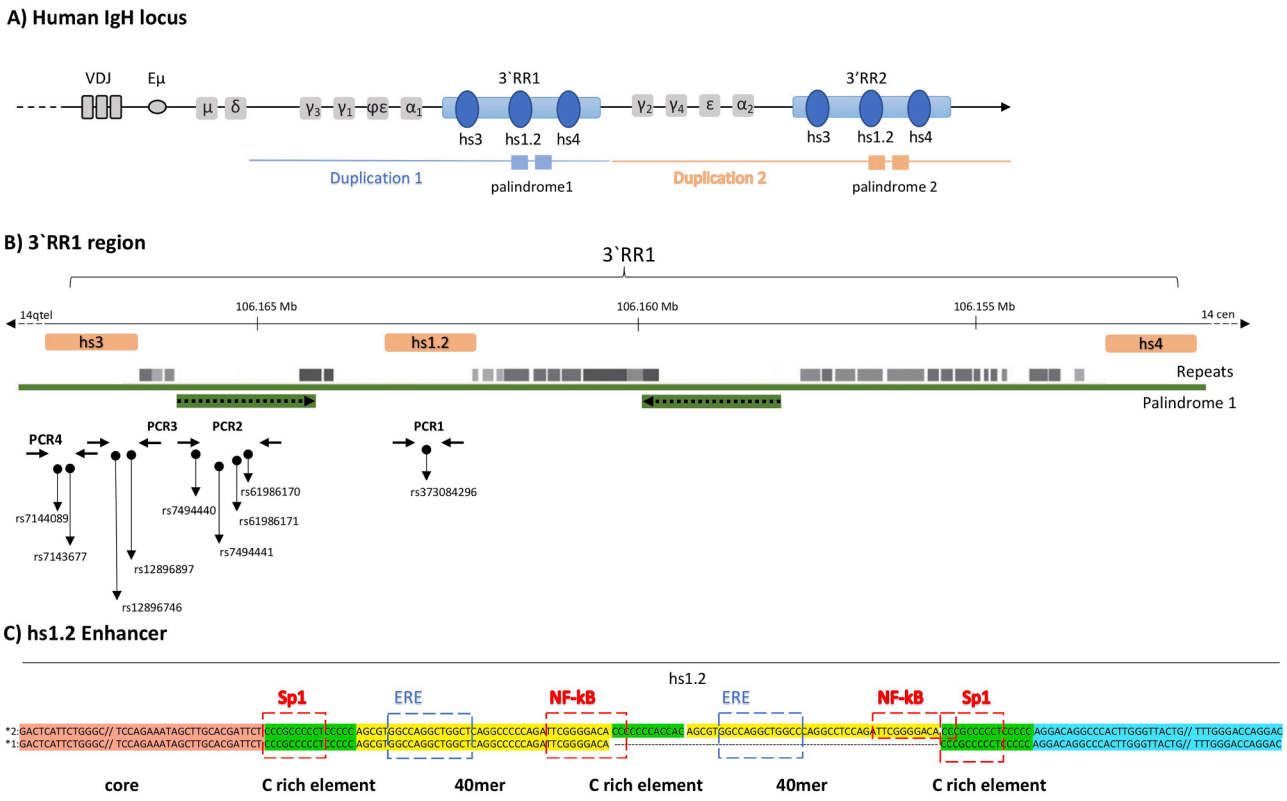

Fig. 1. Schematic maps of human immunoglobulin heavy chain (IgH). Human IgH locus (A), 3′ Regulatory Region 1 (3′RR1) (B) and hs1.2 enhancer (C). Nine SNPs (rs373084296, rs7494440, rs7494441, rs61986170, rs61986171, rs12896746, rs12896897, rs7144089 and rs7143677) within the 3′RR1 have been identified after amplification of long PCR followed by four different nested PCRs, highlighted by the arrows. The DNA fragment generated by PCR1 included the hs1.2 enhancer.

initially amplified by a long PCR assay using the custom DNA oligo primers, SA2.5 (5′-GGATCCCTGTTCCTGATCACT G-3′) and A2R (5′-GCCCTTCCTGCCAACCTG-3′), and the Expand Long Template PCR System (Roche, Basel, Switzerland). The long PCR reaction conditions were: 3.75 units of Taq DNA polymerase, Expanded Long Template buffer 1 (10X conc. with 17.5 mM $MgCl_2$), dNTP mix (350 μM), primers (300 nM), DNA (100 ng) and Betaine (0,9M) in the final volume of 50 μl. The PCR reaction was performed at 94 °C for 2 min, followed by 10 cycles at 94 °C for 10 s, 64 °C for 30 s, 68 °C for 4 min followed by 30 cycles at 94 °C for 15 s, 64 °C for 30 s, 68 °C for 4 min plus 20 s cycle elongation for each successive cycle and one final extension at 68 °C for 7 min.

Subsequently, four distinctive nested PCRs were performed to selectively amplify the polymorphic regions of IgH 3′RR-1 (Table S1). The nested PCR1 was carried out using the primers P3FFrw (5′-GACTCATTCTGGGCAGACTTG-3′) and D3Rev (5′-GTCCTGGTCCCAAAGATGG −3′) at the following conditions: 10X PCR buffer (minus Mg), 50 mM $MgCl_2$ (1.5 mM final conc.), 0.2 mM dNTP mix, 0.5 μM of each of P3FFrw and D3Rev primers, 1/10 of the volume of the Long PCR reaction and 1 U Taq DNA Polymerase recombinant, Invitrogen™. The thermocycler conditions for the nested PCR1 reaction were initial denaturation at 94 °C for 3 min, 30 cycles at 94 °C for 45 s, 55 °C for 30 s, 72 °C for 33 s and final extension at 72 °C for 10 min. The second, third and fourth Nested PCR amplifications were carried out as described above using the primers: Nested2Frw (5′-TCTCCTGTTCTCTGACCATC-3′) and Nested2Rev (5′- GTCACATCCATACCCACACT-3′) for PCR2; Nested3Frw (5′- TCCCTGTCCCTGTCTCTTAT-3′) and Nested3Rev (5′-AATACCCAAAATAGCCCTGT-3′) for PCR3; and Nested4Frw (5′- ATTCAAGAGGCTTCAGGAGA-3′) and Nested4Rev (5′-TTCCTTTTCTGAGCATGTATC −3′) for PCR4. The PCR products were confirmed by gel electrophoresis in 1.5% agarose gels and Sanger sequenced in service at the Eurofins Genomics (Ebersberg, Germany).

2.6. Haplotype association and statistical analysis

Beagle package (Browning et al., 2021) was used for phasing genotypes and for imputing ungenotyped markers. Linkage Disequilibrium (LD) blocks, haplotypes and frequencies were computed by Haploview v4.2 (Barrett et al., 2005). Pairwise LD coefficients (D′*100) were generated using genotype data of the 9 SNPs and the solid spine method was chosen for the prediction of LD block. This method searches for a "spine" of strong LD running from one marker to another along the legs of the triangle in the LD chart. Only haplotypes with a frequency greater that 1% were reported.

The identified haplotype block was then passed to Plink v1.07 (Purcell et al., 2007) to perform a multi-marker association test between haplotypes and traits (pneumonia and severity of disease). A case/control chi-square test was performed for pneumonia and a linear regression was performed for disease severity, considering all five levels. Sex, age and comorbidity were added as covariates. Additionally, a stratification test for sex was performed by applying the –filter-men and –filter-women options. Allele frequency calculation and other statistics on SNPs were performed in Plink v1.07. Differences in means between stratified groups were evaluated by a Student's *t*-test ($\alpha = 0.05$).

3. Results

The 192 COVID-19 patients included 115 men and 77 women. Men were subdivided as follows: group 0 (N = 10, mean age 52.2 years), group 1 (N = 47, mean age 62.4 years), group 2 (N = 31, mean age 66.4 years), group 3 (N = 17, mean age 67.6 years), group 4 (N = 10, mean age 78 years) (Fig. S1). Of these 115 men, 31 (26.9%) had no comorbidities and no assumption of pharmacological medication at home; one comorbidity was reported in 47 (40.9%) patients (11 with cancer, 6 suffering for cardiac pathologies, 1 with type 1 diabetes, 19 with

hypertension, 5 with obesity, 5 with other diseases), two comorbidities in 17 (14.8%), three or more comorbidities, including neoplasms, cardiac pathologies, obesity, hypertension, chronic lung diseases, diabetes, autoimmune disorders and chronic kidney diseases in 20 (17.4%) patients. At CT scan 134/192 patients (70% of total) had evidence of interstitial pneumonia. Of these, 89 (66.41%) were men. Among them, 15 patients aged < 50 years old and 74 > 50 years old (Fig. S1). Women were subdivided as follows: group 0 (N = 8, mean age 48.1 years), group 1 (N = 35, mean age 69.3 years), group 2 (N = 19, mean age 76.2 years), group 3 (N = 12, mean age 76.2 years), group 4 (N = 3, mean age 80.7 years) (Fig. S1). Of 77 women, 16 (20.8%) had no comorbidities and no assumption of pharmacological medication at home; one comorbidity was reported in 30 (38.9%) patients (4 with cancer, 11 suffering for cardiac pathologies, 1 with chronic obstructive pulmonary disease, 3 with hypertension, 2 with rheumatologic diseases, 9 with other diseases), two comorbidities in 21 (27.3%), three or more comorbidities, including neoplasms, cardiac pathologies, obesity, hypertension, chronic lung diseases, diabetes, autoimmune disorders and chronic kidney diseases in 10 (13%) patients.

At CT scan of 134/192 patients with evidence of interstitial pneumonia, 45 (33.6%) were women. Among them, 5 aged < 50 years old and 40 > 50 years old.

Demographic distribution and clinical features of study population are summarized in Table 1.

Patients with only minor symptoms, no need of oxygen, without risk factors and no evidence of pneumonia did not receive drugs. All patients with mild respiratory symptoms on oxygen treatment, presence of comorbidities, or evidence of pneumonia at CT scan received drug treatment following the "Guidelines for the treatment of people with COVID-19 disease Edition 2.0, 13 March 2020" (Mussini et al., 2021): Hydroxychloroquine 200 mg BID, lopinavir/ritonavir 200/50 mg, 2 pills BID, Azithromycin 500 mg/day. Patients with severe pneumonia, ARDS or overall respiratory failure, hemodynamic failure, need for mechanical (or non-invasive) ventilation received Dexamethasone or Tocilizumab therapy.

3.1. SNPs of 3′RR-1

We determined the allelic distribution of nine Single Nucleotide Polymorphisms (SNPs), located at the 3′RR-1 in the Ig heavy chain locus situated on the palindrome region between the hs3 and hs1.2 enhancers, including *1 and *2 alleles of polymorphic hs1.2 enhancer region. The minor allele frequencies of the 9 SNPs ranged between 18.5% and 46.9% (Table S1). Significant differences were found among 5 allelic frequencies in relationship to different degrees of disease severity, presence or absence of pneumonia and in relation to sex.

Table S2 summarizes the chi-square and p-value of deviations from Hardy–Weinberg equilibrium related to the expected and observed genotypes for each SNP. We observed an increase of homozygosity for Cytosine (C) in the rs7494441 in patients with diseases of increased severity. On the contrary, an increase of homozygosity for Guanine (G) was evident in asymptomatic patients of group 0. In the rs12896746, homozygosity for G was associated with a reduced clinical severity, while heterozygosity for Adenosine (A)-G was associated with a worse disease. Homozygosity for Thymine (T) in rs12896897 was associated to a favourable course, while heterozygosity for CT predicted the worst clinical severity. In the rs7144089, homozygosity for C was associated to a more favourable course of the disease, and homozygosity for G to the worst clinical severity (Table S3). The genotypes associated with the presence of pneumonia displayed homozygosity for G in rs7144089 (Table S4). The other genotypes did not show any specificity.

3.2. Haplotypes

The 9 genotyped SNPs exhibited strong Linkage Disequilibrium (LD) resulting in the same LD block-1 (Fig. S2) and producing 7 specific haplotypes configurations (Table 2). For each haplotype, we carried out an association test to highlight any possible link with pneumonia and with disease severity. Table S5 provides a summary of the case/control results for pneumonia. The frequency of the main haplotype H1 was similar in patients with pneumonia (cases frequency 0.33) and in controls (0.32). Frequencies of haplotypes H2-H7 showed large differences across the groups, but these were not statistically significant. The only statistically significant association (p = 0.045 in Table S5) regarded haplotype H7 (frequency in cases 0.0038 and 0.0272 in controls). However, haplotype H7 was rare, representing the less common among the seven haplotypes. H5 haplotype displayed an interesting pattern. Frequency of cases (0.1107) was higher than the frequency of controls (0.0455) and this haplotype appeared to confer a 2.9 times higher risk of developing pneumonia in patients with COVID.

Associations with disease severity were not statistically significant for any haplotype (Table S6).

As shown in Table 2, each haplotype also exhibited a preferential association with one of the two alleles of hs1.2 locus. We evaluated the degree of association of each haplotype with allele *1 and allele *2 of the hs1.2 locus. Haplotypes H1, H2 and H5 resulted preferentially associated with allele 1 while H3, H4 and H6 with allele 2.

3.3. HS1.2 alleles

Fig. 2 summarizes the pneumonia outcome ratio ("affected"/"affected + not affected") stratified in four groups according to sex and presence of hs1.2 alleles *1 and *2 across the major haplotypes identified. Women displayed a distinctive protection against pneumonia when compared to men especially when they harbour the hs1.2 allele *2. Haplotypes belonging to women hs1.2 allele *2 group revealed the lowest average value (0.42) of pneumonia outcome ratio. Significant differences were found when comparing this group to other groups, namely men hs1.2 allele *2 (p = 0.00008), women hs1.2 allele *1 (0.012) and men hs1.2 allele *1 (0.0009). Although the different haplotypes do not seem to have a clear impact on pneumonia outcome, H1 and H2 showed low pneumonia outcomes values in three groups out of four.

A similar pattern was found for disease severity outcomes in the 4 groups (Fig. S3). However, a significant difference was reported only for the comparison between hs1.2 allele *2 and hs1.2 allele *1 in women (p = 0.04). With regard to pneumonia outcomes, we observed differences between the groups (men vs women, presence of allele *1 or *2 of hs1.2 and age) (Fig. 3). Women with hs1.2 allele *2 exhibit the highest protective arrangement when compared to all other groups (p-value 0.0037). No evidence of a similar pattern could be documented for men. Age (under 50 vs over 50 years) also showed a trend in pneumonia outcome; older patients were more susceptible to develop pneumonia that younger ones, with a statistically significant difference only among men (p-value 0.02). The association between age and disease severity appears stronger (p = 4.63E-09) than that for pneumonia even when

Table 2
The haplotype code, the frequency and the SNPs forming the haplotype (Haplotype sequence) are reported. Since each haplotype results preferentially linked to one of the two alleles of Hs1.2 locus, the percentage of association with each Hs1.2 allele is also reported in the table for each haplotype.

Haplotype code	Haplotype Frequency	Haplotype sequence	Hs1.2 allele2	Hs1.2 allele 1
H1	0.3203	ACCGCACGA	20.13%	79.87%
H2	0.2057	ACCGCGCGA	27.00%	73.00%
H3	0.1692	GGTATGTCA	94.83%	5.17%
H4	0.1328	AGTGCGTCG	91.12%	8.88%
H5	0.09115	ACCGCGTGA	6.33%	93.67%
H6	0.03906	ACCGCGTCG	100.00%	0
H7	0.01042	AGTGCGTGA	ND	ND

Abbreviations: ND = no data for hs1.2.

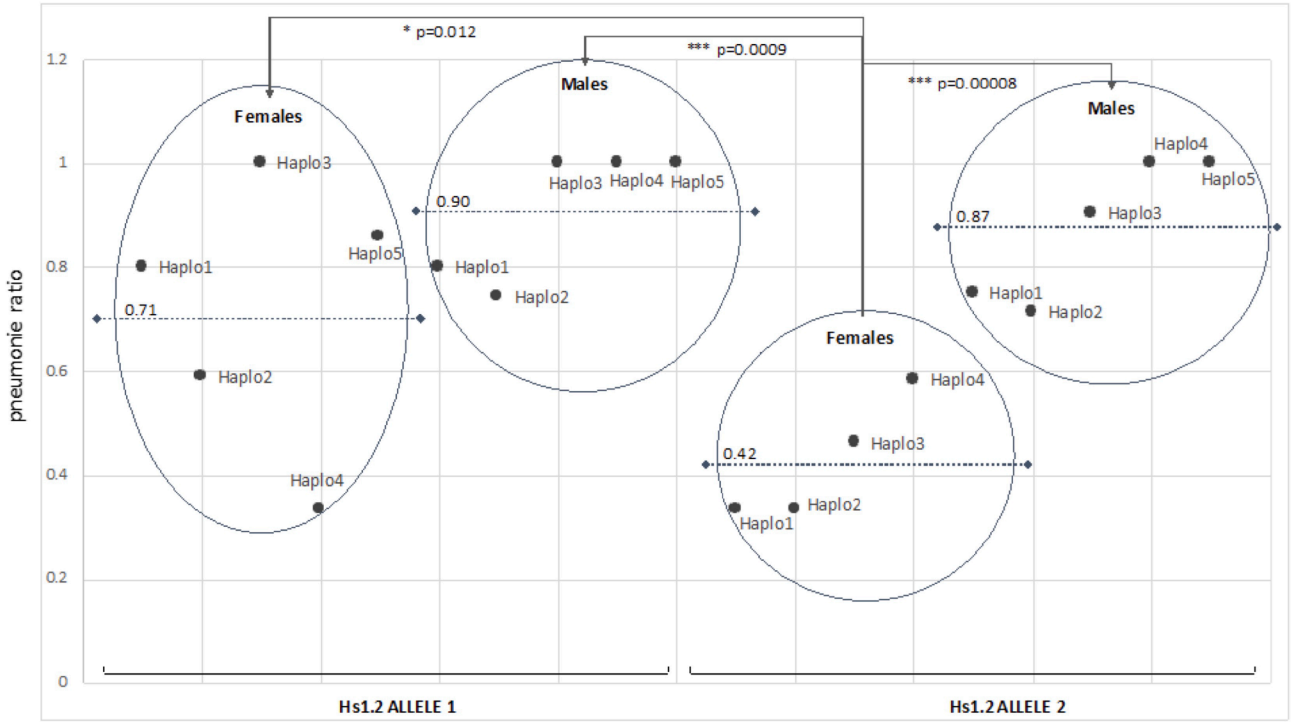

Fig. 2. Pneumonia outcome ratio. Pneumonia outcome ratio (affected/"affected + non affected") according to gender, presence of Hs1.2 alleles and the 5 more frequent Haplotypes (H1-H5). Dotted lines represent the average value of each group. Arrowed lines indicate comparison between patients of each group (*t*-test).

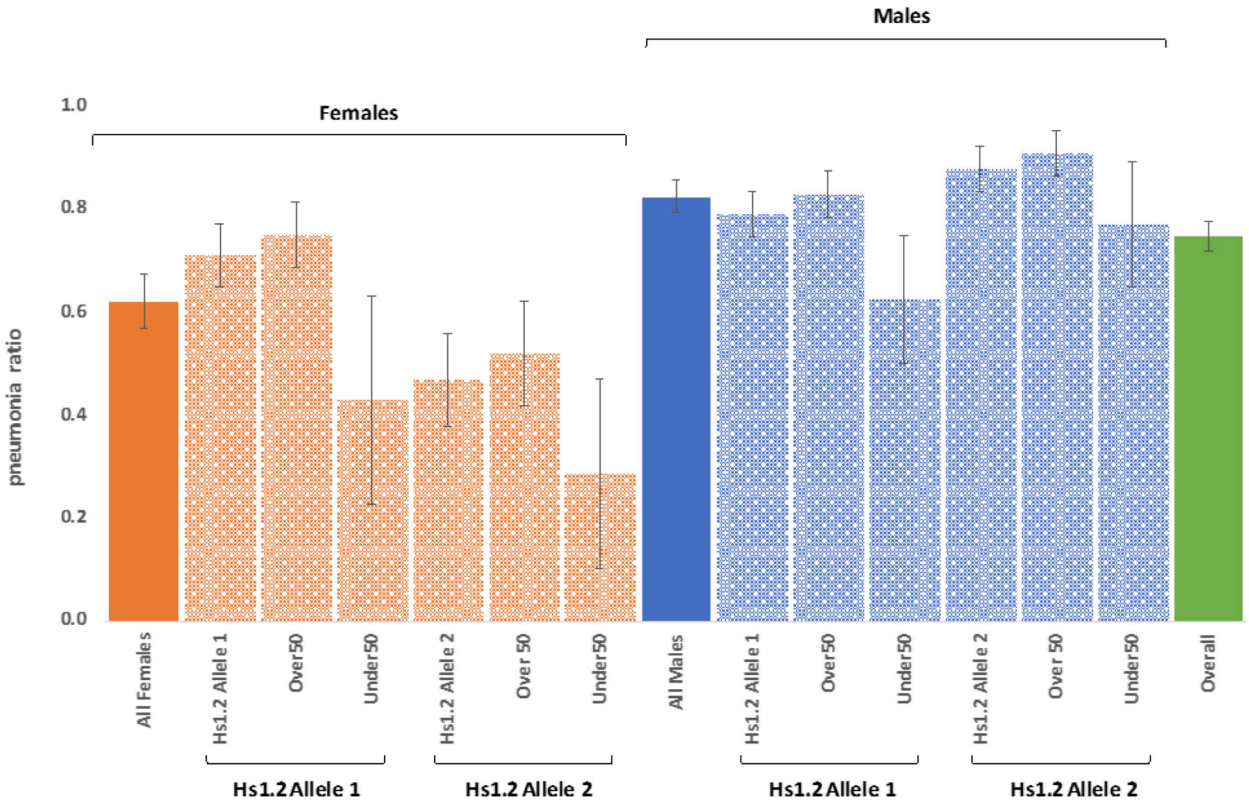

Fig. 3. Histograms of pneumonia ratio. Women (orange bars) and men (blue bars) patients were stratified for Hs1.2 alleles and age. Each bar represents the average value of the group with its standard error. Overall value is also reported (green bar).

considering women (7.8E-06) and men (1.3E-05) separately. In addition, the whole sample of women showed on average a lower disease severity than men (p-value 0.03) although disease severity by itself does not appear to be influenced by the allelic variation of hs1.2 (Fig. S4).

4. Discussion

The findings of the present study confirm a higher susceptibility to COVID 19 disease in men and in patients older than 50 years. Women have a nearly 25% reduced likelihood to develop pneumonia. The presence of allele *2 is associated to a 50% reduced frequency of pneumonia in women younger than 50 years compared to men of same age. The different clinical severity related to the presence of either allele *1 or allele *2 in women of any age suggests that the estrogen response elements (ERE), which are only present in the allele *2 of hs1.2 enhancer in the human Ig heavy chain locus, are predictive of a lower incidence of pneumonia. Interestingly, our study documents that gender differences progressively dissipate with the age, reinforcing the importance of estrogens in the protective immune response against Sars-COV-2 infection. The results also highlight the pivotal role played by the estrogens receptor which binds the polymorphic enhancer hs1.2 of the 3′RR1 with a direct impact on Ig production and B cell maturation (Cianci et al., 2008).

This regulatory activity depends not only on the presence of the hs1.2 polymorphism for ERα consensus but also on the SNPs haplotypes reported. Relevant functions of 3′RR act through a 3D conformational transition induced either by substitution of nucleotides, causing changes for methylation sites of chromatin in the inter-enhancer regions, or by changes for the bond to transcription factors of enhancers. First, DNA binding proteins activate the sterile transcription of the IgH genes, and then, the maturation through the Ig class switch until mature Ig production (Péron et al., 2012).

We hypothesize that age and gender-associated differences in Sars-COV-2 infection could be explained by the presence of the consensus for the ERα lying in two copies in the polymorphic allele *2 of hs1.2 enhancer, and by the SNPs of the 3′RR1 palindrome. In several previous experiments, the immunomodulatory action of the 3′RR was confirmed on mouse B lymphocytes during the class switch recombination as well as during B cell maturation (Jones et al., 2019a, 2020). Additionally, in humans, several genomic variants located in the internal enhancers of the 3′RR1 have been reported to be associated to several immune-disorders and disease status (Cianci et al., 2008).

The 3D conformation of the 3′RR1 contains a palindromic region where the SNPs reported in this study are located. The methylation status of the chromatin can induce a conformational change, and the alternative structure can modulate the Ig heavy chain activity. Both methylation site changes and the consensus for DNA binding proteins as ERα receptor can play a role for the activation of 3′RR1. Taken together, our data establish an essential role of the polymorphic regions of 3′RR1 in the regulation of human Ig heavy chain expression, suggesting a gender-specific effect of estrogens and estrogen receptor binding to the Ig heavy chain locus.

In conclusion, we have identified a novel genetic protective factor, which contains the enhancer hs1.2 in 3′RR-1 with DNA binding sites for the Estrogen receptor alpha (ERα) that is involved in B cell maturation and in Ig heavy chain production. Women harboring *2 allele in hs1.2 enhancer of 3′RR-1 of human Ig heavy chain locus which is enriched with estrogen response elements (ERE), appear to be protected from pneumonia in response to Sars-COV-2 infection when compared to men. These findings highlight key functional genetic variants related to gender-specific responses to Sars-COV-2 infection in humans and the relevance of ERα binding to hs1.2 enhancer and other regulatory region of Ig heavy chain locus in the Ig expression and B cell activity.

CRediT authorship contribution statement

Mattia Colucci: Conceptualization, Methodology, Formal analysis, Validation, Data curation, Writing – original draft. **Domenico Frezza:** Conceptualization, Resources, Data curation, Writing – original draft, Writing – review & editing. **Giovanni Gambassi:** Conceptualization, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing. **Francesco De Vito:** Methodology, Resources. **Angela Iaquinta:** Methodology, Resources. **Maria Grazia Massaro:** Methodology, Resources. **Simona Di Giambenedetto:** Investigation, Resources. **Alberto Borghetti:** Investigation, Resources. **Francesca Lombardi:** Investigation, Resources. **Noemi Panzironi:** Methodology, Formal analysis, Validation. **Valentino Ruggieri:** Conceptualization, Methodology, Formal analysis, Validation, Formal analysis, Data curation, Writing – original draft, Writing – review & editing, Supervision. **Vincenzo Giambra:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Rossella Cianci:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gene.2022.146698.

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