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Research paper

Shorter androgen receptor polyQ alleles protect against life-threatening COVID-19 disease in European males

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ABSTRACT

Background: While SARS-CoV-2 similarly infects men and women, COVID-19 outcome is less favorable in men. Variability in COVID-19 severity may be explained by differences in the host genome.
Methods: We compared poly-amino acids variability from WES data in severely affected COVID-19 patients versus SARS-CoV-2 PCR-positive oligo-asymptomatic subjects.
Findings: Shorter polyQ alleles (≤22) in the androgen receptor (*AR*) conferred protection against severe outcome in COVID-19 in the first tested cohort (both males and females) of 638 Italian subjects. The association between long polyQ alleles (≥23) and severe clinical outcome ($p = 0.024$) was also validated in an independent cohort of Spanish men <60 years of age ($p = 0.014$). Testosterone was higher in subjects with *AR* long-

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Testosterone
COVID-19
LASSO logistic regression
WES
Viral infection and host genome

polyQ, possibly indicating receptor resistance (p = 0.042 Mann-Whitney U test). Inappropriately low serum testosterone level among carriers of the long-polyQ alleles (p = 0.0004 Mann-Whitney U test) predicted the need for intensive care in COVID-19 infected men. In agreement with the known anti-inflammatory action of testosterone, patients with long-polyQ and age ≥60 years had increased levels of CRP (p = 0.018, not accounting for multiple testing).

Interpretation: We identify the first genetic polymorphism that appears to predispose some men to develop more severe disease. Failure of the endocrine feedback to overcome AR signaling defects by increasing testosterone levels during the infection leads to the polyQ tract becoming dominant to serum testosterone levels for the clinical outcome. These results may contribute to designing reliable clinical and public health measures and provide a rationale to test testosterone as adjuvant therapy in men with COVID-19 expressing long *AR* polyQ repeats.

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Research in context

Evidence before this study

We searched on Medline, EMBASE, and Pubmed for articles published from January 2020 to August 2020 using various combinations of the search terms "sex-difference", "gender" AND SARS-Cov-2, or COVID. Epidemiological studies indicate that men and women are similarly infected by COVID-19, but the outcome is less favorable in men, independently of age. Several studies also showed that patients with hypogonadism tend to be more severely affected. A prompt intervention directed toward the most fragile subjects with SARS-Cov-2 infection is currently the only strategy to reduce mortality. Glucocorticoid treatment is a cost-effective measure to improve the outcome of severe cases. Clinical algorithms have been proposed, but little is known on the ability of genetic profiling to predict outcome and disclose novel therapeutic strategies.

Added-value of this study

In a cohort of 1178 men and women with COVID-19, we used a supervised Machine Learning approach on a synthetic representation of genetic variability due to poly-amino acid repeats. Comparing the genotype of patients with extreme manifestations (severe vs. asymptomatic), we found an association between the poly-glutamine repeat number of the androgen receptor (AR) gene, serum testosterone concentrations, and COVID-19 outcome in male patients. Failure of the endocrine feedback to overcome AR signaling defects by increasing testosterone levels during the infection leads to the fact that polyQ ≥ 23 becomes dominant to testosterone levels for the clinical outcome.

Implications of all the available evidence

We identify the first genetic polymorphism predisposing some men to develop a more severe disease irrespectively of age. Based on this, we suggest that sizing the *AR* poly-glutamine repeat has important implications in the diagnostic pipeline of patients affected by life-threatening COVID-19 infection. Most importantly, our studies open to the potential of using testosterone as adjuvant therapy for patients with severe COVID-19 having defective androgen signaling, defined by this study as ≥23 PolyQ repeats, and inappropriately low levels of circulating androgens.

1. Introduction

Alongside the mode of transmission, viral load, comorbidities, and demographic factors (such as age and sex), the host genetic background appears to play an important role in COVID-19 severity and progression [1–8]. We hypothesized that common polymorphisms may contribute to COVID-19 severity, including poly-amino acids repeat polymorphisms, such as the polyQ tract of the Androgen Receptor (*AR*). AR contains in its N-terminus domain a polymorphic polyQ tract, ranging between 9 and 36 repeated CAG units in the normal population [9]. *In vitro* and *in vivo* studies have demonstrated that the transactivation potential of AR is inversely correlated to repeat length, and Q-tract size can significantly influence androgen-dependent physiological functions [9–12].

Several lines of evidence lead to the concept that androgens are relevant to both SARS-CoV-2 infection and COVID-19 disease presentation; however, they seem to have a Janus bifacial way of action [13,14]. On one side, androgens promote the transcription of the *TMPRSS2* gene that encodes a serine protease known to prime the spike (S) protein of coronaviruses, facilitating viral entry into the cells [15]. On the other hand, hypogonadism is known to correlate with severe COVID-19 [16] and other chronic conditions, partly due to the loss of attenuation of the inflammatory immune response exerted by testosterone (T) [17–19].

2. Methods

2.1. Patients

We performed a nested case-control study (NCC). Cases and controls were drawn from the Italian GEN-COVID cohort of 1178 subjects infected with SARS-CoV-2 diagnosed by RT-PCR on nasopharyngeal swab [2]. Demographic characteristics of patients enrolled in the cohort are summarized in Table 1 according to their clinical status. In the current NCC study, cases were selected according to the following inclusion criteria: *i.* CPAP/biPAP ventilation (230 subjects); *ii.* endotracheal intubation (108 subjects). As controls, 300 subjects were selected using the sole criterion of not requiring hospitalization. Exclusion criteria for both cases and controls were *i.* SARS-CoV-2 infection not confirmed by PCR; *ii.* non-caucasian ethnicity. Demographic characteristics of the subjects in the NCC study are summarized in Table 1. A similar Spanish cohort, composed of male COVID-19 patients (117 cases and 41 controls) was used to validate the results in another representative European population highly impacted by COVID-19. All subjects were white European. The Spanish Covid HGE cohort is under IRB approval PR127/20 from Bellvitge University Hospital, Barcelona, Spain.

Table 1
Demographics characteristics of the Italian GEN-COVID Cohort and NCC study.

		Intubation	CPAP/BiPAP Ventilation	Oxygen Therapy	Hospitalized w/o respiratory support	Oligo^-asymptomatics w/o hospitalization
GEN-COVID	Number of Sybjects	108	230	352	188	300
	Male/Female	80/28	157/73	208/144	104/84	116/184
	Age males (years)	61,52±11,43	62,75±13,48	63,41±14,53	55,99±15,44	47,40±13,23
	Age females (years)	63,71±13,96	66,23±15,25	68,40±14,74	52,88±16,39	48,61±11,06
		Cases				**Controls**
NCC study	Number of Subjects	338				300
	Male/Female	237/101				116/184
	Age males (years)	62,34±12,84				47,40±13,23
	Age females (years)	65,53±14,94				48,61±11,06

^ Oligosymtpomatic: individuals with minor symptoms of COVID-19 (mild fever, cough, sore throat, etc.)

2.2. Ethics

The GEN-COVID study was approved by the University Hospital of Siena Ethics Review Board (Protocol n. 16917, dated March 16, 2020). This observational study has been inserted in www.clinicaltrial.org (NCT04549831). The Spanish Covid HGE cohort is under IRB approval PR127/20 from Bellvitge University Hospital, Barcelona Spain. Written informed consent was obtained from all individuals who contributed samples and data.

2.3. Analysis of triplets size in the AR locus

To establish allele sizes of the polymorphic triplet in the AR locus, we used the HUMARA assay with minor modifications [20]. Specifically, we performed a fluorescent PCR followed by capillary electrophoresis on an ABI3130 sequencer. Allele size was established using the Genescan Analysis software.

2.4. Binary representation of WES data

Variants calling was performed according to the GATK4 best practice guidelines, using BWA for mapping, and ANNOVAR for annotating. WES data were represented in a binary mode on a gene-by-gene basis. Poly-amino acids triplet repeats were represented in a binary mode: long and short repeats in respect to the reference sequence on the genome. A total of 40 genes with 43 triplet repeat regions were taken from UniProtKB (**Supplementary Table S1**). In the boolean representation of poly-amino acids triplet repeats, for each of these 40 genes two features were defined, Dij and Iij, with Dij being equal to 1 if gene i in sample j has a repeated region shorter than the reference, 0 otherwise, and Iij being equal to 1 if gene i in sample j has a repeated region longer than the reference, 0 otherwise.

2.5. LASSO logistic regression

We adopted the LASSO logistic regression that provides a feature selection method within the classification tasks able to enforce both the sparsity and the interpretability of the results. The weights of the logistic regression algorithm can be interpreted as the importance of the subset of the most relevant features for the task [21].

The input features of the LASSO logistic regression are the poly-amino acids triplet repeats as well as gender, comorbidity (1 if there is at least one comorbidity) and age, the latter as a continuous variable normalized between 0 and 1. Comorbidities were defined as the presence of one or more clinical conditions (i.e. cardiac, endocrine, neurological, neoplastic diseases) at the time of infection. During the fitting procedure, the class slight unbalancing is tackled by penalizing the misclassification of the minority class with a multiplicative factor inversely proportional to the class frequencies. The data pre-processing was coded in Python, whereas for the logistic regression model we used the scikit-learn module with the liblinear coordinate descent optimization algorithm.

2.6. Total T measurement

Blood samples were collected after an overnight fast, immediately centrifuged at 4 °C and stored at -20 °C until assayed. Serum and plasma total T (TT), SHBG levels in plasma and serum LH were measured following standard procedures.

Serum TT was measured using the Access testosterone assay (Beckman Coulter Inc., Fullerton, CA, USA) with a minimum detection limit of 0.35 nmol/L. Reference range for this assay was 6.07−27.1 nmol/L and liquid chromatography - tandem mass spectrometry (LC-MS/MS) according to a previously validated method provided with reference values between 9.8−28.4 nmol/L [22]. Thawed plasma underwent 15 min incubation at 56 °C for virus inactivation, and TT measured in 100 μl of plasma, with sensitivity limit being 0.270 nmol/L, imprecision ranging 9.8 to 0.7% and accuracy 90.6 to 101.5% at concentration levels between 1.12 and 39.2 nmol/L. A stability test under viral inactivation conditions was performed in 6 samples, revealing a T mean (min-max) % loss of 9.7% (4.6−16.7%).

SHBG levels were measured in plasma samples using Quantikine ELISA Kit (DSHB G0B, R&D Systems, Minneapolis, MN, USA) according to the manufacturers' instructions. Serum LH was measured using "Access LH assay" a chemiluminescenSert, two-step enzyme immunoassay (Beckman Coulter Inc., Fullerton, CA, USA). Sensitivity for the LH determination is 0.2 mIU/mL. Reference range in adult males for this assay is 1.2−8.6 mIU/mL.

2.7. Statistical analysis

Since serum and plasma T values were not normally distributed, the statistical analyses were performed using non-parametric tests. When appropriate, transformation was used for skewed data in regression models. We used the Mann-Whitney U test to compare T levels in males with *AR* long-polyQ (≥23) versus males with short-polyQ repeat (≤22). Logistic regression analysis was performed to test the contribution of age, T, and the number of polyglutamine repetitions on COVID-19 outcome. The only prespecified interaction tested was the T by polyQ (categorical). Box-Tidwell procedure was used to assess linearity and the Hosmer and Lemeshow to assess goodness of fit test. Multicollinearity was assessed by variance inflation factor, and dealt with by dropping the offending variables from the analysis on the basis of clinical grounds.

2.8. Role of funders

The work was financially supported by MIUR project "Dipartimenti di Eccellenza 2018-2020" to Department of Medical Biotechnologies University of Siena, Italy (Italian D.L. n.18 March 17, 2020) and by "Bando Ricerca COVID-19 Toscana" project to Azienda Ospedaliero-Universitaria Senese. It was also funded by private donors for COVID-19 research and charity funds from Intesa San Paolo "Fondo di Beneficenza n. b/2020/0119". The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or

writing of the manuscript. The authors collected the data, and had full access to all of the data in the study. They also had the final decision and responsibility to submit the study results for publication.

3. Results

3.1. Testing the role of common poly-amino acid repeat polymorphisms in COVID-19 outcome

In order to test the role of common poly-amino acid repeat polymorphisms in determining COVID-19 clinical severity, we performed a NCC, selecting the extreme phenotypic ends of our entire GEN-COVID cohort (Table 1 and Fig. 1). Among 18,439 annotated genes, we selected those with amino acid repeats, namely 40 genes, and represented them as a boolean variable. Logistic regression with LASSO regularization analysis identified *AR* as the only protective gene (Fig. 1, panel a). The 10-fold cross-validation provides good performances in terms of accuracy (77%), precision (81%), sensitivity (77%), specificity (78%) and Area Under the Curve (AUC) score (86%) (Fig. 1, panel b). The performances of the logistic regression without LASSO regularization for the selected set of features (age, gender, comorbidity and AR gene) are 79% accuracy, 81% precision, 81% sensitivity, 78% specificity, 88% roc-auc. The model shows a slight decrease of almost all the performance measures when the AR gene is removed from the set (accuracy -1.2%, precision -1.3%, sensitivity -1.4%, specificity -1.2%, roc-auc +0.3%). Finally, the logistic regression

A)

B)

Performance	Average	Standard Deviation
Accuracy	77%	6%
Precision	81%	7%
Sensitivity	77%	7%
Specificity	78%	10%
Roc-auc	86%	6%

Fig. 1. LASSO logistic regression. The bar of the LASSO logistic regression beta coefficients represents the importance of each feature for the classification task (Fig. 1) (**Panel a**). The positive beta coefficients of the LASSO (upward bars) reflect a susceptible behaviour of the features to the target COVID-19 disease, whereas the negative coefficients (downward bars) a protective action. The calculated odd ratio of AR short repeats (≤22) is 0.79 i.e. protective. Therefore, the odd ratio of long repeats (≥23) is 1/0.79 = 1.27 i.e. severity. **Panel b:** Table reporting the averages and the standard deviations of accuracy, precision, sensitivity, specificity, and ROC-AUC scores for the 10-folds of the cross-validation.

on the male cohort with the AR gene alone provides results quite higher than the random guess (accuracy 58%, precision 71%, sensitivity 64%, specificity 55%, roc-auc 55%).

3.2. Validation of polyQ polymorphism by sizing the PolyQ repeat of the AR *gene*

In order to validate the results on *AR* obtained by LASSO logistic regression, we sized the number of triplets in the male subset (351 subjects) using the gold standard technique that uses a fluorescent PCR reaction followed by the use of GeneScan Analysis software® (Applied Biosystems) [20]. We identified a 98% concordance between the results of the two techniques in measuring the polyQ repeats. Based on the *AR* polyQ length, male patients were subdivided into two categories, those having a number of PolyQ repeats less than or equal to 22 repeats, and those having a number of PolyQ repeats greater than or equal to 23 repeats, being 23 repeats the reference sequence on genome browsers and the reported cut-off value [23-24]. We found that PolyQ repeats below 22 are enriched in the asymptomatic cohort of males. The difference was statistically significant in the group of males younger than 60 years of age in which genetic factors are expected to have a major impact (p-value 0.024 by χ^2 test) (Table 2; **Supplementary Table S2**).

3.3. Validation of polyQ polymorphism in the Spanish Cohort

We then sized the polyQ repeat in an independent cohort consisting of 158 <60 years old Spanish males without known comorbidities (117 cases and 41 controls). The association with shorter repeats (≤ 22) and protection was confirmed (p-value 0.014 by χ^2 test) (Table 3).

3.4. Males with longer polyQ have receptor resistance

To functionally link the length of the PolyQ repeats to *AR* functionality, we measured TT in 183 men using LCMS/MS (**Supplementary Table S2**). TT was higher in patients carrying ≥23 vs ≤22 glutamines (13.45 vs 11.23 nmol/L, *p*-value 0.042), reflecting reduced negative feedback from the less active receptors present in patients carrying a PolyQ repeat of ≥23. This difference was evident also comparing the TT value and polyQ repeats in the case and the control group (Fig. 2).

3.5. Unbalanced T-AR axis in males with longer polyQ repeats

The hormonal status of the entire male cohort revealed lower TT and calculated free T levels and higher SHBG levels with increasing age (**Supplementary Table S3**).

To evaluate whether the AR receptor reduced activity resulted in signs and symptoms of hypogonadism, subjects were interviewed, post-infection, using a modified version of the Androstest® [25]. Interviews were available for 61 subjects (43 short and 18 long) representative of the extremes genotypes (≤19 and ≥25 repeats) of the cohort. An Androtest score ≥8 was found in 38% of men with longer repeats as compared to 16% of those with ≤19 glutamines (likelihood ratio, $p = 0.046$). Similarly, cryptorchidism (11% in long repeats vs. 2% in short repeats), and anemia (11% in long repeats vs. 2% in short repeats), two powerful sings of low androgenicity, and severe erectile dysfunction (22% in long repeats vs. 9% in short repeats) were more frequently reported in subjects with longer repeats, but not osteopenia/osteoporosis (6% in long repeats vs. 7% in short repeats) (**Supplementary Table S4**). These results indicate a trend toward clinical hypogonadism for those with longer repeats. Conversely, in the entire male dataset, 6 cases of prostate cancer were found annotated in the past-medical history, all in the ≤22 glutamines group, suggesting an increased prostate sensitivity to androgens in this group. No difference was found in the prevalence of BPH or 5-alpha-reductase inhibitors use.

As the reduced signal transduction of AR might be partially compensated by higher T levels, we tested whether the decreased AR negative feedback was sufficient to overcome larger polyQ repeats size (**Fig. 2**). Logistic regression was performed to investigate the joint effect of T level and polyglutamine receptor length on the likelihood that subjects require intensive care during COVID infections, adjusting for age in the model. The logistic regression model was highly significant (χ^2 (3) = 18,881, $p < 0.0001$), with the model explaining 7.5% (Nagelkerke /R2) of the variance in COVID-19 outcome (**Supplementary Table S5**). To test whether the association between T and the outcome changes when the polyQ is short (≤ 22) or long (≥23), an interaction term was included in the model. A significant interaction was found (*p*-value 0.018), suggesting impaired feedback as a predictor of the worst outcome, namely intubation or CPAP/BiPAP versus hospitalization not requiring respiratory assistance. To provide an intuitive graphical representation, we plotted the ratio between TT serum concentrations and polyQ number vs. clinical outcome (**Supplementary Figure 1**). Results show a decreased mean ratio, a sign of an inappropriate rise of TT for increasing polyglutamine repeats, and association with a worse outcome ($p = 0.0004$).

3.6. Inflammatory phenotype in males with longer polyQ repeats

Finally, we tested the relationship between the *AR* polyQ repeat size and 5 laboratory markers of immunity/inflammation, including CRP, Fibrinogen, IL6, CD4 and NK count. We found that older (≥60) males with *AR* polyQ tract ≥23 have a higher (55.92 versus 48.21 mg/dl) mean value of CRP (p-value 0.018, not accounting for multiple testing) and lower mean value of Fibrinogen and a trend of higher IL6 (Table 4).

4. Discussion

We employed machine learning methodologies to identify a set of genes involved in the severity of COVID-19. In the presence of very high dimensionality, as for instance in a WES study, it is crucial to select the most predictive genes representing patterns of variation (mutations or variants) in subjects with different classes of response (i.e., disease state: from asymptomatic to severe cases). This problem is even more complex in diseases where multiple genes are involved in determining the severity and clinical variability of the pathology. Here, we wanted to represent poly-amino acids repeat

Table 2
PolyQ alleles correlation with COVID-19 outcome - males with age <60.

Males <60	<22	>23	*Marginal Row Totals*
Cases	52 (59,1%)	36 (40,9%)	88 (48,1%)
Controls	71 (74,7%)*	24 (25,3%)	95 (51,9%)
Marginal Column Totals	123 (67,2%)	60 (32,8%)	183 (Grand Total)

* p-value (cases vs controls) =0.024

Table 3
Validation in Spanish cohort

Spanish validation (χ2) Males global	≤22	≥23	*Marginal Row Totals*
Cases	51 (43,6%)	66 (56,4%)	117 (74,1%)
Controls	27 (65,9%)*	14 (34,1%)	41 (25,9%)
Marginal Column Totals	78 (49,4%)	80 (50,6%)	158 (Grand Total)

* **p-value (cases vs controls)=**0.014 (Significant at $p<0.05$)

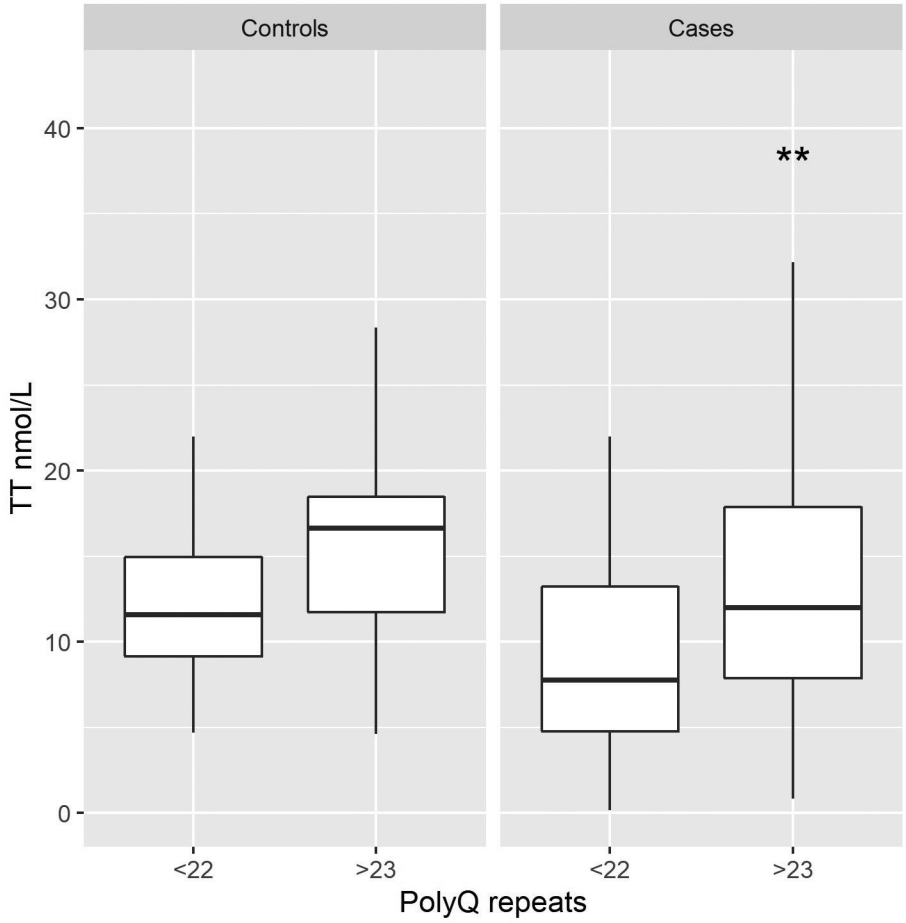

Fig. 2. Relationship between Total Testosterone and polyQ repeats in the case and the control group. Box-plot showing values of Total Testosterone (TT), expressed in nmol/L, in subjects with shorter (≤22) and longer (≥23) polyQ repeats in AR gene grouped between controls (left panel) and cases (right panel). The TT median value, represented by the black horizontal line, is higher in patients with ≥23 polyQ repeats in the case group, (**p-value = 0.023; Mann-Whitney U test). No statistically significant difference was present in the control group (p-value = 0.088; Mann-Whitney U test).

polymorphisms that are typically missed in classical GWAS analysis, which concentrates on bi-allelic polymorphisms.

We used a machine learning approach and logistic regression with a LASSO regularization to test if using such a simplified representation could lead to a reliable prediction of extreme clinical outcomes (asymptomatic versus severely affected). This approach enabled us to predict such clinical outcomes with 77% sensitivity.

AR contains a highly variable polyglutamine repeat (poly-Q) located in the N-terminal domain of the protein, spanning from 9 to 36 glutamine residues in the normal population [5]. *AR* polyQ length correlates with receptor functionality, with shorter polymorphic glutamine repeats typically associated with higher and longer PolyQ tracts with lower receptor activity [5]. *AR* is expressed in both males and females, but the bioavailability of its ligands T and dihydroT (DHT) differs significantly, being much higher in males. As previous studies linked male hypogonadism to a poorer outcome in COVID-19 patients we decided to focus on male patients and demonstrated that shorter polymorphic glutamine repeats (≤22) confer protection against life-threatening COVID-19 in a subpopulation of individuals with age <60 years.

We also confirmed the association between polyQ size and receptor activity. Specifically, we showed that longer polyQ size (≥23) is associated with higher serum T levels, suggestive of impaired negative feedback (p=0.004 at Mann-Whitney U test) at the level of the hypothalamus and pituitary gland. While this is compensated in healthy subjects [26], during non-gonadal illnesses (NGI) such as COVID-19, some patients are unable to compensate for the reduced AR activity with higher T levels [27]. The result is a status of reduced androgenicity even in the presence of apparently normal T values [27].

As T is known to have an immunomodulatory activity attenuating inflammatory immune responses [26–32], we hypothesized that a long PolyQ repeat would lead to a pro-inflammatory status heralded by increased proinflammatory markers [19,33] by conferring decreased AR transcriptional activity. Conversely, men with a more active receptor (short PolyQ tract) would be protected because they can tame the inflammatory response and increase survival regardless of serum T levels. We found that -CRP-, one of the main inflammatory markers, was higher in subjects with a long AR PolyQ tract. This observation not only is in line with the known anti-inflammatory function of T, but also reinforces the functional importance of the AR PolyQ tract and its association with COVID-19 clinical outcome. Furthermore, this observation suggests that CRP is hierarchically more relevant than serum T level, which can be inappropriately normal and mask a status of low androgenicity in men with a long PolyQ repeat.

The allele distribution of the PolyQ repeat length varies among different populations, with the shortest in Africans, medium in Caucasians, and longest in Asians [34]. Interestingly, WHO data on mortality rates during the first pandemic wave indicated a higher fatality rate in China and Italy (https://covid19.who.int/) [35] with respect to African. Hence, *AR* polyQ length variability could represent an

Table 4
Correlation between polyQ repeats in AR gene and laboratory values

CRP M≥60y cases			CRP M<60y cases		
Triplets	Mean	Count	Triplets	Mean	Count
≤**22**	48,21	78	≤**22**	54,5	43
≥**23**	55,92	38	≥**23**	26,41	29
p-value = 0.018 (Significant at p<0,05)			p-value = 0.2		
Fibrinogen M≥60y cases			**Fibrinogen M<60y cases**		
Triplets	Mean	Count	Triplets	Mean	Count
≤**22**	401,33	57	≤**22**	316,93	22
≥**23**	320,34	27	≥**23**	356,91	19
p-value = 0.093			*p*-value = 0.53		
IL6 times the upper limit of normal M≥60y cases			**IL6 times the upper limit of normal M<60y cases**		
Triplets	Mean	Count	Triplets	Mean	Count
≤**22**	54,56	40	≤**22**	40,43	17
≥**23**	75,78	16	≥**23**	31,8	14
p-value = 0,249			*p*-value = 0,81		
CD4 Lymphocytes M≥60y cases			**CD4 Lymphocytes M<60 cases**		
Triplets	Mean	Count	Triplets	Mean	Count
≤**22**	264,06	32	≤**22**	503,68	16
≥**23**	357,52	21	≥**23**	396,13	15
p-value = 0.22			*p*-value = 0.45		
NK Cells M≥60y cases			**NK Cells M<60y cases**		
Triplets	Mean	Count	Triplets	Mean	Count
≤**22**	70,71	28	≤**22**	147,3	13
≥**23**	102,25	16	≥**23**	107,14	14
p-value = 0.179			*p*-value = 0.098		

explanation for the observed differences in death rate. Moreover, Africans seem to be more prone to infection [36]. This observation could be due to a more active AR receptor, leading to a higher expression of *TMPRSS2*, a protease essential for SARS-CoV-2 spread [15].

Different studies have shown an association between hypogonadism or long polyQ repeats and severe COVID-19 [16,37] and other chronic obstructive pulmonary diseases [17,18]. Our results are in line with these initial observations and provide a possible mechanism explaining these associations. The present study brings these observations to the next level, revealing that is the overall androgenic effect -resulting from the interaction of polyQ polymorphism and circulating T levels- that predicts the need for intensive care. In infected men, we observed impaired feedback no longer sufficient to compensate for the reduced AR transcriptional activity, leading to the conclusion that polyQ tract length is hierarchically more important than serum T levels. This concept helps to solve some inconsistencies, including the early reports of a slightly better outcome in prostate cancer patients -who tend to have smaller polyQ repeats, as in our cohort - when compared to other cancers. Interestingly, previous studies failed to link polyQ with mortality, in healthy subjects [26] or individuals with chronic diseases such as diabetes mellitus [38]. Thus, the observed association between low androgenicity and outcome seems related to the hyperinflammatory state present in severe COVID-19.

An improvement in peak oxygen saturation in men receiving T replacement therapy has been demonstrated in a randomized controlled trial [39] and could be one of the mechanisms responsible for the observed protective effect of AR's with shorter polyQ tract in COVID-19 patients. The observations reported in this study prompt organizing a clinical trial where patients selected based on their serum T concentration and polyQ repeat size are randomized to receive T vs. placebo. Such study could introduce the concept that a simple genetic test measuring the *AR* polyQ repeat can be used in male patients to screen for those who are more likely to benefit from T therapy.

Variants of another X-linked gene, *TLR7*, have been associated with severe COVID-19 outcomes in young men [6]. In the 2 reported families, the rare *TLR7* mutations segregated as a highly penetrant monogenic X-linked recessive trait. While variants in *TLR7* gene are expected to account for a small number of severely affected cases, our findings involve a much larger number of subjects, as long polyQ alleles are relatively common (27%) [40]. Overall, X-linked genetic variants keep coming up as important for defining severe COVID-19 cases in males.

In conclusion, we present a method that can predict if subjects infected by SARS-CoV-2 are at risk for life-threatening complications. This approach has 77% accuracy, 81% precision, 77% sensitivity, and 78% specificity. Furthermore, we present evidence suggesting that a more active AR has the potential to confer protection against COVID-19 severity. If confirmed, these observations should be followed by properly conducted clinical trials exploring if T replacement may decrease morbidity and mortality in patients affected by the most severe forms of the disease. Finally, as shown by regression analysis, ORs ranges between 1.26 and 1.45, therefore the risk of carrying a longer *AR* is much smaller than other already known strong predictors such as age and sex, but still is highly significant, relatively common, and among the very few known genetic predictors of COVID-19 outcome.

Declaration of Competing Interest

The authors declare no competing interests.

Additional information

GEN-COVID Multicenter Study (https://sites.google.com/dbm.unisi.it/gen-covid)

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Spanish COVID HGE

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Contributors

EF, FM, AR designed the study. CF and IM, were in charge of biological samples' collection and biobanking. MB, FF were in charge of clinical data collection. MB, FF, AR, and FM performed analysis/interpretation of clinical data. UP, FF and MM performed T measurement by LC-MS/MS. EDG, AS, AP and LPS performed the validation of association between shorter repeats and protection in a Spanish cohort. MM and AI critically reviewed the manuscript and interpreted clinical data/androgen physiopathological processes. SA and MB were in charge of DNA isolations from peripheral blood samples. FV, GD, AG, RT carried the sequencing experiments. EB, NP, SF, CG, MG and MS, performed bioinformatics and statistical analyses. EB, NP, SD, CF, and SC prepared Figures and Tables. EB, NP, AMP, FPC, AR, EF and FM wrote the manuscript. All authors have reviewed and approved the manuscript.

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Data availability and data sharing statement

The samples referenced here are housed in the GEN-COVID Patient Registry and the GEN-COVID Biobank and are available for sharing. The sequencing data are deposited in http://www.nig.cineca.it/, specifically, http://nigdb.cineca.it) and available for consultation. For further information, you may contact the corresponding author, Prof. Alessandra Renieri (e-mail: alessandra.renieri@unisi.it).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ebiom.2021.103246.

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