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Shaping current European mitochondrial haplogroup frequency in response to infection: the case of SARS-CoV-2 severity

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José Luis Cabrera-Alarcon[1,2], Raquel Cruz[3,4], Marina Rosa-Moreno[1], Ana Latorre-Pellicer[5], Silvia Diz de Almeida[3,4], Scourge Cohort Group*, José A. Riancho[2,6,7,8], Augusto Rojas-Martinez[9], Carlos Flores[10,11,12], Pablo Lapunzina[3,13], Fátima Sánchez-Cabo[1], Ángel Carracedo[3,4,14] & José Antonio Enriquez[1,2]

The frequency of mitochondrial DNA haplogroups (mtDNA-HG) in humans is known to be shaped by migration and repopulation. Mounting evidence indicates that mtDNA-HG are not phenotypically neutral, and selection may contribute to its distribution. Haplogroup H, the most abundant in Europe, improved survival in sepsis. Here we developed a random forest trained model for mitochondrial haplogroup calling using data procured from GWAS arrays. Our results reveal that in the context of the SARS-CoV-2 pandemic, HV branch were found to represent protective factors against the development of critical SARS-CoV-2 in an analysis of 14,349 patients. These results highlight the role of mtDNA in the response to infectious diseases and support the proposal that its expansion and population proportion has been influenced by selection through successive pandemics.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) discovered in Wuhan, China, in 2019, represented a global pandemic, responsible for around 18.2 million deaths worldwide, considering only a period between Jan 1st, 2020, and Dec 31st, 2021[1]. Although, being SARS-Cov-2 the latest pandemic with severe consequences, throughout history mankind has been confronted with several infectious agents, which have shaped our genotype. By extension this selective process has also shaped the mitochondrial DNA (mtDNA), and the components encoded by it. From this perspective, mitochondrial haplogroup (HG) markers are variations in mtDNA accumulated in human populations due to matrilineal inheritance, that allows to trace individuals ancestry and the classification of individuals into HGs[2]. Several of these HG markers determine amino acid changes in subunits encoded by mtDNA of the oxidative phosphorylation system (OxPhos). The OxPhos system represent an actual hub of integration of cell metabolism, that must adapt to several physiological situations[3].

Although most genetic studies analyzing susceptibility and severity to human infectious diseases have focused on the immune system[4], there is evidence for the influence of mitochondrial HG on survival to sepsis, relative to the degrees of heating that individuals can afford[5]. It is known that the severity of SARS-CoV-2 is highly correlated with the comorbidities, age and sex of the patients[6,7]. Therefore, in relation to these known risk factors, in this study we demonstrate the relevance of mitochondrial HV branch (HGs H, V and HV) as protective factor for SARS-CoV-2 severity independent of general genetic background, comorbidities, age or sex, reinforcing the idea that mitochondria play a relevant role in the outcome of infectious disease.

Results

Development of a machine learning model to perform HG calling

The mitochondrial HGs were identified using a random forest model trained on the genotypes of 189 positions/probes from our GWAS array, as features. These probes were selected based on two criteria: they cover HG markers as defined by MITOMAP, and they demonstrate sufficient probe quality. This model was initially trained on 61,134 sequences from the MITOMAP database. These sequences were pre-labeled using Haplogrep2, which leverages HG markers across the entire mitochondrial genome, rather than just the 189 positions that constituted our training dataset. We subsequently validated our random forest model externally by applying the same 189 positions to individuals from the 1000 Genomes Project[8], who were similarly labeled using Haplogrep2[9], based on full mitochondrial sequence data (Fig. 1, see methods). This approach allowed us to determine mitochondrial HGs with a level of reliability determined by 3-fold cross-validation (3-fold CV) in training data-set by Cohen's kappa coefficient, $\kappa = 0.98$. On the other hand, the accuracy obtained in external validation was $\kappa=0.95$. Therefore, this machine learning approach has enough accuracy to determine the HG of our samples. Thus, using this ad hoc tool, we performed mitochondrial HG calling of the 14,349 patients participating in this study obtaining the HG distribution (Table 1).

A full list of affiliations appears at the end of the paper. *A list of authors and their affiliations appears at the end of the paper. e-mail: jaenriquez@cnic.es

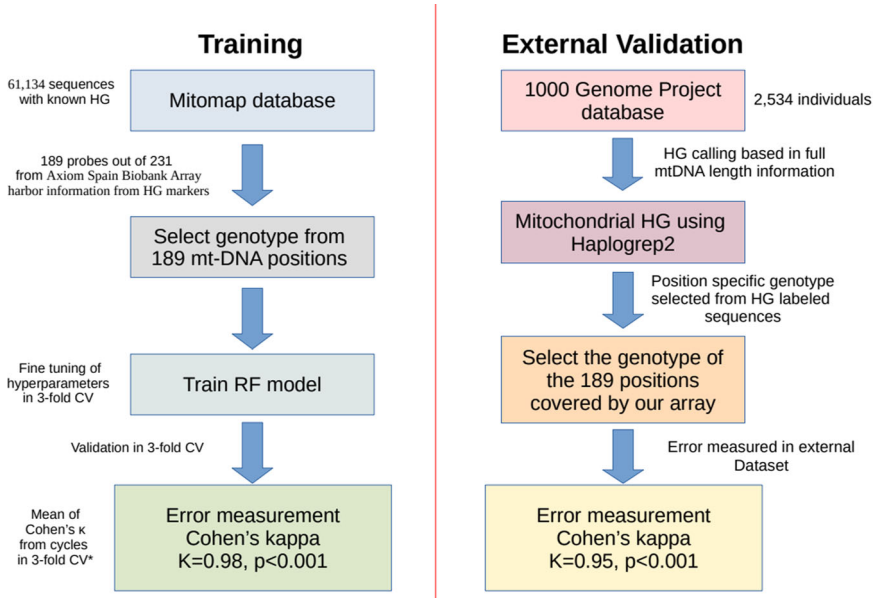

Fig. 1 | Workflow followed to train machine learning model to perform mitochondrial HG calling. The left panel shows the strategy for training a random forest model to classify subjects into mitochondrial HGs, while the right panel outlines how to perform external validation of the trained random forest model. *This process of 3-fold cross-validation (3-fold CV) is different from the 3-fold CV fine-tuning search of hyperparameters.

Analysis of HG as independent risk factors for COVID-19 severity

Then, we evaluated HGs as independent risk/protective factors of COVID-19 severity from comorbidity, genetic background, sex, and age. As we only had comorbidity data for the SCOURGE cohort (cases), the analysis was performed in two ways, first considering the SCOURGE cohort and then including the control patients in the analysis. When analyzing the SCOURGE cohort only 8,778 out of 8,894 have comorbidity information. In a first step, regressors to be considered were filtered out according to their significance in univariate models. This univariate analysis conducted using a logistic regression model as the initial step, revealed that out of all the considered HGs, only the HV branch emerged as a protective factor against severe disease (Supplementary Table S1). Furthermore, the univariate analysis of comorbidities indicated that a history of vascular, digestive, onco-hematologic, and respiratory diseases were risk factors, while a history of neurological disease appeared protective. Cardiac disease history was not significant in our cohort (Supplementary Table S1). Among the 10 principal components representing genetic background, only PC1 and PC3 were significant (Supplementary Table S1). As expected, age was a risk factor for disease severity, as well as sex also emerged as a risk factor in the same direction to that previously described by other authors[7]. Then, to generate a more robust global multivariate model, avoiding possible collinearities and problems derived from the excessive complexity of multivariate models, the global model was built based on the evidence collected from partial multivariate models. Therefore, given the significant risk factors identified before, the independence of the HV branch as a protective factor against severe disease was evaluated initially across four different settings, resulting in four distinct multivariate models. These models assessed the HV branch's independence in relation to comorbidities, genetic background, age, and sex (Supplementary Table S2). Firstly, in multivariate model considering significant comorbidities, the HV branch remained a protective factor against vascular or respiratory comorbidities, while neurological comorbidities also remained protective. The multivariate model for genetic background revealed that both the HV branch and PC1 had protective main effects. Interestingly, when examining the HV branch's independent effect concerning sex, the HV branch was not significant, while the risk effect for sex persisted in this population (Supplementary Table S2). However, a significant female bias for the HV branch was observed (Fisher test: OR=0.898, CI=0.825-0.977, p-value=0.0118). Then, we explore the univariate analysis of the interaction between the HV branch and sex, that showed a similar trend (OR=1.965; CI=1.719-2.242), indicating that males with the HV branch had almost twice probability of developing severe COVID-19. Lastly, in the multivariate model with age, the HV branch maintained its protective effect, and the expected increased risk due to aging was observed (Supplementary Table S2).

By combining the findings from all these multivariate models, the global multivariate model confirmed the same effects for all features, with the HV branch being independently protective against SARS-CoV-2 severity (Table 2). Furthermore, we tested the relevance of HV branch, comparing nested models deleting the HV branch from multivariate model, using ANOVA test, confirmed the relevance and strength of mitochondrial HV branch condition as protective factor ($p<0.01^{**}$).

Once the regressor effects of mitochondrial HGs on the severity of COVID-19 in the SCOURGE cohort were analyzed, we proceeded to their evaluation, also including all the patients (Cases+Controls=14,379 patients), repeating the same strategy. Information on comorbidities is lost in this analysis. Additionally, the models were adjusted to account for potential population stratification arising from the management of different cohorts (with cases represented by the SCOURGE cohort and controls). This was addressed by applying mixed-effects models. These mixed models are crucial for ensuring that the results of HG association are robust, reliable, and accurately reflect true biological relationships rather than being influenced by population structure artifacts. Univariate analysis of all considered features, leave that HGs HV branch, I, U, K and J showed correlation with disease severity, having HV branch and I a protective role, being HGs U, K and J risk factors (Supplementary Table S3). Regarding genetic background as principal components, all PCs were significant but PC2 and PC7, that were discarded for downstream analysis (Supplementary Table S3). Finally, as observed when analyzing SCOURGE cohort, age and sex were risk factors and in the same direction, (Supplementary Table S3). Next, significant HGs were evaluated in multivariate models to check independent effects in 3 frames: regarding sex, age and genetic background, (Supplementary Table S4). Evaluating the independent effects of HGs and sex on disease severity, only the HV branch emerged as a significant protective factor, independent of sex (Supplementary Table S4). Exploring the existence of significant interactions between both features, it was found that there were not significant interactions (ANOVA over nested models: p-value=1). In

Table 1 | Characterization of the patients participating in the study

	Global	Case	Control	Male	Female	≥65y	<65y	Severe	No-Sev
N	14,349	8894	5455	6988	7361	4922	9417	1124	13,225
Age mean±sd	58.7 ±17.3	62.2 ±17.9	53.1 ±14.8	58.6 ±16.3	58.8 ±18.3	78.2 ±9.0	48.6 ±10.7	65.4 ±13.0	58.2 ±17.5
Female	7361	4784	2577	–	–	2478	4883	310	7051
Male	6988	4110	2878	–	–	2444	4544	814	6174
HV branch (%)	7731 (53.88)	4792 (53.88)	2939 (53.88)	3680 (52.66)	4051 (55.03)	2648 (53.8)	5083 (53.91)	572 (50.89)	7159 (54.13)
Hg H (%)	6627 (46.18)	4088 (45.96)	2539 (46.54)	3158 (45.19)	3469 (47,12)	2240 (45.51)	4387 (46.54)	488 (43.42)	6139 (46.42)
Hg U (%)	2035 (14.18)	1278 (14.37)	757 (14.37)	1045 (13.45)	990 (14.95)	687 (13.96)	1348 (14.3)	169 (15.03)	1866 (14.11)
Hg K (%)	985 (6.86)	600 (6.75)	385 (7.06)	482 (6.8)	503 (6.9)	357 (7.25)	628 (6.66)	88 (7.83)	897 (6.78)
Hg J (%)	1277 (8.90)	806 (9.06)	471 (8.63)	622 (8.9)	655 (8.9)	435 (8,84)	842 (8.93)	106 (9.43)	1171 (8.85)
Hg T (%)	1159 (8.08)	684 (7.69)	475 (8.71)	575 (8.23)	584 (7.93)	384 (7.8)	775 (8.22)	97 (8.63)	1062 (8.1)
Hg V (%)	633 (4.41)	402 (4.52)	231 (4.23)	299 (4.28)	334 (4,54)	228 (4.63)	405 (4.3)	49 (4.36)	584 (4.42)
Hg HV (%)	471 (3.28)	302 (3.39)	169 (3.1)	223 (3.19)	248 (3.37)	180 (3.66)	291 (3.1)	35 (3.1)	436 (3.3)
Hg X (%)	224 (1.56)	150 (1.69)	74 (1.36)	111 (1.59)	113 (1.54)	93 (1.89)	131 (1.39)	16 (1.42)	208 (1.57)
Hg I (%)	212 (1.48)	120 (1.35)	92 (1.69)	105 (1.45)	107 (1.5)	61 (1.24)	151 (1.6)	15 (1.33)	197 (1.5)
Hg W (%)	152 (1.06)	109 (1.22)	43 (<1%)	85 (1.22)	67 (<1%)	56 (1.14)	96 (1)	12 (1)	140 (1)
Hg M (<1%)	107	60	47	62	45	38	69	7	100
Hg L3 (<1%)	105	64	41	48	57	36	69	8	97
Hg R (<1%)	87	55	32	44	43	24	63	7	80
Hg L2 (<1%)	76	53	23	40	36	32	44	9	67
Hg L1 (<1%)	68	44	24	36	32	28	40	10	58
Hg N (<1%)	66	37	29	26	40	24	42	4	62
Hg B (<1%)	24	16	8	9	15	8	16	2	22
Hg A (<1%)	10	8	2	7	3	1	9	1	9
Hg D (<1%)	10	6	4	3	7	5	5	0	10
Hg P (<1%)	8	4	4	5	3	0	8	1	7
Hg C (<1%)	4	2	2	1	3	3	1	0	4
Hg L0 (<1%)	3	3	0	1	2	2	1	0	3
Hg F (<1%)	3	1	2	0	3	0	3	0	3
Hg G (<1%)	1	1	0	1	0	0	1	0	1
Hg E (<1%)	1	1	0	0	1	0	1	0	1
Hg L4 (<1%)	1	0	1	0	1	0	1	0	1
Cardiac	–	1055	–	632	423	846	209	154	902
Respiratory	–	901	–	563	338	598	303	190	711
Vascular	–	4,081	–	2200	1881	2730	1351	682	3394
Nervous	–	770	–	340	430	655	115	80	690
Onco-Hem.	–	646	–	410	236	479	167	104	542
Digestive	–	264	–	153	111	172	92	45	219

Mitochondrial HG determined by machine learning.

Table 2 | Results of multivariate logistic regression model for SARS-Cov2 severity in SCOURGE cohort, including mitochondrial HG genotype information

Feature	Coefficient	Std. error	*P*-value	OR	CI 95%
HV branch	−0.70148	0.21793	<0.01**	0.496	0.324–0.760
Vascular	0.45792	0.07472	<0.001***	1.581	1.366–1.831
Neurological	−0.47463	0.12947	<0.001***	0.622	0.480–0.797
Respiratory	0.40721	0.09315	<0.001***	1.503	1.24942659–1.800
PC1	−2.15774	0.49375	<0.001***	0.116	0.044–0.3019
HV branch x PC1	0.45545	0.66205	<0.001***	6.714	1.836–24.612
sex	1.14988	0.07176	<0.001***	3.158	2.746–3.639
age	1.90422	0.22709	<0.05*	6.714	1.836–24.612

Level of significance represented as ***$p < 0.001$, **$p < 0.01$ and *$p < 0.05$.

partial scenario analyzing HGs and age, again only HV branch was selected as protective feature in the multivariate model obtained, not showing interaction with age (ANOVA for nested models: p-value=0.424). Finally, in multivariate model considering genetic background context, again HV branch was the only HG explanatory for SARS-Cov2 severity, joined with PC1 and PC3, showing interaction with PC1 (ANOVA over nested models: p-value<0.01**), as also was observed when analyzing only SCOURGE cohort (Supplementary Table S4). Gathering the performance of regressors in these three frames, a global multivariate model was assembled, confirming protective role of HV branch in this disease, as well as the fact that its effect is different depending on the genetic background, as observed by the significance of the interaction between PC1 and HV branch (Table 3).

Analysis of energy changes in in silico models determined by major haplogroup markers

Next, to assess the potential structural impact of missense variants identified by major HG markers, we analyzed the energy variations they cause within in silico models following rosetta Flex-ddG protocol[10]. Regarding these in silico results calculated with respect to the reference mtDNA sequence[11], in HGs J and T respiratory complex I (CI) showed a significant stabilization, while HG K presented a significant destabilization, remaining HV branch HG U and T unaltered (Fig. 2, left panel). All analyzed HGs presented significant levels of destabilization for complex III ($CIII_2$), except for HV branch (Fig. 2, middle panel). Finally, in complex V (CV), only HG K presented a significant destabilization (Fig. 2, right panel). No major HG markers causing missense variations are affecting complex IV genes.

Discussion

Since 2019, the global pandemic caused by the SARS-Cov-2 virus (COVID-19) spread rapidly with serious implications all around the world. However, humans have faced numerous pandemics and epidemics, which likely acted as a selective force in human evolution. By the same token, HG H (largest representation of the HV branch) is the most frequent HG in European population, representing 37–58%[12]. However, in a study performed in 54 individuals from Upper Paleolithic and Early Neolithic from Northern Spain, ancient hunter-gatherer samples were mainly from HG U (50-80%), while later Neolithic samples resulted more heterogeneous differing on their proportions in HGs J, U and H[13]. Viewed in this way, since the HG H arrived in Europe from the Near East (22,000 BP), HG H increased its proportion, being almost 19% in Linear Pottery Culture (Neolithic), increasing the frequency to a 44% during Neolithic as observed in samples from the Basque Country and Navarre[13,14]. Nevertheless, the HG H has undergone a multifaceted and dynamic history in Europe, shaped by migrations, demographic shifts, and possibly selective pressures such diseases. In this study we explore the hypothetical contribution of disease linked selective pressures. Thus, in Europe, HG H has become in a relatively short evolutionary period, the most frequent one. To preserve such a high frequency, HG H may provide some evolutionary advantage, constituting a clear example of evolutionary selection during historical time in human evolution. In this process of selective sweep, pandemic/epidemic events must have been an important keystone. In this context, Yersinia pestis is one of the deadliest pathogens for humans. During the second pandemic (Black Death) alone, it wiped out at least 30% of the European population, illustrating how a pandemic can influence the genetic landscape related to immune response. However, to date, there is no consistent evidence linking this to mitochondrial HGs[4].

In this regard, although survival to infectious diseases is a multifactorial issue, the advantage conferred by HG H in pandemics/epidemics could be a relevant factor. As an example, Chinnery et al, described an overcome in survival to sepsis event in ICU patients by HG H patients compared against remaining HGs[5]. They reported that HG H patients could withstand a higher core temperature than the rest of the HGs, so they proposed fever as a possible cause of the survival differences.

Interferon release by virus infected cells is part of the innate immune response, that promotes several pathways to control virus replication/infection[15]. Bearing this in mind, it is known that fever enhances the immune response against virus infection, boosting both innate and adaptive immune response to virus[16–20]. In the same direction, it is known that the use of antipyretics is associated with increased mortality[21–23]. The fever produces shivering as part of the strategy of increasing core temperature, that increases metabolic rate sixfold above basal levels[24], where mitochondria have a key role in heat production. It is known, that sustained high temperature above the physiological threshold (heat stress) can induce permanent mitochondrial dysfunction that leads to cytotoxic ROS production triggering cell death[25,26]. Furthermore, it has been described recently, that respiratory complexes, especially complex I and structures derived from respiratory complexes assembly called supercomplexes are unstable at temperatures above 43ºC, both in intact cells and isolated mitochondria[27].

Table 3 | Results of multivariate logistic regression model for SARS-Cov2 severity in SCOURGE+Control cohort, including mitochondrial HG genotype information

Feature	Coefficient	Std. error	*P*-value	OR	CI 95%
HV branch	−0.72041	0.22033	<0.01**	0.487	0.316–0.749
PC1	−2.35113	0.49937	<0.001***	0.095	0.036–0.25
PC3	−1.62667	0.36593	<0.001***	0.197	0.096–0.403
HV branch x PC1	1.97794	0.67010	<0.01**	7.228	1.944–26.878
sex	1.22007	0.07102	<0.001***	3.387	2.947–3.893
age	1.05173	0.19806	<0.001***	2.863	1.942–4.220

Level of significance represented as ***$p < 0.001$, **$p < 0.01$ and *$p < 0.05$.

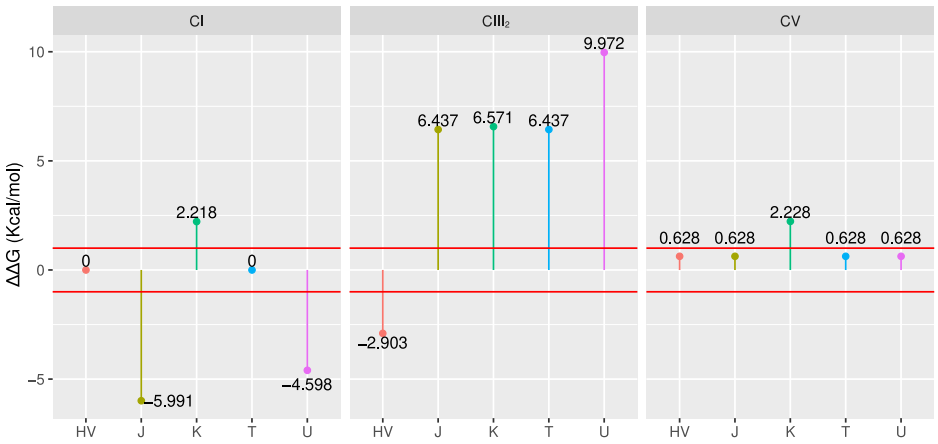

Fig. 2 | Stability changes by respiratory complex, determined by major mitochondrial HG markers. All HGs are compared to a reference model determined according to the Cambridge reference sequence (NC_012920). Only mitochondrial HGs that were significant risk modulators for SARS-Cov2 severity are represented. According to the developers of the rosetta Flex.ddG protocol, significant energetic changes can be considered above or below ± 1 Kcal/mol (red lines). Although CIV also contains three subunits encoded by mtDNA, this respiratory complex is not represented, as none of the described mitochondrial HG markers produce amino acidic changes.

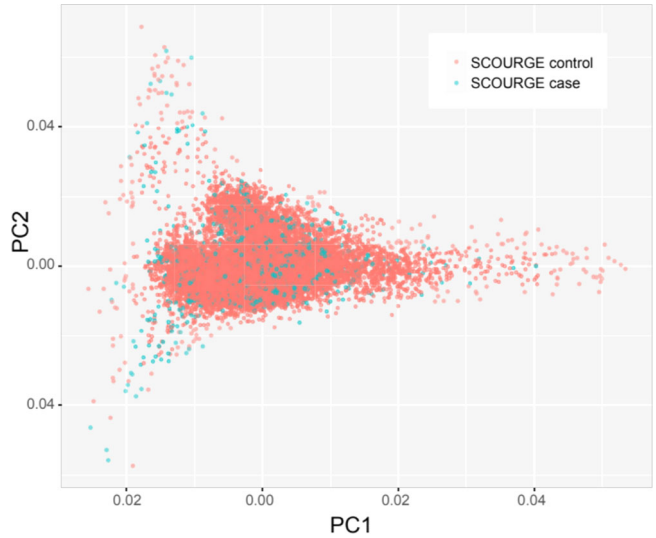

Fig. 3 | Principal component (PC) analysis summarizing genetic variability Cases Vs Controls. A plot depicting the two main PC, cases (n=8894) Vs controls (n=5455), that gathers that main amount of overall genetic variability based in GWAS array.

As a result, greater resilience to sustained high temperatures could be evolutionarily favored for combating infectious diseases, particularly through changes that reduce OxPhos capacity fatigue, such as mutations in OxPhos structures associated with certain HG markers. In this context, a recent study has shown that elevated temperature (39 ºC) significantly influences the metabolism of the electron transport chain (ETC), particularly impacting complex I. This leads to increased ROS production and the activation of selective apoptosis in TH_1 lymphocytes[28]. TH_1 cells play a critical role in cellular immunity against intracellular pathogens, especially through the production of INF-γ in response to viral infections. As a result, variations in tolerance to hyperthermia, caused by small structural differences determined by mitochondrial HGs, could directly affect the effectiveness of TH_1-mediated antiviral responses.

In this study, we confirmed that HGs significantly influence the severity of SARS-CoV-2, with the HV branch specifically having a protective role. This remains relevant today, as its effects are still evident, despite significant medical advances in combating infectious diseases that have reduced the correlation between susceptibility, severity, and survival.

Regarding the flex-ddG analysis, it is important to note that the HG markers analyzed are highly prevalent in human populations, so that under normal physiological conditions they would not represent any decrease in OxPhos fitness. However, under extreme conditions such as hyperthermia, tolerable differences in RCs' stability under normal physiological conditions, could represent a real decrease in stability and a key role in disease outcome. Interestingly, only the HV branch shows no significant destabilization in any RC induced by top-level HG markers. It is important to notice that Flex-ddG in silico analysis has the limitation that the same genetic background is used, making it impossible to rule out that the observed effects are due to specific mito-nuclear interaction with this nuclear environment of the OxPhos system. However, this same limitation is accepted for the use of cybrid models, a technology widely used to precisely determine mitonuclear interactions. However, the goal of our Flex-ddG modeling was to generate in silico mechanistic hypotheses based on observations of the analyzed population.

In the same line of our outcomes, it has been described that HG markers linked to HG H as 7028C is protective against severe COVID-19 disease[29]. The results provided by our multivariate model, based on data drawn from such a large cohort of patients, provide important evidence for the role of mitochondrial HGs as modulating factors in the risk of developing the severe form of the disease, regardless of the genetic background, comorbidities, age or sex of the patients. In addition, from an evolutionary perspective, these outcomes confirm the relevance of H[30] and furthermore OxPhos genotype in the defense to an infectious pathogen.

Methods

Sample processing and genotyping

Data from a total of 11,977 COVID-19-positive cases were recruited as part of the SCOURGE study (https://www.scourge-covid.org) from 34 hospital or research centers across Spain between March and December 2020. Samples and data were collected by the participating centers through their respective biobanks after informed consent. The whole project was approved by the Galician Ethical Committee, ref.: 2020/197. Additionally, 5,943 people with unknown COVID-19 status were included as population controls: 3,437 samples from the Spanish DNA biobank (https://www.bancoadn.org) and 2,506 samples from the GR@CE consortium. All ethical regulations relevant to human research participants were followed.

Genomic DNA was obtained from peripheral blood and isolated using the Chemagic DNA blood100 kit (PerkinElmer Chemagen Technologies GmbH), following the manufacturer's recommendations. Genotyping was performed using the Axiom Spain Biobank Array (Thermo Fisher scientific, Waltham, MA, USA) according to the manufacturer's instructions in the Santiago de Compostela Node of the National Genotyping Center (CeGen-ISCIII). This array contains 757,836 markers and is enriched in rare variants selected in the Spanish population.

Details concerning the sample processing and quality control can be found in the first report describing the European GWAS of this consortium[31]. All individuals included in the analysis were of European ancestry. Ancestry was inferred with Admixture[32] using defined 1KGP superpopulations. Those individuals with an estimated probability >80% of pertaining to European ancestry were defined as European (N=15,571)[31]. After down-sampling individuals with missing values for disease severity, sex or age, we obtained an effective dataset of 14,349 individuals (8,894 COVID-19 positive cases and 5,455 population controls). In these individuals' genomic principal components (PCs) were computed using a LD-pruned ($r^2 < 0.1$ with a window size of 1000 markers) subset of genotyped SNPs passing quality check for controlling the population structure in the posterior analyses (Fig. 3).

HG calling using machine learning

We developed an ad hoc method to perform mitochondrial HG calling from GWAS array data, based in machine learning (Fig. 1). The Axiom Spain Biobank Array covers up to 231 mitochondrial confident positions. Around 189 out of these 231 positions were positions linked to HG markers and the probes that define the genotype in our array have enough quality. Our goal was to train a random forest classifier using 189 positions (which define the variables for the random forest) from 61,134 HG-labeled sequences obtained from the Mitomap database. We employed 3-fold cross-validation to fine-tune the model's hyperparameters (Fig. 1, left panel). The hyperparameters were restricted to the number of variables randomly sampled as candidates at each split (5, 10, 20, 40, or 60). Model error was first assessed using a 3-fold cross-validation loop on the training dataset, with hyperparameter fine-tuning conducted within each cycle (obtained by nested cycles of 3-fold CV, mentioned before). Thus, the error was measured as the mean of Cohen's kappa calculated for each cross-validation cycle. Next, external validation of this model was undertook using Publicly available data from the third phase of 1,000 genome project[8]. In this samples, the SNPs collected from full length mtDNA were used to call HG using haplogrep2[9]. Finally, we use our model to predict HGs in our 14,749 patients, based on genotype information for the 189 positions. (Fig. 1, right panel). An app powered by shiny (https://shiny.posit.co/) will be available at https://github.com/Cabrera-alarcon/GENOXPHOS.

Analysis of HGs as independent risk factors

To assess the value of HGs as independent risk factor for the severity of SARS-CoV-2, we analyzed only HGs with frequencies >1% (H, HV, V, J, T,

U, K, I, W and X). Severe disease development was considered for patients with fatal outcome, admission to the ICU or the need for mechanical ventilation (invasive or noninvasive). Additionally, HGs were connected by branches based on top-level MITOMAP HG markers (present in ≥80% of HGs) that result in amino acid changes in mitochondrial DNA encoded OxPhos subunits. The missense status of these top-level HG markers was predicted using the variant effect predictor[33] (Supplementary Data 1). As a result, HGs H, V, and HV were grouped together under the HV branch.

Then we assessed explanatory meaning of HGs for SARS-Cov2 severity in two groups, the SCOURGE cohort (those are our case group, for which we have comorbidity information for 8,778 out of 8,894) and the global group of patients represented by the SCOURGE+Control patients (8,894+5,455).

Initially, we examined the impact of potential explanatory variables by fitting a univariate logistic regression model to study these effects in the SCOURGE cohort. For the analysis across all patients, we employed a mixed-effects logistic regression model to account for the population stratification into cases and controls as a random effect. To fit such mixed-effects models we used lme4 v-1.-35.3 R-package[34]. Five groups of features were considered, HGs, comorbidities, genetic background, sex and age. The comorbidities were represented by the patient's cardiac history (ischemic heart disease, heart failure, cardiac arrhythmia or peripheral vascular disease), vascular history (arterial hypertension, hypercholesterolemia, uncomplicated diabetes mellitus, diabetes mellitus with visceral repercussions, obesity), digestive antecedents (Peptide ulcer, Chronic liver disease without portal hypertension, Chronic liver disease with portal hypertension), nervous system antecedents (Cerebrovascular disease such as infarction or hemorrhage without sequelae or minimal sequelae, with hemiplegia or paraplegia, dementia or other neurological disease), respiratory history (Chronic Obstructive Pulmonary Disease or other chronic respiratory disease) and oncological or oncohematological history (localized solid tumor, metastatic solid tumor, leukemia, lymphoma or bone marrow/hematopoietic precursor transplant). The genetic background was estimated as to summarize genetic variability as the 10 principal components determined from genotype matrix. Since many of these variables were dichotomous and the quantitative variables (genetic background and age) were on very different scales, a min-max normalization of the data was performed.

Next, the regressors that were significant in univariate models were evaluated by assembling multivariate models. Initially, we assessed the significant HGs from the univariate models across four different scenarios, resulting in four distinct multivariate models. These models tested the independent effects of the HGs: one considering only comorbidities (in the SCOURGE cohort), one accounting for genetic background, another checking for independent effects with sex and age. During this process, feature selection was conducted using a stepwise backward strategy, reducing the Akaike information criterion (AIC) for the main effects. Once the features were selected, potential interactions between HGs and other features were explored by analyzing the relevance of their inclusion in the model through ANOVA tests comparing nested models. The same strategy was applied to evaluate the importance of including HGs in the final models. Finally, based on the information obtained from these partial multivariate models, a global model was fitted, both when considering only SCOURGE cohort and when studying SCOURGE+Controls. In these models, stratification of analyzed population was considered as aleatory effect. In all models a threshold for significance of 0.05 was adopted.

Analysis of energy changes in in silico models determined by major HG markers

Further analyses were performed to evaluate in our in-silico models (Cabrera-Alarcon & Enriquez, manuscript submitted), to assess whether observed results of significant HGs from multivariate mixed effect logistic regression correlate with changes in OxPhos complexes stability by analyzing residue changes determined by major HG markers gathered from MITOMAP. Structural consequences oh HG markers were determined using Variant Effect Predictor[33]. For this purpose, changes in the strength that bind subunits assembled in OxPhos complexes due to residue changes were studied following the rosetta Flex-ddG protocol[10]. According to developers of this tool significant energy changes can be considered from ±1 Kcal/mol.

Data availability

All data used to develop machine learning models are publicly available in Mitomap: (https://www.mitomap.org/foswiki/bin/view/Main/WebHome) and The International Genome Sample Resource (https://www.internationalgenome.org), and materials that are not available in the main text, the supplementary materials or github repository will be available upon request. Summary statistics from the SCOURGE Latin-American GWAS will be available at https://github.com/CIBERER/Scourge-COVID19. Raw genotype or phenotype data cannot be made available due to restrictions imposed by the ethics approval.

Code availability

Code use in this study will be available at https://github.com/Cabrera-alarcon/GENOXPHOS.

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Author contributions

Conceptualization: J.L.C.-A., R.C., F.S.-C., A.C., J.A.E. Methodology: J.L.C.-A., M.R.-M., R.C., A.L.-P. Investigation: J.L.C.-A., M.R.-M. Visualization: J.L.C.-A., J.A.E. Funding acquisition: F.S.-C., J.A.E. Project administration: S.D.A., J.A.R., A.R.M., C.F., P.L. Sample Providers: S.C.G. Supervision: A.C., J.A.E. Writing—original draft: J.L.C.-A., F.S.-C., J.A.E. Writing—review and editing: All Authors

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The authors declare no competing interests.

Additional information

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Correspondence and requests for materials should be addressed to José Antonio Enriquez.

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[1]Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid E-28029, Spain. [2]Centro de Investigación Biomédica en Red de Fragilidad y Envejecimiento Saludable.(CIBERFES) Instituto de Salud Carlos III, Madrid E-28029, Spain. [3]Centro de Investigación Biomédica en Red en Enfermedades Raras (CIBERER), Instituto de Salud Carlos III, Madrid E-28029, Spain. [4]Grupo de Medicina Xenómica-CIMUS-Universidade de Santiago de Compostela, Santiago de Compostela, Spain. [5]Grupo de Genética Clínica y Genómica Funcional, Facultad de Medicina, Universidad de Zaragoza, IIS Aragón, CIBERER-GCV02, E-50009 Zaragoza, Spain. [6]Universidad de Cantabria, Cantabria, Spain. [7]Hospital U M Valdecilla, Cantabria, Spain. [8]DIVAL, Cantabria, Spain. [9]Tecnológico de Monterrey, Escuela de Medicina y Ciencias de la Salud, Monterrey, México. [10]Centre for Biomedical Network Research on Respiratory Diseases (CIBERES), Instituto de Salud Carlos III, Madrid, Spain. [11]Genomics Division, Instituto Tecnológico y de Energías Renovables, Santa Cruz de Tenerife, Spain. [12]Research Unit, Hospital Universitario Nuestra Señora de Candelaria, Santa Cruz de Tenerife, Spain. [13]Instituto de Genética Médica y Molecular (INGEMM), Hospital Universitario La Paz-IDIPAZ, ERN-ITHACA-European Reference Network, Madrid, Spain. [14]Fundación Pública Galega de Medicina Xenómica (SERGAS), Health Research Institute of Santiago de Compostela (IDIS), Santiago de Compostela, Spain. ✉e-mail: jaenriquez@cnic.es

Scourge Cohort Group

Javier Abellán[15,16], René Acosta-Isaac[17], Jose María Aguado[18,19,20,21], Carlos Aguilar[22], Sergio Aguilera-Albesa[23,24], Abdolah Ahmadi Sabbagh[25], Jorge Alba[26], Sergiu Albu[27,28,29], Karla A. M. Alcalá-Gallardo[30], Julia Alcoba-Florez[31], Sergio Alcolea Batres[32], Holmes Rafael Algarin-Lara[33,34], Virginia Almadana[35], Kelliane A. Medeiros[36,37], Julia Almeida[38,39], Berta Almoguera[3,40], María R. Alonso[41], Nuria Álvarez[41], Rodolfo Álvarez-Sala Walther[32], Yady Álvarez-Benítez[33,34], Felipe Álvarez-Navia[42,43], Katiusse A. dos Santos[44], Álvaro Andreu-Bernabeu[20,45], Maria Rosa Antonijoan[46], Eleno Martínez-Aquino[47], Eunate Arana-Arri[48,49], Carlos Aranda[50,51], Celso Arango[20,45,52], Carolina Araque[53,54], Nathalia K. Araujo[55], Ana C. Arcanjo[56,57,58], Ana Arnaiz[59,60], Francisco Arnalich Fernández[61], María J. Arranz[62], José Ramón Arribas López[61], Maria-Jesús Artiga[63], Yubelly Avello-Malaver[64], Carmen Ayuso[3,40], Belén Ballina Martín[25], Raúl C. Baptista-Rosas[65,66,67], Ana María Baldion[64], Andrea Barranco-Díaz[34], María Barreda-Sánchez[68,69], Viviana Barrera-Penagos[64], Moncef Belhassen-Garcia[43,70], David Bernal-Bello[71], Enrique Bernal[68], Joao F. Bezerra[72], Marcos A. C. Bezerra[73], Natalia Blanca-López[74], Rafael Blancas[75], Lucía Boix-Palop[76], Alberto Borobia[77], Elsa Bravo[78], María Brion[79,80], Óscar Brochado-Kith[81], Ramón Brugada[80,82,83,84], Matilde Bustos[85], Alfonso Cabello[86], Alejandro Cáceres[87,88,89], Juan J. Cáceres-Agra[90], Esther Calbo[76], Enrique J. Calderón[14,91,92], Shirley Camacho[93], Francisco C. Ceballos[81], Yolanda Cañadas[51], Cristina Carbonell[42,43], Servando Cardona-Huerta[94], María Sánchez-Carpintero Abad[50,51], Carlos Carpio-Segura[32], José Antonio Carrillo-Avila[95], Marcela C. Campos[56], Carlos Casasnovas[3,96,97], Luis Castaño[3,48,98,99,100], Carlos F. Castaño[50,51], Jose E. Castelao[101], Aranzazu Castellano Candalija[102], María A. Castillo[93], Walter G. Chaves-Santiago[54,103], Sylena Chiquillo-Gómez[33,34], Marco A. Cid-López[30], Óscar Cienfuegos-Jiménez[94], Rosa Conde-Vicente[104], Gabriela C. R. Cunha[105], M. Lourdes Cordero-Lorenzana[106], Dolores Corella[107,108], Almudena Corrales[10,109], Jose L. Cortés-Sánchez[94,110], Marta Corton[3,40], Karla S. C. Souza[111], Fabiola T. C. Silva[56], Raquel Cruz[3,6,7,9], Luisa Cuesta[112], Nathali A. C. Tavares[113], Maria C. C. Carvalho[114], David Dalmau[62,76], Raquel C. S. Dantas-Komatsu[115], M. Teresa Darnaude[116], Raimundo de Andrés[117], Carmen de Juan[118], Juan J. de la Cruz Troca[14,119,120], Carmen de la Horra[92], Ana B. de la Hoz[48], Alba De Martino-Rodríguez[121,122], Marina S. Cruz[123], Julianna Lys de Sousa Alves Neri[124], Victor del Campo-Pérez[125], Juan Delgado-Cuesta[126], Aranzazu Diaz de Bustamante[116], Anderson Díaz-Pérez[34], Beatriz Dietl[76], Silvia Diz-de Almeida[3,9], Manoella do Monte Alves[127,128], Elena Domínguez-Garrido[129], Lidia S. Rosa[130], Andre D. Luchessi[131], Jose Echave-Sustaeta[132], Rocío Eiros[133], César O. Enciso-Olivera[53,54], Gabriela Escudero[134], Pedro Pablo España[135], Gladys Estigarribia Sanabria[136], María Carmen Fariñas[59,60,137], Ramón Fernández[59,138], Lidia Fernández-Caballero[3,40], Ana Fernández-Cruz[139], Silvia Fernández-Ferrero[25], Yolanda Fernández Martínez[25], María J. Fernandez-Nestosa[140], Uxía Fernández-Robelo[141], Amanda Fernández-Rodríguez[81], Marta Fernández-Sampedro[59,60,137], Ruth Fernández[3,40], Tania Fernández-Villa[142], Carmen Fernández-Capitán[102], Antonio Augusto F. Carioca[143], Patricia Flores-Pérez[144], Lácides Fuenmayor-Hernández[34], Marta Fuertes-Núñez[25], Victoria Fumadó[145], Ignacio Gadea[146], Lidia Gagliardi[50,51], Manuela Gago-Domínguez[7,10], Natalia Gallego[11], Cristina Galoppo[147], Ana García-Soidán[148], Carlos García-Cerrada[15,16], Aitor García-de-Vicuña[48,98], Josefina Garcia-García[68], Irene García-García[77], Carmen García-Ibarbia[59,60,137], Andrés C. García-Montero[149], Leticia García[50,51], Mercedes García[50,51], María Carmen García Torrejón[16,150], Inés García[3,40], Elisa García-Vázquez[68], Emiliano Garza-Frias[94], Angela Gentile[147], Belén Gil-Fournier[151], Jéssica N. G. de Araújo[152], Mario Gómez-Duque[54,103], Javier Gómez-Arrue[121,122], Luis Gómez Carrera[32], María Gómez García[6], Ángela Gómez Sacristán[153], Juan R. González[4,5,13,14], Anna González-Neira[41], Beatriz González Álvarez[121,122], Fernán González Bernaldo de Quirós[154], Rafaela González-Montelongo[155], Javier González-Peñas[20,45,52], Manuel Gonzalez-Sagrado[104], Hugo Gonzalo-Benito[156], Oscar Gorgojo-Galindo[157], Miguel Górgolas[86], Florencia Guaragna[147], Jessica G. Chaux[54], Encarna Guillén-Navarro[68,158,159,160], Beatriz Guillén-Guío[109], Pablo Guisado-Vasco[132], Luz D. Gutiérrez-Castañeda[54,161], Juan F. Gutiérrez-Bautista[162], Sara Heili-Frades[163], Rafael H. Jacomo[164], Estefania Hernández[165], Cristina Hernández-Moro[25], Luis D. Hernández-Ortega[166,167], Guillermo Hernández-Pérez[42], Rebeca Hernández-Vaquero[168], Belén Herráez[41], M. Teresa Herranz[68], María Herrera[50,51], María José Herrero[169,170], Antonio Herrero-González[171], Juan P. Horcajada[28,172,173,174], Natale Imaz-Ayo[48], Maider Intxausti-Urrutibeaskoa[175], Antonio Íñigo-

Campos[155], María Íñiguez[176], Rubén Jara[68], Ángel Jiménez[50,51,177], Pilar Jiménez[162], María A. Jiménez-Sousa[81], Iolanda Jordan[14,178], Ignacio Jiménez-Alfaro[179], Rocío Laguna-Goya[180,181], Daniel Laorden[32], María Lasa-Lázaro[180,181], María Claudia Lattig[93,182], Ailen Lauriente[147], Anabel Liger Borja[183], Lucía Llanos[184], Amparo López-Bernús[42,43], Miguel López de Heredia[3], Esther Lopez-Garcia[14,119,120,185], Eduardo López-Granados[3,186,187], Rosario Lopez-Rodriguez[3,40], Miguel A. López-Ruz[188,189,190], Leonardo Lorente[191], José M. Lorenzo-Salazar[155], José E. Lozano[192], María Lozano-Espinosa[183], Ignacio Mahillo[10,193,194], Esther Mancebo[180,181], Carmen Mar[135], Cristina Marcelo Calvo[102], Alba Marcos-Delgado[195], Miguel Marcos[42,43], Alicia Marín-Candón[77], Pablo Mariscal-Aguilar[32], Laura Martin-Pedraza[74], Marta Martin-Fernandez[196], Caridad Martín-López[183], José-Ángel Martín-Oterino[42,43], María Dolores Martín[197], Vicente Martín[14,195], María M. Martín[198], María Martín-Vicente[81], Amalia Martinez[199], Óscar Martínez-González[75], Ricardo Martínez[165], Pedro Martinez-Paz[156], Covadonga M. Díaz-Caneja[20,45,52], Óscar Martínez-Nieto[64,182], Iciar Martínez-López[200,201], Michel F. Martínez-Reséndez[94], Silvia Martínez[59,137], Juan José Martínez[3,97], Ángel Martínez-Pérez[202], Andrea Martínez-Ramas[3,40], Violeta Martínez-Robles[25], Laura Marzal[3,40], Juliana F. Mazzeu[203,204,205], Francisco J. Medrano[14,91,92], Xose M. Meijome[206,207], Natalia Mejuto-Montero[208], Ingrid Mendes[3], Alice L. Duarte[111], Ana Méndez-Echevarría[209], Humberto Mendoza Charris[34,78], Eleuterio Merayo Macías[210], Fátima Mercadillo[211], Arieh R. Mercado-Sesma[166,167], Pablo Mínguez[3,40], Elena Molina-Roldán[212], Antonio J. J. Molina[195], Juan José Montoya[165], Susana M. T. Pinho[36,213,214], Patricia Moreira-Escriche[118], Xenia Morelos-Arnedo[34,78], Rocío Moreno[3], Víctor Moreno Cuerda[15,16], Antonio Moreno-Docón[68], Junior Moreno-Escalante[34], Alberto Moreno Fernández[102], Patricia Muñoz García[10,20,215], Pablo Neira[147], Julián Nevado[3,11,12], Israel Nieto-Gañán[148], Vivian N. Silbiger[131], Rocío Nuñez-Torres[41], Antònia Obrador-Hevia[216,217], J. Gonzalo Ocejo-Vinyals[59,137], Virginia Olivar[147], Silviene F. Oliveira[56,205,218,219], Lorena Ondo[3,40], Alberto Orfao[38,39], Eva Ortega-Paino[63], Luis Ortega[220], Rocío Ortiz-López[94], Fernando Ortiz-Flores[59,137], José A. Oteo[26,176], Manuel Pacheco[165], Fredy Javier Pacheco-Miranda[34], Irene Padilla-Conejo[25], Sonia Panadero-Fajardo[95], Mara Parellada[20,45,52], Roberto Pariente-Rodríguez[148], Vicente Friaza[14,92], Estela Paz-Artal[180,181,221], Germán Peces-Barba[10,222], Miguel S. Pedromingo Kus[223], Celia Perales[146], Ney P. C. Santos[224], Genilson P. Guegel[225], María Jazmín Pérez[147], Alexandra Pérez[80,82], Patricia Pérez-Matute[176], César Pérez[226], Gustavo Pérez-de-Nanclares[48,98], Felipe Pérez-García[227,228], Patricia Pérez[229], Luis A. Pérez-Jurado[1,2,3,87], M. Elena Pérez-Tomás[68], Teresa Perucho[230], Lisbeth A. Pichardo[25], Adriana P. Ribeiro[36,37,214], Mel·lina Pinsach-Abuin[80,82], Luz Adriana Pinzón[54,103], Jeane F. P. Medeiros[231], Guillermo Pita[41], Francesc Pla-Juncà[3,232], Laura Planas-Serra[3,97], Ericka N. Pompa-Mera[233], Gloria L. Porras-Hurtado[165], Aurora Pujol[3,97,234], María Eugenia Quevedo-Chávez[33,34], Maria Angeles Quijada[46,235], Inés Quintela[6], Soraya Ramiro-León[151], Pedro Rascado Sedes[236], Joana F. R. Nunes[56], Delia Recalde[121,122], Emma Recio-Fernández[176], Salvador Resino[81], Renata R. Sousa[214], Carlos S. Rivadeneira-Chamorro[54], Diana Roa-Agudelo[64], Montserrat Robelo Pardo[236], Marianne R. Fernandes[224,237], María A. Rodríguez-Hernández[85], Agustí Rodriguez-Palmero[97,238], Emilio Rodríguez-Ruiz[7,236], Marilyn Johanna Rodriguez[54], Fernando Rodríguez-Artalejo[14,119,120,185], Marena Rodríguez-Ferrer[34], Carlos Rodríguez-Gallego[239,240], José A. Rodríguez-García[25], Belén Rodríguez Maya[15], Antonio Rodriguez-Nicolas[162], German Ezequiel Rodríguez-Novoa[147], Paula A. Rodriguez-Urrego[64], Federico Rojo[241,242], Andrea Romero-Coronado[34], Rubén Morilla[92,243], Filomeno Rondón-García[25], Antonio Rosales-Castillo[244], Cladelis Rubio[245], María Rubio Olivera[50,51], Francisco Ruiz-Cabello[162,189,246], Eva Ruiz-Casares[230], Juan J. Ruiz-Cubillan[59,137], Javier Ruiz-Hornillos[51,247,248], Montserrat Ruiz[3,97], Pablo Ryan[249,250,251], Hector D. Salamanca[53,54], Lorena Salazar-García[93], Giorgina Gabriela Salgueiro-Origlia[102], Anna Sangil[76], Olga Sánchez-Pernaute[252], Pedro-Luis Sánchez[43,133], Antonio J. Sánchez López[253], Clara Sánchez-Pablo[133], María Concepción Sánchez-Prados[32], Javier Sánchez-Real[25], Jorge Sánchez-Redondo[15,254], Cristina Sancho-Sainz[175], Esther Sande[226], Arnoldo Santos[226], Agatha Schlüter[3,97], Sonia Segovia[232,255,256], Alex Serra-Llovich[62], Fernando Sevil-Puras[22], Marta Sevilla-Porras[3,11], Miguel A. Sicolo[257,258], Cristina Silván-Fuentes[3], Vitor M. S. Moraes[259], Vanessa S. Souza[105], Jordi Solé-Violán[10,260], José Manuel Soria[202], Jose V. Sorlí[107,108], Nayara S. Silva[261], Juan Carlos Souto[17], John J. Sprockel[54,103], José Javier Suárez-Rama[6], David A. Suárez-Zamora[64], Xiana Taboada-Fraga[208], Eduardo Tamayo[157,262], Alvaro Tamayo-Velasco[263], Juan Carlos Taracido-Fernández[171], Romero H. T. Vasconcelos[113], Carlos Tellería[121,122], Thássia M. T. Carratto[259], Jair Antonio Tenorio-Castaño[3,11,12], Alejandro Teper[147], Izabel M. T. Araujo[111], Juan Torres-Macho[264], Lilian Torres-Tobar[265], Ronald P. Torres-Gutiérrez[223], Jesús Troya[249], Miguel Urioste[211], Juan Valencia-Ramos[266], Agustín Valido[35,267], Juan Pablo Vargas-Gallo[268,269], Belén Varón[270], Tomas Vega[271], Santiago Velasco-Quirce[272], Valentina Vélez-Santamaría[96,97], Virginia Víctor[50,51], Julia Vidán-Estévez[25], Gabriela V. Silva[111], Miriam Vieitez-Santiago[59,137], Carlos Vilches[273], Lavinia Villalobos[25], Felipe Villar[222], Judit Villar-Garcia[274,275,276], Cristina Villaverde[3,40], Pablo Villoslada-Blanco[176], Ana Virseda-Berdices[81], Tatiana X. Costa[277], Zuleima Yáñez[34], Antonio Zapatero-Gaviria[278], Ruth Zarate[279], Sandra Zazo[241], Carlos Flores[10,109,155,240], José A. Riancho[59,60,137], Augusto Rojas-Martinez[280], Pablo Lapunzina[3,11,12] & Ángel Carracedo[3,6,7,9,10]

[15]Hospital Universitario Mostoles, Medicina Interna, Madrid, Spain. [16]Universidad Francisco de Vitoria, Madrid, Spain. [17]Haemostasis and Thrombosis Unit, Hospital de la Santa Creu I Sant Pau, IIB Sant Pau, Barcelona, Spain. [18]Unit of Infectious Diseases, Hospital Universitario 12 de Octubre, Instituto de Investigación Sanitaria Hospital 12 de Octubre (imas12), Madrid, Spain. [19]Spanish Network for Research in Infectious Diseases (REIPI RD16/0016/0002), Instituto de Salud Carlos III, Madrid, Spain. [20]School of Medicine, Universidad Complutense, Madrid, Spain. [21]Centre for Biomedical Network Research on Infectious Diseases, Instituto de Salud Carlos III, Madrid, Spain. [22]Hospital General Santa Bárbara de Soria, Soria, Spain. [23]Pediatric Neurology Unit, Department of Pediatrics, Navarra Health Service Hospital,

Pamplona, Spain. [24]Navarra Health Service, NavarraBioMed Research Group, Pamplona, Spain. [25]Complejo Asistencial Universitario de León, León, Spain. [26]Hospital Universitario San Pedro, Infectious Diseases Department, Logroño, Spain. [27]Fundación Institut Guttmann, Institut Universitari de Neurorehabilitació ofounde a la UAB, Hospital de Neurorehabilitació, Barcelona, Spain. [28]Universitat Autònoma de Barcelona (UAB), Barcelona, Spain. [29]Fundació Institut d'Investigació en Ciències de la Salut Germans Trias I Pujol, Barcelona, Spain. [30]Hospital General de Occidente, Guadalajara, Mexico. [31]Microbiology Unit, Hospital Universitario N. S. de Candelaria, Santa Cruz de Tenerife, Spain. [32]Hospital Universitario La Paz-IDIPAZ, Servicio de Neumología, Madrid, Spain. [33]Camino Universitario Adelita de Char, Mired IPS, Barranquilla, Colombia. [34]Universidad Simón Bolívar, Facultad de Ciencias de la Salud, Barranquilla, Colombia. [35]Hospital Universitario Virgen Macarena, Neumología, Seville, Spain. [36]Hospital das Forças Armadas, Brasília, Brazil. [37]Exército Brasileiro, Brasília, Brazil. [38]Departamento de Medicina, Universidad de Salamanca, Salamanca, Spain. [39]Centro de Investigación del Cáncer (IBMCC) Universidad de Salamanca – CSIC, Salamanca, Spain. [40]Department of Genetics & Genomics, Instituto de Investigación Sanitaria-Fundación Jiménez Díaz University Hospital – Universidad Autónoma de Madrid (IIS-FJD, UAM), Madrid, Spain. [41]Spanish National Cancer Research Centre, Human Genotyping-CEGEN Unit, Madrid, Spain. [42]Hospital Universitario de Salamanca-IBSAL, Servicio de Medicina Interna, Salamanca, Spain. [43]Universidad de Salamanca, Salamanca, Spain. [44]Universidade Federal do Rio Grande do Norte, Programa de Pós-Graduação em Ciências Farmacêuticas, Natal, Brazil. [45]Department of Child and Adolescent Psychiatry, Institute of Psychiatry and Mental Health, Hospital General Universitario Gregorio Marañón (liSGM), Madrid, Spain. [46]Clinical Pharmacology Service, Hospital de la Santa Creu I Sant Pau, IIB Sant Pau, Barcelona, Spain. [47]Servicio de Medicina Interna, Sanatorio Franchin, Buenos Aires, Argentina. [48]Biocruces Bizkai HRI, Bizkaia, Spain. [49]Cruces University Hospital, Osakidetza, Bizkaia, Spain. [50]Hospital Infanta Elena, Valdemoro, Madrid, Spain. [51]Instituto de Investigación Sanitaria-Fundación Jiménez Díaz University Hospital – Universidad Autónoma de Madrid (IIS-FJD, UAM), Madrid, Spain. [52]Centre for Biomedical Network Research on Mental Health (CIBERSAM), Instituto de Salud Carlos III, Madrid, Spain. [53]Fundación Hospital Infantil Universitario de San José, Bogotá, Colombia. [54]Fundación Universitaria de Ciencias de la Salud, Bogotá, Colombia. [55]Universidade Federal do Rio Grande do Norte, Departamento de Analises Clínicas e Toxicológicas, Natal, Brazil. [56]Departamento de Genética e Morfologia, Instituto de Ciências Biológicas, Universidade de Brasília, Brasília, Brazil. [57]Colégio Marista de Brasilia, Brasília, Brazil. [58]Associação Brasileira de Educação de Cultura, Ribeirão Preto, Brazil. [59]IDIVAL, Santander, Spain. [60]Universidad de Cantabria, Santander, Spain. [61]Hospital Universitario La Paz-IDIPAZ, Servicio de Medicina Interna, Madrid, Spain. [62]Fundació Docència I Recerca Mutua Terrassa, Barcelona, Spain. [63]Spanish National Cancer Research Center, CNIO Biobank, Madrid, Spain. [64]Fundación Santa Fe de Bogota, Departamento Patologia y Laboratorios, Bogotá, Colombia. [65]Hospital General de Occidente, Zapopan, Jalisco, Mexico. [66]Centro Universitario de Tonalá, Universidad de Guadalajara, Tonalá, Jalisco, Mexico. [67]Centro de Investigación Multidisciplinario en Salud, Universidad de Guadalajara, Tonalá, Jalisco, Mexico. [68]Instituto Murciano de Investigación Biosanitaria (IMIB-Arrixaca), Murcia, Spain. [69]Universidad Católica San Antonio de Murcia (UCAM), Murcia, Spain. [70]Hospital Universitario de Salamanca-IBSAL, Servicio de Medicina Interna-Unidad de Enfermedades Infecciosas, Salamanca, Spain. [71]Hospital Universitario de Fuenlabrada, Department of Internal Medicine, Madrid, Spain. [72]Escola Tecnica de Saúde, Laboratorio de Vigilancia Molecular Aplicada, Recife, Brazil. [73]Federal University of Pernambuco, Genetics Postgraduate Program, Recife, Pernambuco, Brazil. [74]Hospital Universitario Infanta Leonor, Servicio de Alergia, Madrid, Spain. [75]Hospital Universitario del Tajo, Servicio de Medicina Intensiva, Toledo, Spain. [76]Hospital Universitario Mutua Terrassa, Barcelona, Spain. [77]Hospital Universitario La Paz-IDIPAZ, Servicio de Farmacología, Madrid, Spain. [78]Alcaldía de Barranquilla, Secretaría de Salud, Barranquilla, Colombia. [79]Instituto de Investigación Sanitaria de Santiago (IDIS), Xenética Cardiovascular, Santiago de Compostela, Spain. [80]Centre for Biomedical Network Research on Cardiovascular Diseases (CIBERCV), Instituto de Salud Carlos III, Madrid, Spain. [81]Unidad de Infección Viral e Inmunidad, Centro Nacional de Microbiología (CNM), Instituto de Salud Carlos III (ISCIII), Madrid, Spain. [82]Cardiovascular Genetics Center, Institut d'Investigació Biomèdica Girona (IDIBGI), Girona, Spain. [83]Medical Science Department, School of Medicine, University of Girona, Girona, Spain. [84]Hospital Josep Trueta, Cardiology Service, Girona, Spain. [85]Institute of Biomedicine of Seville (IbiS), Consejo Superior de Investigaciones Científicas (CSIC)- University of Seville- Virgen del Rocio University Hospital, Seville, Spain. [86]Division of Infectious Diseases, Instituto de Investigación Sanitaria-Fundación Jiménez Díaz University Hospital – Universidad Autónoma de Madrid (IIS-FJD, UAM), Madrid, Spain. [87]ISGlobal, Barcelona, Spain. [88]Universitat Pompeu Fabra (UPF), Barcelona, Spain. [89]Centre for Biomedical Network Research on Epidemiology and Public Health (CIBERESP), Instituto de Salud Carlos III, Madrid, Spain. [90]Intensive Care Unit, Hospital Universitario Insular de Gran Canaria, Las Palmas de Gran Canaria, Spain. [91]Departemento de Medicina, Hospital Universitario Virgen del Rocío, Universidad de Sevilla, Seville, Spain. [92]Instituto de Biomedicina de Sevilla, Seville, Spain. [93]Universidad de los Andes, Facultad de Ciencias, Bogotá, Colombia. [94]Tecnológico de Monterrey, Monterrey, Mexico. [95]Andalusian Public Health System Biobank, Granada, Spain. [96]Neuromuscular Unit, Neurology Department, Hospital Universitari de Bellvitge, L'Hospitalet de Llobregat, Barcelona, Spain. [97]Bellvitge Biomedical Research Institute (IDIBELL), Neurometabolic Diseases Laboratory, L'Hospitalet de Llobregat, Barcelona, Spain. [98]Osakidetza, Cruces University Hospital, Barakaldo, Spain. [99]Centre for Biomedical Network Research on Diabetes and Metabolic Associated Diseases (CIBERDEM), Instituto de Salud Carlos III, Madrid, Spain. [100]University of Pais Vasco, UPV/EHU, Bilbao, Spain. [101]Oncology and Genetics Unit, Instituto de Investigacion Sanitaria Galicia Sur, Xerencia de Xestion Integrada de Vigo-Servizo Galego de Saúde, Vigo, Spain. [102]Hospital Universitario La Paz, Hospital Carlos III, Madrid, Spain. [103]Hospital de San José, Sociedad de Cirugía de Bogota, Bogotá, Colombia. [104]Hospital Universitario Río Hortega, Valladolid, Spain. [105]Programa de Pós Graduação em Ciências da Saúde, Faculdade de Medicina, Universidade de Brasília, Brasília, Brazil. [106]Servicio de Medicina ofounded, Complejo Hospitalario Universitario de A Coruña (CHUAC), Sistema Galego de Saúde (SERGAS), A Coruña, Spain. [107]Valencia University, Preventive Medicine Department, Valencia, Spain. [108]Centre for Biomedical Network Research on Physiopatology of Obesity and Nutrition (CIBEROBN), Instituto de Salud Carlos III, Madrid, Spain. [109]Research Unit, Hospital Universitario N.S. de Candelaria, Santa Cruz de Tenerife, Spain. [110]Otto von Guericke University, Departament of Microgravity and Translational Regenerative Medicine, Magdeburg, Germany. [111]Universidade Federal do Rio Grande do Norte, Depar- tamento de Analises Clinicais e Toxicologias, Natal, Brazil. [112]Institute of Psychiatry and Mental Health, Hospital General Universitario Gregorio Marañón (liSGM), Madrid, Spain. [113]Hospital Universitario Lauro Wanderley, João Pessoa, Brazil. [114]Programa de Pós Graduação em Ciências Farmacêuticas (PPgCF), Natal, Brazil. [115]Universidade Federal do Rio Grande do Norte, Programa de Pós-graduação em Ciências da Saúde, Natal, Brazil. [116]Hospital Universitario Mostoles, Unidad de Genética, Madrid, Spain. [117]Internal Medicine Department, Instituto de Investigación Sanitaria-Fundación Jiménez Díaz University Hospital – Universidad Autónoma de Madrid (IIS-FJD, UAM), Madrid, Spain. [118]Hospital Universitario Severo Ochoa, Servicio de Medicina Interna, Madrid, Spain. [119]Department of Preventive Medicine and Public Health, School of Medicine, Universidad Autónoma de Madrid, Madrid, Spain. [120]IdiPaz (Instituto de Investigación Sanitaria Hospital Universitario La Paz), Madrid, Spain. [121]Instituto Aragonés de Ciencias de la Salud (IACS), Zaragoza, Spain. [122]Instituto Investigación Sanitaria Aragón (IIS-Aragon), Zaragoza, Spain. [123]Universidade Federal do Rio Grande do Norte, Programa de Pós-Graduação em Ciências da Saúde, Natal, Brazil. [124]Universidade Federal do Rio Grande do Norte, Programa de Pós Graduação em Nutrição, Natal, Brazil. [125]Preventive Medicine Department, Instituto de Investigacion Sanitaria Galicia Sur, Xerencia de Xestion Integrada de Vigo-Servizo Galego de Saúde, Vigo, Spain. [126]Hospital Universitario Virgen del Rocío, Servicio de Medicina Interna, Seville, Spain. [127]Universidade Federal do Rio Grande do Norte, Departamento de Infectologia, Natal, Brazil. [128]Hospital de Doenças Infecciosas Giselda Trigueiro, Rio Grande do Norte, Natal, Brazil. [129]Unidad Diagnóstico Molecular. Fundación Rioja Salud, Logroño, Spain. [130]Faculdade de Ciências da Saúde, Universidade de Brasília, Brasília, Brazil. [131]Universidade Federal do Rio Grande do Norte, Departamento de Analises Clinicas e Toxicologicas, Natal, Brazil. [132]Hospital Universitario Quironsalud Madrid, Madrid, Spain. [133]Hospital Universitario de Salamanca-IBSAL, Servicio de Cardiología, Salamanca, Spain. [134]Hospital Universitario Puerta de Hierro, Servicio de Medicina Interna, Majadahonda, Spain. [135]Biocruces Bizkaia Health Research Institute, Galdakao University Hospital, Osakidetza, Barakaldo, Spain. [136]Instituto Regional de Investigación en Salud-Universidad Nacional de Caaguazú, Caaguazú, Paraguay. [137]Hospital U M Valdecilla, Santander, Spain. [138]Fundación Asilo San Jose, Santander, Spain. [139]Unidad de Enfermedades Infecciosas, Servicio de Medicina Interna,

Hospital Universitario Puerta de Hierro, Instituto de Investigación Sanitaria Puerta de Hierro – Segovia de Arana, Madrid, Spain. [140]Universidad Nacional de Asunción, Facultad de Politécnica, San Lorenzo, Paraguay. [141]Urgencias Hospitalarias, Complejo Hospitalario Universitario de A Coruña (CHUAC), Sistema Galego de Saúde (SERGAS), A Coruña, Spain. [142]Grupo de Investigación en Interacciones Gen-Ambiente y Salud (GIIGAS) – Instituto de Biomedicina (IBIOMED), Universidad de León, León, Spain. [143]Universidade de Fortaleza, Natal, Brazil. [144]Hospital Universitario Niño Jesús, Pediatrics Department, Madrid, Spain. [145]Unitat de Malalties Infeccioses I Importades, Servei de Pediatría, Infectious and Imported Diseases, Pediatric Unit, Hospital Universitari Sant Joan de Deú, Barcelona, Spain. [146]Microbiology Department, Instituto de Investigación Sanitaria-Fundación Jiménez Díaz University Hospital – Universidad Autónoma de Madrid (IIS-FJD, UAM), Madrid, Spain. [147]Hospital de Niños Ricardo Gutierrez, Buenos Aires, Argentina. [148]Department of Immunology, IRYCIS, Hospital Universitario Ramón y Cajal, Madrid, Spain. [149]University of Salamanca, Biomedical Research Institute of Salamanca (IBSAL), Salamanca, Spain. [150]Hospital Infanta Elena, Servicio de Medicina Intensiva, Valdemoro, Madrid, Spain. [151]Hospital Universitario de Getafe, Unidad de Genética, Madrid, Spain. [152]Pro- grama de pós-graduação em biotecnologia – Rede Nordeste de Biotecnologia (RENORBIO), Universidade Federal do Rio Grande do Norte, Natal, Brazil. [153]Pneumology Department, Hospital General Universitario Gregorio Marañón (iiSGM), Madrid, Spain. [154]Ministerio de Salud Ciudad de Buenos Aires, Buenos Aires, Argentina. [155]Genomics Division, Instituto Tecnológico y de Energías Renovables, Santa Cruz de Tenerife, Spain. [156]Hospital Clinico Universitario de Valladolid, Unidad de Apoyo a la Investigación, Valladolid, Spain. [157]Universidad de Valladolid, Departamento de Cirugía, Valladolid, Spain. [158]Sección Genética Médica – Servicio de Pediatría, Hospital Clínico Universitario Virgen de la Arrixaca, Servicio Murciano de Salud, Murcia, Spain. [159]Departamento Cirugía, Pediatría, Obstetricia y Ginecología, Facultad de Medicina, Universidad de Murcia (UMU), Murcia, Spain. [160]Grupo Clínico Vinculado, Centre for Biomedical Network Research on Rare Diseases (CIBERER), Instituto de Salud Carlos III, Madrid, Spain. [161]Hospital Universitario Centro Dermatológico Federico Lleras Acosta, Bogotá, Colombia. [162]Hospital Universitario Virgen de las Nieves, Servicio de Análisis Clínicos e Inmunología, Granada, Spain. [163]Intermediate Respiratory Care Unit, Department of Neumology, Instituto de Investigación Sanitaria-Fundación Jiménez Díaz University Hospital – Universidad Autónoma de Madrid (IIS-FJD, UAM), Madrid, Spain. [164]Sabin Medicina Diagnóstica, Santa Catarina, Brazil. [165]Clinica Comfamiliar Risaralda, Pereira, Colombia. [166]Centro Universitario de Tonalá, Universidad de Guadalajara, Guadalajara, Mexico. [167]Centro de Investigación Multidisciplinario en Salud, Universidad de Guadalajara, Guadalajara, Mexico. [168]Unidad de Cuidados, Intensivos Hospital Clínico Universitario de Santiago (CHUS), Sistema Galego de Saúde (SERGAS), Santiago de Compostela, Spain. [169]IIS La Fe, Plataforma de Farmacogenética, Valencia, Spain. [170]Universidad de Valencia, Departamento de Farmacología, Valencia, Spain. [171]Data Analysis Department, Instituto de Investigación Sanitaria-Fundación Jiménez Díaz University Hospital – Universidad Autónoma de Madrid (IIS-FJD, UAM), Madrid, Spain. [172]Hospital del Mar, Infectious Diseases Service, Barcelona, Spain. [173]Institut Hospital del Mar d'Investigacions Mèdiques (IMIM), Barcelona, Spain. [174]CEXS-Universitat Pompeu Fabra, Spanish Network for Research in Infectious Diseases (REIPI), Barcelona, Spain. [175]Biocruces Bizkaia Health Research Institute, Basurto University Hospital, Osakidetza, Basurto, Bizkaia, Spain. [176]Infectious Diseases, Microbiota and Metabolism Unit, Center for Biomedical Research of La Rioja (CIBIR), Logroño, Spain. [177]Opthalmology Department, Instituto de Investigación Sanitaria-Fundación Jiménez Díaz University Hospital – Universidad Autónoma de Madrid (IIS-FJD, UAM), Madrid, Spain. [178]Hospital Sant Joan de Deu, Pediatric Critical Care Unit, Barcelona, Spain. [179]Paediatric Intensive Care Unit, Agrupación Hospitalaria Clínic-Sant Joan de Déu, Esplugues de Llobregat, Barcelona, Spain. [180]Hospital Uni- versitario 12 de Octubre, Department of Immunology, Madrid, Spain. [181]Instituto de Investigación Sanitaria Hospital 12 de Octubre (imas12), Transplant Immunology and Immunodeficiencies Group, Madrid, Spain. [182]SIGEN Alianza Universidad de los Andes – Fundación Santa Fe de Bogotá, Bogotá, Colombia. [183]Hospital General de Segovia, Medicina Intensiva, Segovia, Spain. [184]Clinical Trials Unit, Instituto de Investigación Sanitaria-Fundación Jiménez Díaz University Hospital – Universidad Autónoma de Madrid (IIS-FJD, UAM), Madrid, Spain. [185]IMDEA-Food Institute, CEI UAM + CSIC, Madrid, Spain. [186]Hospital Universitario La Paz-IDIPAZ, Servicio de Inmunología, Madrid, Spain. [187]La Paz Institute for Health Research (IdiPAZ), Lymphocyte Pathophysiology in Immunodeficiencies Group, Madrid, Spain. [188]Hospital Universitario Virgen de las Nieves, Servicio de Enfermedades Infecciosas, Granada, Spain. [189]Instituto de Investigación Biosanitaria de Granada (ibs GRANADA), Granada, Spain. [190]Universidad de Granada, Departamento de Medicina, Granada, Spain. [191]Intensive Care Unit, Hospital Universitario de Canarias, La Laguna, Spain. [192]Dirección General de Salud Pública, Consejería de Sanidad, Junta de Castilla y León, Valladolid, Spain. [193]Fundación Jiménez Díaz, Epidemiology, Madrid, Spain. [194]Universidad Autónoma de Madrid, Department of Medicine, Madrid, Spain. [195]Instituto de Biomedicina (IBIOMED), Universidad de León, León, Spain. [196]Universidad de Valladolid, Departamento de Medicina, Valladolid, Spain. [197]Preventive Medicine Department, Instituto de Investigación Sanitaria-Fundación Jiménez Díaz University Hospital – Universidad Autónoma de Madrid (IIS-FJD, UAM), Madrid, Spain. [198]Intensive Care Unit, Hospital Universitario N. S. de Candelaria, Santa Cruz de Tenerife, Spain. [199]Hospital Universitario Infanta Leonor, Servicio de Medicina Intensiva, Madrid, Spain. [200]Unidad de Genética y Genómica Islas Baleares, Palma de Mallorca, Spain. [201]Hospital Universitario Son Espases, Unidad de Diagnóstico Molecular y Genética Clínica, Palma de Mallorca, Spain. [202]Genomics of Complex Diseases Unit, Research Institute of Hospital de la Santa Creu I Sant Pau, IIB Sant Pau, Barcelona, Spain. [203]Faculdade de Medicina, Universidade de Brasília, Brasília, Brazil. [204]Programa de Pós-Graduação em Ciências Médicas, Universidade de Brasília, Brasília, Brazil. [205]Programa de Pós-Graduação em Ciências da Saúde, Universidade de Brasília, Brasília, Brazil. [206]Hospital El Bierzo, Gerencia de Asistencia Sanitaria del Bierzo (GASBI), Gerencia Regional de Salud (SACYL), Ponferrada, Spain. [207]Grupo INVESTEN, Instituto de Salud Carlos III, Madrid, Spain. [208]Unidad de Cuidados Intensivos, Complejo Universitario de A Coruña (CHUAC), Sistema Galego de Saúde (SERGAS), A Coruña, Spain. [209]Hospital Universitario La Paz-IDIPAZ, Servicio de Pediatría, Madrid, Spain. [210]Hospital El Bierzo, Unidad Cuidados Intensivos, León, Spain. [211]Spanish National Cancer Research Centre, Familial Cancer Clinical Unit, Madrid, Spain. [212]Instituto de Investigación Sanitaria San Carlos (IdISSC), Hospital Clínico San Carlos (HCSC), Madrid, Spain. [213]Marinha do Brasil, Brasil, Brazil. [214]Universidade de Brasília, Brasília, Brazil. [215]Hospital General Universitario Gregorio Marañón (IiSGM), Madrid, Spain. [216]Unidad de Genética y Genómica Islas Baleares, Unidad de Diagnóstico Molecular y Genética Clínica, Hospital Universitario Son Espases, Palma de Mallorca, Spain. [217]Instituto de Investigación Sanitaria Islas Baleares (IdISBa), Palma de Mallorca, Spain. [218]Programa de Pós- Graduação em Biologia Animal (UnB), Brasília, Brazil. [219]Programa de Pós-Graduação Profissional em Ensino de Biologia (UnB), Brasília, Brazil. [220]Anatomía Patológica, Instituto de Investigación Sanitaria San Carlos (IdISSC), Hospital Clínico San Carlos (HCSC), Madrid, Spain. [221]Universidad Complutense de Madrid, Department of Immunology, Ophthalmology and ENT, Madrid, Spain. [222]Department of Neumology, Instituto de Investigación Sanitaria-Fundación Jiménez Díaz University Hospital – Universidad Autónoma de Madrid (IIS-FJD, UAM), Madrid, Spain. [223]Hospital Nuestra Señora de Sonsoles, Ávila, Spain. [224]Universidade Federal do Pará, Núcleo de Pesquisas em Oncologia, Belém, Pará, Brazil. [225]Secretaria Municipal de Saude de Apodi, Natal, Brazil. [226]Intensive Care Department, Instituto de Investigación Sanitaria-Fundación Jiménez Díaz University Hospital – Universidad Autónoma de Madrid (IIS-FJD, UAM), Madrid, Spain. [227]Hospital Universitario Príncipe de Asturias, Servicio de Microbiología Clínica, Madrid, Spain. [228]Universidad de Alcalá de Henares, Departamento de Biomedicina y Biotecnología, Facultad de Medicina y Ciencias de la Salud, Madrid, Spain. [229]Inditex, A Coruña, Spain. [230]GENYCA, Madrid, Spain. [231]Universidade Federal do Rio Grande do Norte, Departamento de Análises Clínicas e Toxicológicas, Natal, Brazil. [232]Neuromuscular Diseases Unit, Department of Neurology, Hospital de la Santa Creu I Sant Pau, Universitat Autònoma de Barcelona, Barcelona, Spain. [233]Instituto Mexicano del Seguro Social (IMSS), Centro Médico Nacional Siglo XXI, Unidad de Investigación Médica en Enfermedades Infecciosas y Parasitarias, Mexico City, Mexico. [234]Catalan Institution of Research and Advanced Studies (ICREA), Barcelona, Spain. [235]Drug Research Centre, Institut d'Investigació Biomèdica Sant Pau, IIB-Sant Pau, Barcelona, Spain. [236]Unidad de Cuidados Intensivos, Hospital Clínico Universitario de Santiago (CHUS), Sistema Galego de Saúde (SERGAS), Santiago de Compostela, Spain. [237]Hospital Ophir Loyola, Departamento de Ensino e Pesquisa, Belém, Pará, Brazil. [238]University Hospital Germans Trias I Pujol, Pediatrics Department, Badalona, Spain. [239]Department of Immunology, Hospital Universitario de Gran Canaria Dr. Negrín, Las Palmas de Gran Canaria, Spain. [240]Department of Clinical Sciences, University Fernando Pessoa Canarias, Las Palmas de Gran Canaria, Spain. [241]Department of Pathology, Biobank, Instituto de Investigación Sanitaria-

Fundación Jiménez Díaz University Hospital – Universidad Autónoma de Madrid (IIS-FJD, UAM), Madrid, Spain. [242]Centre for Biomedical Network Research on Cancer (CIBERONC), Instituto de Salud Carlos III, Madrid, Spain. [243]Universidad de Sevilla, Departamento de Enfermería, Seville, Spain. [244]Hospital Universitario Virgen de las Nieves, Servicio de Medicina Interna, Granada, Spain. [245]Fundación Universitaria de Ciencias de la Salud, Grupo de Ciencias Básicas en Salud (CBS), Bogotá, Colombia. [246]Universidad de Granada, Departamento Bioquímica, Biología Molecular e Inmunología III, Granada, Spain. [247]Hospital Infanta Elena, Allergy Unit, Valdemoro, Madrid, Spain. [248]Faculty of Medicine, Universidad Francisco de Vitoria, Madrid, Spain. [249]Hospital Universitario Infanta Leonor, Madrid, Spain. [250]Complutense University of Madrid, Madrid, Spain. [251]Gregorio Marañón Health Research Institute (IiSGM), Madrid, Spain. [252]Reu- mathology Service, Instituto de Investigación Sanitaria-Fundación Jiménez Díaz University Hospital – Universidad Autónoma de Madrid (IIS-FJD, UAM), Madrid, Spain. [253]Biobank, Puerta de Hierro-Segovia de Arana Health Research Institute, Madrid, Spain. [254]Universidad Rey Juan Carlos, Madrid, Spain. [255]The John Walton Muscular Dystrophy Research Centre, Newcastle University and Newcastle Hospitals NHS Foundation Trust, Newcastle upon Tyne, England. [256]Neuromuscular Unit, Neuro- pediatrics Department, Institut de Recerca Sant Joan de Déu, Hospital Sant Joan de Déu, Barcelona, Spain. [257]Casa de Saúde São Lucas, Natal, Brazil. [258]Hospital Rio Grande, Natal, Brazil. [259]Departamento de Química, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, São Paulo, Brazil. [260]Intensive Care Unit, Hospital Universitario de Gran Canaria Dr. Negrín, Las Palmas de Gran Canaria, Spain. [261]Universidade Federal do Rio Grande do Norte, Pós- graduação em Biotecnologia – Rede de Biotecnologia do Nordeste (Renorbio), Natal, Brazil. [262]Hospital Clinico Universitario de Valladolid, Servicio de Anestesiologia y Reanimación, Valladolid, Spain. [263]Hospital Clinico Universitario de Valladolid, Servicio de Hematologia y Hemoterapia, Valladolid, Spain. [264]Hospital Universitario Infanta Leonor, Servicio de Medicina Interna, Madrid, Spain. [265]Sociedad de Cirugía de Bogotá, Hospital de San José, Bogotá, Colombia. [266]University Hospital of Burgos, Burgos, Spain. [267]Universidad de Sevilla, Seville, Spain. [268]Fundación Santa Fe de Bogota, Instituto de servicios medicos de Emergencia y trauma, Bogotá, Colombia. [269]Universidad de los Andes, Bogotá, Colombia. [270]Quironprevención, A Coruña, Spain. [271]Junta de Castilla y León, Consejería de Sanidad, Valladolid, Spain. [272]Gerencia Atención Primaria de Burgos, Burgos, Spain. [273]Immunogenetics-Histocompatibility group, Servicio de Inmunología, Instituto de Investigación Sanitaria Puerta de Hierro – Segovia de Arana, Madrid, Spain. [274]Hospital del Mar, Department of Infectious Diseases, Barcelona, Spain. [275]Hospital del Mar Medical Research Institute (IMIM), Barcelona, Spain. [276]Universitat Autònoma de Barcelona, Department of Medicine, Barcelona, Spain. [277]Maternidade Escola Janário Cicco, Natal, Brazil. [278]Consejería de Sanidad, Comunidad de Madrid, Madrid, Spain. [279]Centro para el Desarrollo de la Investigación Científica, Caaguazú, Paraguay. [280]Tecnológico de Monterrey, Escuela de Medicina y Ciencias de la Salud, Monterrey, Mexico.