Clonal chromosomal mosaicism and loss of chromosome Y in men are risk factors for SARS-CoV-2 vulnerability in the elderly

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Abstract

The ongoing pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2, COVID-19) has an estimated overall case fatality ratio of 1.38% (pre-vaccination), being 53% higher in males and increasing exponentially with age. Among 9578 individuals diagnosed with COVID-19 in the SCOURGE study, we found 133 cases (1.42%) with detectable clonal mosaicism for chromosome events (CME) and 226 males (5.08%) with acquired loss of chromosome Y (LOY). Individuals with clonal mosaic events (CME and/or LOY) showed a 54% increase in the risk of COVID-19 lethality. LOY is associated with transcriptomic biomarkers of immune dysfunction, pro-coagulation activity and cardiovascular risk. Interferon-induced genes involved in the initial immune response to SARS-CoV-2 are also down-regulated in LOY. Thus, CME and LOY underlie at least part of the sex-biased severity and mortality of COVID-19 in aging patients. Given its potential therapeutic and prognostic relevance, evaluation of clonal mosaicism should be implemented as biomarker of COVID-19 severity in elderly people.

KEYWORDS: Clonal mosaicism, chromosomal mosaic events, loss of chromosome Y, COVID-19, SARS-CoV-2, elderly people, mortality.

Background

The ongoing pandemic of coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) represents a major health threat to the entire world.[1] As of December, 2021, there have been over 400 million confirmed cases of COVID-19 worldwide, with more than 5.7 million deaths reported. A best estimate of the overall case fatality ratio after adjusting for demography and under-ascertainment in the initial outbreak in China was 1.38% (95% confidence interval 1.23–1.53), being significantly higher in aging people (6.4% in ≥60 and 13.4% in ≥80 age groups) and in males.[2] World-wide data of the age-stratified case fatality ratio and infection fatality ratio show a similar pattern with a remarkable sex-bias increasing with advanced age, with 60% overall deaths reported in men (estimated hazard ratio of 1.59, 95% confidence interval 1.53-1.65).[3] Interestingly, sex-dependent differences in disease outcomes were also found during the past SARS-CoV and MERS-CoV epidemics[4,5] and also in mice infected with the virus.[6]

Understanding the underlying basis of this different sex and age vulnerability is crucial because aging men and women are likely to have fundamentally different reactions to the SARS-CoV-2 virus infection, treatments, and vaccines. Male patients with COVID-19 have higher plasma levels of innate immune cytokines (IL-8 and IL-18) and stronger induction of non-classical monocytes, while females had more robust T cell activation during infection. Proposed causes include different case definition of disease, different environmental and social factors (such as lifestyle, smoking history or work-environment) and sex-specific immune-defense factors. The X chromosome harbors multiple genes important for immunity and there are many X-linked immunodeficiencies, so males have greater susceptibility to infections starting at birth.[6] More specifically, SARS viruses use the angiotensin converting enzyme (ACE2), encoded by an X-linked gene, as a receptor to enter and infect ACE-2 expressing cells.[1] Sex variation in the expression of this gene with paradoxically higher expression and higher circulating levels in men than in women has also been proposed as a candidate mechanism.[7] However, ascertainment bias and environmental factors are unlikely to prevail in different populations while the gender-specific immune factors or ACE2 variation would not fully explain the increased risk and sex-divergence with aging. The analysis of previously untreated patients with moderate COVID-19 disease revealed that male patients have higher levels of innate immune cytokines and more robust induction of non-classical monocytes, while female patients have more robust T-cell activation, which is sustained in old age.[8] A B-cell autoimmune disorder present in about 10% of individuals with life-threatening COVID-19 pneumonia has been reported, 5 times more common in males than females, characterized by detection of neutralizing immunoglobulin G autoantibodies against interferon type 1.[9] Finally, a meta-analysis of genome-wide association studies searching for host-specific genetic factors has revealed 13 loci significantly associated with SARS-Cov2 infection or severe manifestations of COVID-19, but do not fully explain the gender differences.[10]

Chromosomal mosaic events (CME) detectable in blood, including deletions, gains or copy neutral changes, are age-related somatic alterations that indicate clonal hematopoiesis when detectable and have been associated with increased risk for cancer, cardiovascular disease and overall mortality.[11-15] Expanded CMEs have also been recently associated with increased risk for incident infections, including COVID-19 hospitalization.[16] Multiple germline genetic alleles involved in susceptibility to clonally expanded CME have been identified, with enrichment at regulatory sites for the immune system.[16] In men, mosaic X chromosome monosomy (XCM), acquired by somatic loss of the Y chromosome (LOY), is the most common copy number alteration in male leukocytes, estimated to occur in <2% men under 60 years of age, but exponentially increasing with aging to 15–40% in 70–85 year-old males and >50% at 93 years of age.[17] LOY has also been associated with a wide spectrum of human diseases including cancer, Alzheimer's disease, cardiovascular disease, and reduced overall life expectancy in men.[18-21] Genetic variation in multiple loci is involved in the inherited susceptibility to LOY, which can also be driven by smoking and other environmental exposures.[17] Extreme down-regulation of chromosome Y gene expression mainly driven by genes with X-chromosome homologs that escape X-inactivation seems to be the functional mediator of the reported association between LOY and disease.[22,23]

In women, developmental (causing Turner syndrome) or late onset XCM detectable in leukocytes, usually with loss of the inactive X-chromosome, is found with lower frequency than in men but also increasing with age (0.05% in 50-year old; 0.25% in 75-year old).[24] Females with XCM have an increased risk for autoimmune disease, recurrent viral infections and earlier cardiovascular mortality,[25] which is associated with excessive production of pro-inflammatory cytokines (IL-6), decrease in anti-inflammatory cytokines (IL-10, TGF-β) and a lower CD4:CD8 ratio.[26]

We have tested here the hypothesis that CME/XCM/LOY could be underlying factors for the increased severity and mortality of COVID-19 in the elderly and mainly in men. Overall, we have associated clonal mosaicism with a 50% increase in the risk of COVID-19 lethality. We have also correlated LOY in aging males with multiple parameters of cardiovascular dysfunction, and defined the transcriptomic deregulation that underlies disease risks, including signatures of immune system dysfunction and increased coagulation activity. We have finally studied how some of the genes deregulated by LOY are involved in the response to SARS-CoV-2 infection.

Methods

Covid-19 infection, mortality data, CME and LOY prevalence estimates
Accumulated data until July 21st, 2021 was obtained from the Spanish National Epidemiological

Registry

(https://www.isciii.es/QueHacemos/Servicios/VigilanciaSaludPublicaRENAVE/EnfermedadesTra nsmisibles/Paginas/InformesCOVID-19.aspx). Hospitalization rates, intensive care admission rates, and mortality stratified by age and sex was obtained from this report. Prevalence estimates of CME and LOY by age were obtained from the general population.[14,21]

EGCUT subjects, phenotype and genotype data

LOY was assessed in a total of 530 adult men belonging to the Estonian Gene Expression Cohort (EGCUT, www.biobank.ee) that comprises a large cohort of 53,000 samples of the Estonian Genome Center Biobank, University of Tartu.[27] Detailed phenotypic information from all the individuals studied, including clinical analysis (blood cell counts and general biochemistry) and follow-up until June 2020, was available in ICD-10 codes. Patients selected in this study were genotyped using OmniX array. All individuals had genotyping success rate above 95%. All studies were performed in accordance with the ethical standards of the responsible committee on human experimentation, and with proper informed consent from all individuals tested.

SCOURGE subjects, phenotype and genotype data

A total of 9578 (5134 females and 4444 males) patients diagnosed with COVID-19 and recruited to the SCOURGE study were included in this study.[28] Mean age was 62.58 years, 61.06 for females and 64.34 for males. Available phenotype data included age, sex, some clinical variables of past clinical history, several defined measures of COVID-19 severity and vital status (alive or dead) 90 days after diagnosis. The severity variables classified individuals in five levels called Asymptomatic (A), Mild (light: L), Moderate (M), Severe (G), and Critical (C). Additional information about pre-existing conditions as categorical variables was also available for most cases, including history of vascular disorders, cardiac problems, neurologic conditions, gastrointestinal disorders, onco-hematologic conditions, respiratory issues, and pulmonary thrombo-embolism. Blood DNA was genotyped using a customized Affymetrix SNP microarray.[28] Genotype data passed quality controls for GWAS analysis.

Detection of mosaic CME and LOY

The genotype CEL files from everyone were used to extract the log-R ratio (LRR) and B-allele (BAF) frequency from SNP probes. We used the *apt* software for quality control (QC) and the extraction of the array intensity signals. Following the QC pipeline with filters *axiom-dishqc-DQC>0.82* and *call-rate>0.97*, we observed that all individuals could be included. The signals were obtained from CNV calling pipeline with default parameters *mapd-max=0.35* and *waviness-sd-max=0.1*. We also called mosaicisms in autosomes and chromosome X with the MAD algorithm.[29] The method uses the fixed deviation from the expected BAF value of 0.5 for heterozygous SNPs (Bdev) to call allelic imbalances by using a segmentation procedure. The segmentation was performed using the three different parameters of MAD: *T>8, aAlpha=0.8,*

minSegLength>100. Some false positive alterations were detected in bad quality arrays. Therefore, curation via visual inspection, considering variability of LRR and BAF mean values in the segment, was performed by two independent investigators. Each mosaic alteration was classified as copy-loss, copy-gain or copy-neutral. The estimated percentage of abnormal cells was computed based on the B-deviation as previously reported.[10]

Mosaic LOY detection and quantification was performed using the *MADloy* tool which implements LOY calling using the mean LRR (mLRRY) and B-deviation derived-measures from chromosome Y across subjects.[30] For each sample, *MADloy* first estimates the normalized mLRRY given by its ratio with the trimmed-mean of mLRRY values in the autosomes to discard regions with copy number alterations. B-deviation is calculated for the pseudoautosomal regions 1 and 2 (PAR1, 0-2.5Mb on both Xp and Yp; PAR2, 300kb on distal Xq and Yq, Mb 155 and 59 respectively), and the XY transposed region (88-92Mb on X, 2.5-6.5Mb on Y). The method is calibrated to detect mosaicism when the proportion of affected cells is above 10%. We then plotted the values of the mLRRY signals for males and females. A signal from chromosome Y in females is observed due to the background noise of the array and some cross-hybridization. While we observed variability of the mLRRY signal, numerous males were identified with extreme low values of mLLRY, suggesting loss of chromosome Y. We categorized the level of LOY status into three groups according to the magnitude of the decrease in mLRRY, believed to be a function of XCM/LOY cellularity.

Bulk transcriptome data

Gene expression was obtained with Illumina whole-genome expression BeadChips (HT12v3) from peripheral blood RNA in the EGCUT cohort. Low quality samples were excluded. All probes with primer polymorphisms were left out, leaving 34,282 probes. The expression dataset is publicly available at GEO (Gene Expression Omnibus) under the accession number GSE48348.[31] In this dataset, a total of 11 individuals with LOY were identified. In order to consider the effect of aging on LOY detection and to have the maximum power, 32 age and gender-paired normal samples without LOY (3 controls per case) were selected for the transcriptomic analyses.

The effect of SARS-CoV-2 infection on gene expression was assessed in independent biological triplicates of two different cell lines that were mock treated or infected with SARS-CoV-2 (USA-WA1/2020). One corresponds to primary human lung epithelium (NHBE) and the other to transformed lung alveolar cells (A549). These data are available at GEO under the accession number GSE147507.

Statistical data analyses

Gene expression data was quantile-normalized to the median. We analyzed linear regression residuals of gene expression data on forty multidimensional scaling components, to correct for

possible unwanted variability. Array quality was assessed using *arrayQualityMetrics* Bioconductor package. *genefilter* Bioconductor package was used to filter for features without annotation and/or exhibiting little variation and low signal across samples, leaving a total of 15,592 probes from 34,282. Differential expression (DE) between individuals with and without LOY was then performed using *limma* Bioconductor package. Significant DE genes were considered at false discovery rate (FDR) <0.05. Significant DE genes at p<0.001 level were selected for Gene Ontology (GO) and KEGG (Kyoto Encyclopedia of Genes and Genomes) enrichment analysis with *clusterProfiler* Bioconductor package. Over-representation of DE genes in the gene set obtained from the analysis of SARS-CoV-2 infected cell lines (p<0.001 and log-foldchange >0.5) was performed using exact Fisher test. Cell-type composition of the 43 individuals with bulk transcriptomic data (11 LOY, 32 normal) was estimated using the 'xcell' method implemented in the *immunodeconv* R package.[32]

Association analysis between CME or LOY status and clinical data, including blood cell counts and biochemical parameters, was assessed using linear models adjusted by age. All statistical analyses were performed using the statistical software R version 3.6.3 (http://www.r-project.org).

Results

Higher Covid-19 severity and mortality in males, a sex-bias that increases with aging

Accumulated data on the age-stratified case fatality ratio and infection fatality ratio in a large sample from Spain, show a pattern with a remarkable sex-bias increase with advancing age (Figure 1). Available reports, mostly based on hospital records, show the same tendency in other countries. COVID-19 lethality, CME prevalence and LOY prevalence in men, as previously reported in multiple reports including the UK biobank dataset, appear to increase exponentially with age (Figure 1).[18-21]

COVID-19 severity variables and their association with age

We first studied the SCOURGE clinical data. Phenotype data was available from all 9578 individuals (5134 females and 4444 males) patients diagnosed with COVID-19 and recruited to the SCOURGE study (Table 1). According to disease severity, there were 607 cases asymptomatic (6.8% A), 2727 individuals with mild symptoms (30% L), 2141 patients with moderate disease (23.6% M), 2449 with severe manifestations (27% G), 1157 critical (12.7% C). We visually inspected the contrasts defined together with the level of severity and the age of the patients. Mean age was 62.58 years, 64.34 for males and 61.06 for females, with an age difference between sexes that was statistically significant ($P = 4.1 \times 10^{-19}$). All clinical categories and variables correlated with age except for "critical" and "history of pulmonary thromboembolism".

Association between CME and COVID-19 severity (lethality)

The algorithm followed by manual curation finally detected 133 individuals (1.42%), 61 males and 72 females, carrying CMEs in blood affecting the autosomes and/or the X chromosome (Table 1, Table S1, Figures 2A & 2B and Figure S1). Globally, 95 individuals had a single CME while 38 of them had more than one event, for a total of 213 CMEs. There were 88 deletions, 5 whole chromosome monosomies, 20 segmental gains and 21 whole chromosome trisomies, along with 78 copy-neutral changes (somatic segmental uniparental disomies), and a few complex rearrangements. Mean age for individuals with CMEs was 75.04±12.7. We then performed association analyses across the different outcome variables related to COVID-19 severity and the presence of mosaicism. We first confirmed the strong association between mosaicism and age (year) ($OR = 1.051$, $P = 1.05 \times 10^{-16}$), as previously reported. We then observed a significant association between the presence of CME and COVID-19 lethality (1-survival, $OR = 1.77$, $P = 0.015$), after adjusting for sex and age (Figure 3).

Association between LOY and COVID-19 severity

Among all male cases, we detected 226 individuals with LOY (mean age 82.0±7.9), a 5,08% prevalence of LOY in this cohort (Table 1, Table S2). According to the estimated proportion of cells with XCM/LOY, 162 individuals had mild LOY (<25% cells with XCM), 43 moderate LOY (25-65% cells with XCM) and 21 had extreme LOY (>65% cells with XCM) (Figure 2C). We also identified three women with detectable chromosome Y in a proportion of cells, then likely corresponding to X0/XY mosaicism and a possible diagnosis of Turner syndrome, as well as three individuals with non-mosaic XYY (Figure 2C). We observed 6 men with both LOY and CME, 220 with LOY and no CME, and 55 with CME and no LOY, which resulted in no significant correlation between the presence of LOY and CME.

We first confirmed a strong association between XCM/LOY in males and age ($OR = 1.11$, $P = 5.65 \times 10^{-51}$). We then fitted a series of models between LOY and the contrast *CG > MLA*, for which we had observed a strong association with age. We first observed a significant association between the contrast and LOY, primarily due to its association with age (not significant after adjusting by age, $OR = 1.25$, $P = 0.15$). We also performed association tests for all the contrasts and clinical variables adjusting only by age and we observed some significant associations. LOY was associated with reduction in survival ($OR = 0.713$, $P = 0.045$) and with clinical history of vascular disease ($OR = 0.627$, $P = 0.001$) and lung thromboembolism ($OR = 0.271$, $P = 0.042$). While associations with severity were not significant, we observed a consistent estimate of their risk given by LOY.

We then tested the association with the continuous value for mLRRY across all severity contrast and clinical variables. We found a significant association with survival for higher relative levels of chromosome Y content ($\beta = 0.86$, $P = 0.0054$).

We then performed a joint analysis for all mosaicisms, CMEs and LOY, confirming their strong association with age ($OR = 1.08$ $P = 1.95 \times 10^{-62}$) and also with COVID-19 lethality ($OR = 1.53$, $P = 0.004$) after corrections, including adjustment for other clinical variables (Figure 3). The associations of all type mosaicism with severity contrasts were not significant but consistent across all contrasts.

Germline aneuploidies and COVID-19

In addition to 6 individuals with XCM and likely Turner syndrome, 3 cases with 45,X0/46,XY mosaicism mentioned above, 2 more cases with 45,X0/46,XX mosaicism and one with likely 45,X0/46,XY/46,XX mosaicism, the algorithm also detected a total of 25 individuals with germline (non-mosaic) aneuploidies. We detected 7 cases with Down syndrome (trisomy 21) and 18 with gonosomal aneuploidies, including 9 with Klinefelter syndrome (47,XXY), 6 with triple X syndrome (47,XXX) and 3 with XYY syndrome (47,XYY) (Table S3). We found an association of aneuploidies with the presence of CMEs ($OR = 9.90$, $P = 0.0047$).

We then performed association tests of phenotypic features with all the aneuploidies, removing individuals with CMEs. We did not find any significant association between COVID-19 severity parameters and any type of aneuploidy given this small sample size, although previous history of cardiopathy was significantly associated, as expected (OR = 4.02, $P = 0.004$).

Correlation of LOY with cellular and biochemical phenotypes in EGCUT individuals

We analyzed SNP microarray data with MADloy of a selected sample of 530 apparently healthy adult men from the Estonian Genome Center of the University of Tartu cohort (EGCUT) and classified them as having (n=28) or not having LOY (n=502). We then correlated genotype classification with several clinical parameters. Individuals with LOY had significantly age-adjusted decrease in red cell counts, decrease in mean corpuscular hemoglobin concentration and higher red cell distribution width, low basophil counts and borderline low lymphocyte proportions. Biochemical parameters revealed low albumin levels, low triglycerides and elevated homocysteine and urea levels (Table S3).

Blood transcriptome in individuals with LOY reveals immune defects and cardiovascular risk

We also compared blood transcriptome from 11 men with LOY (median age: 69, range: 58-84) and 32 age-paired men without LOY (median age: 68, range: 60-87) as controls. Multiple genes differentially expressed between groups were found, including autosomal and gonosomal genes (Tables S4-S6 and Figs S2-S3), providing insight into the mechanisms of disease susceptibility caused by LOY with implications for COVID-19. *CSF2RA,* located on the X-Y chromosome pseudoautosomal 1 (PAR1) region, is one of the most significantly down-regulated genes in

LOY (Fig 4A), along with other multiple Y chromosome genes with homologs on the X chromosome that escape X inactivation and with known function in immunity (Table S6).

Top autosomal genes overexpressed in LOY, such as *VWF and MYL9* (Fig 4A), are associated with cardiovascular risk. *VWF* codes for the von Willebrand factor (vWF), a pro-coagulant protein that promotes platelet adhesion and smooth muscle cell proliferation, while *MYL9* encodes Myosin Light Chain 9, regulatory, important in inflammatory immune responses.

Since changes in gene expression may reflect differences in cell-type composition and functionality, we estimated the average cell-type functional composition of samples from individuals with LOY compared to those without LOY using bulk transcriptome data (Table S7). The results were consistent with LOY individuals having significantly decreased GM-progenitors and B cell naïve cells, along with increased counts of endothelial cells (Fig 4B). Enrichment gene set analysis using differentially expressed genes revealed a few categories significantly over-enriched, most notably the coagulation and cellular detoxification, the leukocyte migration and neutrophil activation (Fig 4C, Tables S8-S9). Overall, gene expression in LOY individuals leads to a down-regulated immune score.

Down-regulated genes in LOY involved in response to SARS-CoV-2 infection

We tested whether the genes that participate in the primary response to SARS-CoV-2 infection were significantly deregulated in blood cells of individuals with LOY. We obtained 249 deregulated genes with SARS-CoV-2 infection in primary human lung epithelium (NHBE) and 130 for transformed lung alveolar (A549) (339 unique genes for the two cell lines). This gene set is highly over-represented in several pathways including defense response to virus, IL-17, type I interferon and NF-Kappa B signaling (Table S10). From the deregulated genes in cells infected with SARS-CoV-2 13 were also deregulated in individuals with LOY (Figure 5A and Table S11) indicating a strong significant over-representation (OR of enrichment = 7.23, p=1.5x10^{-7}). Most of these genes are interferon response genes (*IFIT3, IFI44L, ITFT1, IFI6*), which are down-regulated in individuals with LOY (Figure 5B-D).

Discussion

We have shown in the SCOURGE study that clonal detectable CMEs, including XCM, are relatively common in blood of aging individuals, as previously reported,[14] with much higher frequency in males due to somatic LOY.[18] In addition to a risk factor for cancer, cardiovascular complications, incidental infections and all cause early mortality,[12,16,18,33] clonal hematopoiesis with CME and/or XCM due to LOY is a risk factor for COVID-19 lethality with a combined odds ratio of 1.53. Despite some limitations of our study due to a relatively small sample size and the possibility of uncontrolled confounding factors, similar results have been recently reported in the

UK biobank revealing increased risk for diverse incident infections and COVID-19 hospitalization in people with clonal hematopoiesis.[16,34] Our data indicate that these two types of chromosomal mosaicism underlie at least part of the aging-related and sex-biased severity and mortality of COVID-19. Therefore, identification of CME and LOY in blood cells is likely to have an immediate clinical relevance in the management of aged patients with COVID-19.

The mechanisms predisposing to autosomal events and LOY seem be mostly unrelated, as no significant association has been found between both types of events in our cohort and the germline loci reported to predispose to autosomal CMEs and LOY are different.[16,19,21] While only 10% of autosomal CMEs correspond to whole chromosome aneuploidies (mainly trisomies 8, 12 and 15 and monosomy 7) likely mediated by mitotic non-disjunction, this is the main mechanism for XCM and LOY. Mitotic non-disjunction of Y chromosomes may be facilitated by the higher rate of cellular turnover of aging men. In mice, while the Y chromosome is stably transmitted during meiotic cell divisions, there is a high frequency of non-disjunction in mitosis, mainly in the earliest cleavage divisions.[35]

A possible pathogenetic mechanism that could be common to clonal CMEs and XCM is immunosenescence, which involves modifications of humoral and cellular immunity. One aspect of immunosenescence is a decline in the absolute number of peripheral blood lymphocytes with locus-dependent reduction of HLA class-I cell surface expression, related with increased risk of subsequent mortality. T-lymphocytes also play a central role in the effector and regulatory mechanisms of the adaptive immune response.[36]

Many of the biochemical and transcriptomic alterations found in individuals with LOY have been already associated to poor prognosis for SARS-CoV-2 infection.[37-40] Several genes located on the Y chromosome with relevant functions in the immune system have functional homologs on the X chromosome that escape X inactivation in females (Table S4). Cells with XCM are likely haploinsufficient for many of those genes, which are downregulated in individuals with mosaic XCM due to LOY. In this regard, we observed low expression of *CSF2RA* in individuals with LOY, who also have low GM progenitors. *CSF2RA* codes for the alpha subunit of the heterodimeric receptor for colony stimulating factor 2, a cytokine that regulates the production, differentiation, and function of granulocytes and macrophages (GM-CSF), key cells for antigen presentation in infections, and is also critical for T cell function. GM-CSF increases IL-2R and IL-2 signaling, which can increase expansion of lymphocytes and IFN-γ production important for anti-viral response. Therefore, GM-CSF leads to enhanced protective responses.[41] Loss or inactivation of both copies of the *CSF2RA* gene is associated with surfactant metabolism dysfunction-4 and pulmonary alveolar proteinosis, a primary immunodeficiency (OMIM 300770).[42] As Leukine® (sargramostim, rhu-GM-CSF) is currently being assessed in the SARPAC trial because of its potential positive effect on antiviral immunity and contribution to restore immune homeostasis in the lungs (https://clinicaltrials.gov/ct2/show/NCT04326920), our

data suggest that patients with LOY might be predictive of a poor response due to their low expression of one of the receptor subunits for GM-CSF (*CSF2RA*).[43]

Patients severely affected with COVID-19 have lower lymphocyte counts, especially T cells, higher leukocyte counts and neutrophil-lymphocyte-ratio, lower percentages of monocytes, eosinophils, and basophils, along with generally elevated levels of infection-related biomarkers and inflammatory cytokines, including IL-6. Helper, suppressor and regulatory T cells were all below normal levels in the severe group, with increased naïve helper T cells and decreased memory helper T cells.[1,44] We observed a significant overlap of deregulated genes in LOY individuals that participate in the immediate immune response elicited by SARS-CoV-2 virus infection. Some of these genes clearly activated in both studied cell types infected by SARS-CoV-2 are markedly under expressed in individuals with LOY (*SLPI, IFI6, IFIT1, IFIT3,* and *IFI44L*) (Figure 3B-D). Secretory leukocyte protease inhibitor (SLPI) is a regulator of innate and adaptive immunity that protects the host from excessive inflammation in infectious disease, while the other four genes encode interferon induced proteins of the innate immune system that participate in the immediate host response to viral infections.[45] Dysfunctions of the adaptive immunity and interferon-mediated immediate host response in individuals with XCM/LOY are consistent with the observed sexual dimorphism in human immune system aging, and might underlie a poor immune response to SARS-CoV-2 infection.[46] This patterns along with the increased severity in older males, suggests that XCM due to LOY may be one underlying factor for susceptibility to COVID-19 in a proportion of patients.

In addition to depleted hematopoietic progenitor cells and possible immunodeficiency, individuals with LOY may have increased levels of circulating endothelial cells, which are known biomarkers for endothelial dysfunction and cardiovascular disease.[47] We observed up-regulation of *VWF* and *MYL9* in LOY. Pro-coagulant vWF promotes platelet adhesion and smooth muscle cell proliferation, and elevated levels of vWF have been associated with higher risk for thrombosis and cardiovascular disease.[48] MYL9 is a ligand for CD69 to form a net-like structure inside blood vessels in inflamed lungs and is also a risk factor for cardiovascular disease risk found over-expressed in aged versus young injured arteries.[49,50] Through these mechanisms, XCM/LOY seems to contribute to COVID-19 lethality by its associated cardiovascular risk.

Conclusion

In summary, clonal detectable CME & XCM are relatively common in aging individuals with much higher frequency in males due to somatic LOY. LOY is associated to decreased progenitors and stem cells, along with immune system dysfunction and increased coagulation and cardiovascular risk, as revealed by biochemical and gene expression data. Our data indicate that this type of mosaicism underlies at least part of the sex-biased severity and

mortality of COVID-19 in aging patients. Given its potential relevance for modulating prognosis, therapeutic intervention, and immunization responses, we propose that evaluation of CME/LOY by currently established methods should be implemented in both, retrospective studies and all prospective and currently ongoing clinical trials with different medications and vaccines for COVID-19. Testing for CME/LOY at large scale in elderly people may also be helpful to evaluate vaccination response and to identify still unexposed people who may be especially vulnerable to severe COVID-19 disease.

Declarations

Ethics approval and consent to participate: All studies were performed in accordance with the ethical standards of the responsible committee on human experimentation, and with proper informed consent from all individuals tested. The whole SCOURGE project was approved by the Galician Ethical Committee Ref 2020/197, along with the Ethics and Scientific Committees of all participating centers.

Availability of data and materials: Transcriptomic data from EGCUT individuals are available at GEO repository (see Methods). LOY and CME status obtained from genomic data are available upon request.

Conflicts of Interest: LAPJ is founding partner and scientific advisor of qGenomics laboratories. The other authors declare no conflict of interest.
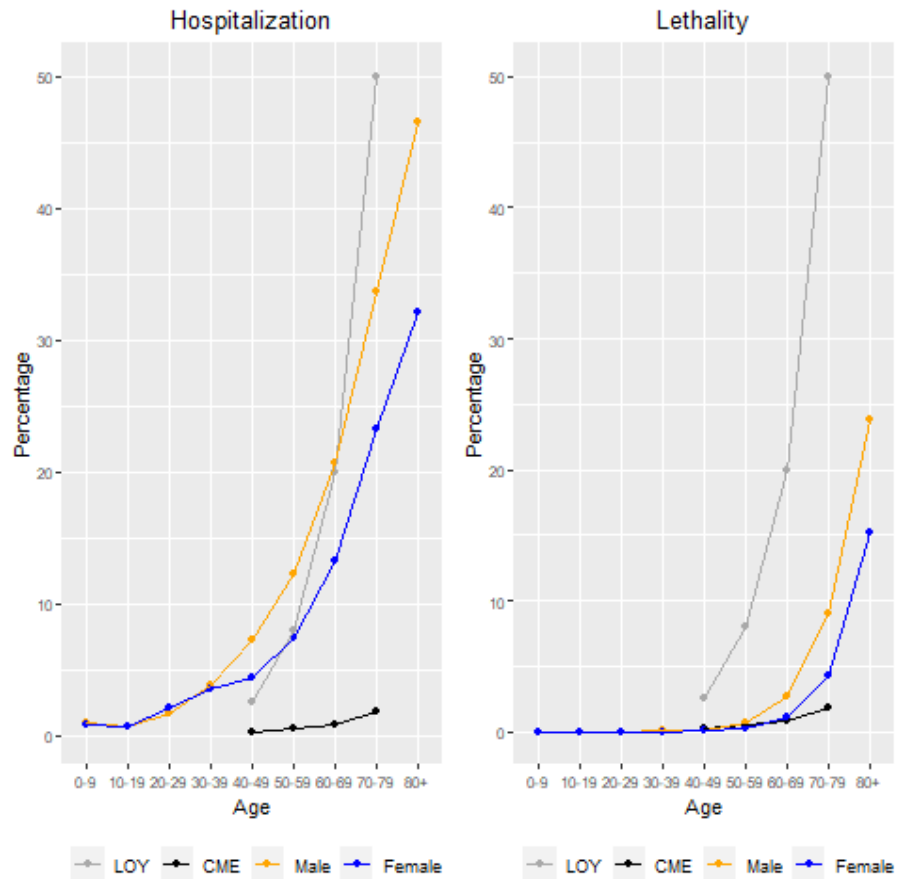
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FIGURES

Figure 1: Increasing male (orange) to female (blue) differences in hospitalization and mortality rates for COVID-19 in Spain in the different age intervals (updated December 2021). Estimated prevalence by age in the population of detectable CME (black) or LOY in men (grey) in blood is also shown.[13,14]
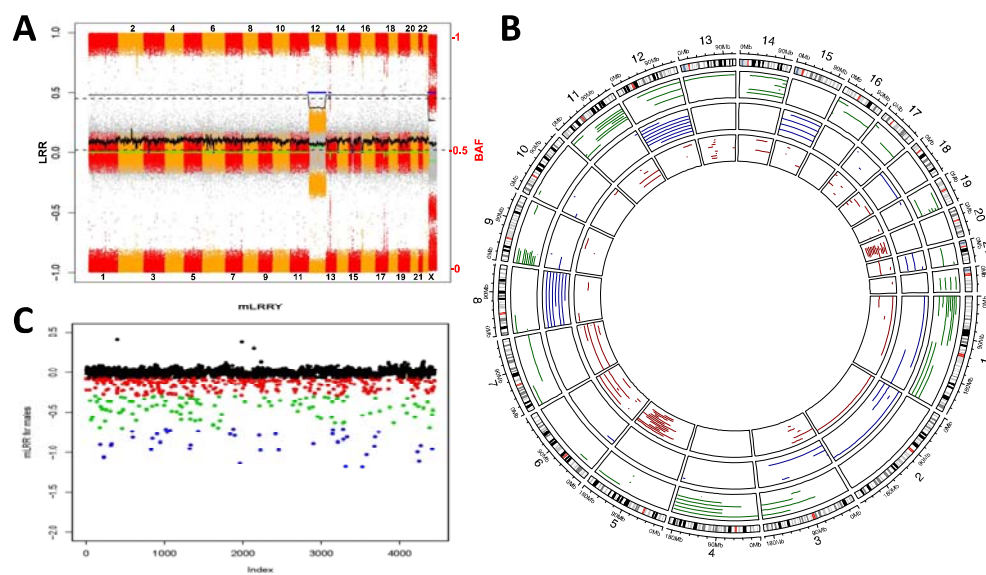
Figure 2: (**A**) Plot representing the whole-genome molecular karyotype obtained by SNParray of blood DNA from an individual with several CMEs. Dots in grey are LRR values (average per widow shown by a green lane), while colored dots are BAF values of homozygous and heterozygous SNPs from odd (red) and even number (orange) chromosomes, respectively. Abnormal BAF and average LRR values in three regions (blue lanes interrupting the black lane in the upper part) correspond to mosaicism for trisomy 12, a small interstitial deletion in 13q and X-chromosome monosomy. The blue lanes interrupting the green lane at LRR=0 correspond to small regions of homozygosity. (**B**) Circus plots showing all detected CMEs in the SCOURGE dataset. In red deletions, in blue gains and in green copy neutral events. (**C**) Analysis of LOY in male individuals in the SCOURGE study based on mean LRR from chromosome Y (mLRRY: relative amount of DNA from the Y chromosome with respect to autosomes). Blue dots correspond to males with mosaic LOY in more than 65% of cells (XCM>65%), green dots to males with LOY/XCM between 25%-65%, and red dots to males with LOY/XCM in less than 25% of cells. The three individuals with top mLRRY values have apparently non-mosaic gains of chromosome Y (47,XYY).
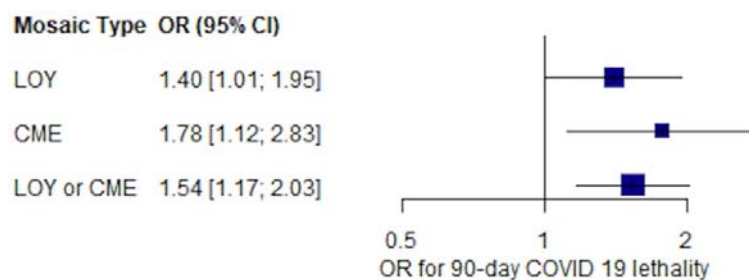
Figure 3: Associations of detectable CMEs and LOY with COVID-19-related mortality (<90 days after infection). Analyses are adjusted for age, sex, and 10 principal components of ancestry. Individuals with prevalent hematologic cancer were excluded from the analysis.
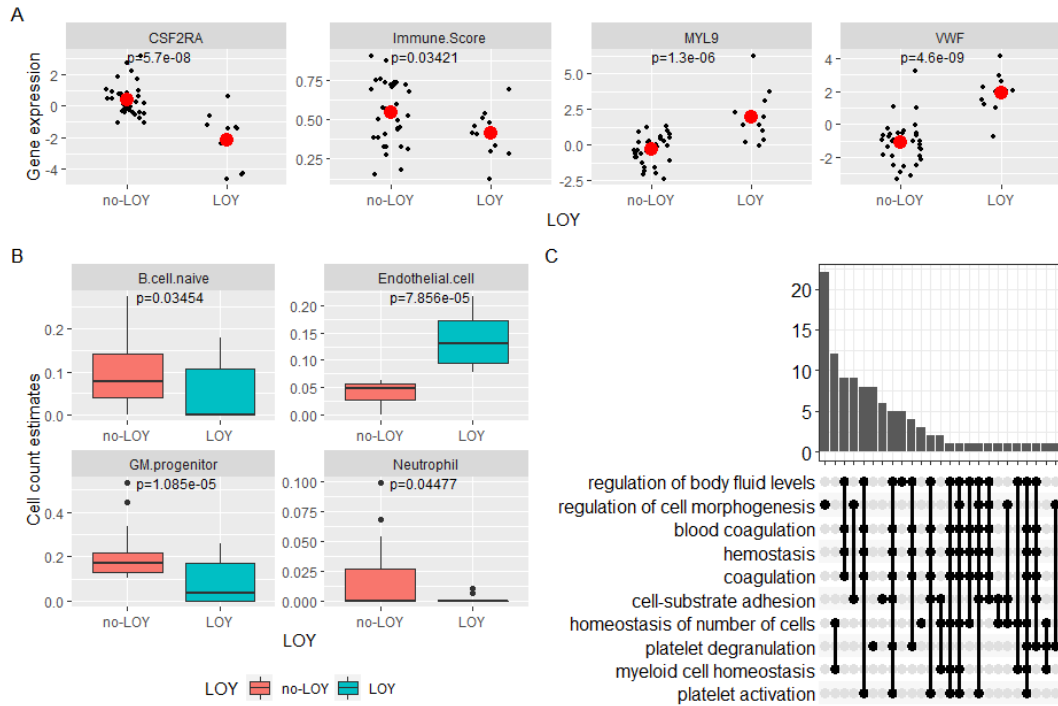
Figure 4: (**A**) Decreased expression of *CSF2RA* mRNA and increased expression of *MYL9* and *VWF* in individuals with LOY compared with controls with no LOY (mean gene expression in red dot). (**B**) Different predicted cell counts underlying the transcriptomic differences between cases with LOY and control individuals (no-LOY). (**C**) Gene Ontology (GO) enrichment of top differentially expressed genes.
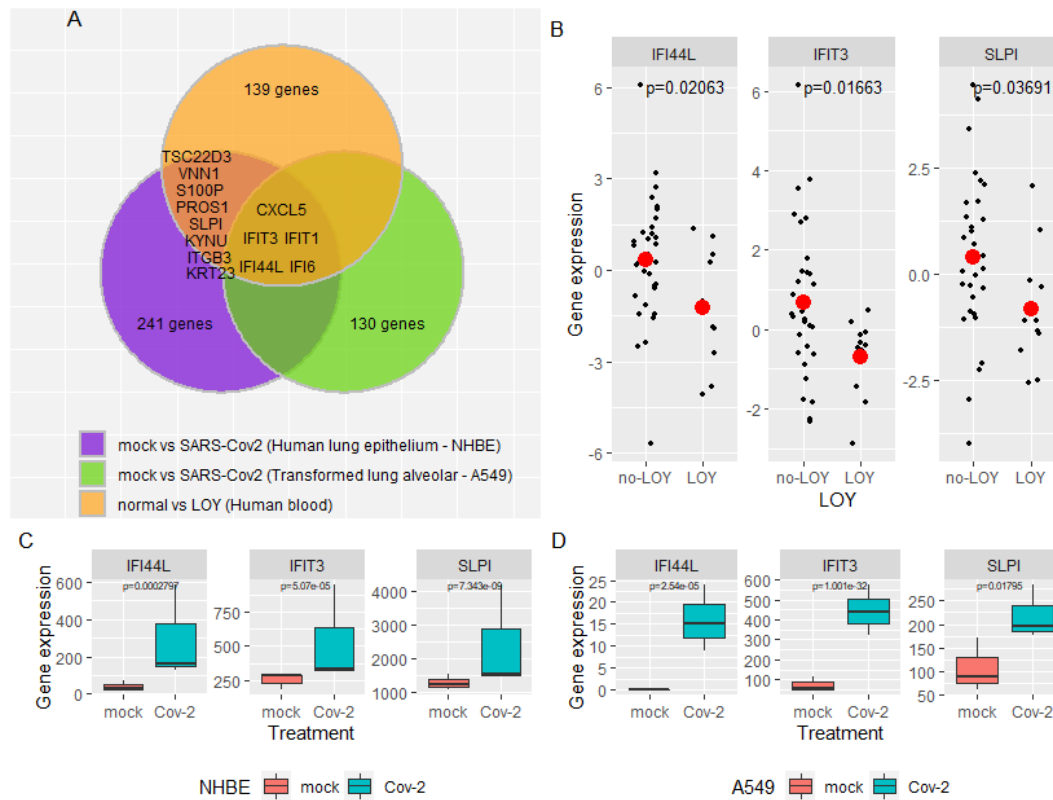
Figure 5: (A) Overlap between top differentially expressed genes in individuals with LOY and deregulated genes in SARS-CoV-2 infected cells. Panels B, C and D show detailed gene expression patterns of some of these overlapping genes, including down-regulated in individuals with LOY (B), and over-expressed in NHBE (C) and A549 (D) cell lines infected with SARS-CoV-2.

		Asymptomatic (0)	Mild (1)	Moderate (2)	Severe (3)	Critical (4)	Subtotal	No data	Dead*	Alive*	Subtotal	Total	
Cohort (no CME)	cases	607	2727	2141	2449	1157	9081	499	809	8050	8859	9445	
	%	6.8	30.0	23.6	27.0	12.7			9.1	90.9			
	age	53.3	51.3	66.9	70.6	65.4			79.9	60.5			
CMEs	cases	4	17	33	44	16	114	19	28	86	114	133	9578
	%	3.5	14.9	28.9	38.5	14.0			24.5	75.4			
	age	80.9	65.6	78.2	79.5	71.4			81.1	74.1			
Males (no LOY)	cases	163	743	1068	1241	792	4007	323	398	3494	3892	4218	
	%	4.1	18.6	26.7	31.0	19.8			10.2	89.8			
	age	53.2	51.9	64.8	69.1	64.0			77.2	61.5			
Males LOY	cases	4	13	46	96	47	208	20	65	143	208	226	4444
	%	1.9	6.2	22.1	46.1	22.6			31.2	68.7		5.1	
	age	81.2	78.5	81.0	83.5	79.3			84.1	81.5			
*>90 days after COVID-19													

Table 1: Number, proportion and mean age of patients in the different clinical categories of COVID-19 severity in the SCOURGE study, with and without detectable CMEs or LOY (males).

Legends of the Supplemental Material

Table S1:
Patients with CMEs. In each case with a patient identifier (ID), detected CMEs are indicated by chromosome (chr), start and end coordinates of the event, estimated proportion of cells carrying the CME (cellularity) and type of event (gain, loss, or copy neutral loss of heterozygosity – cnloh). Patient age, COVID-19 severity, and patient status (dead or alive) 90 days after disease are shown in the three last columns. NA: data not available.

Table S2:
Male patients with XCM due to LOY. In addition to the patient identifier (ID) and patient age at diagnosis of COVID-19, columns show the estimated proportion of cells with XCM (LOY proportion), COVID-19 severity, and patient status (dead or alive) 90 days after disease. NA: data not available.

Table S3:
Individuals with germline (non-mosaic) complete aneuploidies (chromosome 21 and gonosomes), and developmental mosaic X-chromosome aneuploidies.

Table S4:
Comparison of analytical parameters between age-matched individuals with and without LOY in the EGCUT biobank.

Table S5:
Top 30 differentially expressed genes in blood of individuals with LOY.

Table S6:
Top 30 differentially expressed Y-linked genes in blood of individuals with LOY.

Table S7a and S7b:
Y-linked genes with homologs in the X-chromosome and a possible role in immunity.

Table S8:
Association between cell-type composition estimated using bulk transcriptomic data (immunedecov R package) and LOY status.

Table S9:
GO enrichment analysis of differentially expressed genes in individuals with LOY. GO terms significant at 5% FDR are shown.

Table S10:
KEGG enrichment analysis of differentially expressed genes in individuals with LOY. KEGG terms significant at 5% FDR are shown.

Table S11:
Top GO enrichment of differentially expressed genes in the two cell lines infected with SAS-CoV-2 (NHBE and A549).

Table S12:
Intersection between significant genes in the blood transcriptomic comparison of LOY versus no LOY individuals and in the two cell lines infected with SAS-CoV-2 (NHBE and A549).

Figure S1:
Plots of the whole-genome molecular karyotype obtained by SNParray of blood DNA from all 133 individuals of SCOURGE with detectable CMEs.

Figure S2:
Top differentially expressed genes in blood between individual with LOY and controls at genome level. The plots show the gene expression for individuals with (LOY) and without LOY

(normal). The p-values correspond to a linear model adjusted for age and surrogate variables using limma.

Figure S3:

Top differentially expressed chromosome Y genes between individual with LOY and controls. The plots show the gene expression for individuals with (LOY) and without LOY (normal). The p-values correspond to a linear model adjusted for age and surrogate variables using limma.

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