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COVID-19 AND RETINITIS PIGMENTOSA INTERACTING GENES COULD REVEAL NEW MOLECULAR PATHWAYS INVOLVING UNKNOWN SARS-COV-2 BIOLOGICAL MECHANISMS TRYING TO CLARIFY VIRUS ENTRY AND SYSTEMIC DIFFUSION

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Abstract

The pandemic COVID-19, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has become a widespread problem for world population health. The novel coronavirus manifested a highly contagiousness, causing severe respiratory syndrome able to lead to death, especially in risk patients with preexisting diseases (e.g. cardiovascular, gastrointestinal and respiratory diseases, cancer). About ophthalmic comorbidities, only relationship with conjunctivitis and clinical retinal findings were reported in COVID-19 patients. Furthermore, even if detection of SARS-CoV-2 in human retinas was proved, the molecular mechanisms by which the virus could alter the homeostasis of retinal cells is still unknown. We analyzed common biological pathways genes shared by retinitis pigmentosa and the COVID-19 by newest bioinformatics tools, trying to unveil new biological mechanisms which might involve the SARS-CoV-2 (e.g. entry in retinal host cells or passage to nervous system and bloodstream) and to identify new biomarkers useful to predict the increased susceptibility towards SARS-CoV-2.

Keywords

COVID-19; Retinitis pigmentosa; High-Throughput Nucleotide Sequencing; Inflammation; Retina

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) represents a world widespread problem for population healthy, since its related disease (COVID-19) was declared pandemic by the WHO on March 11, 2020 [1]. The novel coronavirus manifested a highly contagiousness, causing severe respiratory distress syndrome able to lead to death especially in risk patients with preexisting diseases, as cardiovascular and gastrointestinal [2]. However, very little is known about ophthalmic comorbidities related to COVID-19. The most proved ocular involvement in COVID-19 affected individuals is linked to conjunctivitis. Patients with such disease showed high expression levels of both Angiotensin Converting Enzyme 2 (ACE2) and Transmembrane Serine Protease 2 (TMPRSS2), two key proteins for SARS-CoV-2 entry into host cells [3]. Recently, ACE2 was detected in the retina of rodent, porcine and human eyes. In detail, in rodent retina, ACE2 activity was found in the inner nuclear layer, especially in the Müller cells. In porcine ocular tissue, ACE2 was expressed in the ciliary body, vitreous body and in the retina. Interestingly, the existence of SARS-CoV-2 nucleic acid was assessed in the human retina, where it was also seen that activation of ACE2 can alleviate inflammatory response in retinal pigment epithelium cells [4]. Even if detection of SARS-CoV-2 in human retinas was defined, the molecular mechanisms by which the virus could alter the homeostasis of retinal cells is still unknown. Only a few clinical retinal findings emerged from instrumental exams in COVID-19 patients, such as hyper-reflective lesions at the level of ganglion cell and inner plexiform layers, more prominently at the papillomacular bundle in both eyes, as well as subtle cotton wool spots and microhemorrhages along the retinal arcade [5]. Such phenotypes are typical of several retinal diseases, as the rare heterogenous group of Retinitis pigmentosa.

Our hypothesis is based on this group of eye diseases especially by two reasons: 1) High inflammation and oxidative stress constitute the most prevalent physiopathological scenario of all retinitis pigmentosa forms, as well as the known predominant consequences of SARS-CoV-2 infection; 2) both in feline and mouse models, different coronaviruses are known to imply several ocular defects, mainly retinitis.

Retinitis pigmentosa disease group, as genetically heterogeneous as phenotypically homogeneous, is mainly determined by rod and cone death which, however, represents only the final stage of impairment in many molecular pathways involving, among the others, phototransduction,

visual cycle cascade, connecting cilium trafficking, fatty acid metabolism, photoreceptor outer segment assembly, interphotoreceptor matrix and inflammasome [6-9]. We think that the inflammatory status might increase the susceptibility of RP affected patients to SARS-CoV-2 infection, also worsening the clinical picture in these subjects. Moreover, we would like to discover potential interacting proteins encoded by genes common to RP and COVID-19, trying to unveil new biological mechanisms which might involve the SARS-CoV-2. In this way, we aim to find new virus possibilities to entry into retinal host cells and, after infection, how the creating scenario might worsen the RP phenotype and facilitate the passage of the SAR-CoV-2 to the bloodstream, reaching a systemic diffusion. Then we would like to investigate any genetic variants carried by identified genes by NGS and in silico analyses, to determine a genotype-phenotype correlation in a cohort of symptomatic and asymptomatic COVID-19 patients, affected or not by RP, with the final discovery of new biomarkers useful to predict the increased susceptibility towards SARS-CoV-2. Such detection might permit us to produce a custom NGS panel useful to predict the susceptibility to COVID-19 infection, especially by entering through the eye and diffusing to bloodstream via retina.

Materials and Methods

We performed an in-silico pathway analysis trying to validate the sustainability of our hypothesis. Our pipeline was based on the use of Cytoscape 3.9.0 software (National Institute of General Medical Sciences, Bethesda, MD, USA) [10], together with its plug-ins GeneMANIA (v. 3.5.2) (University of Toronto, Toronto, Canada) [11], ClusterMaker2 (v. 2.0) (University of California, CA, USA) [12] and ClueGO (v. 2.5.8) (INSERM, Paris, France) [13]. GeneMANIA permitted the identification of the most related genes in a query gene set, made of RP, SARS and Coronavirus infection known causative/associated genes, applying a guilt-by-association approach. Output genes, clustered by ClusterMaker2 and linked by pathway, plus genetic and physical interactions were submitted to ClueGO, which performed a GO term enrichment analysis to highlight putative shared biological pathways. GeneMANIA has been set to find the top 20 related genes and at most attributes using automatic weighting. ClusterMaker2 performed a clustering based on BestNeighbor Filter, set with the Proportion of node edges in cluster = 0.5. ClueGO options have been set as follow: CLINVAR, GO (Biological Process, Cellular Component, Molecular Function and Immune System Process), INTERPRO, KEGG, REACTOME (Pathways and Reactions),

WIKIPATHWAYS and CORUM 3.0 as selected ontologies; GO Tree Interval Min Level = 3 and Max Level = 8; GO Term/Pathway Selection Min # Genes = 3 and % Genes = 4.000; GO Term/Pathway Network Connectivity (Kappa Score) = 0.4; Statistics Options set on Enrichment/Depletion (Two-Sided hypergeometric test), with pV correction = Bonferroni step-down. Finally, only GO terms with $p < 0.01$ were selected.

Results and Discussion

RP known/associated genes from RetNet (https://sph.uth.edu/retnet/) resulted interestingly connected with Coronavirus and SARS infection related genes (from Malacards database, https://www.malacards.org), as well as with genes involved in already known comorbidities of COVID-19 [2]. In detail, about 80 RP genes were connected with 16 coronavirus infection related genes, about 50 RP with 12 SARS, and about 60 RP genes with 38 COVID-19 related comorbidities (Figure 1).

The ACE2 gene deserves a special mention, as already described as the most involved in SARS-CoV-2 infection. It highlighted connections with about 60 RP genes (Figure 2).

More than 80 clustered pathways resulted from enrichment of previously filtered genes (Figure 3). Among them, the “Expression of IFN-induced genes” and “Regulation of mononuclear cell migration” emerged from most significant pathways involving genes from the Coronavirus and SARS infection clusters, and resulted directly linked to “Syncytium formation by plasma membrane fusion” arose from several RP clustered genes analysis. Furthermore, the main pathway “Regulation of response to food” from filtered and cluster genes related to COVID-19 comorbidities resulted strongly linked with RP clusters, showing shared genes involved in many biochemical mechanisms such as “brain morphogenesis”, “cilium movement”, “cilium movement in cell motility regulation of cilium”, “beat frequency involved in ciliary motility”, “leptin-mediated signaling pathway”, “striatum development”, “cytokinesis”, “spindle” and “spindle pole”.

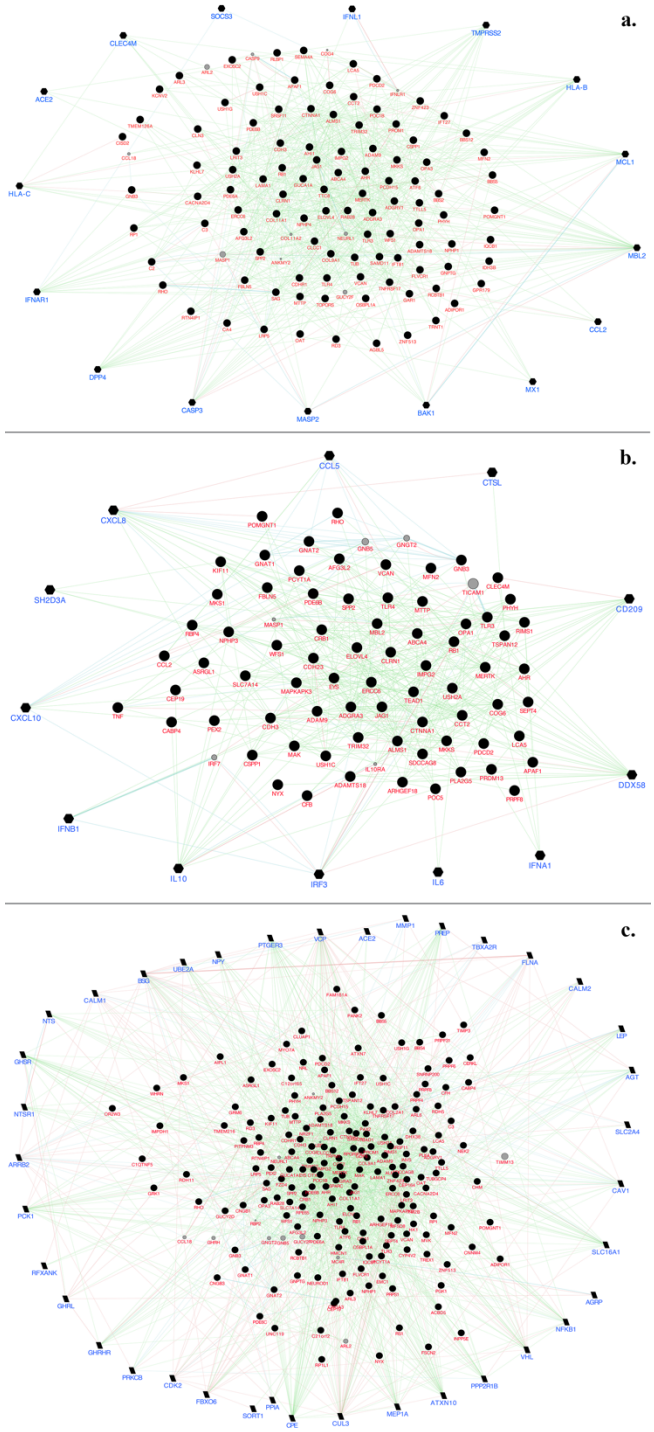

Figure 1. Interaction of RP genes with SARS-CoV-2 possible related genes. The represented network highlights known RP causative/associated genes in relationship with their best neighbors among coronavirus_infection (a), SARS (b) and known comorbidities (c) correlated genes in host cells, emerged from GENEMANIA analysis. Green edges indicate genetic interactions. Pink edges indicate physical interactions.

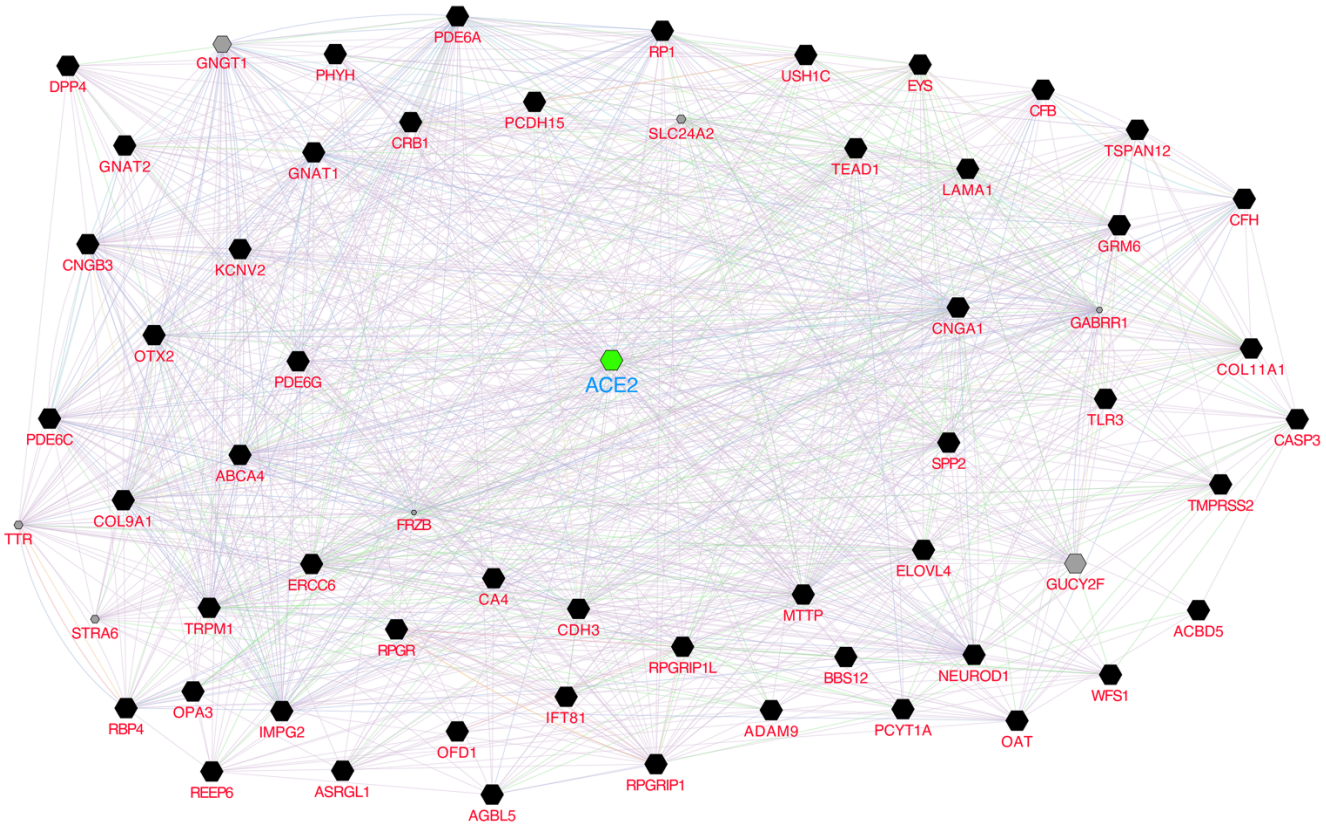

Figure 2. Interaction of RP genes with ACE2 gene. The represented network highlights known RP causative/associated genes in relationship with ACE2 gene, emerged from GENEMANIA analysis. Green edges indicate genetic interactions. Pink edges indicate physical interactions.

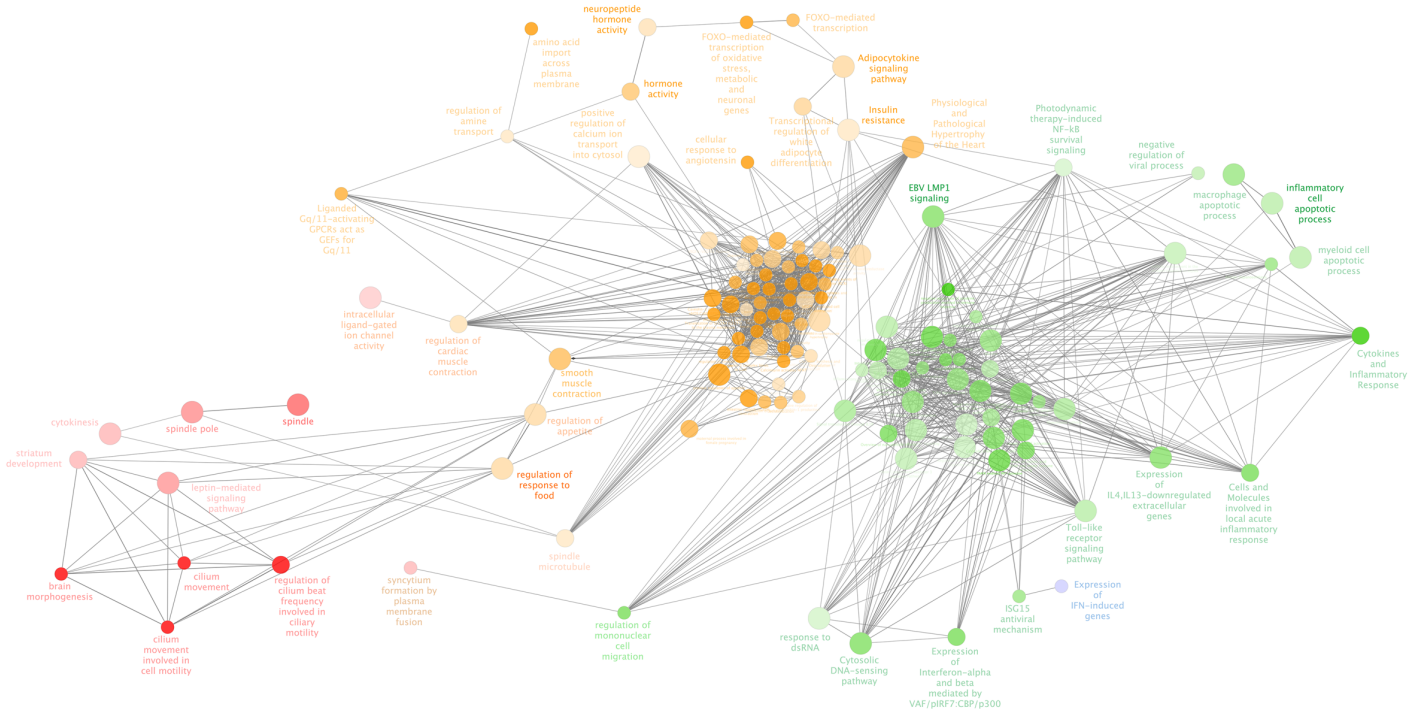

Figure 3. ClueGO enrichment of RP genes with SARS-CoV-2 possible related genes. The represented network highlights connections between clusters of shared genes between RP and coronavirus_infection, SARS and known comorbidities, generated by ClueGO analysis. Red = RP clusters, Blue = Coronavirus Infection clusters, Green = SARS clusters, Orange = Comorbidities associated to Covid-19.

Conclusions and Perspectives

Obtained preliminary discoveries could represent, in our opinion, an important starting point to further steps. Once identified most significant linked genes and proteins, we would like to investigate any genetic variants carried by identified genes by Next Generation Sequencing (NGS) techniques and in silico supporting analyses, in order to determine a genotype-phenotype correlation in a cohort of symptomatic and asymptomatic Covid-19 patients, maybe affected or not by RP. Detection of variants in selected genes might permit us to finally create a custom NGS panel useful to predict the susceptibility to Covid-19 infection, especially by entering through the eye and diffusing to bloodstream via its nervous part, the retina. In the meantime, the use of developed panel could help individuals affected by retinitis pigmentosa to their ocular susceptibility. This approach could decrease the costs for the National Health System, because it might reduce the number of swab and intensive care access.

References

1. Baloch, S., M.A. Baloch, T. Zheng, and X. Pei, The Coronavirus Disease 2019 (COVID-19) Pandemic. Tohoku J Exp Med, 2020. 250(4): p. 271-278.
2. Broto, C., D. Dibyajyoti, B. Gopalakrishnan, and R. Arijit, Network-Based Analysis of Fatal Comorbidities of COVID-19 and Potential Therapeutics. 2020.
3. Zhang, B.N., Q. Wang, T. Liu, S.Q. Dou, X. Qi, H. Jiang, B.X. Qi, B. Zhang, and Q.J. Zhou, [Expression analysis of 2019-nCoV related ACE2 and TMPRSS2 in eye tissues]. Zhonghua Yan Ke Za Zhi, 2020. 56(0): p. E011.
4. Casagrande, M., A. Fitzek, K. Puschel, G. Aleshcheva, H.P. Schultheiss, L. Berneking, M.S. Spitzer, and M. Schultheiss, Detection of SARS-CoV-2 in Human Retinal Biopsies of Deceased COVID-19 Patients. Ocul Immunol Inflamm, 2020: p. 1-5.
5. Marinho, P.M., A.A.A. Marcos, A.C. Romano, H. Nascimento, and R. Belfort, Jr., Retinal findings in patients with COVID-19. Lancet, 2020. 395(10237): p. 1610.
6. Donato, L., E.M. Abdalla, C. Scimone, S. Alibrandi, C. Rinaldi, K.M. Nabil, R. D'Angelo, and A. Sidoti, Impairments of Photoreceptor Outer Segments Renewal and Phototransduction Due to a Peripherin Rare Haplotype Variant: Insights from Molecular Modeling. Int J Mol Sci, 2021. 22(7).
7. Donato, L., R. D'Angelo, S. Alibrandi, C. Rinaldi, A. Sidoti, and C. Scimone, Effects of A2E-Induced Oxidative Stress on Retinal Epithelial Cells: New Insights on Differential Gene Response and Retinal Dystrophies. Antioxidants (Basel), 2020. 9(4).
8. Donato, L., C. Scimone, S. Alibrandi, E.M. Abdalla, K.M. Nabil, R. D'Angelo, and A. Sidoti, New Omics-Derived Perspectives on Retinal Dystrophies: Could Ion Channels-Encoding or Related Genes Act

as Modifier of Pathological Phenotype? Int J Mol Sci, 2020. 22(1).
9. Donato, L., C. Scimone, S. Alibrandi, C. Rinaldi, A. Sidoti, and R. D'Angelo, Transcriptome Analyses of lncRNAs in A2E-Stressed Retinal Epithelial Cells Unveil Advanced Links between Metabolic Impairments Related to Oxidative Stress and Retinitis Pigmentosa. Antioxidants (Basel), 2020. 9(4).
10. Shannon, P., A. Markiel, O. Ozier, N.S. Baliga, J.T. Wang, D. Ramage, N. Amin, B. Schwikowski, and T. Ideker, Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res, 2003. 13(11): p. 2498-504.
11. Franz, M., H. Rodriguez, C. Lopes, K. Zuberi, J. Montojo, G.D. Bader, and Q. Morris, GeneMANIA update 2018. Nucleic Acids Res, 2018. 46(W1): p. W60-W64.
12. Morris, J.H., L. Apeltsin, A.M. Newman, J. Baumbach, T. Wittkop, G. Su, G.D. Bader, and T.E. Ferrin, clusterMaker: a multi-algorithm clustering plugin for Cytoscape. BMC Bioinformatics, 2011. 12: p. 436.
13. Bindea, G., B. Mlecnik, H. Hackl, P. Charoentong, M. Tosolini, A. Kirilovsky, W.H. Fridman, F. Pages, Z. Trajanoski, and J. Galon, ClueGO: a Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. Bioinformatics, 2009. 25(8): p. 1091-3.