Companion diagnostic for the chloroquine use in the treatment of COVID-19: systems biology report of candidate markers.

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Research

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Abstract

BACKGROUND

Chloroquine is used for the treatment of COVID-19 patients. However, efficacy of the chloroquine has been under discussion. Variability of clinical outputs of the drug application requires implementation of a companion diagnostic that would allow monitoring responsiveness to chloroquine. The first line of such markers would be markers already used in clinics. Analysis of reported mechanisms of COVID-19 and chloroquine may lead to such markers.

METHODS

Systemic analysis of molecular mechanisms and markers engaged by chloroquine and COVID-19 virus was performed. The networks of regulatory mechanisms were explored for an intersection and relevance to clinical markers.

RESULTS

Reported here systemic analysis describes the intersection of molecular mechanisms of chloroquine and processes engaged by COVID-19. 266 nodes provide insight into the mechanisms of chloroquine impact on the infection and represent a pool of companion diagnostic markers. As an example, an intersection with the markers of heart arrhythmia retrieved 19 nodes. Thirteen of them were reported in human plasma: levels of albumin, amyloid precursor protein, and endoglin correlate with adverse cardiac effects.

CONCLUSIONS

Reported nodes are the candidate markers for companion diagnostic of the chloroquine application to COVID-19 patients. Some of these markers are already used in the clinic and their interpretation may contribute to monitoring for adverse effects of chloroquine.

Background

The use of chloroquine for the treatment of COVID-19 patients has been under discussion (1–3). To be effective, chloroquine has to act on its targets that would lead to a therapeutic response. To discriminate between responding, non-responding and adverse effects-prone patients, there is a need of a companion diagnostic for chloroquine. Such markers are routine in oncology (4, 5). These markers inform clinicians whether a drug would be useful for a given patient. Without these markers, an effect of drugs is frequently non-conclusive when evaluated at the population level. Companion diagnostics allows selection of responsive patients and prediction of the disease development.
Chloroquine has been used since the 1940th. Studies of this remedy generated information about molecular mechanisms of its action. An international DrugBank depository (drugbank.ca) is an example of a curated and proven drug target database (6). The studies of COVID-19 are not yet as extensive as studies of chloroquine, but there are already reports of COVID-19 targets in human cells (7–9). Identified targets reflect molecular mechanisms engaged by chloroquine and COVID-19, and systems biology allows identification of these regulatory processes. A number of network building tools and high-quality databases are available for systemic analysis of molecular mechanisms engaged by COVID-19 and chloroquine (7, 10–13). An analysis of regulatory networks is the most comprehensive way to explore mechanisms that are initiated or dependent on the targets of COVID-19 and chloroquine. Comprehensiveness is ensured by the incorporation of the experimental data from hundreds to thousands of reports. For example, UniProt database contains 562,755 records of experimental data (uniprot.org) (14). This a rich source for systemic network analysis.

COVID-19 infection manifests in many different clinical symptoms (15–17). It indicates that the virus employs different molecular mechanisms and attacks different types of cells. Here we report an identification of potential markers to evaluate the efficacy of chloroquine in the treatment of COVID-19 patients. Our systemic analysis identified 266 nodes, i.e. genes and proteins that represent common molecular mechanisms engaged by chloroquine and COVID-19. An example of cardiac arrhythmia showed 19 potential companion diagnostic markers for chloroquine use and prediction of cardiac adverse effects.

**Methods**

The datasets for building networks were collected as follows, and are listed in Supplementary Table S1. For chloroquine, the targets were retrieved from the Drug Bank depository (drugbank.ca) (6). For COVID-19 interacting proteins, 322 interactors were reported by Gordon et al., and ACE2 and TMPRSS2 were used (7, 18). For arrhythmia, markers described by Bose et al. were used (19).

The networks building and analysis was performed in Cytoscape (10). The significance for the inclusion of nodes and edges was set to p < 0.05. For the building of the networks, we used the UniProt database (14). For extraction of intersections, the “Network Analysis” tool of Cytoscape was used. Statistical significance of network building (inclusion of nodes and confidence of edges) was set on p < 0.05. BiNGO tool was used for the analysis of affected biological processes. For statistical significance, the level was set at p < 0.05, and the hypergeometric statistical test was used, with Benjamini and Hochberg false discovery rate correction.

A cross-validation analysis of identified nodes with published reports about their clinical values and a role in physiology was performed. We searched PubMed with the Medical Subject Headings (MeSH) of a node and words “COVID-19”, “chloroquine”, and “heart”. Retrieved publications were scrutinized for information about clinical values of the nodes as markers and for involvement of the nodes in molecular mechanisms and biological processes of relevance for a virus infection, predictive marker value, correlation with clinical outputs and adverse effects, and a role in crucial intracellular regulatory mechanisms, e.g. proliferation, death and differentiation of cells.

**Results**
Identification of common targets of COVID-19 and chloroquine

For chloroquine, there have been reported 11 direct targets, i.e. GSTA2, TNF, TLR9, GST, HMGB1, GSTM1, CYP2C8, CYP3A4, CYP3A5, CYP2D6 and CYP1A1 (Supplementary Table S1). Chloroquine impact on these targets may lead to engagement of a regulatory network containing 1,336 nodes and 2,526 edges (Supplementary Figure S1; Supplementary File S1, network “Chloroquine_UniProt”). The network was built with the retrieval of interaction data from the UniProt database. The same database was used to build networks of angiotensin-converting enzyme 2 (ACE2) and type 2 transmembrane serine protease (TMPRSS2) and COVID-19 interactors that are listed in Supplementary Table S1. The structure of the networks are shown in Supplementary Figures S2 and S3, and the networks are presented in Supplementary File S1 (networks “Cov_UniProt” and “ACE2TMPRSS2_UniProt”). The ACE2/TMPRSS2 network contains 15 nodes and 19 edges, and the COVID-19 network contains 828 nodes and 1,545 edges. These 3 networks represent molecular mechanisms engaged by chloroquine and COVID-19 directly or via ACE2-TMPRSS2. Note that the graphical presentation of the networks is to illustrate structure of the networks. Cytoscape Session file (Supplementary File S1) provides access to the networks and allows exploration of the networks, zooming on identifiers, perform selection of sub-networks, clustering and search for biological processes of clinical relevance.

To identify mechanisms shared by COVID-19 and chloroquine, we searched for intersections between these 3 networks. The intersection of the chloroquine and ACE2/TMPRSS2 networks extracted only 2 nodes, i.e. albumin and 14-3-3 zeta/delta. This shows that chloroquine has rather a narrow impact on ACE2 and TMPRSS2-dependent mechanisms. The intersection of the chloroquine and COVID-19 target networks extracted 266 nodes interconnected by 347 edges (Figure 1A; Supplementary Table S2, Supplementary File S1, network “Intersection_ChloroqUniProt_CovUniProt...”). This large number of common nodes indicates a significant molecular cross-talk between chloroquine and COVID-19. One hundred nine of these nodes were also detected in the human plasma (Table 1). These intersections identify mechanisms of chloroquine interference with COVID-19 action and list potential plasma markers (Figure 2). The intersection nodes may represent markers of companion diagnostic for chloroquine use. If these nodes are affected in a patient infected with the virus, then the chloroquine prescription may be of help, as chloroquine would markers act on/via these affected nodes.

COVID-19 and cardiac arrhythmia markers

To evaluate whether the intersection nodes would lead to the identification of clinically relevant markers, we used an example of cardiac arrhythmia. Markers of arrhythmia were used to generate a network (Supplementary Figure S4). The arrhythmia markers are OPN, ANXA5, GDF15, MPO, LGALS3, TNNT2, TNNI3, ANFB, REN, IL6 and CRP (Supplementary Table S1) (19). The arrhythmia network was explored further for the intersection with common nodes of chloroquine and COVID-19 regulatory mechanisms (Figure 1B; Supplementary File S1 network “Intersection_Arhythmia_Cov19...”). There were no edges retrieved between these nodes and amyloid precursor protein was retrieved with 3 different accession numbers. We identified 19 nodes linking arrhythmia markers to chloroquine and COVID-19 (Table 2). Analysis of these 19 nodes showed an engagement of processes affecting the heart and regulation of cell death and proliferation.
Detection of proteins in serum or plasma suggest their suitability as makers for repeatable sampling by blood collection. We used a database of proteins detected in plasma (http://www.plasmaproteomedatabase.org) and retrieved 13 proteins (Table 2). Then, we searched for reports of clinical applications of these 13 proteins as markers of cardiac conditions. Levels of human serum albumin (ALB), amyloid proteins (APP) and soluble endoglin (ENG) correlate with cardiovascular diseases (Figure 2). Albumin concentration below 10 g/L correlates with cardiovascular diseases (20). Levels of amyloid precursor protein (APP) higher than 150 pg/mL correlate with cardiomyopathy (21). Amyloid-beta (1-40) protein was associated with the incidence of coronary heart failure (22). Two of other identified by us proteins, i.e. microtubule-associated protein tau (MART) and prion protein (PRNP) are also associated with the onset of cellular degeneration (23-25). Endoglin is involved in the development and regulation of vasculature. Elevated levels of soluble endoglin in plasma correlate with enhanced left ventricular filling pressure (26). 14-3-3zeta/delta (YWHAZ) is one of the 10 genes enhanced in ischemic stroke (27).

The systems biology approach allowed us to explore published original experimental data in the search for companion diagnostic markers for chloroquine. Reported here 109 nodes represent a pool of these markers. The example of the search for markers to guide the use of chloroquine and preventing cardiac arrhythmia identified 19 candidates. Four of these were reported to correlate with adverse effects, thus confirming the clinical value of our approach.

**Discussion**

Systemic network analysis becomes a potent and efficient tool for the investigation of correlations and molecular mechanisms (8, 12, 13). Well-developed and curated databases contain large volumes of original experimental data. This data are available for analysis with a number of tools. Here, we used Cytoscape that allows retrieval of molecular interactions, functional dependencies, correlation and clinical data (10). Used by us the UniProt database contains more than 500,000 curated entries (14). This rich source of data in combination with the efficient analysis tool, i.e. Cytoscape, leads to unveiling novel dependencies. Two hundred sixty-six nodes common for COVID-19 and chloroquine show an extensive impact of chloroquine on the infection (Figs. 1 and 2; Supplementary Table S2). That may explain the clinical efficacy of chloroquine. However, changes in expression and/or activity of many of these nodes may also have undesirable consequences, leading to adverse effects of chloroquine.

This manuscript reports the identification of potential companion markers of chloroquine. As an example of applicability of our data, we report 19 marker candidates for guiding chloroquine treatment of COVID-19-infected patients and monitoring for cardiac arrhythmia (Table 2). Four of these markers are already known to affect cardiac conditions. The decrease in albumin to concentrations below 10 g/L correlates with cardiac adverse effects (20). Albumin levels have been recommended for clinical monitoring of COVID-19 patients (20, 28–31). Hypoalbuminemia with the albumin levels lower than 35 g/L was associated with the 2-time higher risk of the long-term mortality in heart failure (31). Chloroquine was described as a drug against prion and Alzheimer’s diseases (32). Prion protein and amyloid beta peptide are likely to be components of the innate immune system (33). Amyloid-beta protein association with coronary heart disease and amyloidosis-
related heart disease was reported (21, 22). Identification of amyloid precursor protein, microtubule-associated tau and prion proteins indicate a link of cell damage and degeneration to cardiac conditions.

Similar observations were made for other nodes annotated in Table 2. For example, proliferating cell nuclear antigen (PCNA) level increases in arrhythmia, and when chloroquine has an effect, it prevents PCNA increase (34). CD177 was reported to contribute to blocking atrial fibrillation (35). Chloroquine inhibits autophagy and promotes apoptosis, and METTL2, SHLD3, TP53BP1 are engaged nodes in these processes (Table 2) (36–38). Cardiomyocyte proliferation is regulated by another identified node, disabled homolog 2 (Dab2) (39). Dab2 is involved in suppression of apoptosis by Epstein-Barr virus (EBV) (40). Two nodes, mitochondrial antiviral signaling protein (MAVS) and DExD/H-Box Helicase 58 (DDX58) were reported as antiviral proteins (41, 42). Inhibition of MAVS expression decreased efficacy of hydroxychloroquine against dengue virus (42). These examples show that the identified nodes have a high probability to be markers for a companion diagnostic. The 19 markers annotated in Table 2 are the example of using the pool of 266 common nodes of COVID-19 and chloroquine. Our report provides a basis for further clinical studies of the potential markers.

Reported by us results can be used in clinical practice already now, as some of identified by us nodes are used in routine clinical diagnostics, e.g. albumin, soluble endoglin and amyloid precursor protein. Repurposing of their use for COVID-19 patients treated with chloroquine can be applied now. For example, a higher risk of adverse cardiac effects would be indicated by downregulation of albumin and up-regulation of amyloid precursor protein, tau protein, prion protein and soluble endoglin (21, 22, 26, 43).

**Conclusion**

Presented here network analysis describes potential markers of a companion diagnostic for the use of chloroquine for the treatment of COVID-19 patients. Some of the markers, e.g. albumin, soluble endoglin and amyloid precursor protein have records of clinical correlations of their expression and cardiac adverse effects. Other proteins are candidates for companion diagnostic in clinical trials of chloroquine in the treatment of COVID-19 infection.

**Abbreviations**

ACE2, angiotensin-converting enzyme 2; TMPRSS2, type 2 transmembrane serine protease; MeSH, Medical Subject Headings; ALB, human serum albumin; APP, amyloid proteins; ENG, soluble endoglin; PCNA, proliferating cell nuclear antigen; MART, microtubule-associated protein tau; PRNP, prion protein; YWHAZ, 14-3-3zeta/delta; MAVS, mitochondrial antiviral signaling protein; DDX58, DExD/H-box helicase 58; disabled homolog 2 (Dab2); EBV, Epstein-Barr virus.

**Declarations**

**Ethics approval and consent to participate:** Not required for this work.

**Consent to publication:** Not required for this work.
Availability of data and material: All data are freely available. The .cys file of the networks and analysis is also available without restrictions (Supplementary File S1). The .cys file is available for download at https://figshare.com/articles/online_resource/SupplementaryFileS1_Cytoscape_DataNetwork_cys/12793580. This file would allow further clinical use of data reported in this publication. The systemic network analysis file (.cys file) may be useful for clinicians who want to use this publication for clinical applications. The .cys file allows clinicians to do own analysis of reported here data, e.g. search for markers and evaluate clinical conditions of their interest.

Competing interests: The authors declare no conflict of interest in relation to the subject matter and financial interests of this publication. Contribution from Oranta CancerDiagnostics AB was pro bono.

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References

1. Funck-Brentano C, Salem JE. Chloroquine or hydroxychloroquine for COVID-19: why might they be hazardous? Lancet 2020;S0140-6736(20)31174-0. doi: 10.1016/S0140-6736(20)31174-0.

2. Hernandez AV, Roman YM, Pasupuleti V, Barboza JJ, White CM Hydroxychloroquine or Chloroquine for Treatment or Prophylaxis of COVID-19: A Living Systematic Review. Ann. Intern. Med. 2020; Online May 27. doi: 10.7326/M20-2496, 2020.

3. Borba MGS, Val FFA, Sampaio VS, Alexandre MAA, Melo GC, et al. CloroCovid-19 Team: Effect of High vs Low Doses of Chloroquine Diphosphate as Adjunctive Therapy for Patients Hospitalized With Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Infection: A Randomized Clinical Trial. JAMA Netw. Open. 2020; 3(4):e208857. doi: 10.1001/jamanetworkopen.2020.8857.

4. Jørgensen JT. A paradigm shift in biomarker guided oncology drug development. Ann. Transl. Med. 2019; 7(7):148. doi: 10.21037/atm.2019.03.36.

5. McLachlan J, George A, Banerjee S. The Current Status of PARP Inhibitors in Ovarian Cancer. Tumori 2016;102(5):433-440. doi: 10.5301/tj.5000558.

6. Wishart DS, Feunang YD, Guo AC, Lo EJ, Marcu A, Grant JR, et al. DrugBank 5.0: a major update to the DrugBank database for 2018. Nucleic Acids Res. 2018;46(D1): D1074-D1082. doi: 10.1093/nar/gkx1037.

7. Gordon GE, Jang GM, Bouhaddou M, Xu J, Obernier K, O’Meara MJ, et al. A SARS-CoV-2-Human Protein-Protein Interaction Map Reveals Drug Targets and Potential Drug Repurposing. 2020; bioRxiv March 27: https://doi.org/10.1101/2020.03.22.002386.

8. Ostaszewski M, Mazein A, Gillespie ME, Kuperstein I, Niarakis A, et al. COVID-19 Disease Map, building a computational repository of SARS-CoV-2 virus-host interaction mechanisms. Sci. Data 2020;7(1):136.
9. Du M, Cai G, Chen F, Christiani DC, Zhang Z, Wang M. Multi-omics Evaluation of Gastrointestinal and Other Clinical Characteristics of SARS-CoV-2 and COVID-19. Gastroenterology 2020; pii: S0016-5085(20)30399-1. doi: 10.1053/j.gastro.2020.03.045.

10. Shannon P, Shannon P, Markiel A, Ozier O, O, Baliga NS, Wang JT, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Research 2003;13(11): 2498-504.

11. Souchelytskyi S, Nera A, Souchelytskyi N. COVID-19 engages clinical markers for the management of cancer and cancer-relevant regulators of cell proliferation, death, migration and immune response. Sci. Rep. 2020; August.

12. Silverman EK, Schmidt HHHW, Anastasiadou E, Altucci L, Angelini M, Badimon L, et al. Molecular networks in Network Medicine: Development and applications. Wiley Interdiscip. Rev. Syst. Biol. Med. 2020; e1489. doi: 10.1002/wsbm.1489.

13. Bowen JR, Ferris MT, Suthar MS. Systems biology: A tool for charting the antiviral landscape. Virus Res. 2016;218: 2-9. doi: 10.1016/j.viruses.2016.01.005.

14. The UniProt Consortium: UniProt: a worldwide hub of protein knowledge. Nucleic Acids Res. 2019;47: D506-515.

15. Extance A. Covid-19 and long term conditions: what if you have cancer, diabetes, or chronic kidney disease? BMJ 2020; Mar 25: 368:m1174. doi: 10.1136/bmj.m1174.

16. Patel KP, Patel PA, Vunnam RR, Hewlett AT, Jain R, Jing R, Vunnam SR. Gastrointestinal, hepatobiliary, and pancreatic manifestations of COVID-19. J. Clin. Virol. 2020;128: 104386. doi: 10.1016/j.jcv.2020.104386.

17. Henry BM, Santos de Oliveira MH, Benoit S, Plebani M, Lippi G. Hematologic, Biochemical and Immune Biomarker Abnormalities Associated With Severe Illness and Mortality in Coronavirus Disease 2019 (COVID-19): A Meta-Analysis. Clin. Chem. Lab. Med. 2020;58(7): 1021-28.

18. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, et al. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell 2020;181(2): 271-80.e8. doi: 10.1016/j.cell.2020.02.052.

19. Bose A, Truong QA, Singh JP. Biomarkers in electrophysiology: role in arrhythmias and resynchronization therapy. J. Inter. Card. Electrophysiol. 2015;43(1):31-44. doi: 10.1007/s10840-015-9982-7.

20. Ronit A, Kirkegaard-Klitbo DM, Dohlmann TL, Lundgren J, Sabin CA, Phillips AN, Nordestgaard BG, Afzal S. Plasma albumin and incident cardiovascular disease: results from the CGPS and an updated meta-analysis, Arterioscler. Thromb. Vasc. Biol. 2020;40(2): 473-82. doi: 10.1161/ATVBAHA.119.313681.

21. Wolfson AW, Shah KS and Patel JK: Amyloid and the heart. Curr. Cardiol. Rep. 2019;21(12): 164. doi: 10.1007/s11886-019-1230-9.

22. Stamatelopoulos K, Mueller-Hennessen M, Georgiopoulos G, Sachse M, Boedinghaus J, Sopova K, et al. Amyloid-beta (1-40) and the Risk of Death From Cardiovascular Causes in Patients With Coronary Heart Disease. J. Am. Coll. Cardiol.2015; 65(9): 904-16. doi: 10.1016/j.jacc.2014.12.035.
23. Mehrpour M, Codogno P. Prion Protein: From Physiology to Cancer Biology. Cancer Lett. 2010;290(1):1-23. doi: 10.1016/j.canlet.2009.07.009.

24. Galenko O, Jacobs V, Knight S, Bride D, Cutler MJ, Muhlestein JB, Carlquist JL, Anderson JL, Knowlton KU, Bunch J. Circulating Levels of Biomarkers of Cerebral Injury in Patients With Atrial Fibrillation. Am. J. Cardiol. 2019;124(11):1697-1700. doi: 10.1016/j.amjcard.2019.08.027.

25. Murakami N, Oyama F, Gu Y, McLennan IS, Nonaka I, Ihara Y. Accumulation of Tau in Autophagic Vacuoles in Chloroquine Myopathy. J. Neuropathol. Exp. Neurol. 1998;57(7):664-673. doi: 10.1097/00005072-199807000-00003.

26. Meluzín J, Tomandl J. Can biomarkers help to diagnose early heart failure with preserved ejection fraction? Dis. Markers 2015;426045. doi: 10.1155/2015/426045.

27. Eyileten C, Wicik Z, De Rosa S, Mirowska-Guzel D, Soplinska A, Indolfi C, Jastrzebska-Kurkowska I, Czlonkowska A, Postula M. MicroRNAs as Diagnostic and Prognostic Biomarkers in Ischemic Stroke-A Comprehensive Review and Bioinformatic Analysis. Cells 2018;7(12):249. doi: 10.3390/cells7120249.

28. Kavsak PA, de Wit K, Worster A. Clinical Chemistry Tests for Patients With COVID-19 - Important Caveats for Interpretation. Clin. Chem. Lab. Med. 2020;Apr 16;j/cclm-ahead-of-print/cclm-2020-0436/cclm-2020-0436.xml. doi: 10.1515/cclm-2020-0436.

29. Kavsak PA, de Wit K, Worster A. Emerging Key Laboratory Tests for Patients With COVID-19. Clin. Biochem. 2020;Apr 30;S0009-9120(20)30391-X. doi: 10.1016/j.clinbiochem.2020.04.009.

30. Suzuki S, Hashizume N, Kanzaki Y, Maruyama T, Kozuka A, Yahikozawa K. Prognostic Significance of Serum Albumin in Patients With Stable Coronary Artery Disease Treated by Percutaneous Coronary Intervention. PLoS One 2019;14(7):e0219044. doi: 10.1371/journal.pone.0219044. eCollection 2019.

31. Ancio A, Allepaerts S, Robinet S, Oury C, Pierard LA, Lancellotti P. Serum Albumin Level and Long-Term Outcome in Acute Heart Failure. Acta Cardiol. 2019;74(6):465-71. doi: 10.1080/00015385.2018.1521557.

32. Gay M, Carato P, Coevoet M, Renault N, Larchanché PE, Barczyk A, Yous S, Buée L, Sergeant N, Melnyk P. New Phenylaniline Derivatives as Modulators of Amyloid Protein Precursor Metabolism. Bioorg. Med. Chem. 2018;26(8):2151-2164. doi: 10.1016/j.bmc.2018.03.016.

33. Lathe R, Darlix JL. Prion Protein PRNP: A New Player in Innate Immunity? The Aβ Connection. J. Alzheimers Dis. Rep. 2017;1(1):263-75. doi: 10.3233/ADR-170037.

34. Zhang S, Zhu C, Liu Q, Wang W. Effects of Chloroquine on GFAP, PCNA and Cyclin D1 in Hippocampus and Cerebral Cortex of Rats With Seizures Induced by Pentylenetetrazole. J. Huazhong Univ. Sci. Technolog. Med. Sci. 2005;25(6):625-28. doi: 10.1007/BF02896153.

35. Yue H, Liang W, Gu J, Zhao X, Zhang T, Qin X, Zhu G, Wu Z. Comparative transcriptome analysis to elucidate the therapeutic mechanism of colchicine against atrial fibrillation. Biomed. Pharmacother. 2019;109422. doi: 10.1016/j.biopharma.2019.109422, 2019.

36. Song H, Feng X, Zhang H, Luo Y, Huang J, Lin M, et al. METTL3 and ALKBH5 Oppositely Regulate M 6 A Modification of TFEB mRNA, Which Dictates the Fate of Hypoxia/Reoxygenation-Treated Cardiomyocytes. Autophagy 2019;15(8):1419-1437. doi: 10.1080/15548627.2019.1586246.
37. Noordermeer SM, Adam S, Setiaputra D, Barazas M, Pettitt SJ, Ling AK, et al. The Shieldin Complex Mediates 53BP1-dependent DNA Repair. Nature 2018;560(7716):117-21. doi: 10.1038/s41586-018-0340-7.

38. Vucicevic L, Misirkic-Marjanovic M, Paunovic V, Kravic-Stevovic T, Martinovic T, Ciric D, et al. Autophagy inhibition uncovers the neurotoxic action of the antipsychotic drug olanzapine. Autophagy 2014;10(12):2362-78. doi: 10.4161/15548627.2014.984270.

39. Hofsteen P, Robitaille AM, Chapman DP, Moon RT, Murry CE. Quantitative Proteomics Identify DAB2 as a Cardiac Developmental Regulator That Inhibits WNT/β-catenin Signaling. Proc. Natl. Acad. Sci. U S A 2016;113(4):1002-7. doi: 10.1073/pnas.1523930113.

40. Min K, Kim JY, Lee SK. Epstein-Barr Virus miR-BART1-3p Suppresses Apoptosis and Promotes Migration of Gastric Carcinoma Cells by Targeting DAB2. Int. J. Biol. Sci. 2020;16(4):694-707. doi: 10.7150/ijbs.36595.

41. Wang R, Zhu Y, Lin X, Ren C, Zhao J, Wang F, Gao X, Xiao R, Zhao L. et al.: Influenza M2 Protein Regulates MAVS-mediated Signaling Pathway Through Interacting With MAVS and Increasing ROS Production. Autophagy 2019;15(7):1163-1181. doi: 10.1080/15548627.2019.1580089.

42. Wang LF, Lin YS, Huang NC, Yu CY, Tsai WL, Chen JJ, et al. Hydroxychloroquine-inhibited Dengue Virus Is Associated With Host Defense Machinery. J. Interferon Cytokine Res. 2015;35(3):143-156. doi: 10.1089/jir.2014.0038.

43. Vitverova B, Najmanova I, Vicen M, Tripska K, Igreja Sa IC, et al. Long Term Effects of Soluble Endoglin and Mild Hypercholesterolemia in Mice Hearts. PLoS One 2020;15(5):e0233725. doi: 10.1371/journal.pone.0233725.

Tables

Table 1 List of nodes common for COVID-19 and chloroquine that have been observed in the human plasma. These 109 nodes are candidate plasma or serum markers for assessment of chloroquine efficacy in treating COVID-19 infection.

At the end of the table are listed 35 nodes that were not observed in the human plasma.
| Gene symbol   | Gene name                                      |
|--------------|-----------------------------------------------|
| HPRD_01228   | HMGB1 high mobility group box 1               |
| HPRD_01456   | PCNA proliferating cell nuclear antigen       |
| HPRD_02717   | NCBP1 nuclear cap binding protein subunit 1, 80kDa |
| HPRD_01592   | RPS6 ribosomal protein S6                     |
| HPRD_10941   | RPS3 ribosomal protein S3                     |
| HPRD_01245   | NCL nucleolin                                 |
| HPRD_00883   | HTT huntingtin                                 |
| HPRD_04323   | TLR2 toll-like receptor 2                     |
| HPRD_02514   | SYK spleen tyrosine kinase                    |
| HPRD_03703   | MYD88 myeloid differentiation primary response 88 |
| HPRD_13847   | MAVS mitochondrial antiviral signaling protein |
| HPRD_04462   | IKKB inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase beta |
| HPRD_00660   | FUS fused in sarcoma                           |
| HPRD_01142   | MAPT microtubule-associated protein tau        |
| HPRD_11299   | USE1 unconventional SNARE in the ER 1 homolog (S. cerevisiae) |
| HPRD_14389   | METTL3 methyltransferase like 3                |
| HPRD_00087   | PSEN1 presenilin 1                            |
| HPRD_00100   | APP amyloid beta (A4) precursor protein        |
| HPRD_01222   | CD177 CD177 molecule                           |
| HPRD_03333   | ATXN1 ataxin 1                                |
| HPRD_00831   | SIRT1 sirtuin 1                               |
| HPRD_02391   | IL2RG interleukin 2 receptor, gamma           |
| HPRD_00989   | IL4 interleukin 4                              |
| HPRD_07259   | RTN4 reticulin 4                               |
| HPRD_04087   | APBB1 amyloid beta (A4) precursor protein-binding, family B, member 1 (Fe65) |
| HPRD_01861   | TNFRSF1A tumor necrosis factor receptor superfamily, member 1A |
| HPRD_04583   | RIPK1 receptor (TNFRSF)-interacting serine-threonine kinase 1 |
| HPRD_02739   | CSNK1A1 casein kinase 1, alpha 1              |
| HPRD_02217   | IKKBG inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase gamma |
| HPRD_05155   | PPARGC1A peroxisome proliferator-activated receptor gamma, coactivator 1 alpha |
| HPRD_05258   | AKAP8 A kinase (PRKA) anchor protein 8         |
| HPRD_01238   | NFKB1 nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 |
| HPRD_02799   | CASP3 caspase 3, apoptosis-related cysteine peptidase |
| HPRD_03538   | TRAF2 TNF receptor-associated factor 2         |
| HPRD_03685   | PA2G4 proliferation-associated 2G4, 38kDa     |
| HPRD_05521   | HDAC2 histone deacetylase 2                   |
| HPRD_01453   | PRNP prion protein                            |
| HPRD_01470   | PTPN11 protein tyrosine phosphatase, non-receptor type 11 |
| HPRD_02480   | INSM1 insulinoma-associated 1                 |
| HPRD_08950   | HDAC3 histone deacetylase 3                   |
| HPRD_03143   | HDAC1 histone deacetylase 1                   |
| HPRD_06942   | AGO1 argonaute RISC catalytic component 1     |
| HPRD_06943   | AGO2 argonaute RISC catalytic component 2     |
| HPRD_01494   | EPHA2 EPH receptor A2                         |
| HPRD_09694   | TET1 tet methylcytosine dioxygenase 1         |
| HPRD_01242   | HNRNPA1 heterogeneous nuclear ribonucleoprotein A1 |
| HPRD_02911   | NCOR1 nuclear receptor corepressor 1          |
| HPRD_04078   | EP300 E1A binding protein p300                |
| HPRD_07211   | NR1H3 nuclear receptor subfamily 1, group H, member 3 |
| HPRD_02660   | NR1H2 nuclear receptor subfamily 1, group H, member 2 |
| HPRD_01574   | RB1 retinoblastoma 1                          |
| HPRD_08406 | MYCBP | c-myc binding protein |
| HPRD_09709 | DACT1 | dishevelled-binding antagonist of beta-catenin 1 |
| HPRD_03388 | PRKACA | protein kinase, cAMP-dependent, catalytic, alpha |
| HPRD_01615 | DDX5 | DEAD (Asp-Glu-Ala-Asp) box helicase 5 |
| HPRD_03402 | RPS6KA1 | ribosomal protein S6 kinase, 90kDa, polypeptide 1 |
| HPRD_00303 | MCM2 | minichromosome maintenance complex component 2 |
| HPRD_10641 | AKAP8L | A kinase (PRKA) anchor protein 8-like |
| HPRD_05397 | AURKB | aurora kinase B |
| HPRD_02910 | NCOR2 | nuclear receptor corepressor 2 |
| HPRD_10566 | SSX32 | synovial sarcoma, X breakpoint 2 interacting protein |
| HPRD_11331 | SNX33 | sorting nexin 33 |
| HPRD_12072 | SNX9 | sorting nexin 9 |
| HPRD_02786 | TEC | tec protein tyrosine kinase |
| HPRD_15407 | SNX18 | sorting nexin 18 |
| HPRD_01835 | ZFP36 | ZFP36 ring finger protein |
| HPRD_05390 | NFKBIA | nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha |
| HPRD_05759 | TNFRSF21 | tumor necrosis factor receptor superfamily, member 21 |
| HPRD_14732 | MOV10 | M0v10, Moloney leukemia virus 10, homolog (mouse) |
| HPRD_05247 | PABPC1 | poly(A) binding protein, cytoplasmic 1 |
| HPRD_04703 | ADAM17 | ADAM metallopeptidase domain 17 |
| HPRD_01903 | ITGAV | integrin, alpha V |
| HPRD_00628 | ITGB1 | integrin, beta 1 (fibronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12) |
| HPRD_05936 | PACSIN1 | protein kinase C and casein kinase substrate in neurons 1 |
| HPRD_05390 | PACSIN2 | protein kinase C and casein kinase substrate in neurons 2 |
| HPRD_05937 | PACSIN3 | protein kinase C and casein kinase substrate in neurons 3 |
| HPRD_03254 | UPF1 | UPF1 regulator of nonsense transcripts homolog (yeast) |
| HPRD_03570 | NCOA3 | nuclear receptor coactivator 3 |
| HPRD_02534 | CREBBP | CREB binding protein |
| HPRD_03274 | MAD2L1 | MAD2 mitotic arrest deficient-like 1 (yeast) |
| HPRD_04541 | IQGAP1 | IQ motif containing GTPase activating protein 1 |
| HPRD_06343 | LRPPRC | leucine-rich pentatricopeptide repeat containing |
| HPRD_05944 | HDAC9 | histone deacetylase 9 |
| HPRD_01197 | NGFR | nerve growth factor receptor |
| HPRD_00532 | COMT | catechol-O-methyltransferase |
| HPRD_04870 | TANK | TRAF family member-associated NFKB activator |
| HPRD_05367 | CNOT2 | CCR4-NOT transcription complex, subunit 2 |
| HPRD_02698 | FABP4 | fatty acid binding protein 4, adipocyte |
| HPRD_02811 | CHUK | conserved helix-loop-helix ubiquitous kinase |
| HPRD_00589 | ESR1 | Estrogen receptor alpha |
| HPRD_01166 | MYD1 | myogenic differentiation 1 |
| HPRD_01320 | CBL | Cbl proto-oncogene, E3 ubiquitin protein ligase |
| HPRD_06780 | KAT2B | K(lysine) acetyltransferase 2B |
| HPRD_02557 | TLE1 | transducin-like enhancer of split 1 (E(sp1) homolog, Drosophila) |
| HPRD_03139 | DAB2 | Dab, mitogen-responsive phosphoprotein, homolog 2 (Drosophila) |
| HPRD_13006 | CBX8 | chromobox homolog 8 |
| HPRD_05569 | TP53BP1 | tumor protein p53 binding protein 1 |
| HPRD_00565 | ENG | endoglin |
| HPRD_00279 | CSNK2A2 | casein kinase 2, alpha prime polypeptide |
| HPRD_00532 | DNMT1 | DNA (cytosine-5-)methyltransferase 1 |
| HPRD_01812 | TFCR | transferrin receptor (p90, CD71) |
| HPRD_01296 | RAB8A | RAB8A, member RAS oncogene family |
Table 2. 19 common nodes for arrhythmia, COVID-19 and chloroquine.

13 nodes were described in human plasma. 6 nodes at the end of the table are nodes that were not described in human plasma. Figure 2 describes reported information about these nodes as clinical markers.

| PPD ID   | Gene symbol | Gene name                                                                 |
|----------|-------------|---------------------------------------------------------------------------|
| HPRD_01456 | PCNA        | proliferating cell nuclear antigen                                         |
| HPRD_13847 | MAVS        | mitochondrial antiviral signaling protein                                 |
| HPRD_01142 | MAPT        | microtubule-associated protein tau                                        |
| HPRD_14389 | METTL3      | methyltransferase like 3                                                  |
| HPRD_00100 | APP         | amyloid beta (A4) precursor protein                                       |
| HPRD_01222 | CD177       | CD177                                                                      |
| HPRD_03333 | ATXN1       | ataxin 1                                                                  |
| HPRD_01453 | PRNP        | prion protein                                                             |
| HPRD_03139 | DAB2        | Dab, mitogen-responsive phosphoprotein, homolog 2 (Drosophila)            |
| HPRD_05569 | TP53BP1     | tumor protein p53 binding protein 1                                       |
| HPRD_00565 | ENG         | endoglin                                                                   |
| HPRD_00062 | ALB         | albumin                                                                    |
| HPRD_03183 | YWHAZ       | tyrosine 3-monoxygenase/tryptophan 5-monoxygenase activation protein, zeta polypeptide |

Nodes not detected in plasma

- MAD2L2
- DDX58
- SHLD3
- METTL14
- PRKN
- ATAD3A

Supplementary Materials Legends

Supplementary Table S1. List of GO terms used for the networks COVID-19, chloroquine and arrhythmia markers.

Supplementary Table S2. List of the nodes of intersection of COVID-19 and chloroquine (264 nodes) and ACE2/TMPRSS2 and chloroquine (2 nodes).
Supplementary Figures show structure of the networks. To explore the networks and zoom on identifiers, edges or search for clusters, please use the Cytoscape Session file available at https://figshare.com/articles/online_resource/SupplementaryFileS1_Cytoscape_DataNetwork_cys/12793580.

Supplementary Figure S1. Network generated with the targets of chloroquine (A) and biological processes represented by that network (B). The network was built with Cytoscape and UniProt database. Biological processes were retrieved with BiNGO tool.

Supplementary Figure S2. Network generated with the targets of COVID-19 (A) and biological processes represented by that network (B). The network was built with Cytoscape and UniProt database. Biological processes were retrieved with BiNGO tool.

Supplementary Figure S3. Network generated with the targets of ACE2 and TMPRSS2 (A) and biological processes represented by that network (B). The network was built with Cytoscape and UniProt database. Biological processes were retrieved with BiNGO tool.

Supplementary Figure S4. Network generated with the targets of arrhythmia (A) and biological processes represented by that network (B). The network was built with Cytoscape and UniProt database. Biological processes were retrieved with BiNGO tool.

Supplementary File S1. Cytoscape Session file (.cys) contains networks used in this study. These networks can be evaluated for nodes and edges of interest, e.g. to see identifiers. See the text for annotation of the networks. The file is uploaded at FigShare for free access; link is https://figshare.com/articles/online_resource/SupplementaryFileS1_Cytoscape_DataNetwork_cys/12793580.

**Figures**
Figure 1

Structure of the network formed by common targets of chloroquine and COVID-19 (A) and common nodes retrieved by intersection of the networks of markers of arrhythmia and targets of chloroquine and COVID-19 (B). The networks were built with Cytoscape and UniProt database, as described in the text. Numbers of nodes and edges are indicated for (A). Common nodes of arrhythmia markers and targets of chloroquine and COVID-19 did not show connections/edges. The retrieved nodes are shown in (B). The network (A) and nodes (B) are shown to illustrate the structure of the network (A) or absence of it (B). For zooming in the networks for identifiers (nodes and edges identities) and the networks analysis, the networks are in Supplementary File S1 as a Cytoscape Session file (.cys file), available for download at https://figshare.com/articles/online_resource/SupplementaryFileS1_Cytoscape_DataNetwork_cys/12793580.
Figure 2

Workflow of selection of potential companion diagnostic markers. Two hundred sixty-six common COVID-19 and chloroquine nodes were evaluated for representation of biological functions and relevance to adverse effects. Retrieved with BiNGO tool biological processes and the nodes of the relevance to the heart arrhythmia markers are annotated.

Supplementary Files
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- SupplMaterialsALL200821.pdf