Chloroquine use in the Treatment of COVID-19: Systems Biology Report of Common Targets of SARS-CoV-2 and Chloroquine.

Serhiy Souchelnytskyi (serhiy@qu.edu.qa)
Qatar University https://orcid.org/0000-0001-8243-9276

Nazariy Souchelnytskyi
Uppsala Universitet

Research

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Abstract

BACKGROUND: Chloroquine use for treatment of COVID-19 patients has been under discussion and recommendations have been shifting from positive to caution or non-conclusive. Variability of clinical outputs requires understanding of mechanisms of the differences. Implementation of a companion diagnostic would allow selecting patients who may benefit from the drug. The first line would be markers already used in clinics. Systems biology opens for an opportunity to identify targets common for chloroquine and SARS-CoV-2. These common targets would be candidates for the companion diagnostic.

METHODS: Systemic analysis of molecular mechanisms and markers engaged by chloroquine and SARS-CoV-2 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 intersection nodes of SARS-CoV-2 and chloroquine are the candidate markers for companion diagnostic of the chloroquine application. 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. That may explain recent reports of non-conclusive benefit from use of chloroquine and hydroxychloroquine (www.who.int/publications/m/item/targeted-update-safety-and-efficacy-of-hydroxychloroquine-or-chloroquine-for-treatment-of-covid-19). Chloroquine and hydroxyl chloroquine are used as immunomodulators and showed promising data in in vitro studies of COVID-19 management (1–3). However, the number of clinical evidence is still not sufficient to claim a high certainty conclusion. The systems biology approach may offer the way to identification of markers that would identify patients who may benefit from the drug and those patients who would not. Companion diagnostics with the predicted markers 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 (www.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 SARS-CoV-2 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 1. For chloroquine, the targets were retrieved from the Drug Bank depository (drugbank.ca) (6). For SARS-CoV-2 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 SARS-CoV-2 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 1). Chloroquine impact on these targets may lead to engagement of a regulatory network containing 1,336 nodes and 2,526 edges (Supplementary Fig. 1; Supplementary File 1, 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 SARS-CoV-2 interactors that are listed in Supplementary Table 1. The structure of the networks are shown in Supplementary Figs. 2 and 3, and the networks are presented in Supplementary File 1 (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 SARS-CoV-2 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 1) 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 SARS-CoV-2 target networks extracted 266 nodes interconnected by 347 edges (Fig. 1A; Supplementary Table 2, Supplementary File 1, 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 SARS-CoV-2 action and list potential plasma markers (Fig. 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 Fig. 4). The arrhythmia markers are OPN, ANXA5, GDF15, MPO, LGALS3, TNNT2, TNNI3, ANFB, REN, IL6 and CRP (Supplementary Table 1) (19). The arrhythmia network was explored further for the intersection with common nodes of chloroquine and COVID-19 regulatory mechanisms (Fig. 1B; Supplementary File 1 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 (Fig. 2). It has to be noted that these markers have also been associated with general conditions and not only cardiac, e.g. hypoalbuminemia associated with liver and kidney diseases, or had a limited use in clinics, e.g. APP or ENG. 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 intersection 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 potential clinical value of our approach. Monitoring of the described here markers may help in preventing severe side effects in COVID-19 patients, even if some of the markers are considered as general, or not-frequently used or even novel. The general (ALB) or not-frequently used (APP, ENG, MART, PRNP and YWHAZ) may be applied in clinics already now, as they are approved as markers. Novel candidate markers from the list of 19 nodes would have to be evaluated in clinical trials, and this work contributes with rationale for such trials.

**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 2). 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. The complexity of chloroquine molecular
mechanisms and differences in representation of these mechanisms in different individuals may lead to
different clinical outputs.

This manuscript reports the identification of nodes (genes and proteins) common for SARS-CoV-2 and
chloroquine. These interaction nodes may be influenced by both the virus and the drug. Therefore, they would
reflect whether and how chloroquine may influence SARS-CoV-2-engaged mechanisms. Such nodes can be
potential companion diagnostic markers of chloroquine, even if these markers are known for use for other
clinical conditions. As an example of applicability of our data, we report 19 marker candidates for guiding
chloroquine treatment of SARS-CoV-2-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 clinical diagnostics, e.g. albumin, or testing is available, even if not-frequently, e.g. soluble endoglin
and amyloid precursor protein. These markers are used for non-COVID-19 conditions, and 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 nodes common for SARS-CoV-2 and chloroquine. The common
nodes are intersections of molecular mechanisms of the virus and the drug. Having two inputs, these nodes
are potential markers of a companion diagnostic of chloroquine for the treatment of COVID-19 patients.
Some of the intersection nodes, 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:**

This study was approved by the ethics committee of the Orotta College of Medicine and Health Sciences and the health facility management division of the Ministry of Health. All patients provided written informed consent to participate in this study.

**Consent for Publication:**

Since, it is case report. Consent for publication is not applicable here. Personal or identifying information of study participants is not disclosed in any form in this paper.

**Availability of data and materials**

All datasets used for this study are available from corresponding author on reasonable request.

**Competing interests**

The authors declare no competing interests.

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**Authors’ contributions**
MEH, SMR, YS, IME and FT conceived and designed the study. MEH, SMR, YP and MW analyzed the data and revised the paper. MEH and SMR wrote the manuscript. All authors read and approved the final manuscript.

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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.
| PPD ID     | 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 | IKBKB       | 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_08381 | SIRT1       | sirtuin 1                                                     |
| HPRD_02391 | IL2RG       | interleukin 2 receptor, gamma                                 |
| HPRD_00989 | IL4         | interleukin 4                                                 |
| HPRD_07259 | RTN4        | reticulon 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 | IKBKG       | inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase |
| 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_03382 | 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 | SSX2IP | synovial sarcoma, X breakpoint 2 interacting protein |
| HPRD_01484 | PRKAR2A | protein kinase, cAMP-dependent, regulatory, type II, alpha |
| 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_01235 | 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 | Mov10, 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_00284 | 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 | MYOD1 | 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 | TFRC | transferrin receptor (p90, CD71) |
| HPRD_01296 | RAB8A | RAB8A, member RAS oncogene family |
| HPRD_05373 | MAP4K3 | mitogen-activated protein kinase kinase kinase kinase 3 |
| HPRD_04091 | ADAM9 | ADAM metalloproteinase domain 9 |
| HPRD_00561 | EEF2 | eukaryotic translation elongation factor 2 |
| HPRD_15942 | PPP1CA | protein phosphatase 1, catalytic subunit, alpha isozyme |
| HPRD_0062 | ALB | albumin |
| HPRD_03183 | YWHAZ | tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide |

**Nodes not observed in human plasma.**

These 35 nodes are unlikely to be plasma or serum markers.

MAD2L2, EMD, IFIH1, RAB39A, Ccnd1, TSPAN12, TSPAN15, P14340, SORBS2, CBX2, CREBZF, TICAM1, DDX58, JMJD6, TBK1, AZ12, E7, SHLD3, METTL14, Tlr4, MDC1, CREB3, GPS2, TNIP2, P; TSPAN5, PHLPP1, CSNK2A1, IRF7, CCND1, VACWR196, PTEN, TLR4, NCF1, Dact2.

**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-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide |

Nodes not detected in plasma

|                |                        |
|----------------|------------------------|
| MAD2L2         | Mitotic arrest deficient 2-like protein 2 |
| DDX58          | DEAD box protein 58    |
| SHLD3          | Shield complex subunit 3 |
| METTL14        | Methyltransferase-like protein 14 |
| PRKN           | parkin RBR E3 ubiquitin protein ligase    |
| ATAD3A         | ATPase family AAA domain containing 3A   |

*PPD, Plasma Proteome Database.

**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 1 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
This is a list of supplementary files associated with this preprint. Click to download.

- 01SupplTableS1.docx
- 01SupplTableS2.docx
- 1SupplFigureS1AB.tif
- 1SupplFigureS2AB.tif
- 1SupplFigureS3AB.tif
- 1SupplFigureS4AB.tif