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

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Research

Keywords: COVID-19, SARS-CoV-2, chloroquine, common targets, companion diagnostic, systems biology

Posted Date: November 3rd, 2020

DOI: https://doi.org/10.21203/rs.3.rs-99139/v1

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.

Funding

This research did not receive any grant from funding agencies in the public, commercial, or not-for-profit sectors.

Acknowledgements

We would like to thank the members of the Immunoserology Department at Eritrean National Health Laboratory, Eritrean National Higher Education and Research Institutes, and National Animal and Plant Health Laboratory.

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

		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_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 metallopeptidase domain 9
HPRD_00561	EEF2	eukaryotic translation elongation factor 2
HPRD_15942	PPP1CA	protein phosphatase 1, catalytic subunit, alpha isozyme
HPRD_00062	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, AZI2, 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

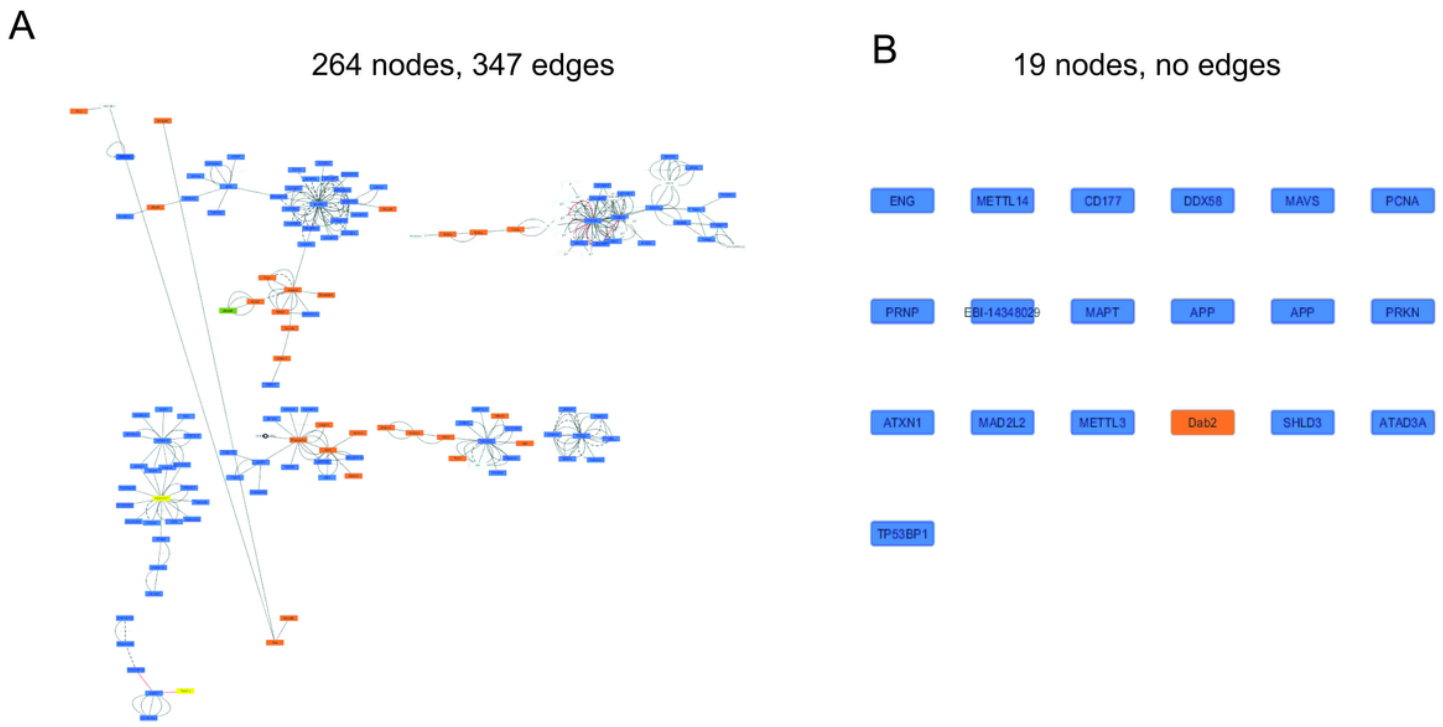

Figure 1

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

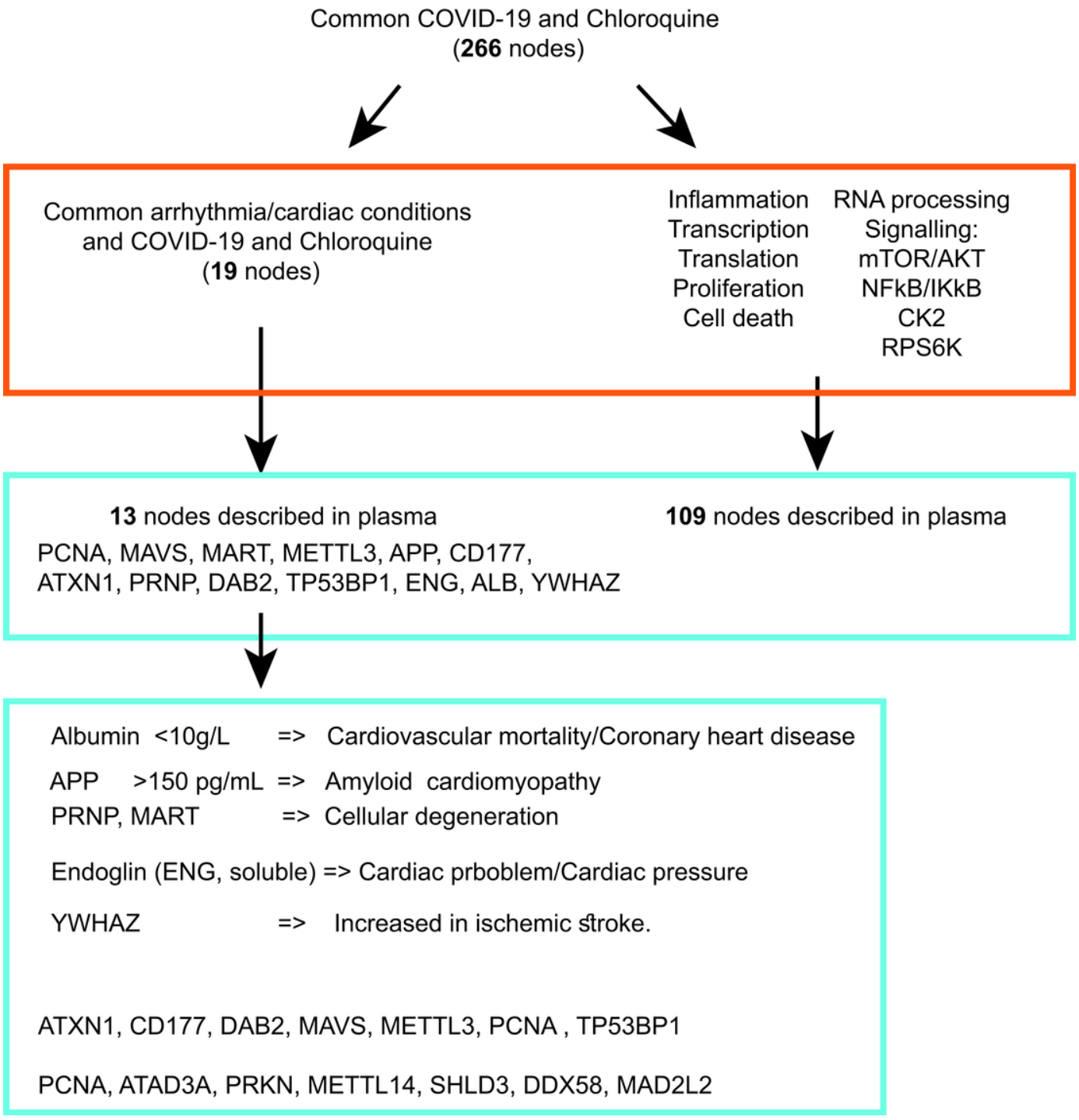

Figure 2

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