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Uncovering the interplay between hypertension and the inflammatory response for the patient affected by COVID-19 via mathematical modeling and computer-based analysis

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Abstract

We explore here the systems-based regulatory mechanisms that determine human blood pressure patterns. This in the context of the reported negative association between hypertension and COVID-19 disease. We are particularly interested in the key role that plays angiotensin converting enzyme 2 (ACE2), one of the first identified receptors that enable the entry of the SARS-CoV-2 virus into a cell. Taking into account the two main systems involved in the regulation of blood pressure, that is, the Renin-Angiotensin system and the Kallikrein-Kinin system, we follow a Bottom-Up systems biology modeling approach in order to built the discrete Boolean model of the gene regulatory network that underlies both the typical hypertensive phenotype and the hypotensive/normotensive phenotype. These phenotypes correspond to the dynamic attractors of the regulatory network modeled on the basis of publicly available experimental information. Our model recovers the observed phenotypes and shows the key role played by the inflammatory response in the emergence of hypertension. Source code go to the next url: https://github.com/cxro-cc/red_ras_kks

1 Introduction

At the end of December 2019, an outbreak of a respiratory disease with similar characteristics to the severe acute respiratory syndrome (SARS) was reported in Wuhan, China. This disease

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was later named as COVID-19, which is as an acronym for Coronavirus Disease 2019, with its causative agent being identified as a new positive-sense single-stranded RNA virus just named "SARS-CoV-2" [8]. Among the first identified receptors that enable the entry of the virus into a cell is angiotensin converting enzyme 2 (ACE2). According to reports, an unfavorable diagnosis in patients with COVID-19 occurs when there are one or more previous comorbidities, such as hypertension, diabetes, obesity, among other [8]. In this paper we are interested in the association between hypertension and COVID-19. Hypertension is an abnormal state of blood pressure (BP) that can be diagnosed when high frequency of either elevated systolic and/or diastolic blood pressure is found [9]. BP is a complex process regulated by mainly two systems, where the ACE2 enzyme plays an important and correlative role, known as [22, 6]:

RAS: Renin-angiotensin. This system consists of a series of steps that generate different ligands with angiotensinogen, which is a glycoprotein that is cleaved by the renin enzyme to form angiotensin I (Ang I). Then, the Ang I peptide is cleaved by the ACE enzyme (Angiotensin-converting enzyme) to form angiotensin II (Ang II), with the latter being a ligand for two receptors called AT1R and AT2R. Among the AT1R signals is the vasoconstriction associated with a hypertensive phenotype. On the contrary, the AT2R receptor generates vasodilatation and hypotension signals. In turn, the enzyme ACE2 (Angiotensin-converting enzyme 2) can cleaved both angiotensin I (Ang I) and angiotensin II (Ang II) peptides into angiotensin 1–9 [Ang-(1-9)] and angiotensin 1-7 [Ang-(1-7)] respectively. The effects of Ang-(1-7) such as vasodilation are exerted via the MasR receptor and Ang-(1-9) have been reported to be a ligand for the AT2R receptor [22].

KKS: Kallikrein-Kinin. This system with high molecular weight kininogen (HK), and the low molecular weight kininogen (LK) derive from alternative splicing of the kininogen gene (KNG1). The kininogen HK is cleaved by kallikrein plasma to form bradykinin (BK); this ligand is admitted by the B2 receptor (B2R or BDKRB2) resulting in a vasodilation signal. Bradykinin can be cleaved by carboxypeptidase N (CPN) mainly, generating des-Arg9-arginin. It has been informed that the des-Arg9-arginin is admitted by the B1 receptor (B1R or BDKRB1), leading to vasoconstriction, which generates a hypertensive response. Other transformations are carried out by the bradikinin peptide and LK kininogen, which also generate ligands for B1R and B2R. The ACE and ACE2 enzymes play important regulatory roles in this system, ACE2 deactivates des-Arg9-dradykinin, compared to ACE, which inactivates bradykinin [6].

Due to the interaction of these two systems with the immune system, there is a very strong connection between hypertension and the inflammatory response. In what follows we address this aspect.

Crosstalk between inflammation and hypertension

In addition to the former factors, inflammation contributes to the hypertension processes and part of the mechanisms that interrelate the RAS and KKS pathways with the immune system have been defined [22, 19]. Experimental evidence in animals shows an increase in blood pressure after the administration of Ang II peptide, along with an increase in the invasion by immune cells towards organs such as the kidneys, the heart, among others; and as a consequence of this, we can see an increase in the expression levels of pro-inflammatory cytokines such as interleukin (IL-6), interferon gamma (IFNG), TNFa (tumor necrosis factor-alpha) and others. On the other hand, feedback between processes can be observed, for example, in the regulation

of the ACE enzyme through the NFkB factor that is stimulated by TNFa and IL-6. AT2R receptor activation is associated with low levels of TNFa and IL-6 [22]. Interestingly, the later activation is also related to the expression of anti-inflammatory cytokines such as interleukin (IL-10) [7]. Evidence reports that TNFa is involved in the expression of B1 receptor [18].

We can at this point discuss the dynamic relationship that exists between hypertension and COVID-19.

COVID-19 and hypertension

Clinical and epidemiological data from patients with COVID-19 suggests that specific comorbidities increase the risk and severity of infection. In China, hypertension was the main morbidity associated with severity [8], as well as in Mexico [2]. In addition, SARS-CoV-2 infection leads to a hyper-inflammatory response and with it, to the release of several cytokines, including IL-1B, IL-6, IFNG, among other. This response increases even more in patients with previous morbidity such as hypertension, diabetes, cardiovascular disease, and chronic obstructive pulmonary disease, generating a "Cytokine-storm" [12].

Systems biology approach

The fundamental objective of medical systems biology is focused on providing efficient and reliable formal methods, essentially of a mathematical and computational nature, that make sense of the growing experimental information that emanates today from basic biomedical research [21]. Consequently, medical systems biology explores, via systems-based methods, the intrinsic and extrinsic biomolecular circumstances that govern the disruption of the normal dynamics of gene regulatory networks. The emergence and progression of the disease results from the affectation of gene regulatory processes that in normal situations ensure biological development. In this, the patient's lifestyle is decisive, since it largely determines the cellular micro-environment. Any preventive approach to health requires an understanding of the biomolecular phenomenology that underlies disease. In our case, we follow a Bottom-Up approach, recognizing gene regulatory networks as dynamic systems, making extensive use of both mathematical modeling and the state-space approach as fundamental tools [21]. Then, we interpret the disease in terms of phenotypic cellular transitions, which we model in discrete Boolean terms through the application of the notion of *dynamic attractor* in the context of formal dynamical systems [5].

2 Methods and results

In what follows we briefly present both our systems-based methodology we have followed and our qualitative results.

Methods

The proposed Boolean model was inferred from experimental data available in literature, which characterizes the key molecules and interactions of the RAS-KKS-inflammation systems through a Bottom-Up approach, of the generic principles that underlie the gene regulation of blood pressure.

In Boolean regulatory network models, each variable or network element represents genes, transcription factors (FTs), RNA sequences or hormones. Each element of this network can take two values, 1 (on or active) or 0 (repressed or inactive) representing its state or concentration.

Thus a gene regulatory network can be represented via a discrete Boolean network over a set of n nodes. Each one representing proteins, genes, miRNAs or transcription factors. Each node, say x_i, has in general n regulators. The binary value of the i-th node at discrete time $t+1$ is determined by its n regulators at discrete time t through a Boolean function f_i. Which is to say:

$$x_i\left(t+1\right) = f_i\left(x_1\left(t\right), x_2\left(t\right) \ldots x_n\left(t\right)\right), \quad i = 1, 2, \ldots, n. \tag{1}$$

Thus, the set of all possible configurations, expressed by $\Omega = 2^n$, forms the state space of the network. The evolution time of the system is represented by a trajectory within the state space, and since, the state space is a finite set and the dynamics of the network is deterministic, eventually the dynamics of the network converges to a single state or to a cyclic set of states which are referred to as fixed point attractor and cyclic attractor respectively (see review [11]). Note that we are not assuming in our description of the regulatory network the presence of exogenous inputs. This because we are interested in the necessary and sufficient regulatory dynamics that explain the experimentally characterized cellular phenotypes. For more information on the dynamic behavior of the network, gain-and loss-of-function mutations were simulated and the behavior of the resulting attractors was evaluated by comparing them with those obtained for wild type. In parallel, the results were contrasted with the experimental evidence reported. Loss-of-function mutations were simulated by setting the node value to 0, conversely, gain-of-function with the value 1. To answer the question of phenotypic occurrence frequency, we randomly perturbed the logical functions that define the regulatory network. We perform this perturbation by randomly altering the output of the corresponding Boolean function, *e.g.*, when the output of the function is equal to 1 it is changed to 0 and vice versa. Also proceeded to randomly modify the logical operators that constitute a given logical function. We computed these modifications 1000 times in the wild-type and the mutated network. For each modification, we computed 50 replicates and calculated the attractors to which it led by evaluating their logic functions and subsequently counted the frequency of phenotypes [15], in this case the hypertensive and hypotensive phenotypes and we proceeded with a statistical analysis. We performed an Aligned Rank Transform for Nonparametric Factorial ANOVAs followed by a Bonferroni post-hoc test to evaluate the statistical differences between genotypes and phenotypes with a $p\ value < 0.05$. If there is a significant difference different letters were assigned to bars and if there is no significant difference the same letters were assigned.

2.1 Results

In order to understand how the typical expression patterns are established during a hypertension process, or hypotension/normotension where inflammation plays an important role, a discrete Boolean model was built based on the experimental evidence reported on the literature. A network with 29 nodes and 69 regulatory interactions was obtained. We must point out that CPN (carboxypeptidase N subunit 1) and Renin are considered in our model as constitutive nodes. Table 1 shows the logical rules that incorporate the experimental publicly available information that determines the activation of each network node based on the relationship among its regulators, which can be activated or inhibited.

We explore the dynamics of the model via the the package BoolNet from the R project [16]. Two attractors were found, each of them corresponding to the gene expression patterns typical of experimentally observed hypertensive, and hypotensive/normotensive phenotypes for the wild type (Figure 1). Interestingly, the attractor corresponding to the hypotensive phenotype where the ACE2-Ang-(1-9)-AT2R axis, was found to be active. However, the pathway through Ang-(1-7) peptide and MasR receptor associated with a hypotensive phenotype remained inactive

Nodes	Logical rules
ACE	NFKB ∧ ¬Ang17
ACE2	(IFNa ∧ ¬AT1R) ∨ (AT2R ∧ ¬AT1R)
Aldosterone	AT1R ∧ ¬ANP
Ang17	(Ang19 ∧ ACE) ∨ (ACE2 ∧ AngII) ∨ (NEP ∧ AngI)
Ang19	AngI ∧ ACE2
AngI	Renin
AngII	AngI ∧ ACE ∧ ¬NEP
ANP	(¬NEP ∧ ¬AT1R ∧ AT2R) ∨ (¬NEP ∧ ¬AT1R ∧ MasR) ∨ (¬NEP ∧ AT2R ∧ MasR) ∨ (¬AT1R ∧ AT2R ∧ MasR)
AT1R	AngII
AT2R	Ang19 ∨ AngII
BDKRB1	(IL1B* ∧ TNFa* ∧ NFKB) ∨ desArg9BK
BDKRB2	Bradykinin
Bradykinin	KLKB1 ∧ ¬ACE
CPN	CPN**
desArg9BK	Bradykinin ∧ CPN ∧ ¬ACE2
IFNa	¬IL10
IFNG	(IL12 ∧ IL1B) ∨ (AT1R ∧ ¬IL10)
IL10	(TNFa ∧ ¬IFNG) ∨ (AT2R ∧ ¬IFNG)
IL12	(BDKRB2 ∧ ¬IL10) ∨ AngII
IL1B	AT1R ∧ NFKB
IL6	AT1R ∧ ¬MasR
KLKB1	PRCP
MasR	Ang17
NEP	IL1B* ∧ ¬IFNG*
NFKB	(IL6 ∧ ¬IL10) ∨ (BDKRB1 ∧ ¬IL10) ∨ (IL1B ∧ ¬IL10) ∨ (TNFa ∧ ¬IL10) ∨ (AT1R ∧ ¬ IL10)
PGE2	BDKRB1
PRCP	AT2R
Renin	Renin**
TNFa	¬IL10 ∧ AT1R

Table 1: **Logical regulatory rules of the model.** This table shows the regulatory logical rules that define the dynamics of our discrete Boolean model. Symbols ∧, ∨, and ¬, stand for logical conjunction, logical dijunction, and logical negation, respectively. Moreover, symbol * stands for prediction of relevant interactions and ** stands for constitutive nodes.

(see Figure 1). According to the published experimental evidence, the regulation of Ang-(1-7) expression can occur through the following interactions: Ang-(1-9) with ACE, ACE2 with Ang II, and NEP with Ang I [20].

For more information on the dynamic behavior of the model network, gain- and loss-of-function mutations were simulated and the behavior of the resulting attractors was evaluated by comparing them with those obtained for wild type. In parallel, the results were also contrasted with the reported experimental evidence. The summary of the results that we obtained is show in Table 2.

While performing mutation simulations, the gain-of-function of interleukin IL-1B favors

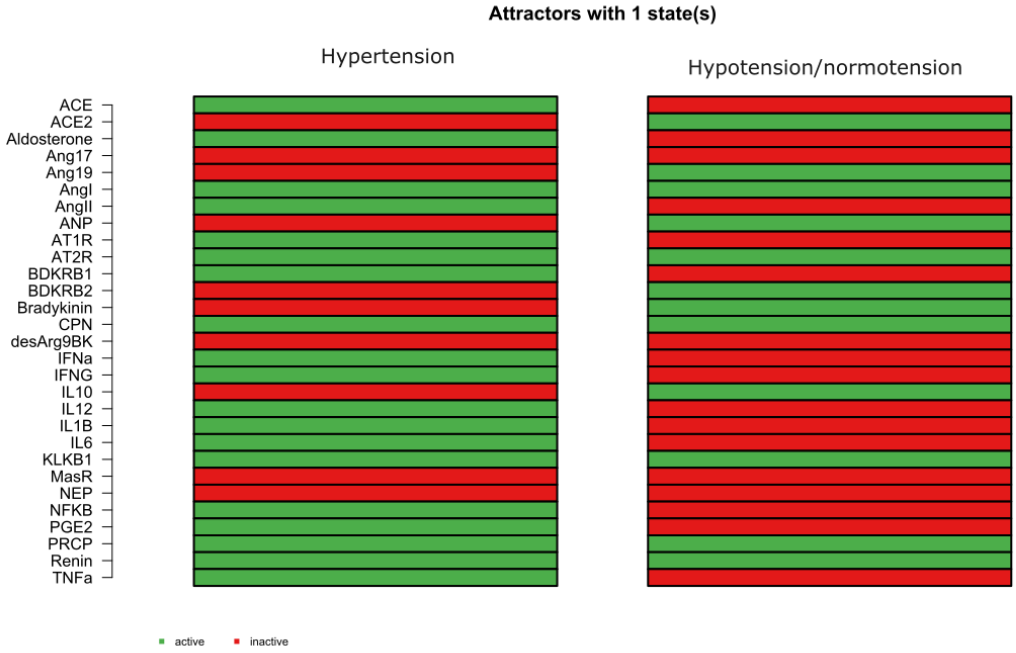

Figure 1: **Attractors of the regulatory network.** This figure shows the two attractors that we obtained when exploring the dynamics of our discrete Boolean model.

Nodes mutated	Attractors obtained after the mutants
Ang II^{-}, ACE^{-}, NFKB^{-}, IFNG^{-}, AT1R^{-}, ACE2^{+}, Ang-(1-9)$^{+}$, Ang-(1-7)$^{+}$, IL-10^{+}, NEP^{+}	Loss of hypertension attractor
ACE2^{-}, Ang-(1-9)$^{-}$, AT2R^{-} Ang II^{-}, ACE^{+}, NFkB^{+}, AT1R^{+}	Loss of hypotension attactor
Ang I^{-}	Loss of hypotension and hypertension attractors
PRCP$^{-\text{ or }+}$, KLKB1$^{-\text{ or }+}$, DesArg9BK$^{-\text{ or }+}$, BDKRB2$^{-\text{ or }+}$, BDKRB1$^{-\text{ or }+}$, IL-6$^{-\text{ or }+}$, IL-12$^{-\text{ or }+}$, TNFa$^{-\text{ or }+}$, IFNa$^{-\text{ or }+}$	Did not affect the expresion pattern of the attractors

Table 2: **Summary of simulated mutations.** In this table we show what results when we test the discrete Boolean model with loss (-) or gain (+) of function mutations, when compared to the wild-type model.

the expression of NEP, also known as MME (membrane metalloendopeptidase), which had remained inactive in both attractors in the wild type. Consequently, it resulted in the activation of the Ang-(1-7)-MasR receptor, along with Ang-(1-9) and AT2R staying active as well. NEP is positively regulated by interleukin IL-1B and negatively regulated by IFNG [27]. Therefore,

the activation of Ang-(1-7) was done by the activation of Ang I together with NEP. Furthermore, NEP is a negative regulator of ANP or also known as ANF (atrial natriuretic peptide), the later has vasodilatory and diuretic effects [13]; after simulations, it remains active even when NEP is present; this is probably favored by signaling from two active ANP regulators, such as AT2R and MasR receptors featured in the hypotension attractor [23, 26]. This result requires further experimental validation. In parallel, the activation of the B1 receptor in the hypertension attractor was observed, even without activation of desArg9BK, this activation was attributed to the expression of cytokynes such as IL-1B and TNFa, and the transcription factor NFkB [18]. After evaluating the mutation simulated from all nodes, desArg9BK was activated when the gain-of-function of bradykinin was performed, because bradykinin was not active in the hypertension attractor in the wild type. ACE2 is one of the main enzymes that degrade desArg9BK [6]. Simulations of both gain- and loss-of-function of the nodes such as, PRCP, KLKB1, Bradykinin and B2 receptor did not affect the expression patterns compared to wild type (Table 2). Which suggests a minimal participation in this phenotype where the NEP-Ang-(1-7)-MasR axis is involved, however, it does not exclude them from having a greater participation in the hypotension process, where ACE2-AngII-AT2R axis participates, among others. In addition, B1 receptor mutations did not alter the establishment of expression patterns, including cytokines, compared to wild type. Among wild type attractors, it was possible to observe the active expression of IL-10, and the inactive expression of IFNG in the hypotension attractor; this expression pattern is found in the opposite way regarding the hypertension attractor (Figure 1). Simulations of the loss-of-function of IFNG show the loss of the hypertension attractor [25], and similarly, the gain-of-function of the IL-10 node promotes the loss of the attractor related to hypertension [24] (see Table 2).

According to a preliminary search for existing drugs able to inhibit IFNG, emapalumab is a monoclonal antibody that binds and neutralizes IFNG, said antibody has already been approved by the FDA, together with Mesopram, which is a phosphodiesterase that also decreases the production of IFNG, however, this last drug also decreases the expression of IL-10. Thanks to data available at the free access program AlphaFold Protein Structure Database, the structure of the Tissue-resident T-cell transcription regulator protein ZNF683, also known as Hobit, was ascertained [1]. Hobit, is a positive regulator of IFNG, which in turn acts as a transcriptional repressor for the motifs of the transcriptional factor called Blimp-1. The Blimp-1 factor acts directly on the IL-10 locus, resulting in increased IL-10 expression. Therefore, Hobit is proposed as a possible study target due to its potential in the regulation of the two key cytokines; IFNG and IL-10, which are involved in morbidities such as hypertension according to the Boolean model (citations found in [1]). Finally, according to the stochasticity analysis the percentage of appearance of the phenotypes is shown in Figure 2. In the wild type version the presence of both phenotypes (hypertensive and hypotensive/normotensive) is shown, with a higher probability of appearance of the hypotensive phenotype as consequence of the connectivity between the nodes of the model. Six nodes were selected to carry out the mutation simulations and the percentages of the phenotypes were obtained. The mutations of loss-of-function of ACE^{-}, $IFNG^{-}$ and gain-of-function of $ACE2^{+}$, $IL\text{-}10^{+}$ nodes will alter the multi-stability of the system so that the hypertension attractor disappears and this result agrees with what been reported experimentally [10, 3, 25, 24]. In ANP node loss-of-function, both phenotypes appear with a greater tendency for the appearance of the hypertensive phenotype and also corroborate the experimental evidence [14]. With respect to the gain-of-function of NEP^{+}, the mutation produced the loss of the hypertension attractor, these contradicts what has been reported in the literature [17]. We must point out that there is controversy regarding the participation of NEP in the regulation of blood pressure due to its duality in the process of hypertension (is

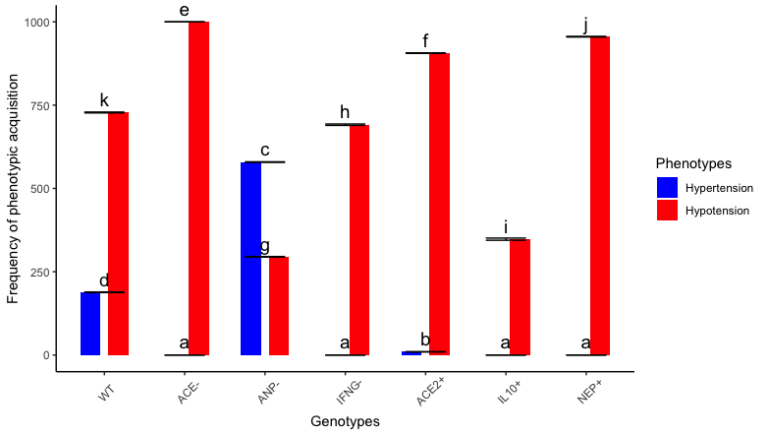

Figure 2: **Stochasticity analysis and percentages of the resulting phenotypes.** In the wild type (WT) and after the simulated mutation (-) loss-of-function and (+) gain-of-function of 6 nodes. Different letters indicate significant differences between genotypes and phenotypes ($p<0.05$) and the same letters indicate no significant difference

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a negative regulator of ANP that has vasodilatory and diuretic effects)[13] and hypotension (participate in the regulation of Ang-(1-7) through the interaction of NEP with Ang I)[20].

3 Discussion and final comments

The goal of the proposed Boolean model was to integrate the information reported among the three systems involved in the regulation of blood pressure, that are the Renin-angiotensin system (RAS), Kalikrein-kinin (KKS) and the inflammation process. With this model, only two stable gene profile configuration (attractors) were identified, those were found to be typical to the expression patterns reported for the hypertensive and hypotensive/normotensive phenotypes. This result shows the relevance of the model reported here. Although only the hypotension attractor associated with the ACE2-Ang-(1-9)-AT2R axis was detected in the wild type, and the pathway given by Ang-(1-7) y MasR, which is associated with a hypotensive/normotensive phenotype was detected only when the gain-of-function of NEP and IL-1B (as NEP regulator) was performed. Furthermore, by evaluating both inflammatory and anti-inflammatory cytokines in an integrated manner, provided data that requires experimental validation in order to propose them as alternative treatments for morbidity such as hypertension. It is clear to us that the qualitative nature of our study requires empirical verification of our results. This is of paramount importance when contemplating the design of therapeutic intervention strategies.

We must point out that Zivile Bekassy et al [4], offer an approach that integrates existing information from the previously mentioned three systems through a review that describes the interactive role of the Renin–Angiotensin, the Kallikrein–Kinin, and the complement system; which are involved in different biological processes, such as hereditary angioedema (HAE), COVID-19, among others. Going deeper into the search for experimental evidence of regulators and transcriptional control of ACE and NEP, a remarkable scarcity was found. The model has also led to an approach to describe relevant aspects of the transition from a hypertensive to hypotensive/normotensive phenotype or vice versa, interconnecting the three systems. In

addition, to provide predictions about interactions among molecules that, up to now, have not been experimentally evaluated for the process of blood regulation in particular. Finally, the inflammation plays a relevant role in the regulation of blood pressure, effectively considering that the relationship between hypertension and inflammation is known, it cannot be ignored that SARS-CoV-2 favors an inflammatory response that, according to the model, contributes to a hypertensive process mainly through IFNG. On the contrary, an overexpression of IL-10 favors a hypotensive phenotype. Therapies should focus on reducing inflammation.

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