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A Static Analysis of *Wnt/ β-Catenin* and *Wnt/Ca*$^{2+}$ Biological Regulatory Networks for ARVC Using Automata Network Model

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ABSTRACT The *Wnt*-activated *β-catenin* and Ca^{2+} ions play a critical role in the regulation of physiology of cardiomyocytes. The dysregulation of both the components causes the replacement of myocardial mass right ventricle with fibrous and adipose tissue which results in the condition of Arrhythmogenic Right ventricular Cardiomyopathy (ARVC). The major hurdle in ARVC treatment is lack of effective therapies targeting the underlying root molecular biomarkers of pathogenesis. Despite major advancements in interpreting the mechanism of ARVC etiology, the dynamics of molecular links in underlying biological machinery are still being delineated. Previously, formal methods based computational modeling techniques including kinetic logic, Petri Nets, hybrid automata and static analysis have greatly contributed in increasing our comprehension to decipher the molecular systems dynamics. It has allowed the identification of biomarkers which can be utilized for target-based therapies owing to meticulous biological abstractions along with implementing reference map that confines together the discrete biological insights. In this study, we have performed the static analysis of the Biological Regulatory Networks (BRNs) of the *Wnt/β-catenin* and *Wnt/Ca*$^{2+}$ signaling pathways to identify the significant biomarkers for ARVC. The abstracted qualitative models of afore mentioned BRNs are first constructed in GINsim software tool and then these models are converted into Automata Network (ANs) Models using Pint software tool. The fix point analysis is performed which contributed in pinpointing the possible therapeutic strategies for ARVC treatment by identification of drug targets such as *Gsk3, Ck1 and Axin* in *Wnt/β-catenin AN* and *Bak & Bax, Parp, mCalpain, JNk and CIn* in *Wnt/Ca*$^{2+}$ AN. Moreover, the *Bcl2* gene is identified as novel therapeutic remedy in both the ANs of ARVC. The *Bcl2* gene prevents the cardiac apoptosis via positive regulation of *Wnt* in *Wnt/β-catenin* AN and through inhibition of *Bak & Bax* (apoptotic component) in *Wnt/Ca*$^{2+}$ AN. The current study tends to fulfill the scientific gap between wet lab studies and provides cost effective and time saving computational strategies for an effectual treatment for deadly diseases like ARVC.

INDEX TERMS Automata network model, ARVC, biomarkers, pint tool.

I. INTRODUCTION

Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC) refers to a heritable autosomal dominant mayocardiopathy. ARVC is characterized by the replacement of myocardial mass with the fibrous and adipose tissue, hence known as myocardial dystrophy. As a consequence, the ventricle enlarges and the volume of blood pumped decrease. It causes arrythmias, syncope, [1] and has been a leading cause of sudden cardiac death accounting for about 20% of deaths of

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individuals aging 30 years or less. ARVC is a progressive disease which is difficult to differentiate from dilated cardiomyopathy [2]. All the hallmarks of ARVC are attributed to *β-catenin* degradation in *Wnt/β-catenin* pathway and to the imbalance of Ca^{2+} homeostasis in *Wnt/*Ca^{2+} [3]. Due to mutations, the canonical*Wnt β-catenin* pathway is downregulated via *Tcf/Lef* transcription factors [4]. Furthermore, calcium homeostasis is also perturbed which leads to inadequate relaxation and decreased contraction force during systole [5]. Ultimately, it increases the expression of fibrogenic and adipogenic genes causes the deposition of fat in heart muscle leading to the pathophysiology of ARVC [1]

The biological signaling pathways make a complex network system which makes it a difficult task to decipher the flow of events [6]. Due to this, there is still a lot to be discovered and understood about the molecular mechanisms of the condition. These networks of molecular interaction and cellular signaling can be analyzed and interpreted using the techniques of system biology for therapeutic purpose [7].

This study focuses on describing the pathology and cellular mechanism of ARVC by analyzing the underlying dynamics of the system [8]. BRNs are used for modeling the regulatory mechanisms of biological activities in living organisms. The regulatory mechanisms are depicted in the form of interaction graphs with nodes representing the biological components (genes and proteins) and the edges indicating the interaction of these components [9]. These interactions are stochastic and continuous in nature having variable underlying dynamics. A variety of formal approaches have been invented for modeling the topology of BRNs to predict these dynamics [10]–[12]. There are several approaches used to model BRNs. Typically, differential equations have been used for the purpose. However, differential equations fail to depict the complex non-linear biological interactions [13]. In contrast, classical discrete modeling approaches such as Boolean models [14]–[16] and Petri nets [17], [18] are intractable for large systems because the size of the state space grows exponentially with the number of components. These approaches rely on global search for state graph (in case of Boolean models) and marking graph (in case of Petri nets) and suffer from reachability problem. Previous studies have demonstrated that reachability problem of Petri net is exponential time-hard and exponential space [19]. On the other hand, for Boolean models formal checking of dynamical models is not the only drawback as it also suffers from combinatory of parameters as well when numbers of variables are increased. Thus, verifying the reachability properties of the discrete models becomes extremely difficult as the number of components increases. We refer our readers to excellent reviews [20]–[22] on limitations, benefits and comparisons of some of the classical methods used for modeling of biological regulatory networks which is itself out of scope of the current work.

This work utilizes a highly scalable analytical framework of Process Hitting (PH) already established for the static analysis of large scale BRNs. Process Hitting approach [9], [23], [24] approach can analyze properties of very large scale biological regulatory networks of up to 10,000 biological entities [25], [26] without constructing their state graph. This approach is capable of handling the networks of thousands of genes. Scalability of this approach is attributed to two main factors. Instead of the whole state space, only the most permissible dynamics are considered. Moreover, if two or more genes have a combined effect on a third gene, the general dynamics are refined via cooperatively among the genes. It is based on stochastic π-Calculus and allows for synthesis of temporal and stochastic parameters, which enables the simulation of dynamical behavior to some extent.

Hence, the Process Hitting approach is the best suited for Static analysis such as the determination of stable states, successive reachability and inferring the K parameters of the BRN [11].

To model the dynamical systems which results from the interlinks of entities, the Automata networks are used. Every entity is presented by an automaton with many internal states of other automaton in the network. Richness of transpiring dynamics emerges from several factors which include presence of feedback loops, the topology of interactions, and the concurrency of transitions. The biological systems including signaling and gene regulatory networks are modeled using Automata networks as shown in Figure 1 [27].

In formal modeling, various properties of biological systems can be expressed as reachability properties in computation model such as the prime state or set of states, sequence of transition leading to the required state or set of states. The initial state may be representing a set of signaling perturbations the required states may be depicting the set of states where the subject transcription factor is active. The activity of the subject factor can be verified by considering mutations as the automata freezing of some stable states which can be modeled. Another option is the deletion of some transitions [28].

Attributing to the advancements in biological accuracy and increasing number of the interacting entities, reachability in automata networks is a PSPACE-complete problem limiting its scalability. Hence, larger systems are almost impossible to tract. The PINT software implements static analysis for the formal approximation of reachability properties and facilitates with the said complexity. PINT verifies the vital or enough conditions and makes it possible to tract the complex networks [29].

To identify the local states of interacting components in a finite state model. The Cut N-sets algorithm is used and the activation of these states are vital to reach a certain goal as presented in Figure 2. The reachability of concerned state is possible only if all the local states of such set are not disabled. These sets are called cut sets which are computed from specific causality structure referred as Graph of Local causality. The resulting cut sets of dynamics from the under-approximation of local states may contain lesser or additional cut sets for the said reachability. When implemented in biological systems for qualitative models, these cut

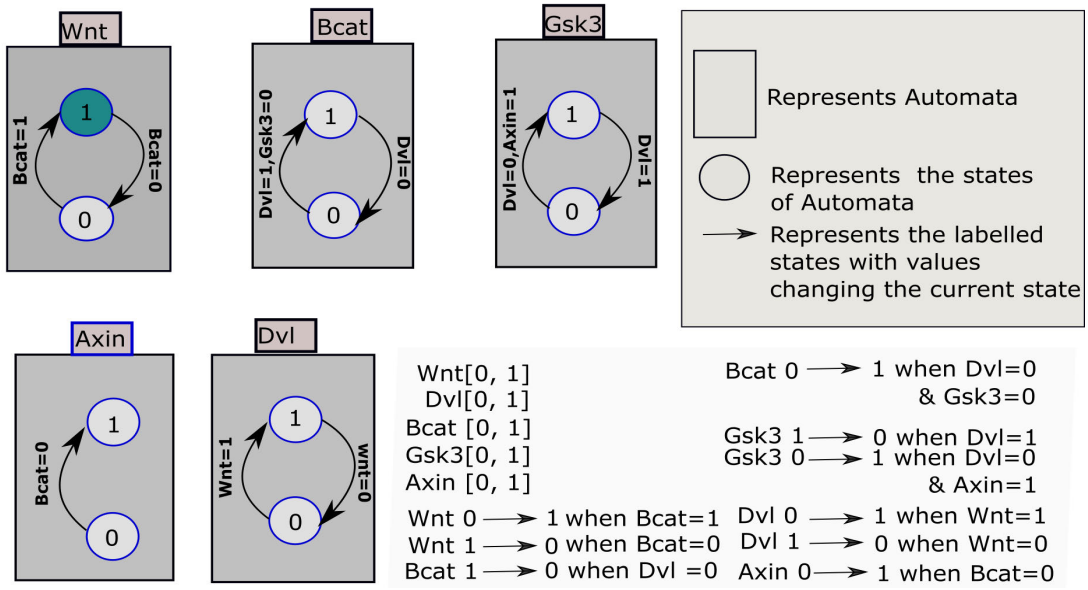

FIGURE 1. Represents the transition graph and schematic of automata network. Automata are shown by labeled boxes, and circles show the local states where the automaton have ticks as their identifier– for example, wnt0 local state is a circle having tick 0 in box Wnt. In the same automaton two local states having a directed edge is a transition. Which can be marked with set of other automaton's local states [31].

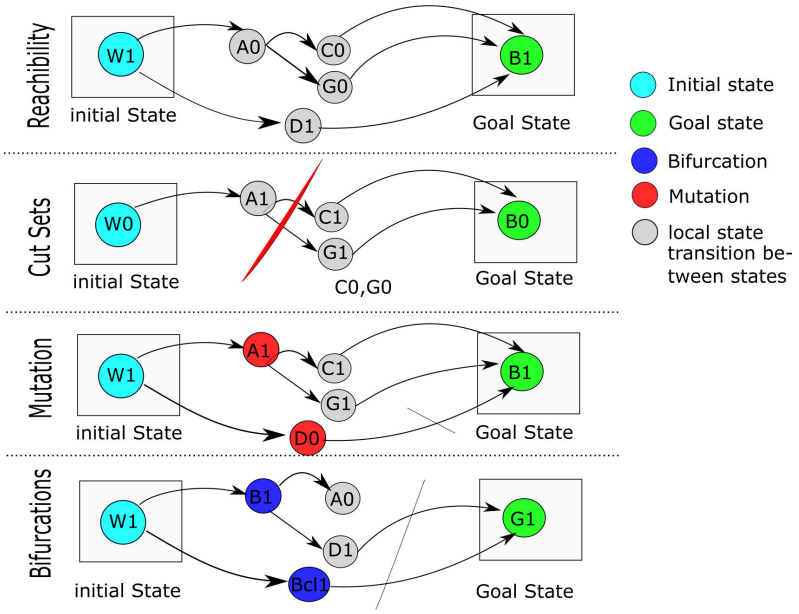

FIGURE 2. The illustration of important features of Pint associated to the transient reachability from set of initial states to the set of goal states and the arrows show the transitions. The Arc in red color crossing the transitions shows the cut sets [34].

sets can be used in identification of therapeutic targets. These therapeutics are proposed to inhibit the concerned molecules from activation up to the model correctness [30].

However, even with the PINT software the reachability problem persists because of the bifurcations (local states) the ability to reach a goal after a specific transition, it is called bifurcation as shown in above Figure 2. Bifurcations are identified by a scalable method of programming with Answer-Set Programming (ASP). The identification relies on PSPACE-complete of ANs and the relevant framework. For large and complex biological pathways several techniques of static analysis have been combined to decrease the bifurcation problem such as ANs dynamics, concurrency and constraint programming [31].

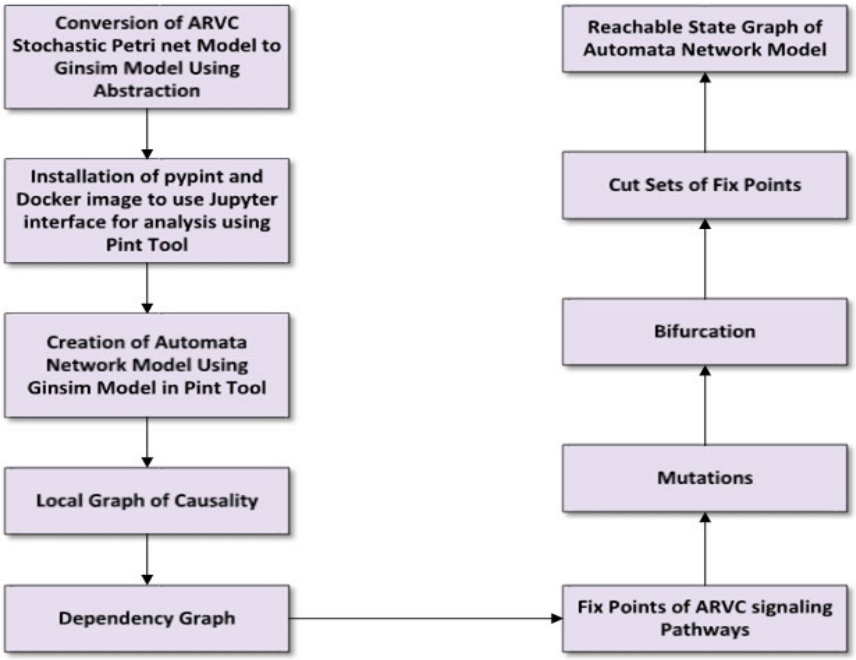

FIGURE 3. The Logical modeling and analysis workflow. The illustration of steps implemented in methodology.

In this study, we have conducted the static analysis of ARVC to predict drugs for the disease using computer-aided drug designing techniques. In this paper the fixed point, cut sets analysis and mutations are performed on AN model of BRNs for ARVC signaling pathways [32].

In*Wntβ-ncatenin* AN Model, fixed point analysis gives the two deadlock states, where one deadlock state is caused by the up regulation of *CK1*, *Gsk3* and*Axi*n proteins and the other deadlock state results from the down regulation of *Bcat, Dvl, Bcl*2 proteins and*Wnt* ligand. These states are verified through cut sets analysis by applying cut sets on biomarkers *(Ck1, Gsk3*, *and Axin*) identified in fixed point analysis. The analysis of *Wnt/*Ca^{2+} AN model predicted *Bax & Bak, CIn, JNk, Parp*, *mCalpain* as biomarkers which are evaluated further by applying cut sets. The mutations were considered for both the AN models of ARVC for verification of the results of fixed points and cut sets. Moreover, the reachability of the desired states (im) possibility is presented by Dependency Graph, GLC, and Reachable State Graphs. Hence, therapeutic components are identified by extensive analysis techniques in ARVC AN models.

II. METHODOLOGY

The graphical representation of methodology adopted in study, to describe the Process Hitting Framework used for the static analysis of large scale biological signaling pathways (*Wnt/β-catenin* and *Wnt/*Ca^{2+}) of ARVC [32] is illustrated in the methodological steps followed in this study to describe the Process Hitting Framework which is used for the Figure 3 and explained in the following sections. All the Figures and source codes are provided as supplementary file 1 and 2[1] (Wnt.html and Ca, Ca1.html).

A. DIRECTED GRAPHS

According to graph theory, directed graph D (V, E) is a tuple of two elements where V is a set of vertices or nodes and E is organized pairs of vertices representing directed arcs or directed edges. Each edge of a BRN can be characterized with a pair of the level of qualitative threshold (positive integer) and a sign of interaction (''+'' or ''–'' signs i.e., ''activation'' or ''inhibition'', respectively) [33].

B. PINT

The software Pint [34] is robust for the evaluation of trajectories within very extensive Boolean, asynchronous and multiplex biological systems. Jupyter notebook provides a suitable framework for performing bioinformatics analysis and has an aptitude of being applicable to perplexed computational biological pathways [35]–[37]. Pint through its Python module pypint makes an impeccable integration with Jupyter notebook enabling an effortless reclaim, and procreation to assuage the model's management and summons to Pint [28]. Its central attribute is its validation upon the presence of a trajectory extending out to the concerned state along with discerning of recurrent points among all those trajectories cardinal to that significant state specified as the cut sets and the legitimate prognosis of mutations impeding the outstretch of any path of states to that significant state [38]. Based on abstract elucidation and causality of trajectories, Pint enables

[1]https://github.com/nazia83/pint

static explication of the traces within the biological systems. In either case, the yielded findings have the capacity fulfilling the requisites of adequate and necessary constraints [34].

The Docker image pauleve/pint[2] provides a ready-to-use Pint environment for all usual operating systems (Windows, Mac OS X, Linux), and notably the Jupyter web interface. Such distributions have become a standard for providing accessible and reproducible analyses in bioinformatics, e.g., *BioContainers*. Pint can automatically convert models expressed as Boolean or multi-valued networks using the pint-import command or Pypint.load() python function. Most of the conversions are performed using GINsim [39], enabling the support for SBML-qual, GINsim, as well as various text formats. Models for the respective purpose, can be directly fetched from URLs or from CellCollective Database [40]. Furthermore, Pint supports the BIOCHAM reaction networks following their Boolean semantics as well [41].

C. AUTOMATA NETWORKS

"An automata network is defined by a tuple $(\sum, S, L, T)$ where:

$\sum$ is the finite set of automata identifiers;
S is the finite set of global states;
L is the finite set of transition labels, and;
T is the mapping from automata to their finite set of local transitions", [30].

Automata network is a discrete definable-state model of collaborating components that are extensively administered for qualitative modeling of discrete, Boolean systems [14], [42]–[44]. Automata network conducts the qualitative modeling of these components considering the synchronous or chronic transitions caused by the state of another automaton within biological signaling systems. At any instance, the individual automaton exists in merely one local state thus, forming global state of the system by congregating the local states of formulated automata [28]. Hence, in this study, the automata network of three entities is taken into account where each entity is referred to as single automata that is interconnected with others through transitions within the complex system.

D. FIX POINTS

Within the confines of Process Hitting substructure, static analysis is performed primarily for procuring the complete checklist of stable states of Process Hitting dynamics and a value is assigned to each vertex of the network [9]. The Process Hitting framework is applied to the non-specific Boolean dynamical biological systems with the help of detailed interaction graphs. The evaluation of these interaction graphs provides us with the disease states [45]. Such disease states are the dead lock states in all viable dynamics, having no further outreach trajectories passing through. The derivation of fix points for the automata network encompassing 4 entities A, B, C and D after implementation of static analysis through Pint software has been enlightened in Figure 1 of the supplementary file 1.

E. CUT SETS

The current technique of using pint software allows formal consideration of large scale networks encompassing colossal number of entities with two local states either 0 or 1 being Boolean in nature. After static reachability speculation, pint comes up with the listing of cut sets applied on the transitions that are generally interpreted upon the interacting graph comprising of sets of nodes. Projection of these nodes is significant in order to reach the specific state such as stable state or a disease state from an initial global state in a finite biological automata network. Now, impeding all the local states from the list within the model, the model is analyzed for the concerned reachability is still correct [34]. Computation of list of entities in the cut sets function for the automata network of 4 entities as in a, b, c and d. Knockdown of these entities obstructs the reachability towards fix point which is shown in the figure 2 of supplementary file 1.

F. MUTATIONS

Formal presentation and effective static analysis run by the Pint software indicates exhaustive evaluation of conjugated biological automata networks and allows strict reasoning for temporal attributes such as reachability towards definite state along with abstraction of sets of variations that fortify evasion of the fix point. By intercepting the path to the goal, the provided catalogue of mutations obstruct any of the transient triggers of the vertex and thus, limits the passage of any trajectory towards disease state or stable state [45]. Inference of list of entities that undergo changes in their expression levels and impede the reachability towards a stable state due to any mutation is represented in the Figure 3 in supplementary file 1.

G. BIFURCATIONS

Pint employs static analysis of the trajectories within Boolean multiplex networks following the goal defined as stable state which is not obtainable any further. Bifurcation transitions comprehend the states and their concerned routes that exhibit major contribution towards the diseased state. Generation of bifurcation indicates the obstruction of the reachability of the concerned state [31]. The Figure 4 refers to the alternative trajectories in the form of bifurcations for the states involved in the path leading towards stable state.

H. DEPENDENCY GRAPH

Dependency Graph refers to a directed graph that is obtained by the aggregation of all the local states and their trajectories within a biological system. This directed graph is prone to further amendments and it can be manifested on the Jupyter notebook. In this regard, activation of all the arcs within a dependency graph is a prerequisite to permit a node to process, whose dependencies maybe Boolean function of other

[2]https://hub.docker.com/r/pauleve/pint/

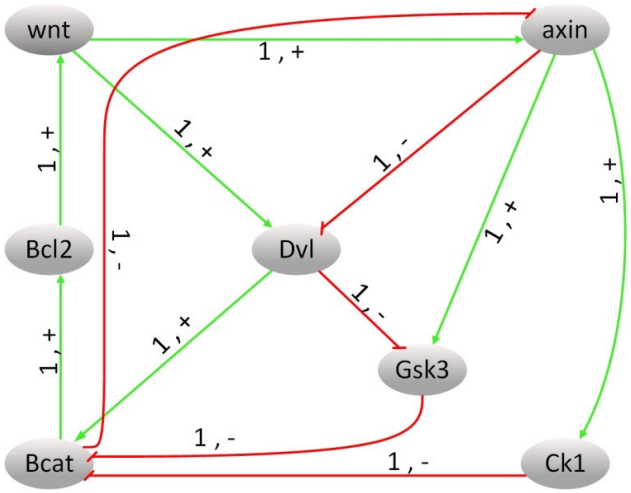

FIGURE 4. Biological regulatory network of *Wnt/β-catenin* pathway. The pathway consists of 7 nodes which depict the biological entities of the complex pathway. These seven nodes tend to have trajectories interconnecting one node to other through vertices. The kinetic parameters are evaluated with an in depth literature review that defines the overall interactions in the biological pathway of *Wnt/β-catenin*.

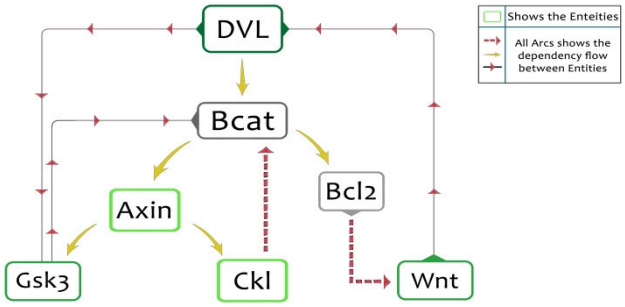

FIGURE 5. The dependency of node *Dvl*, for activation is shown by trajectory (*Bcat, Bcl2, Wnt*) which makes a positive feed loop and the *Bcat* deactivation depends on routs *Axin* to *Gsk3* and *Axin* to *Ck1* in the model as shown in Figure-4 as negative feedback loop in the network.

vertices in the automata network [9]. Here the trajectory from entity A to B depicts that any single path of B is dependent on the state of entity A which is depicted in Figure 5 of supplementary file 1.

I. GRAPH OF LOCAL CAUSALITY

The graph of local causality enables the elucidation of distinct complex regulatory models by abstracting the dynamical restraints. Thus, it casts the underlying basis of static analysis which is one of the major attributes of Process Hitting framework. It averts high-priced classification of the prospective candidates and seeks tractability of substantially complex systems [30].

There are well assorted structures of Graph of Local Causality that primarily build upon the semantics of the definite system. The graph characterizes the local states whose initial incidence is imperative to the reachability of notable local state within an automata network that is traced by causality links between objectives to resolve that reachability [28], as shown in the Figure 6 of supplementary file 1.

J. REACHABLE STATE GRAPH

The Pint software enables a distinct reachable state graph assessment from a definite set of initial states in the model via static analysis. It is known that such perspective is bound in terms of extensibility. Hence, from an initial state, it indicates the reachability towards all the possible states in the given complex model of *Wnt/ β-catenin* and *Wnt/Ca*$^{2+}$. The state graph for the automata network of A, B, C and D entities representing the reachability based on the causality of states [31] has been shown in the Figure 7 of supplementary file 1.

III. RESULTS AND DISCUSSIONS

This section discusses the static analysis performed on Automata Network models of signaling pathways in ARVC. The effective treatment of ARVC is possible at molecular level using computational methods. However, advancements in the molecular targeted therapy depend on thorough understanding and learning of basic mechanism of the *Wnt/β-catenin* and *Wnt/ca*$^{2+}$ signaling pathways. Recently, various computational techniques have been employed to decode the complex biological system in less time and cost-effective manner.

A. CONSTRUCTION OF BRN OF WNT/ β-CATENIN SIGNALING PATHWAY

An abstracted BRN of *Wnt/β-catenin* pathway as shown in Figure 4 below is fabricated on the GINsim tool from detailed *Wnt/β-catenin* pathway [32]. It is worth mentioning that the Biological Regulatory Network of*Wntβ-ncatenin and Wnt/C*$^{a2+}$ pathways are novel contribution in itself as we created it after thorough literature search of molecular entities an interactions involved followed by its systematic abstraction as discussed in our previous work [32].

B. AUTOMATA NETWORK OF WNT/ β-CATENIN

The Automata network is constructed from the GINsim model of *Wnt/ β-catenin* pathway as shown in Figure 4.

The Automata Network of *Wnt/β-catenin* pathway in Table-1 listed before shows the set of all possible local states of automatons (*Wnt, Dvl, Gsk3, β-catenin, Axin, Bcl2* and *Ck1*) based on the conditions of local states of other automata in the network.

C. STABLE STATES (FIX POINT ANALYSIS) FOR WNT/ β-CATENIN PATHWAY

The abstract-based effective analysis of *Wnt/β-catenin* pathways is performed and it can also undertake these integrated complex systems. In the current research, many novel biomarkers are identified based on diseased state or stable state using Automata Network as shown in Table-2 below.

The Automata Network of *Wnt/β-catenin* pathway is used to find the fixed points or stable states of model, the results obtained by the tool are mentioned in above Table-2 it

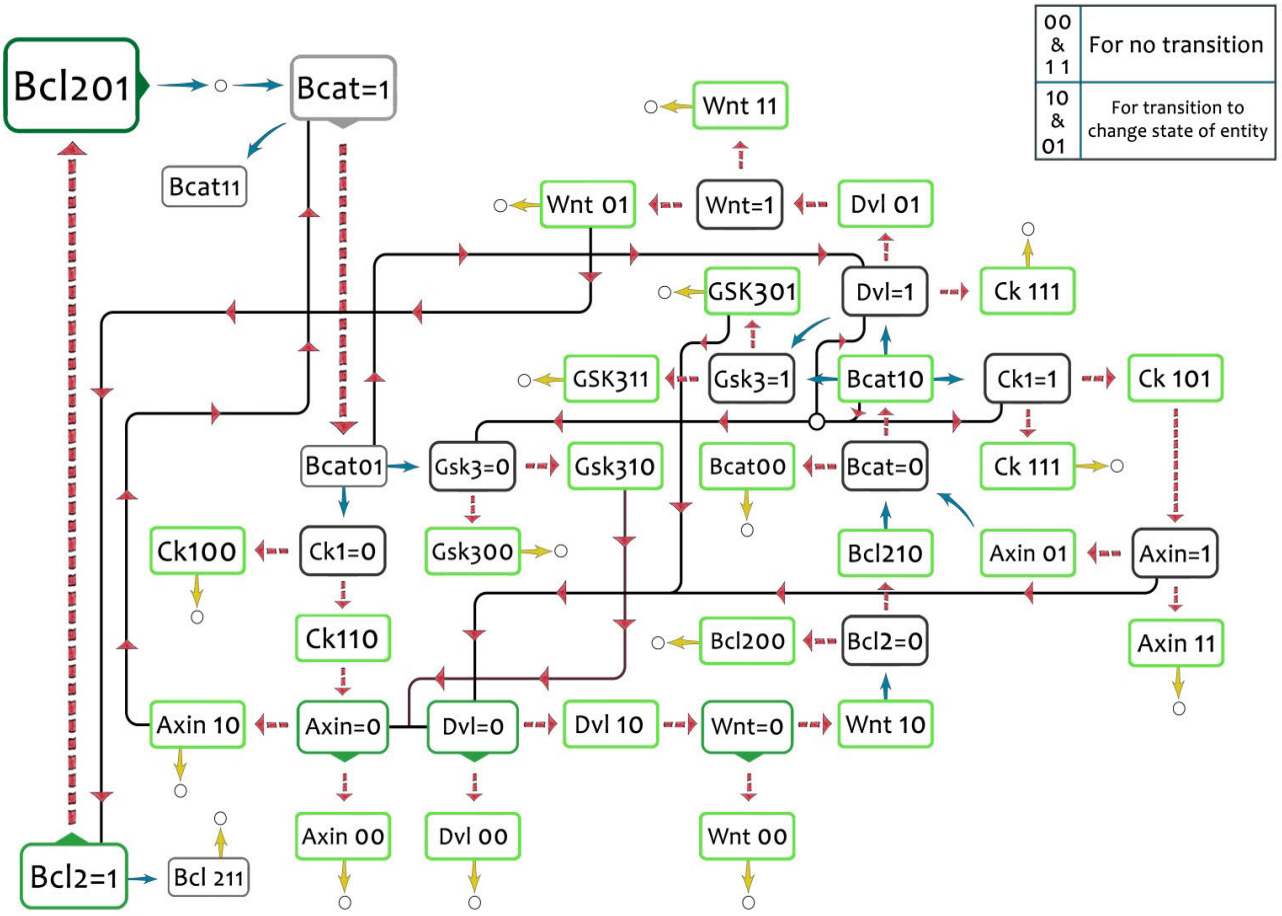

FIGURE 6. Representation of underlying relationship of the components in the network, There are two (0, 1) possibilities for each components where 1 or 0 leads the pathway to different paths.

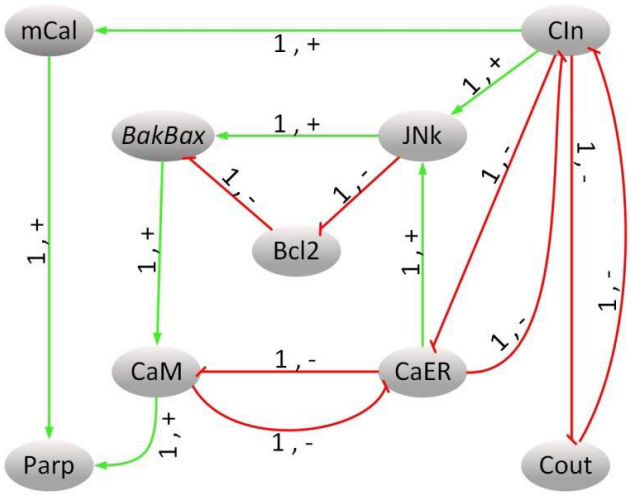

FIGURE 7. Biological regulatory network of *Wnt/Ca*$^{2+}$ Pathway consists of nine nodes, which are interconnected via vertices.

includes the entities *Bcat, Bcl2, Dvl, Gsk3, Axin, Ck1* and *Wnt* and shows two stable states; diseased state in first row and healthy state in the second row. In the diseased state the *Gsk3*, *Axin and Ck1 are* overexpressed but the down regulation of these entities keeps the system in healthy state. Thus, *Gsk3, Ck1* and the *Axin* are marked as biomarkers of the ARVC and can be used as drug target [34]. Here a clear differentiation has been observed in the functional attributes of a normal biological system of *Wnt/β-catenin* pathway and the genetically altered disease pathway of *Wnt/β-catenin*. It can be viewed from the stable state below which is generated with non-mutated *Wnt/β-catenin* pathway for elucidation of normal functional attributes.

The homeostatic state (Non-mutated) of entities in Column-2 highlights the effect of absence of mutations and column 3 shows the presence of mutations on *Wnt/β-catenin* pathway in Table-3. The preconditioned mutations in the entities such as *Wnt, Dvl, Bcat* and *Bcl2* lead towards the diseased state. Their normal regulation steer the pathway towards physiological state. The entities column 3 in Table-3 represents the stable state generated in diseased state (Mutated) (column 3) represent the stable state generated in case of the mutated *Wnt/β-catenin* pathway, resulted by

TABLE 1. Automata network model's nodes and interconnections.

Nodes with states	Interconnections
Wnt [0,1]	*Bca t : 0→1 when Dvl=1 and Gsk3=0 and Ck1=0*
Dvl [0,1]	*Gsk3 : 0→1 when Dvl=0 and Axin=1*
Axin [0,1]	*Axin : 0→1 when Bcat=0*
Gsk3 [0,1]	*Wn t : 1→0 when bcl2=0*
Bcat [0,1]	*Bcl2 :0→1when Bcat=1*
Bcl2 [0,1]	*Ck1 : 1→0 when Axin=0*
Ck1 [0,1]	*Gsk3 : 1→0 when Dvl=0 and Axin=0*
	Gsk3 : 1→0 when Dvl=1
	Ck1 : 0→1 when Axin=1
	Bcl2 : 1→0 when Bcat=0
	Dvl : 1→0 when Wnt=0
	Dvl : 0→1 when Wnt=1
	Wnt : 0→1 when Bcl2=1
	Axin : 1→0 when Bcat=1
	Bcat : 1→0 when Dvl=0
	Bcat : 1→0 when Dvl=1 and Gsk3=0 and Ck1=1
	Bcat : 1→0 when Dvl=1 and Gsk3=1
	Bca t : 0→1 when Dvl=1 and Gsk3=0 and Ck1=0

TABLE 2. Fix point analysis of *Wnt/β-catenin model*.

	Bcat	*Bcl2*	*Ck1*	*Dvl*	*Gsk3*	*Axin*	*Wnt*
Diseased State(0)	0	0	1	0	1	1	0
Homeostatic State(1)	1	1	0	1	0	0	1

the static analysis extensively carried out over the complete pathway of *Wnt/β-catenin* by the Pint software in Table-3. Pathways encompassing states that are disease triggering entities are up-regulated and highly expressed on the basis of mutations that over all derive the *Wnt/β-catenin* pathway.

D. CUT SETS IN WNT/ β-CATENIN MODEL

The current technique allows formal consideration of large scale networks encompassing colossal number of entities with two local states either 0 or 1 being Boolean in nature.

TABLE 3. Stable states in case of non-mutated and mutated *Wntβ-catenin* signaling pathway.

Expression level	meostatic State (Nor Mutated) of Entities	Diseased State Mutated) of Entities
Up Regulated	*Wnt,Dvl,Bcat,Bcl2*	*Gsk3,Axin,Ck1*
Down Regulated	*Gsk3,Axin,Ck1*	*Wnt,Dvl,Bcat,Bcl2*

TABLE 4. Cut sets analysis.

SNO	Biomarkers	Cut sets
1	*Gsk3*	*Dvl: 0, Axin: 1, Bcat : 0*
2	*Axin*	*Bcat: 0*
3	*Ck1*	*Axin:1 , Bcat:0*

After static reachability speculation, Pint comes up with the listing of cut sets applied on the transitions that are generally interpreted upon the interacting graph as set of nodes whose projection is significant in order to reach the concerned state as stable state or disease state from an initial global state in a finite biological automata network. Then, impeding all these local states within the model, execute model analysis was conducted to check if the concerned reachability is still possible. If the reachability is impeded, it indicates that the definite cut set play a role in disrupting the reachability towards goal [34].

Computation of list of entities using cut sets function for the Automata Network has given us four entitie; these include Gsk*3*, *Axin, Dvl and Bca*t given in Table 4. The cut down on pathways leading to activation of *Dv*l and *Bcat* along with deactivation of *Axin* can save the system and can help them land in a healthy state. Their knockdown obstructs the reachability towards fix points, has been shown in Table 5. Another way is to knockdown*Bca*t which will support*Axin.* Analysis of cut sets has given us two entities *(Gsk3*, *Ck*1 and *Axin*) promising enough that they can act as potential Biomarkers, as shown in Table 4.

Boolean values of the cut sets *Gsk3, Axin* and *Ck1* are biomarkers indicate the states don't lead towards the reachability (diseased state) as shown in Table-5.

The reachability towards the diseased state is true or false by disabling the given cut set with state 0 or 1 of biomarkers *Gsk3, Axin* and *Ck1* are presented in Table-5 above.

E. IDENTIFICATION OF MUTATIONS TO CONTROL GOAL REACHABILITY

The Pint tool helps us to identify most effective entities which can be mutated to change their way of action in betterment of system. The induction of mutation can save the system from fatal conditions. There are three proposed mutated genes namely *Dvl*, *Axin* and *Bcat*. If we keep *Dvl* and *Bcat* de activated, it will activate *Axin* and the goal to keep *GSK3* activated will be achieved. The *Gsk3* value '1' causes the

TABLE 5. Cut sets effect on reachability.

Gsk3	*Dvl*: 0 →False *Dvl:* 1 →True *Axin*: 0 →True *Axin*: 1 →False *Bcat:* 0 →False *Bcat*: 1 →True
Axin	*Bcat:* 0 →False *Bcat*: 1 →True
Ck1	*Axin:* 0 →True *Axin*: 1 →False *Bcat:* 1 →False *Bcat:* 0 →True

TABLE 6. Mutations effect on reachability.

Goal	Mutation
Gsk3	*Dvl: 1, Axin: 0, Bcat : 1*

TABLE 7. Bifurcations effect on reachability.

Goal	Bifurcation-1	Bifurcation-2
Bcat=1	Dvl: 1 → 0 when Wnt=0	Ck1:0 →1 when Axin=1
Gsk3=1	Bcl2:0 →1 when Bcat=1	Wnt:0 →1 when Bcl2=1

TABLE 8. The automata network model of *Wnt/Ca*$^{2+}$.

CIn [0,1]	*JNK:1 → 0 when CIn=0*
	JNk:0 → 1 when CIn=1
	mCalpain:0 → 1 when CIn=1
CaER [0,1]	*Cout:1- → 0 when CIn=1*
	CaER:1 → 0 when CIn=1 and CaM=0
Cout [0,1]	*CaM:0 → 1 when CaER=1 and Bak&Bax=1*
JNk [0,1]	*mCalpain:1 → 0 when CIn=0*
Bcl2 [0,1]	*Parp:1 → 0 when CaM=0 and mCalpain=0*
Bax&Bak[0,1]	*Bak&Bax:0 → 1 when JNk=1 and Bcl2=0*
CaM [0,1]	*Cout:0 → 1 when CIn=0*
	Bak&Bax:1 → 0 when JNk=0
Parp [0,1]	*Bak&Bax:1 → 0 when JNk=1 and Bcl2=1*
mCalpain[0,1]	*Bcl2:1 → 0 when JNk=1*
	CIn:0 → 1 when CaER=0 and Cout=0
	CaM:1 → 0 when CaER=0
	CaM:1 → 0 when CaER=1 and Bak&Bax
	CIn:1 → 0 when CaER=0 and Cout=1
	CIn:1 → 0 when CaER=1

degradation of *Bcat* and thus results in apoptosis. These are the positive regulators of the network.

The desired state $Gsk3 = 1$ shows the diseased state of the pathway which will be prevented by mutations in the entities having values ($Dvl = 1$, $Axin = 0$, $Bcat = 1$) and vice versa are denoted in Table-6 and the respective output figures of Jupyter Notebook using Pint code are mentioned in the Supplementary file.

F. BIFURCATION OF WNT/β-CATENIN MODEL

The bifurcations are hidden remedies in the pathways which when adopted can save the system from deadlock state. We have identified two trajectories which are deviating from the diseased state towards the desired state. The bifurcations in Table-7 showed that we can digress the pathway through *Gsk3* and *Ck1* by keeping expression level of *Wnt* and *Axin* as 0 and 1 respectively. It can contribute towards a healthy state by moving away from diseased state. The other bifurcation can be achieved by activating *Bcl2* when *Bcat* is 1 and through *Wnt* if *Bcl2* are kept 1.

There are two goals one is homeostatic state ($Bcat = 1$) and other is diseased state ($Gsk3 = 1$) in the *Wnt/β-Catenin* pathway its s bifurcations are presented in the above Table-7 which prevent them from respective desired states. The Bcat = 1 showing the healthy state while diseased state shown by Gsk3 = 1.

G. DEPENDENCY GRAPH OF WNT/β-CATENIN MODEL

The dependency graph of *Wnt/β-catenin* pathway gives the transparent view of all the possible trajectories among the components in the network to support the drug targets analysis has been performed in above sections and presented in the Figure 5 below.

H. LOCAL GRAPH OF CAUSALITY OF WNT/β-CATENIN MODEL

Here, we can find the prior states and their concentration levels required to move towards the occurrence of desired state. Based on the understanding of the local causality graph, we have explored pathway for transitions which can divert the system from diseased to healthy state. The promising entities in this system are *Wnt, Bcl2, Bcat* and *Dvl*. The physiology of *Wnt* depends on activation of *Dvl, Bcat* and *Bcl2* in a successive manner.

There is another complex pathway which involves *Wnt* regulation and revolves around *Bcat* entity. In this pathway, activation of *Bcl2, Bcat* and *Dvl* leads to activation of *Wnt*. One of the promising entities here is *Bcat*, as it is controlling four entities directly *Dvl, Gsk3, Axin* and *Ck1*. The activation of the former is moving towards healthy physiology while last three are inhibited by *Bcat*. So, inhibition of three pathological entities is required to keep the *Bcat* working, as shown in

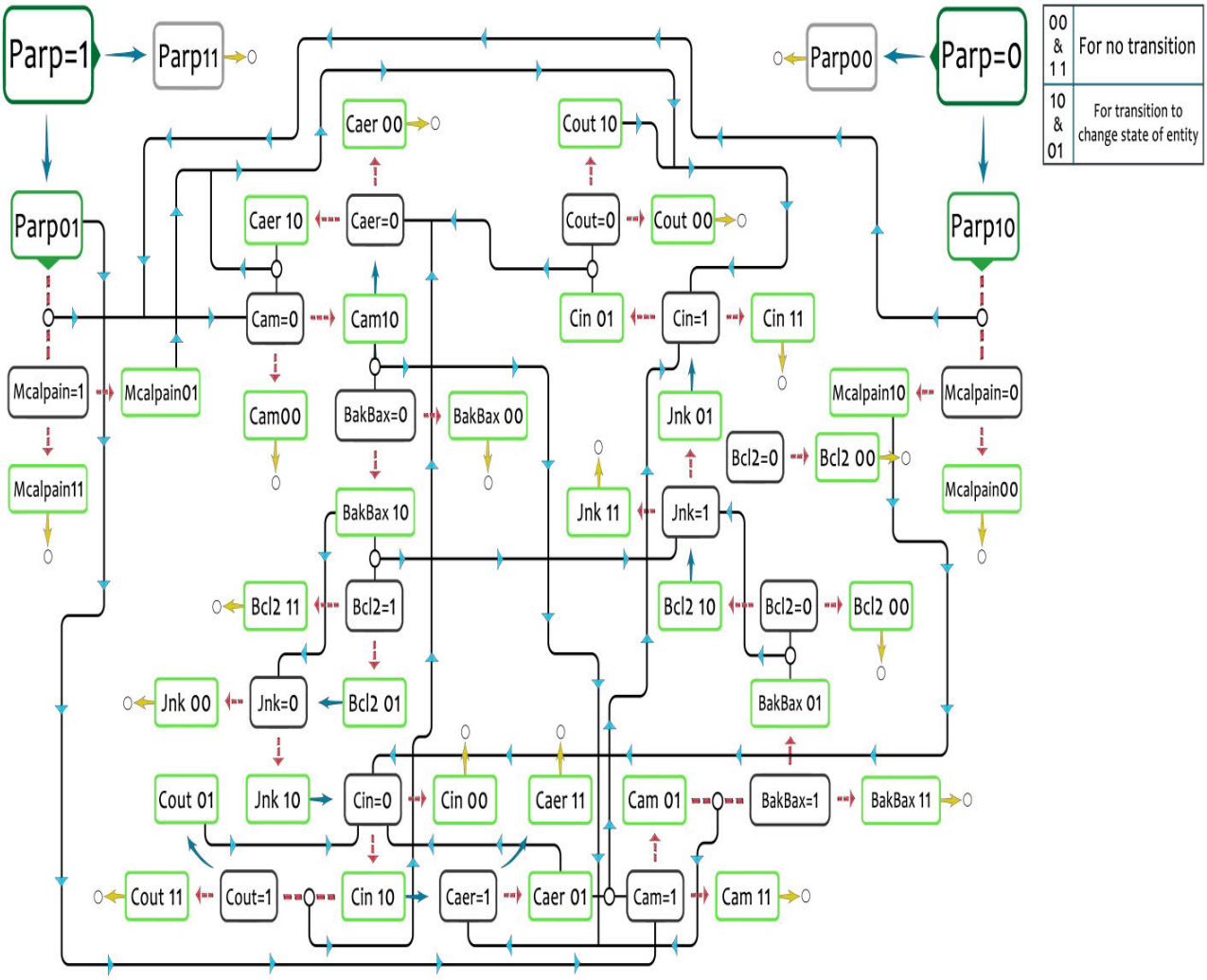

FIGURE 8. Representation of underlying relationship of the components in the network, There are two (0, 1) possibilities for each components where 1 or 0 leads the pathway to different paths.

the figure-6. another, important entity is a negative regulator i.e, *Gsk3*, activation of *Dvl*, *Wnt*, *Bcl2* and *Bcat* trigger inhibition of *Gsk3*. *Axin* is inhibited in the pathway by activating *Bcat* and inhibiting *Ck1(Axin activator of ck1)*. This transition system is critical as *Bcat* is controlling two negative entities (*Axin* and *Ck1*) simulateously.

I. REACHABLE STATE GRAPH OF WNT/β-CATENIN MODEL

The reachable state graph of wnt/β-catenin automata network model is shown in supplementary file figure 8, depicting two deadlock states after which there is no transition occurring. These deadlock states are shown by fixed point analysis in table-2 and verified by cut sets in table-4 and table-5 as well as by literature.

J. THE BRN OF WNT/CA^{2+} MODEL

Further, the ARVC's another BRN of *Wnt/*Ca^{2+} pathway as shown in Figure 7 below, is generated on GINsim which is abstracted from biological pathway in [32], is used for construction of its Stochastic Petri Net Model. In the light of detailed literature review the parameters are adjusted and evaluated that explains the overall interactions within the BRN of *Wnt/*Ca^{2+} pathway.

K. THE AUTOMATA NETWORK MODEL OF WNT/CA^{2+} PATHWAY

Further, the ARVC's another BRN of *Wnt/*Ca^{2+} pathway is generated on GINsim which is abstracted from biological pathway at [21]. In the light of detailed literature review the parameters are adjusted and evaluated that explains the overall interactions within the BRN of *Wnt/*Ca^{2+} pathway.

The equivalent Pint plain text results for Automata Network are represented in Table-8 here to show all the possible links of the local states.

TABLE 9. The fix points analysis of *Wnt/Ca*$^{2++}$ model.

	Bak&Bax	*Bcl2*	*CIn*	*CaER*	*CaM*	*Cout*	*JNk*	*Parp*	*mCalpain*
Diseased State(0)	1	0	1	0	0	0	1	1	1
Homeostatic State(1)	0	1	0	1	0	1	0	0	0

TABLE 10. Stable states in case of non mutated and mutated ARVC pathway.

Expression Level	Homeostatic State (Non Mutated) of Entities	Diseased State (Mutated) of Entities
Up Regulated	*Bcl2, CaER, Cout*	*Bak&Bax, CIn, JNk, Parp, mCalpain*
Down Regulated	*Bak&Bax, CIn, JNk, Parp, mCalpain*	*Bcl2, CaER, Cout*

TABLE 11. Cut sets analysis of *Wnt/Ca*$^{2+}$ model.

SNO	Biomarkers	Cut sets
1	*Parp=1*	*CIn:1, CaER:0, Cout : 0, CIn:0 and CaM:0, CIn:0 and mCalpain:1, CaER:1 and CaM:0, CaER:1 and mCalpain:1, Bcl2:0 and CaM:0, Bcl2:0 and mCalpain:1, Bak&Bax:1 and CaM:0, BakBax:1 and mCalpain:1, CaM[0,1],CaM:1 and mCalpain:1 .*
2	*CIn=1*	*CaER: 0, Cout: 0*
3	*JNk=1*	*CIn:1, CaER: 0, Cout: 0*
4	*Bak&Bax=1*	*CIn: 1, CaER: 0, Cout: 0, JNk: 1, Bcl2: 0*
5	*mCalpain=1*	*CIn: 1, CaER: 0, Cout: 0*

L. STABLE STATES (FIX POINT ANALYSIS) FOR WNT/CA^{2+} SIGNALING PATHWAY

The fix point analysis of Automata Network Model for *Wnt/Ca*$^{2+}$ signaling identified many biomarkers, which can be used further for therapeutic purpose. These biomarkers are shown in Table 9.

The Fix point Analysis which includes the list of all entities that are down regulated constantly to recover from disease to homeostatic state. The expression levels of entities as *Bak & Bax*, *CIn*, *JNk*, *Parp* and *mCalpain* are reduced which leads to the homeostatic state of the network.

The Non-Mutated and Mutated list of entities of *Wnt/Ca*$^{2+}$ pathway is shown in Table-9. When *CIn* is not overloaded in cytoplasm and the *Bcl2* is inhibited the *JNk*, the apoptotic components in the second row of the Table-10 are down regulated hence prevent the activation of apoptotic pathways. The stable states in Mutated *Wnt/Ca*$^{2+}$ pathway generated by Pint tool static analysis are represented in Table-10. The Ca^{2+} level in cytoplasm increased which will causing the activation of apoptotic pathways by the up regulation of given components in the first row of the table.

M. THE CUT SETS IN WNT/CA^{2+} MODEL

The Cut sets shows all the possible states reachable towards the goal and by disabling any state in the list makes it impossible to reach the goal/desired state as shown in Table 11 below.

The *Parp*, *CIn*, *JNk*, *Bak & Bax*, and *mCalpain* are the biomarkers that give the cut sets with Boolean values showing that the respective its state does not lead towards the reachability (diseased state) as mentioned in Table-11. The reachability towards the diseased state is confirmed to be true or false by disabling the given cut set with state 0 or 1 as shown in the above Table-12.

N. IDENTIFICATION OF MUTATIONS TO CONTROL GOAL REACHABILITY IN WNT/CA^{2+} MODEL

The Goals (*Parp and JNk*) are not reachable when the Ca^{2+} value is mutated as *CIn* to '0', *CaER* to '1' and *Cout* to '1' so the inhibition of *Parp* and *JNk* the pro apoptotic components causes the network recovery from disease and the anti-apoptosis gene *Bcl2* inhibited by *JNk* activation as shown in Table 13.

The change in Ca^{2+} level in the *CIn*, *CaER* and *Cout* entities will cause the activation of the apoptotic components *Parp* and *JNk*. And the $Bcl2 = 1$ is the goal to reach the homeostatic state of the pathway which is prevented by mutations in the entities with the values ($JNk = 1$) that are presented in the above table.

O. BIFURCATIONS IN WNT/CA^{2+} MODEL

The bifurcations shown in table-14 shows the alternate paths. We can save the system in case of higher *CIn* by digressing the pathway leading to *JNk* this will help to shut out concentration of *Bcl2* and hence system can move towards heathy state. The *Bcl2* anti-apoptotic component is not reachable when *CIn* is '1' overloaded into cytoplasm and the trajectory doesn't lead it to goal. another bifurcation is applied on diseased state when *JNk* is high, by maintaining homeostasis of Ca^{2+} ions in between *CIn*, *Cout* and *CaER*. this will save from diseased state.

TABLE 12. Cut sets effect on reachability of biomarkers.

	Cut sets Disability effect on Reachability
Parp	*CIn:1* → False, *CaER:0* → False, *Cout : 0* → False, *CIn:0 and CaM:0* → False, *CIn:0 and mCalpain:1* → False, *CaER:1 and CaM:0* → False, *CaER:1 and mCalpain:1* → False, *Bcl2:0 and CaM:0* → False, *Bcl2:0 and mCalpain:1* → False, *Bak&Bax:1 and CaM:0* → False, *BakBax:1 and mCalpain:1* → False, *CaM[0,1]* → False, *CaM:1 and mCalpain:1* → False, *BakBax:0 and mCalpain:0* → True.
CIn	*CaER:0* → False, *Cout: 0* → False
JNk	*CIn:1* → False, *CaER: 0* → False, *Cout: 0* → False *Cout: 1* → True.
Bak&Bax	*CIn: 1* → False, *CaER: 0-* → False, *Cout: 0* → False, *JNk: 1* → False, *Bcl2: 0* → False *CaER: 1* → True, *JNk: 1* → True.
mCalpain	*CIn: 1* → False, *CaER: 0* → False, *Cout: 0* → False *Cout: 1* → True.

TABLE 13. Mutations effect on reachability.

Goal	Mutation
Parp=1 and JNk=1	*CIn:0, CaER:1, Cout : 1*
Bcl2=1	*JNk: 1*

TABLE 14. Bifurcations effect on reachability.

Goal	Bifurcation-1	Bifurcation-2
Bcl2=1	*JNk: 0 → 1 when CIn=1*	*Nil*
JNk=1	*Cout:0 → 1 when CIn=0*	*CaER: 0 → 1when CIn=0*

P. LOCAL GRAPH OF CAUSALITY OF WNT/CA2+

The local graph of casuality help us observe all the possible trajectories towards a healthy state and diseased state. Starting with Calcium regulation, it is controlled by *CIn*, *Cout*, *CaM* and *CaER*. There should be an equilibrated concentration gradient between *CIn* and *Cout*. Concentration level of *CIn* is maintained by movement of Ca^{2+} ions into extracellular space i.e, into *Cout* Figure 8. If $CIn = 1$, (intracellular Calcium) it stimulates movement of extra Ca^{2+} ions into *Cout* (extracellular space) as well as to *CaER* (endoplasmic reticulum). Extra Ca^{2+} ions can be stored into *CaER*, they can move to *CaM* (Mitochondria) and can move back to *CIn* in case of pathological condition, Figure 8. As Ca^{2+} ions in *CIn* increased, they can stimulate *Bak & Bax*, *JNk* and inhibit *Bcl2* and they can contribute to accumulation of calcium in *CIn*. A disease causing gene *PARP* is activated by up regulation of *CIn* and *mCALPAIN*, Figure 9.

Q. DEPENDENCY GRAPH OF WNT/CA2+

The dependency graph of *Wnt/*Ca^{2+} pathway as presented in Figure 9, gives the transparent view of all the possible trajectories among the components in the network to support the drug targets analysis which has been performed in above sections.

R. THE REACHABLE STATE GRAPH OF WNT/CA2+

The reachable state graph of *Wnt/*Ca^{2+} gives two deadlock states after which there is no transition occurred. These dead lock states are shown by fixed point analysis in Table-9 and

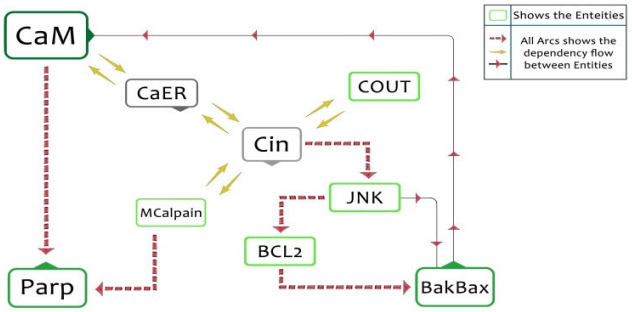

FIGURE 9. The above graph shows the entities of model as shown in Figure-8 are dependent for positive or negative regulation in such manner.

verified by cut sets in Table-11 and Table-12 well as by literature and show graphically in supplementary file Figure 9.

IV. CONCLUSION AND FUTURE WORK

ARVC is considered to be one of the lethal diseases which is growing in number each day. Some of the reasons behind this increase is the unhealthy life style, mutations in genes, dysregulation of signaling networks. Due to the impediment of foregoing computational techniques, the complete dynamics of these extensive signaling pathways could not be studied well previously. The remarkable Process Hitting framework with Pint software contributes in this regard by providing a wide range of Biomarkers for disease. Moreover, the novel drug targets designated by the results of the Pint software accords with a sound therapeutic strategy to combat the disease.

In ARVC the two signaling pathways *Wnt/β-catenin* and *Wnt/Ca*$^{2+}$ involving the responsible components as indicated by detailed literature review and the Static analysis of both the pathways gives some important insights about the therapeutic targets for treatment as well as components for prevention from disease. The qualitative modeling of *Wnt/β-catenin* BRN with the fix points, cut sets, mutations and bifurcations analysis indicate that *Gsk, Axin and Ck1* proteins are potential biomarkers of ARVC disease and proposes that the disease can be prevented by the down regulation of these components and up regulation of *Wnt, β-catenin* and *Dvl* proteins.

The *Wnt/Ca*$^{2+}$ signaling pathway analysis by the same above-mentioned procedure presents that the *Bak & bax, JNk, Parp, mCalpain* and *CIn* are the biomarkers of the ARVC, which can be used as drug targets. Moreover, it is indicated that gene *Bcl2* in both *Wnt/β-catenin* and *Wnt/Ca*$^{2+}$ networks perform a vital role in anti-apoptosis to prevent the occurrence of ARVC. The collective pathway of the *Wnt/β-catenin and Wnt/Ca*$^{2+}$ models can be constructed by the use of *Bcl2* and *Wnt* which are the common positive regulator for ARVC treatment as drug target.

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