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PETRI NET-BASED SIMULATION OF EFFECTIVE TARGETS FOR β-GLOBIN DISORDERS

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Abstract. β-thalassemia, sickle cell anemia and other human β-globin disorders are among major sources of mortality and morbidity in the world. Current options to the treatment of human β-globin disorders, however, have not been progressed to the level of widespread and efficient clinical therapy. Contemporary approaches of target-based drug discovery and gene therapy have made noteworthy, albeit slow, progress in treatment of β-globin disorders. Reactivation of γ-globin gene in adulthood has proven to be efficient measure for improvement of severe forms of β-thalassemia and sickle cell anemia. It has also been observed that reduction of α-globin gene expression is equally plausible approach to alleviating severity of β-thalassemia. In this study, we propose a strategy for β-globin disorders, which is centered upon induction of γ-globin gene expression as well as reduction of free and toxic α-chains. We use Snoopy software tool and hybrid functional Petri nets to construct quantitative model of fetal-to-adult hemoglobin switching network, validate the model with qPCR data available for known treatments, perform simulations to compare the efficacy of the proposed strategy with the ones obtained for already existing drug or gene therapies. The strategies are compared in accordance with $F(\alpha, \beta, \gamma) = \alpha - (\beta + \gamma) + c$, a formula that we devise to measure the effectiveness of a strategy. Simulation results show that our strategy is the optimal as it leads to the minimal value of the function $|F(\alpha, \beta, \gamma)|$ and therefore has more potential beneficial therapeutic effects on β-globin disorders.

Keywords: Quantitative modelling, hybrid functional Petri net, fetal-to-adult hemoglobin switching networks, RNAi-mediated therapy, target-based drug discovery.
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1 Introduction

Although β-thalassemia is one of the first identified molecular diseases, its management is still far from being sufficient. The current curative options for β-thalassemia and sickle-cell disease (SCD) include allogeneic bone marrow transplantation (Michlitsch & Walters, 2008), regular lifelong red blood cell transfusion (Porter & Shah, 2010) and gene therapy (Persons, 2010). Unfortunately, none of these options has progressed to the level of worldwide successful clinical therapy (Porter & Shah, 2010). Therefore, search for efficient therapy of β-globin disorders is still ongoing.

Fetal hemoglobin (HbF), which is the major hemoglobin during fetal life, is gradually replaced by adult hemoglobin (HbA) during infancy. One way to alleviate the severity of β-thalassemia is through substitution of defectively or insufficiently produced β-globin chains in HbA by an increase of γ-globin chains in HbF. Target-based drug therapy and RNAi-based gene therapy are existing options for induction of γ-globin gene and HbF expression. A number of

drugs currently available or in clinical trials have been tested for induction of γ-globin gene expression (Macari et al., 2013; Dahllöf et al., 2015; Rao-Bindal et al., 2013; Dai et al., 2014; Shearstone et al., 2013). Using Petri net involved quantitative modeling we compare the effect of these drugs on γ-globin gene induction and show that multiprotein complex of Erythroid Transcription Factors (ETF) turns out to be more efficient drug target as its silencing leads to greater induction of γ-globin gene compared to the ones discussed in aforesaid research works Mehraei et al. (2016). RNAi-mediated therapeutic strategies inducing γ-globin gene expression include: reducing methyl-binding domain (MBD2) mRNA expression by siRNA-mediated knockdown of MBD2 (Gnanapragasam et al., 2011); shRNA-mediated knockdown of myeloblastosis (Myb) followed by silencing of Kruppel-like transcription factor 1 (KLF1) and B-cell lymphoma/leukemia 11A (BCL11A) mRNAs (Roosjen et al., 2014); shRNA-mediated knockdown of BCL11A followed by silencing of KLF1 and BCL11A mRNAs (Roosjen et al., 2014); siRNA-mediated knockdown of chromodomain helicase DNA binding protein 4 (CHD4) followed by silencing of KLF1 and BCL11A mRNAs (Amaya et al., 2013). Using Petri net technologies we propose a potential RNAi-mediated strategy of inhibiting BCL11A, friend of GATA1 (FOG1) protein and HDAC1/2 mRNAs and show that this strategy is more efficient compared to the ones described above as it yields induction of more γ-globin gene (Bashirov & Mehraei, 2017).

The strategies mentioned above might give reasonable assurance of the reactivation of HbF and be helpful for treating β-globin gene disorders in many cases, but they are not sufficient alone to create a convincing treatment option. One important fact that we should take into account as well, is related to excess of free α-globin chains in patients with β-globin gene disorders. Thus, decreasing the concentration of toxic free α-globin chains should be considered as potential pathway to reach improved treatment option for such diseases. It was reported that natural reduction of free α-globin chains in patients with β-thalassemia was in their advantage in terms of side effect of their lack of β-globin production (Mettananda et al., 2015).

α-hemoglobin stabilizing protein (AHSP) is a chaperone molecule which binds to free α-globin chain of HbA and decreases level of free toxic monomeric α-subunits of HbA and avoids ineffective erythropoiesis consequently Mollan et al. (2013). Therefore, AHSP can be considered as a target to be beneficial for those who suffer from β-globin gene related diseases such as β-thalassemia and SCD.

In this research, we measure comparative efficacy of three strategies first for a minor case, β^+, and then for a severe case, β^0, of β-globin gene disorder. These strategies (1) knock down KLF1 gene expression by Simvastatin and tBHQ, the two drugs in clinical trials (Macari et al., 2013), (2) down regulate BCL11A and SOX6 gene expression using ACY957, a drug in clinical trials (Rao-Bindal et al., 2013), and (3) knock down MBD2 gene expression using MBD2 siRNA (Gnanapragasam et al., 2011). In order to reach more efficient potential treatment, we propose a compound strategy which not only induces γ-globin gene expression, but also avoids excessive free toxic α-globin chains by increasing AHSP gene expression with RNA activation (RNAa) method. Then we use Snoopy tool (Heiner et al., 2012) to create the closest approximation of fetal-to-adult hemoglobin switching network in terms of quantitative modeling with hybrid functional Petri nets (HFPN), validate the model with available qPCR data, and finally perform simulations to identify optimal compound strategy leading to improved treatment of β-globin gene disorders. We devise a function $F(\alpha, \beta, \gamma) = \alpha - (\beta + \gamma) + c$ to measure the effectiveness of the strategies including the compound ones and justify the reason why this formula provides unique criteria for measurement.

The paper is organized as follows. We start with introducing the molecular mechanism driving human fetal-to-adult hemoglobin switch network. After that, we present our HFPN model of human fetal-to-adult hemoglobin switch network. Following this, we discuss the computational validation of the model based on known wet lab results and present our target-based drug discovery strategy. Finally, we summarize our findings.

2 Biological context

2.1 Fetal-to-adult hemoglobin switch mechanism

Human fetal-to-adult hemoglobin switching network holds the key to identifying novel targets for gene therapy of β-globin disorders. BCL11A and Myb as transcriptional regulators bind to γ-globin DNA and repress its transcription. It was reported that shRNA-mediated knockdown of BCL11A and Myb separately lead to derepression of γ-globin gene (Roosjen et al., 2014). NuRD, nucleosome remodeling deacetylase complex of HDAC1/2, MBD2 and CHD4 (Mi2β), contributes to γ-globin gene silencing by forming a complex with BCL11A. It is known that siRNA-mediated knockdown of MBD2 increases γ-globin gene expression (Gnanapragasam et al., 2011). It has also been observed that siRNA-mediated knockdown of CHD4 significantly induces γ-globin gene expression (Amaya et al., 2013). SOX6, GATA1, FOG1 as well as HDAC1/2 bind to BCL11A and thereby contributing repression of γ-globin gene expression.

2.2 RNAi-mediated gene knockdown vs RNAa-based gene induction

RNA-interference (RNAi) is a post-transcriptional phenomena that turns off the gene using its own DNA sequence. Small interfering RNA (siRNA) and short hairpin RNA (shRNA) are current approaches of mediating the RNAi effect. RNAi starts with cut of long double stranded RNA (dsRNA) into fragments of 20-25 nucleotide siRNAs. After that specified siRNA enters into the cell and incorporates into RNA-induced silencing complex, which in turn separates its strands. Then a guided strand pairs with a complementary sequence in mRNA, causing subsequent cleavage of the target mRNA. This consequently blocks further accumulation of the protein, resulting in decrease of its levels, and eventual knockdown. shRNA expressed in the nucleus is then exported to the cytoplasm where it is converted into siRNA by removal of unnecessary fragments.

Recent studies have found that dsRNA can also activate gene expression by targeting gene regularity sequences (Huang et al., 2010), a mechanism that has been termed small RNA-induced gene activation and abbreviated as either saRNA or RNAa, for short. RNAa is both transcriptional and post-transcriptional gene silencing mechanism. Although many questions about RNAa mechanism still remain unanswered and the accurate details of its mechanism needs to be clarified, there have been useful studies to put insights on this matter. One of the interesting interpretation of how this phenomenon works is proposed in Portnoy et al. (2011).

To reach an ideal treatment for β-globin gene disorders such as β-thalassemia or SCD reduction of toxic free α-chains must be considered together with induction of γ-globin gene expression. On the other hand, reducing α-chains more than enough may lead to phenotype of patients who are suffering from α-thalassemia where excess of β-chains in HbA and γ-chains in HbF are the reasons for it (Galanello et al., 2011). Thus, while using γ-globin induction approach which is based on fetal-to-adult hemoglobin switching developmental stage, we should be careful about the ratio among α-, β-, γ-chains in order to reach an ideal treatment for β-globin gene disorders.

We exploit hybrid functional Petri net (HFPN) as computational platform to create quantitative model of human fetal-to-adult hemoglobin switch network, and perform a series of simulations in accordance with the above strategies. The simulation results for strategies (1)-(5) show that our model is consistent with available qPCR data, demonstrating expected distribution of mRNA and protein concentrations. The series of simulation results we carried out reflect the major aim of the research which is finding the optimal strategy leading to maximum γ-globin mRNA fold increase. Comparative analysis of the simulation results on γ-globin mRNA upregulation shows that the strategy (6) is the optimal case as it leads to the highest level of γ-globin mRNA concentration.

Table 1: Description of biological components

Entity name	**Entity type**	**Variable**	**Value**	**Type**
MybmRNA	Continuous	m1	0	Double
Myb	Continuous	m2	0	Double
KLF1mRNA	Continuous	m3	0	Double
KLF1	Continuous	m4	0	Double
BCL11AmRNA	Continuous	m5	0	Double
BCL11A	Continuous	m6	0	Double
HDAC1/2mRNA	Continuous	m7	0	Double
HDAC1/2	Continuous	m8	0	Double
MBD2mRNA	Continuous	m9	0	Double
MBD2	Continuous	m10	0	Double
CHD4mRNA	Continuous	m11	0	Double
CHD4	Continuous	m12	0	Double
NuRD	Continuous	m13	0	Double
BCL11A_NuRD	Continuous	m14	0	Double
GATA1mRNA	Continuous	m15	0	Double
GATA1	Continuous	m16	0	Double
FOG1mRNA	Continuous	m17	0	Double
FOG1	Continuous	m18	0	Double
SOX6mRNA	Continuous	m19	0	Double
SOX6	Continuous	m20	0	Double
ETF	Continuous	m21	0	Double
BCL11A_NuRD_ETF	Continuous	m22	0	Double
γ-globin gene	Continuous	m23	0	Double
γ-globin_BCL11A_NuRD_ETF	Continuous	m24	0	Double
γ-globin mRNA	Continuous	m25	0	Double
γ-chain	Continuous	m26	0	Double
β-globin mRNA	Continuous	m27	0	Double
β-chain	Continuous	m28	0	Double
OCT1mRNA	Continuous	m29	0	Double
OCT1	Continuous	m30	0	Double
AHSP mRNA	Continuous	m31	0	Double
AHSP	Continuous	m32	0	Double
α-globin mRNA	Continuous	m33	0	Double
α-chain	Continuous	m34	0	Double
AHSP_α	Continuous	m35	0	Double
Hemoglobin	Continuous	m36	0	Double
β^0	Discrete	m37	0/1	Boolean
β^+	Discrete	m38	0/1	Boolean
Simvastatin+tBHQ	Discrete	m39	0/1	Boolean
ACY-957	Discrete	m40	0/1	Boolean
MBD2 siRNA	Discrete	m41	0/1	Boolean
AHSP RNAa (β^0)	Discrete	m42	0/1	Boolean
AHSP RNAa (β^+)	Discrete	m43	0/1	Boolean
Delay	Discrete	m44	1	Boolean

Table 2: Description of the processes

Phenomenon	**Process**	**Type**	**Rate**
Transcription of Myb mRNA	T1	Continuous	1
Transcription of Myb	T2	Continuous	m1*0.1
Transcription of KLF1 mRNA	T3	Continuous	m2*0.1
Translation of KLF1	T4	Continuous	m3*0.1
Transcription of BCL11A mRNA	T5	Continuous	m4*0.1
Translation of BLC11A	T6	Continuous	m5*0.1
Transcription of HDAC1/2 mRNA	T7	Continuous	1
Translation of HDAC1/2	T8	Continuous	m7*0.1
Transcription of MBD2 mRNA	T9	Continuous	1
Translation of MBD2	T10	Continuous	m9*0.1
Transcription of CHD4 mRNA	T11	Continuous	1
Translation of CHD4	T12	Continuous	m11*0.1
Binding of HDAC1/2, MBD2 and CHD4	T13	Continuous	m8*m10*m12*0.1
Binding of NuRD with BCL11A	T14	Continuous	m6*m13*0.1
Transcription of GATA1 mRNA	T15	Continuous	1
Translation of GATA1	T16	Continuous	m16*0.1
Transcription of FOG1 mRNA	T17	Continuous	1
Translation of FOG1	T18	Continuous	m18*0.1
Transcription of SOX6 mRNA	T19	Continuous	1
Translation of SOX6	T20	Continuous	m20*0.1
Binding of GATA1, FOG1 and SOX6	T21	Continuous	m16*m18*m20*0.1
Binding of ETF with BCL11A_NuRD	T22	Continous	m14*m21*0.1
Activation of γ-globin gene	T23	Continuous	0.0075
Transcription of γ-globin mRNA	T24	Continuous	m23*0.1
Translation of γ-chain	T25	Continuous	m25*0.1
Binding of BCL11A_NuRD_ETF with γ-globin gene	T26	Continuous	m22*m23*5
Activation of β-globin mRNA by KLF1	T27	Continuous	m4*0.00174
Activation of β-globin mRNA by GATA1	T28	Continuous	m16*0.00174
Activation of β-globin mRNA by FOG1	T29	Continuous	m18*0.00174
Translation of β-chain	T30	Continuous	m27*0.1
Transcription of OCT1 mRNA	T31	Continuous	0.0199
Translation of OCT1	T32	Continuous	m29*0.1
Activation of AHSP mRNA by KLF1	T33	Continuous	m4*0.0199
Activation of AHSP mRNA by GATA1	T34	Continuous	m16*0.0199
Activation of AHSP mRNA by OCT1	T35	Continuous	m30*0.000506
Translation of AHSP	T36	Continuous	m31*1
Transcription of α-globin mRNA	T37	Continuous	0.0054
Translation of α-globin chain	T38	Continuous	m33*0.1
Binding of AHSP and α-chain	T39	Continuous	m32*m34*0.1
Binding of AHSP_α-chain and γ-chain	T40	Continuous	m26*m35*0.1
Binding of AHSP_α-chain and β-chain	T41	Continuous	m28*m35*0.1
β-globin gene mutation	T42	Continuous	m27*0.047
Binding of Simvastatin+tBHQ to KLF1 mRNA	T43	Continuous	m3*0.16

Binding of ACY-957 to BCL11A mRNA	T44	Continuous	m5*0.2
Binding of ACY-957 to SOX6 mRNA	T45	Continuous	m19*1.8
Binding of MBD2 siRNA with MBD2 mRNA	T46	Continuous	m9*0.8
Binding of AHSP RNAa with AHSP mRNA (β^0)	T47	Continuous	m31*0.18
Binding of AHSP RNAa with AHSP mRNA (β^+)	T48	Continuous	m31*0.02
Activation of KLF1 mRNA by CHD4	T49	Continuous	m12*0.1
Delay in transcription of β-globin mRNA	T50	Deterministic	25

Table 3: Degradations in the HFPN model of human fetal-to-adult hemoglobin switch.

Phenomenon	**Process**	**Type**	**Rate**
mRNA degradation	d1–d14	Continuous	mi*0.1
Protein degradation	d15–d34	Continuous	mi*0.01

3 Creating the model

3.1 Quantitative modeling with Petri nets

Biological systems are characterized by rapid molecular interactions between genes, mRNAs, proteins and their complexes. It is quite natural to express, interpret and predict the characteristics of biological systems in terms of quantitative change of biological components. Creating quantitative models is therefore crucial to make meaningful deductions regarding the behavior of biological systems.

Over the last two decades Petri net technologies have been extensively used for creating quantitative models of metabolic networks, signal transduction pathways and gene regulatory networks. When modeling biological processes, Petri net components such as places, transitions and arcs are respectively used to represent biological entities, biological phenomena and flow of biological information. Concentration, reaction rate and reaction stoichiometry are usually assigned as parameters to places, transitions and arcs, respectively. Biological systems often comprise continuous, Boolean and discrete processes. Corresponding Petri nets are expected to be continuous, hybrid and functional. Numerous signal transduction pathways, metabolic networks and gene regulatory networks have been successfully modeled and simulated in terms of Petri net technologies. Particularly, we have used HFPN to create a quantitative model of molecular interactions between major regulators of fetal-to-adult hemoglobin switching network (Mehraei et al., 2016; Bashirov & Mehraei, 2017), p16-mediated signaling pathway (Akçay et al., 2015; Bashirov & Akçay, 2018), SMN protein production network (Duranay et al., 2017; Bashirov et al., 2019).

3.2 Model components

We create HFPN model based on fetal-to-adult hemoglobin switching network (Bauer et al., 2012) and qPCR data available to date (Macari et al., 2013; Shearstone et al., 2013; Gnanapragasam et al., 2011). The screenshot of Snoopy software illustrating HFPN model is represented in Fig. 1.

The model is composed of 36 continuous entities (places) and 8 discrete entities (see Table 1). The discrete entities represent presence/absence of β-globin gene mutation, drug treatments with Simvastatin and tBHQ, as well as with ACY-957, and also MBD2 siRNA. The delay in the expression of β-globin gene relatively to expression of α-globin and γ-globin genes is also expressed as discrete entity. The continuous entities represent γ-globin gene, mRNAs, proteins, and their complexes. The model comprises 83 continuous processes; 49 processes representing activation, transcription, translation and binding (see Table 2) and 34 processes standing for natural degradation of mRNAs and proteins (see Table 3). The only discrete process indicates

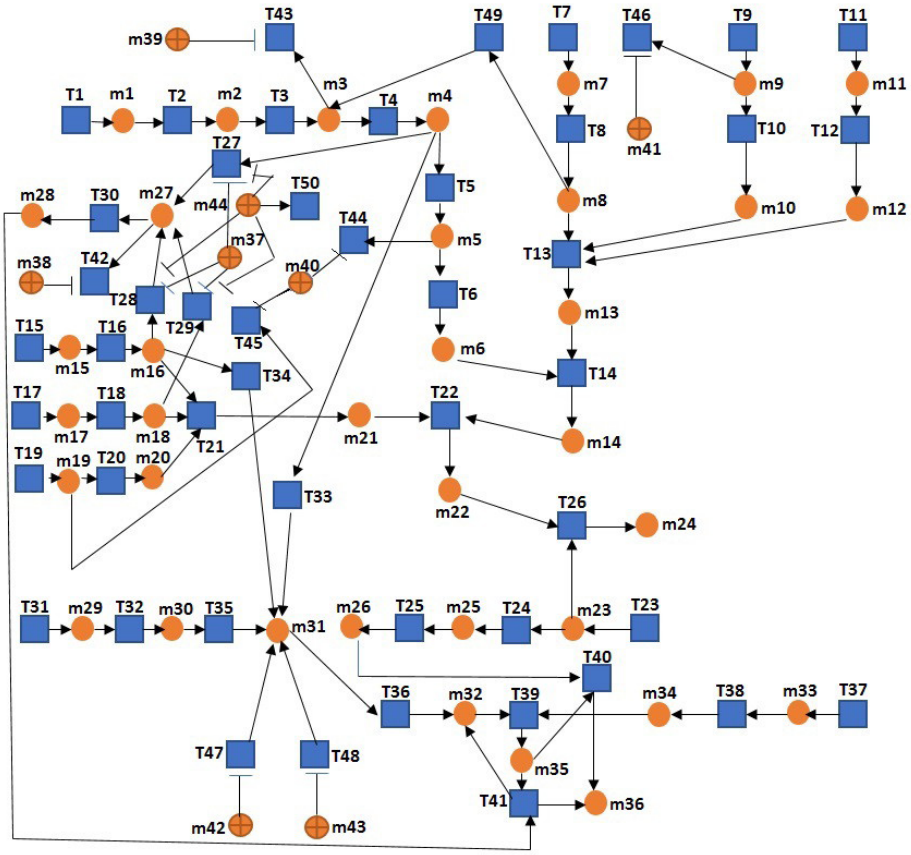

Figure 1: HFPN model of fetal-to-adult hemoglobin switching network

delay in the expression of β-globin gene. The model uses 127 regular arcs (edges) and 13 inhibitor arcs to indicate interactions between components and processes.

In our model we assume that a protein is produced in accordance with the central dogma of biology. This is why, the initial concentrations are set to 0 (see data under "value" in Table 1), and transcription of DNA into mRNA is represented by source transition, which continuously feeds mRNA and consequently the protein. A discrete place takes values of 0 or 1 depending on whether or not specified treatment is applied.

It is quite often the case that due to external conditions two identical wet lab experiments not lead to identical observations. It is hard, therefore, to determine the rates solely based on wet lab observations. We adopt transcription and translation rates described in similar papers (Akçay et al., 2015; Mehraei et al., 2016), but those not following the routine rates are obtained by applying reverse engineering method to reach the closest fit for hemoglobin switching developmental process. In this study, we refer to wet lab observations for Simvastatin together with tBHQ, and ACY-957 drug treatments, and MBD2 siRNA-based treatment to validate our model. We carefully calibrate in silico results wherever and whenever possible to have clear trend and similarity between in silico results and qPCR data (Macari et al., 2013; Shearstone et al., 2013; Gnanapragasam et al., 2011). The process rates adopted in this work are indicated in Tables 2-3.

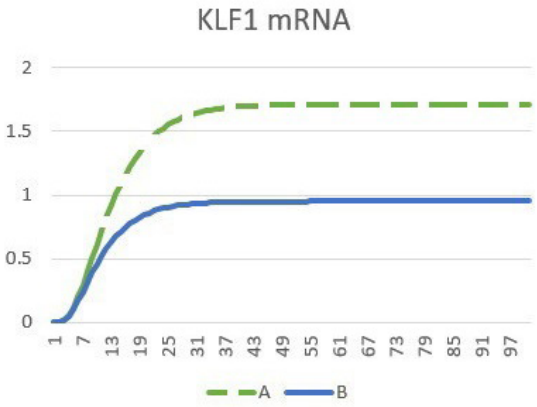

Figure 2: The simulation results for expression of KLF-1 mRNA for both β^0 and β^+ cases with 2 decimal point precision in (A) an untreated cell; (B) a cell treated with a combination of Simvastatin and tBHQ. Simulation results show that combination of Simvastatin and tBHQ decrease KLF1 concentration by 44%

Figure 3: The simulation results for expression of MBD2 mRNA for both Beta0 and β^+ cases with 2 decimal point precision in (A) an untreated cell; (B) a cell treated with MBD2 siRNA. Simulation results for treatment with MBD2 siRNA demonstrate decrease of MBD2 mRNA concentration from 5 to 1 by 5-fold

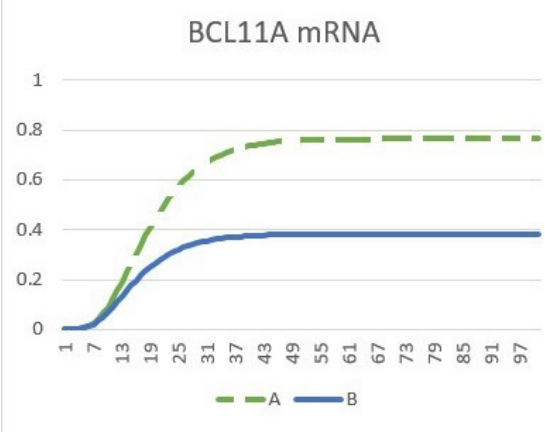

Figure 4: The simulation results for expression of BCL11A mRNA for both β^0 and β^+ cases with 2 decimal point precision in (A) an untreated cell; (B) a cell treated with ACY-957. Simulation results for treatment with ACY-957 show suppression of BCL11A mRNA by 2-fold

Figure 5: The simulation results for expression of SOX6 mRNA for both Beta0 and β^+ cases with 2 decimal point precision in (A) an untreated cell; (B) a cell treated with ACY-957. According to the simulation results, drug treatment with ACY-957 suppresses SOX6 mRNA expression by 10-fold over the untreated control, changing its levels from 5 down to 0.5

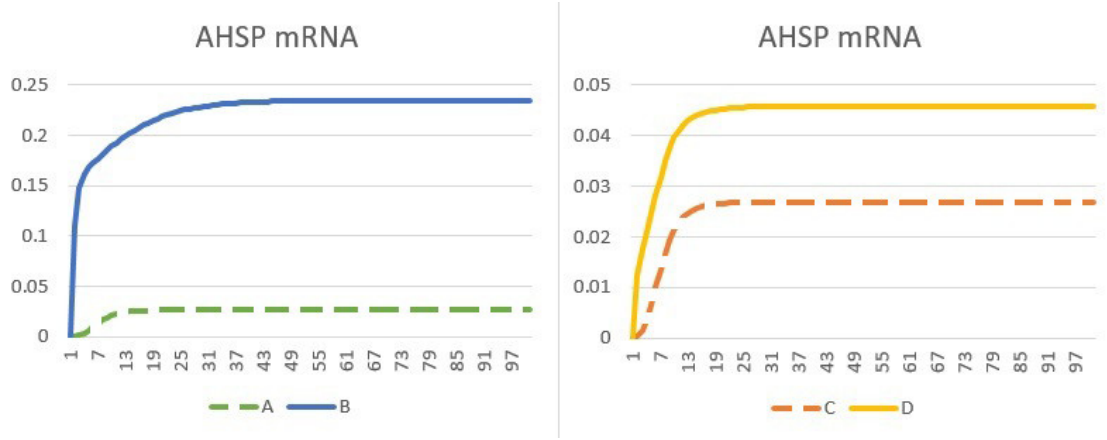

Figure 6: The simulation results for AHSP mRNA expression in a) an untreated cell (β^0); (b) a potential treated with AHSP RNAa (β^0); (c) an untreated cell (β^+); (d) a potential treated with AHSP RNAa (β^+). According to the simulation results, drug treatment with ACY-957 suppresses SOX6 mRNA expression by 10-fold over the untreated control, changing its levels from 5 down to 0.5

4 Numerical validation of the model

We plot the concentrations (on the y axis) versus time (on the x axis) measured in Petri time or pt, for short, with the time interval of 5 pt corresponding to 3 months of gestational age. In these plots, it is assumed that fetal life starts at 15 pt (0 months) and child is born at 30 pt (9 months). We measure a concentration at 70 pt, when its level clearly remains in a stable steady-state.

To find the closest fit to inclinations of β-, γ- and α-globin mRNA levels in fetal-to-adult hemoglobin switching developmental stage we have extrapolated the trends between wild-type β-genes family (Bauer et al., 2012). We consider two cases of β-globin disorder in order to choose the correct treatment. These are severe case, β^0, in which the production of β-chains is almost stopped and a minor one, β^+, in which β-globin chains are suspended, but not fully stopped. Based on simulation results, we find that ratio β/α is 99% for a healthy person, and it is 80% in the case of β^+. These results are in agreement with wet lab observations (Ranjbaran et al., 2014). Simulation results related to the ratios between AHSP, β-, γ- and α-globin gene expressions are

Table 4: Strategies for β-globin gene disorders (β^0).

Strategy	**α-chain**	**β-chain**	**γ-chain**	$\|F\|$
Untreated β^0	0.0213862	0	0.00217587	0.02553206
Simvastatin and tBHQ	0.0215868	0	0.00496968	0.02365175
ACY-957	0.0160068	0	0.0161024	0.00693903
MBD2 siRNA	0.0212424	0	0.00476992	0.02350711
AHSP RNAa + ACY-957	0.00813839	0	0.0153316	0.00015858

Table 5: Strategies for β-globin gene disorders (β^+).

Strategy	**α-chain**	**β-chain**	**γ-chain**	$\|F\|$
Untreated β^+	0.0214002	0.0207425	0.00289782	0.02553206
Simvastatin and tBHQ	0.0216037	0.0203003	0.00497709	0.00281789
ACY-957	0.0160744	0.055084	0.0161972	0.04817217
MBD2 siRNA	0.021255	0.0210743	0.00478446	0.00243087
AHSP RNAa + ACY-957	0.0186321	0.0208394	0.0153316	0.00015858

also validated in accordance with data provided in (Ranjbaran et al., 2014). When there is a lack of β-chains due to β-globin gene mutation drug treatments in primary human erythroid cells with Simvastatin, tBHQ, and combination of Simvastatin and tBHQ decrease KLF1 gene expression by 20%, 25%, and 44%, respectively, and induce γ-globin gene expression (Macari et al., 2013). As it can be seen from Fig. 2, the simulation results for KLF1 mRNA concentration is a good fit to corresponding wet lab observations.

To study human γ-globin gene regulation, experiments were carried out in CID (chemical inducer dimerization)-dependent mouse bone marrow cells carrying β-YAC (β-globin yeast artificial chromosome). It is broadly known that human γ-globin gene is repressed in these adult phenotype erythroid cells. It was also observed that siMBD2 treatment in CID cells reduces expression of MBD2 by approximately 80%, derepressing γ-globin expression (Gnanapragasam et al., 2011). The simulation results for MBD2 gene expression are shown in Fig. 3. By carefully calibrating the rate of binding between siMBD2 and MBD2 mRNA we achieved 5-fold decrease from 5 down to 1 for MBD2 mRNA concentration, which is numerically validated by wet lab observations (Gnanapragasam et al., 2011).

As it was reported, the treatment of CD71lowGlyAneg cells with ACY-957 decreases BCL11A and SOX6 gene expressions by 2- and 10-fold, respectively (Shearstone et al., 2013). The simulation results for BCL11A and SOX6 mRNA levels are respectively illustrated in Fig. 4 and Fig. 5. The simulation results for β^0 show a change of BCL11A mRNA concentration from 0.764096 down to 0.38212 by 2-fold and SOX6 mRNA concentration from 5 down to 0.5 by 10-fold, respectively. In the case of β^+, we observed a change of BCL11A mRNA levels by 2-fold from 0.762913 to 0.381529 and SOX6 mRNA levels by 10-fold from 5 down to 0.5. These results are in strong agreement with qPCR data (Shearstone et al., 2013).

In our proposed strategies, we have increased AHSP gene expression by potential RNAa technique in order to reduce free toxic α-subunits in human adult hemoglobin. The simulation results for our strategies show increase of AHSP levels by 8.7-fold from 0.0269213 to 0.234716 for β^0, and by 1.7-fold from 0.0262398 to 0.045885 in the case of β^+, respectively (see Fig. 6). Due to a lack of a wet lab observations we were not capable to compare and validate The simulation results for MBD2, BCL11A, and SOX6 mRNAs.

5 Identifying potential improved treatment for β-globin disorders

There is an obvious correlation between globin chain synthesis and severity of β-globin disorders. In normal population, globin chain synthesis demonstrates balanced production of α and non-α (β plus γ) chains (Ranjbaran et al., 2014). In patients affected by β-thalassemia and SCD, the balance is achieved by increased γ-globin production which in turn compensates deficit in β-globin mRNA. However, inducing γ-globin gene expression too much can lead to side effects (Roosjen et al., 2014), so that it is reasonable to keep total concentration of β- and γ-globin mRNAs at a constant level. There is also a relationship between the ratio of β- and α-globin mRNAs and β-thalassemia disease severity. It was reported that this ratio is 0.99 in normal controls, but decreases with increase of disease severity (Ranjbaran et al., 2014). In β-thalassemia patients, the balance between α- and non-α-globin expression is achieved by decreased α-globin gene expression. On the other hand, reducing α-globin expression excessively may result in phenotype in patients who deal with excess of γ chains before birth, and β chains after birth (Galanello et al., 2011). Bringing together all these observations we devise a unique function $F(\alpha, \beta, \gamma) = \alpha - (\beta + \gamma) + c$ to measure the effectiveness of a treatment, where c is expected to be a constant related to δ-chain concentration level. As the optimal treatment has to establish potential ideal balance between α-, β- and γ-globin mRNAs the most effective treatment can be found by minimizing $|F|$.

There were several attempts to find effective treatment for β-globin disorders by targeting components of fetal-to-adult hemoglobin switching network. Some of these treatments are discussed in Section 4. To the best of our knowledge the present research is the first work that proposes a compound strategy based on inducing γ-globin gene expression as well as reducing free α-globin chains. We compare the efficacy of treatments discussed in Section 4 with compound treatments by measuring the value of $|F|$ at 70 pt. After validating HFPN model for a healthy person with available qPCR data (Ranjbaran et al., 2014; Forget et al., 2009) and taking into account proportions of the chains in HbA, we find that $c = 0.00703463$. When F is a positive-valued ($F > 0$) there exist excessive free toxic α-subunits in HbA , and when it is a negative-valued ($F < 0$) there might be excessive γ-chains in HbF or β-chains in HbA. A potential optimal strategy is the one that prevents both unwanted cases. This is achieved when $|F|$ approaches to 0. This is main intention behind of considering $|F|$ rather than F and minimizing this function to determine an optimal strategy.

In the case of β^0, the simulation results for treatment with combination of Simvastatin and tBHQ, ACY-947 and MBD2 siRNA demonstrate increase of γ-globin mRNA by 1.9-, 6.9-, and 1.7-fold from 0.00217587 of normal control to 0.00409181, 0.0149824, and 0.00363567, respectively. The corresponding α-, β-, γ-globin chain concentrations and $|F|$ values are illustrated in Table 4. Treatment with ACY-947 obviously induces γ-globin gene expression more than the other two treatments, and also $|F|$ reaches its smallest value. Although the treatment with ACY-947 is more effective comparing to other two treatments, the value of $|F| = 0.00693903$ indicates presence of yet excessive free α chains in HbA. To minimize α chains in HbA, we performed simulations for AHSP RNAa technique followed up by drug treatment with ACY-957. The simulation results for our compound strategy show increase of AHSP mRNA levels by 8.7-fold (see Fig. 6) and decrease of α chains so that we obtained $|F| = 0.00015858$ (see Table 4). This is the minimum possible value of $|F|$ for the treatments considered in the present research including compound ones. Thus, our strategy not only leads to highest γ-globin gene expression induction, but also reduces free toxic α chains as much as possible. The analysis of numerous strategies has shown that our potential therapeutic strategy is the most beneficial as it minimizes $|F|$ (see Table 4).

In the case of β^+, simulation results are illustrated in Table 5. The simulation results for this case show 1.9-, 6.9-, and 1.7-fold induction of γ-globin gene expression from 0.00218212

of normal control to 0.00410155, 0.0150266, and 0.00364474 for treatments with a combination of Simvastatin and tBHQ, ACY-947, and MBD2 siRNA, respectively. We observe that drug treatment with ACY-947 leads to more γ-globin gene induction compared to the other two treatments. However, $|F|$ reaches its minimum value of 0.04817217 for MBD2 siRNA technique. Thus, we conclude that MBD2 siRNA technique along with AHSP RNAa technique the most prominent strategy in the case of β^+. Simulations performed for this compound strategy result in 1.7-fold increase in both γ-globin mRNA and AHSP mRNA levels and $|F| = 0.00015858$.

6 Discussion and conclusions

In the present work we exploit quantitative modeling with HFPN to identify the most prominent therapeutic strategy for minor and severe forms of β-globin disorders, promoting this innovation to the benefit of both reverse pharmacology and quantitative modeling with HFPN.

The present research needs to be assessed from the perspective of the rationalistic approach as we focus on determining optimal targets for target-based drug discovery, RNAi- and RNAa-mediated mechanisms, that leads to the maximum γ-globin gene induction and minimum production of free toxic α-globin chain. In the meantime, we try to shed light on how quantitative modeling with HFPN can be used to recognise targets for target-based treatment of β-globin disorders without specifying how this can be achieved from pharmacological point of view. Although it is not within the scope of this paper, as future prospects, we believe that a more detailed discussion of biological consequences should be considered in collaboration with a pharmacogenetics group. It should also be noted that the present approach can be successfully used for identifying optimal molecular targets not only for β-globin disorders but also for other diseases. By developing deterministic quantitative model we tried to create the closest approximation of underlying biological context based on rigorous review of biological literature available to date. We believe that our model is trustful enough as we tried to be as punctual as possible when constructing molecular interactions between the biological components of fetal-to-adult hemoglobin switching network. As a further work we are planning to integrate stochastic parameters to the model to see the effect of the noise to the underlying biological system.

References

Akçay, N.İ., Bashirov, R. & Tüzmen, Ş. (2015) Validation of signalling pathways: case study of the p16-mediated pathway. *J Bioinform. Comput. Biol.*, *13*(2), 1550007.

Amaya, M., Desai, M., Pnanapragasam, M.N. et al. (2013) Mi2β-mediated silencing of the fetal γ-globin gene in adult erythroid cells. *Blood*, *121*(17), 3493-3501.

Bashirov, R. & Akçay, N. (2018) Stochastic simulation-based prediction of the behavior of the p16-mediated signaling pathway. *Fundam. Inform.*, 160, 167-179.

Bashirov, R., Duranay, R., Şeytanoğlu, A., Mehraei, M. & Akçay, N. (2019) Exploiting stochastic Petri nets with fuzzy parameters to predict efficient drug combinations for Spinal Muscular Atrophy. *Turk. J. Electr. Eng. Co.*, 27, 4009-4022.

Bashirov, R. & Mehraei, M. (2017) Identifying targets for gene therapy of β-globin disorders using quantitative modeling approach. *Inform. Sciences*, 397-398, 37-47.

Bauer, D.E., Kamran, S.C. & Orkin, S.H. (2012) Reawakening fetal hemoglobin: prospects for new therapies for the β-globin disorders. *Blood, 120*(15), 2945-253.

Dahllöf, M.S., Christensen, D.P., Harving, M. et al. (2015) HDAC inhibitor-mediated beta-cell protection against cytokine-induced toxicity is STAT1 Tyr701 phosphorylation independent. *J. Interf. Cytok. Res.*, *35*(1), 63-70.

Dai, Y., Faller, D.V., Sangerman, J.I. et al. (2014) Multiple Oral Therapeutics Suppress Repressors (LSD-1, HDACs, and BCL11A) of Gamma Globin Gene Expression. *Blood*, *124*(21), 2687.

Duranay, R., Bashirov, R. & Şeytanoğlu, A. (2017) Simulation-based identification of optimal combination of drug candidates for spinal muscular atrophy. *Procedia Computer Science*, 120, 253-259.

Forget, B.G. & Hardison, R.C. (2009) The normal structure and regulation of human globin gene clusters, *In: Disorders of hemoglobin.* Cambridge University Press, New York, 46-61.

Heiner, M., Herajy, M., Liu, F. et al. (2012) Snoopy – a unifying Petri net tool. *LNCS*, 7347, 398-407.

Huang, V., Qin, Y., Wang, J. et al. (2010) RNAa is conserved in mammalian cells, *PloS one.* *5*(1), e8848.

Galanello, R. & Cao, A. (2011) Alpha-thalassemia. *Genet. Med.*, *13*(2), 83-88.

Gnanapragasam, M.N., Scarsdale, J.N. Amaya, M.L. et al. (2011) p66α-MBD2 coiled-coil interaction and recruitment of Mi-2 are critical for globin gene silencing by the MBD2-NuRD complex. *PNAS*, *108*(1), 7487-7492.

Macari, E.R., Schaeffer, E.K. West, R.J. et al. (2013) Simvastatin and t-butylhydroquinone suppress KLF1 and BCL11A gene expression and additively increase fetal hemoglobin in primary human erythroid cells. *Blood*, *121*(5), 830-839.

Mehraei, M., Bashirov, R. & Tüzmen, Ş. (2016) Target based drug discovery for β—globin disorders: drug target prediction implementing quantitative modeling with hybrid functional Petri nets. *J. Bioinform. Comput. Biol.*, *14*(4), 1650026.

Mettananda, S., Gibbons, R.J. & Higgs, D.R. (2015) α-globin as a molecular target in the treatment of β-thalassemia. *Blood*, *125*(24), 3694-3701.

Michlitsch, J.G. & Walters, M.C. (2008) Recent advances in bone marrow transplantation in hemoglobinopathies. *Curr. Mol. Med., 8*(7), 675-689.

Mollan, T.L., Banerjee, S., Wu, G. et al. (2013) α-hemoglobin stabilizing protein (AHSP) markedly decreases the redox potential and reactivity of α-subunits of human HbA with hydrogen peroxide. *J. Biol. Chem.*, *288*(6), 4288-4298.

Persons, D.A. (2009) Hematopoietic stem cell gene transfer for the treatment of hemoglobin disorders. *ASH Education Program Bool.*, *2009*(1), 690-697.

Porter, J.B. & Shah, F.T. (2010) Iron overload in thalassemia and related conditions: Therapeutic goals and assessment of response to chelation therapies. *Hematol. Oncol. Clin. North Am.*, *24*(6), 1109-1030.

Portnoy, V., Huang, V., Place, R.F. et al. (2011) Small RNA and transcriptional upregulation. *Wiley Interdisciplinary Reviews: RNA*, 2(5), 748-760.

Ranjbaran, R., Okhovat, M.A., Mobarhanfard, A. et al. (2014) Relationship between AHSP Gene Expression, β/α globin mRNA ratio and clinical severity of the β-thalassemia patients. *Ann. Clin. Lab. Sci.*, *44*(2), 189-193.

Rao-Bindal, K., Koshkina, N.V., Stewart, J. et al. (2013) The histone deacetylase inhibitor, MS-275 (Entinostat), downregulates c-FLIP, sensitizes osteosarcoma cells to FasL, and induces the regression of osteosarcoma lung metastases. *Curr. Cancer Drug Targets, 13*(4), 411-422.

Roosjen, M., McColl, B., Kao, B. et al. (2014) Transcriptional regulators Myb and BCL11A interplay with DNA methyltransferase 1 in developmental silencing of embryonic and fetal β-like globin genes. *The FASEB J.*, *28*(4), 1610-1620.

Shearstone, J.R., Van Duzer, J.H., Jones, S.S. et al. (2013) Mechanistic insights into fetal hemoglobin (HbF) induction through chemical inhibition of histone deacetylase 1 and 2 (HDAC1/2). *Blood*, *122*(21), 2253.