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Research Article

# In Silico Identification of Putative Drug Targets and Analysis of Immuno Thrombocytopenia by using Network Pathway Analysis

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## ABSTRACT

Immune thrombocytopenic purpura (ITP), also known as idiopathic thrombocytopenic purpura, is an immune-mediated acquired disease of adults and children characterized by transient orpersistent decrease of the platelet count and, depending upon the degree of thrombocytopenia, increased risk of bleeding Immune thrombocytopenia (ITP) is an acquired thrombocytopenia, defined as a platelet count ,100 3 109/L, and caused by immune destruction of platelets. ITP is reported in approximately 5 per 100,000 childrenand 2 per 100,000 adults. ITP was called idiopathicthrombocytopenia until recently when several clinical and basic research studies unraveled the pathophysiology of the disease. A lot of bleeding can cause hematomas. A hematoma is a collection of clotted or partially clotted blood under the skin. ITP has no cure, and relapses may occur years after seemingly successful medical or surgical management. Corticosteroids remain the drugs of choice for the initial management of acute ITP. Oral prednisone, IV methyl prednisolone, or high-dose dexamethasone may be used IV immunoglobulin (IVIG) has been the drug of second choice for many years For Rh(D)-positive patients with intact spleens, IV Rho immunoglobulin (RhIG) offers comparable efficacy, less toxicity, greater ease of administration, and a lower cost than IVIG . Platelet transfusions may be required to control clinically significant bleeding but are not recommended for prophylaxis If 6 months of medical management fails to increase the platelet count to a safe range (about 30,000/µL), splenectomy becomes an option.. on the basic of the graph that were plotted 3gene for analyses HLA-DRb5,IGHV-366, FaM212A were resume for the novel drug target out 15 gene

**Key Words:** Immune thrombocytopenia (ITP), Corticosteroids, (IVIG), (RhIG)

## Introduction

Immune thrombocytopenic purpura (ITP), also known as idiopathic thrombocytopenic purpura, is an immune-mediated acquired disease of adults and children characterized by transient or persistent decrease of the platelet count and, depending upon the degree of thrombocytopenia, increased risk of bleeding. (Francesco Rodeghier et al., 2018) Immune thrombocytopenia (ITP) is an acquired thrombocytopenia, defined as a platelet count 100 3 109/L, and caused by immune destruction of platelets. (Michele et al., 2018) ITP is reported in approximately 5 per 100,000 childrenand 2 per 100,000 adults. ITP was called idiopathicthrombocytopenia until recently when several clinical and basic research studies unraveled the pathophysiology of the disease. Thrombocytes, more commonly referred to as platelets, are nonnucleated cellular discs of approximately 2 to 3 µ min diameter that contain mitochondria and express various surface receptors for signaling and intracellular trafficking. (Linden et al., 2013). Although platelets contribute to various important

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functions and processes such as inflammation, atherosclerosis, antimicrobial host defense, angiogenesis, and tumorigenesis (Blair et al., 2009) Immune thrombocytopenia (ITP) is a condition that typically presents with purpura, petechiae, hematoma, nosebleeds, bleeding from the gums, and blood in urine or stool and occurs due to immunologic destruction and or inadequate generation of platelets stool (NHLBI et al.,., 2012) and occurs due to immunologic destruction and or inadequate generation of platelets. (Neunert C, 2012) When ITP is a result of a known condition, such as an autoimmune disease (eg, antiphospholipid antibody syndrome), viral infection (eg, HCV or HIV), or treatment for other conditions (vaccinations, bone marrow transplant, certain drugs), the disease is called secondary ITP. Primary ITP (hereafter ITP) is a diagnosis of exclusion.

In the United States, the prevalence rates of ITP in adults and children are 66 and 50 per million individuals per year, respectively (Silverman et al., 2015) while the overall incidence is about 3-4 per 100,000 people (Terrell et al., 2015). Females are more frequently affected, but the sex difference is less pronounced in ITP compared with most autoimmune diseases, indicating possible involvement of other disease mechanisms in addition to autoimmunity (Ngo et al., 2014). Individuals who have ITP likewise may have nosebleeds, seeping from the gums amid dental work, or other draining that is difficult to stop. Ladies who have ITP may have menstrual draining that is heavier than ordinary.

**Type of immune thrombocytopenia (ITP) –**
The two type of ITP present
1 Acute (temporary or short term)
2 Chronic ( long – lasting)

**Acute-** ITP generally last less than 6 month. It mainly occurs in children- both boy and girls- and is the most common type of ITP. Acute ITP often occurs after a viral infection.

**Chronic-** ITP lasts 6 months or longer and mostly affects adults. However, some teenagers and children do get this type of ITP. Chronic ITP affects women two to three times more often than men.

**Signs, Symptoms, and Complications**

Immune thrombocytopenia (ITP) may not cause any signs or symptoms. However, ITP can cause bleeding inside the body (internal bleeding) or underneath or from the skin (external bleeding). Signs of bleeding may include:

- Bruising or purplish areas on the skin or mucous membranes (such as in the mouth). These bruises are called purpura. They're caused by bleeding under the skin, and they may occur for no known reason.
- Pinpoint red spots on the skin called petechiae. These spots often are found in groups and may look like a rash. Bleeding under the skin causes petechiae.
- A collection of clotted or partially clotted blood under the skin that looks or feels like a lump. This is called a hematoma.
- Nosebleeds or bleeding from the gums (for example, during dental work).
- Blood in the urine or stool (bowel movement).

**Pathophysiology-** As aforementioned, 2 major processes contribute to ITP: decreased platelet production and increased platelet destruction (Nugent et al., 2009). These processes may be affected by multiple pathogenic changes, and they contribute at varying extents to ITP pathogenesis in each patient.

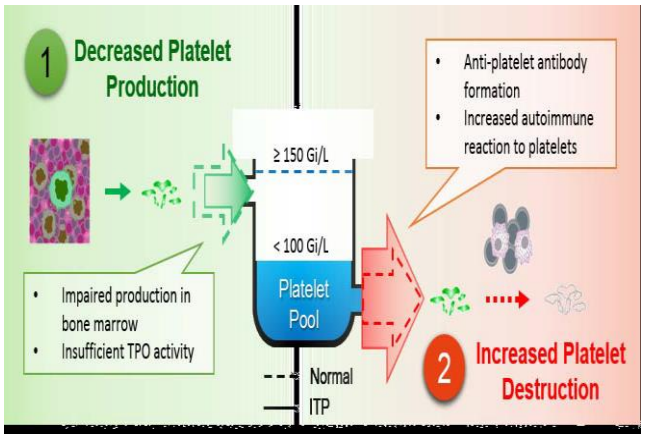
Increased platelet destruction: Abnormally accelerated platelet destruction is a characteristic of ITP (Abadi et al., 2015) Current evidence suggests involvement of a 3-step mechanism. Firstly, immune tolerance is lost due to pathological regulatory and inflammatory T-cell function. Secondly, T-follicular helper cells located primarily in the spleen trigger differentiation of B cells to autoreactive cells (Audia et al., 2014) that produce antiplatelet antibodies (Godeau et al., 2014) Finally antiplatelet antibodies target glycoproteins, primarily glycoprotein IIb/IIIa, on platelets (Winiarski et al., 1986) and cause platelet destruction by macrophages or cytotoxic T cells.

Immune mechanisms that lead to increased platelet destruction may be triggered by many factors. There are over 100 drugs that cause drug-induced

thrombocytopenia (Reese et al., 2010). In addition, vaccines, particularly measles, mumps, and rubella (MMR), and infections have also been associated with thrombocytopenia (Sauvé et al., 2009). There may be common mechanisms by which these factors generally induce accelerated platelet destruction. For instance, the foreign factor (drug or inactivated/ live pathogen) may bind to the surface of the platelet and act as an adapter recruiting anti-foreign factor antibodies, thereby triggering a temporary immune response to platelets (Karpatkin et al., 1992). Thrombocytopenia could be more persistent if anti-foreign factor antibodies cross-react with platelet antigens (Zhang et al., 2009), or if platelet/foreign factor complexes induce internalization and presentation of platelet antigens by antigen presenting cells, leading to development of anti- platelet antibodies (Kuwana et al., 2009)

Decreased platelet production: In patients with ITP, platelet production may not be sufficient to replace the platelets that are destroyed. Since megakaryocytes and platelets share common surface antigens, most anti-platelet antibodies may also target megakaryocytes (Takahashi et al., 1999). Thus, decreased platelet production may be a secondary result of the factors leading to increased platelet destruction, which were discussed above. Other potential causes of decreased platelet production include impaired function of megakaryocytes (Diggs LW et al., 1948), altered megakaryocyte morphology (Tripathi et al., 2014), or abnormal T-cell response in the bone marrow microenvironment (Song et al., 2016). In addition, insufficient TPO levels are considered to be involved in pathogenesis of ITP because increased serum TPO, a typical compensatory response to thrombocytopenia, is not observed in ITP (Imbach et al., 2011).

Platelet production and platelet destruction must be in balance to maintain the size of the circulating platelet pool at normal range (150-400 Gi/L). In physiological conditions, each process can compensate for changes in the other to reestablish the balance; eg, platelet production increases if the platelet pool becomes smaller due to increased platelet destruction. However, in patients with ITP, this balance is disturbed by (1) decreased platelet production, (2) increased platelet destruction, or both, and therapeutic intervention may be needed to reestablish this balance before the decline in the platelet pool becomes life threatening.




## Materials and Methods

**Identification of Putative Drug Targets with Topological Analysis-** Topological parameters have been defined to measure network characteristics using Copesi and String Network and Simulation analyzer plugin. This plugin is a tool that helps to study the topology and the parameters of a network by using descriptive statistics and graphs. Power law fit for the protein network was detected by calculating degree exponent distribution and the coefficient of determination. The shortest path (geodesics) was calculated to evaluate the network and parameters, such as degree (connectivity), between's centrality (BC) and closeness centrality (CC), were used to detect the essential proteins as putative drug targets.

**Targets Analysis for Copesi-**COPASI is an open-source software application for creating and solving mathematical models of biological processes such as metabolic networks, cell-signaling pathways, regulatory networks, infectious diseases, and many others. The COPASI graphical user interface has been written using the Qt toolkit. This allows us to release COPASI on all platforms that Qt supports

To start for the analysis for these steps:

**1. General Model Settings**- If you click on the Model branch of the object tree which was explained in the "COPASI GUI Elements" section, you activate the dialog

that lets you specify certain parameters for your model like its name and the units that are to be used for time, volume and concentration quantities throughout the current model. You can also give a textual description of your model that is more expressive than reactions and equations. You could for example state which part of the metabolism the model describes (e.g. glycolysis) and add some references to articles related to the model. This will help others (and yourself) to understand and identify your models.

**2. Compartments**- There are three methods to add a new compartment to a model, but for all three, we have to navigate to the Compartments branch of the object tree which is located under the Model->Biochemical branch. first open the Model branch and there open the Biochemical branch by clicking on the expansion sign in front of the branch name, or by double clicking on the branch name If you start with a new model and you select the Compartments branch, you will get an empty table with eight columns (see above). The columns are named Status, Name, Type Initial Volume, Volume, Rate, Initial Expression and Expression.

**3. Species** -Adding new species works exactly the same as adding new compartment, so we strongly suggest reading the "Compartments" section if you haven't already done so. Here we will just cover the differences between adding a compartment and adding a species.

**4. Reactions**-Again adding reactions essentially works the same way as adding compartments or species. When you navigate to the Reactions branch of the object tree which is located under the Model->Biochemical branch, you will see a table with five columns. The first two are Status and Name of the reaction. The third column called Equation describes the chemical formula and maybe additional modifiers of the reaction. The fourth column states the name of the kinetics for the reaction which depends on the equation. We will come to this in a second. The last column shows the flux through this reaction.

The easiest way to add a reaction is to type the chemical equation into an empty equation cell in the table. After you typed the equation, you hit the return key and automatically land in the next row where you can type the next reaction equation. This way you can enter all the reactions that make up your model. When you are finished with typing the reaction equations, you commit all the reactions. If any of the reactions contain species that are not already present in the model, they are added automatically. If there was no compartment before, a compartment is also added and all new species get added to this compartment. If there is already one or more compartments, all new species get added to the first compartment that is listed in the object tree.

**5. Parameter View-** The parameter view widget can be displayed by selecting the leaf called Parameter Overview on the Model->Biochemical branch (see below). This widget allows you to view and edit all parameters of the model in one place. This saves you from moving around the model tree if you e.g. first have to edit the initial concentrations for some species and afterward parameters of one or more reactions. The view shows you the initial concentrations for the species at the top followed by the initial time and the volumes of all the compartments and at the bottom the kinetic parameters of all reactions. In order to change a value, you double click on it which lets you input a new value. On hitting the return key or clicking somewhere else, the new value is not written to the object directly, but a'*' character appears in front of the name of the changed parameter. If you now leave this widget or press the Commit button at the bottom of the dialog, the new value is written to the corresponding object in the model

**6. Output Assistant**-The output assistant presents the easiest way to generate your own output definitions which you can later adapt to your wishes using the techniques described in "Manual Definition". Almost all task dialogs in COPASI have a button at the lower right that is labeled Output Assistant. If you click on this button, a new dialog will open with a list of predefined output definitions on the left. If you select one of the output definitions from the list, you will get a short description of what the output does on the right side of the dialog. Above the description is the title of the output definition. This title can be changed in order to be able to identify the different output definitions in case you are planning on creating more than one output definition of a certain type. Using this dialog both plots and reports can be created. Creating an instance of the selected output objects is as easy as clicking on Create! at the bottom of the dialog. Once you clicked this button, a new report or plot, depending on what you selected in the dialog, will appear in the corresponding branch of the Output section of the model tree. The Output branch is the second to last branch in the tree on the left. The name of the output definitions will be the title of the object which you selected from the list. If another output definition in this section already has the same name, a postfix will be appended to the name. The so created output can now be edited or deleted. If the newly created

output is a report it will automatically be selected as the active report for the current task. You then still have to select a filename for the output using the Report button. This is described in the sections about the specific calculation tasks below. How output definitions are created, edit and deleted manually is the topic of the next sections. (Back et al., 1997)

**7. Reports**- This section describes how to create or edit a report definition. Keep in mind that you still have to select this report for the specific calculation task you want to perform. You can do this (and also choose a filename to write to) using the Report button as described below in the sections about the different tasks

**Network analysis by String** -In molecular biology, STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) is a biological database and web resource of known and predicted protein–protein interactions. The STRING database contains information from numerous sources, including experimental data, computational prediction methods and public text collections. It is freely accessible and it is regularly updated. The resource also serves to highlight functional enrichments in user-provided lists of proteins, using a number of functional classification systems such as GO, Pfam and KEGG. The latest version 10.0 contains information on about 9.6 million proteins from more than 2000 organisms. STRING has been developed by a consortium of academic institutions including CPR, EMBL, KU, SIB, TUD and UZH.

**Clustering-** From the 'Clustering' menu it is possible to launch 2 different clustering algorithms (KMEANS and MCL) to cluster the proteins that you are displaying in the network. The only input to these clustering algorithms is the distance matrix obtained from the String global scores (so interacting proteins with an higher global score have more chances to end up in the same cluster). Besides the distance matrix, both the algorithms accept 1 parameter as input:
- KMEANS accepts a parameter to specify the number of clusters that you want to obtain.
- MCL accepts a parameter called "inflation" that it is also indirectly related with the precision of the clustering (higher the inflation -> more clusters you obtain). In order to launch the clustering, just click on the algorithm you want to launch (e.g. 'MCL').
A little panel with a slide-bar will appear below the menu.

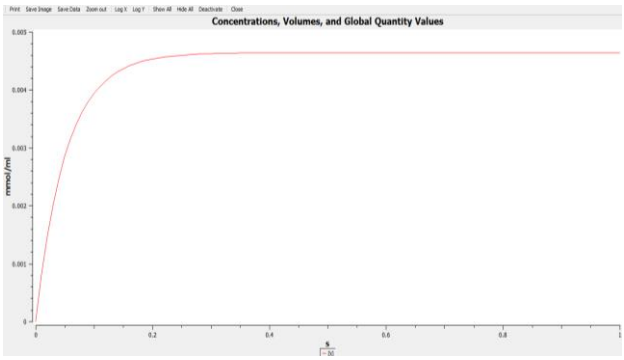
## Results

Preduction of new drug target. Can be suggest by appropriate measures to predict from currently available targets and other data-sets by looking these predictions. it seen to be started forward procedure such as:
1. Similarity of putative two endpoints.
2. Similarity of neighbourhood network.
3. Comparison between the network.
4. Analysis of sequential snapshots of network topology

Immune thrombocytopenia (ITP) is a common hematologic disorder. Its pathogenesis involves both accelerated platelet destruction and impaired platelet production. First-line agents are usually effective initially but do not provide long-term responses.

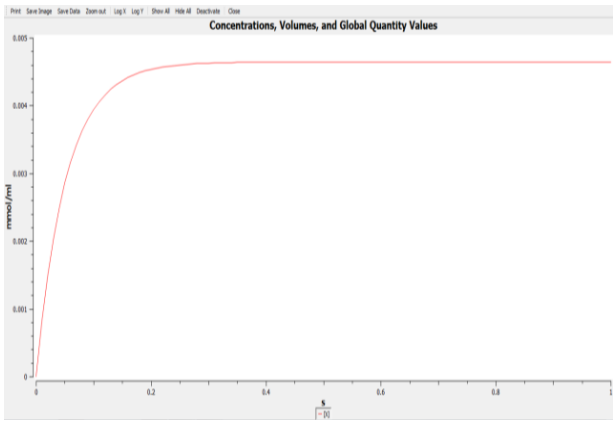
**Time Course result**

| | Time | X |
|---|---|---|
| 1 | 0 | 0 |
| 2 | 0.01 | 0.000815131 |
| 3 | 0.02 | 0.00148273 |
| 4 | 0.03 | 0.00203095 |
| 5 | 0.04 | 0.00248208 |
| 6 | 0.05 | 0.00285396 |
| 7 | 0.06 | 0.00316094 |
| 8 | 0.07 | 0.00341463 |
| 9 | 0.08 | 0.00362449 |
| 10 | 0.09 | 0.00379822 |
| 11 | 0.1 | 0.00394212 |
| 12 | 0.11 | 0.00406139 |
| 13 | 0.12 | 0.00416028 |
| 14 | 0.13 | 0.00424231 |
| 15 | 0.14 | 0.00431037 |
| 16 | 0.15 | 0.00436684 |
| 17 | 0.16 | 0.00441372 |
| 18 | 0.17 | 0.00445265 |
| 19 | 0.18 | 0.00448496 |
| 20 | 0.19 | 0.0045118 |
| 21 | 0.2 | 0.00453408 |

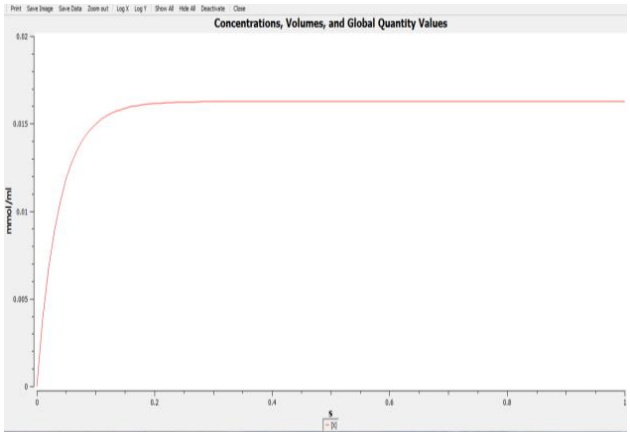


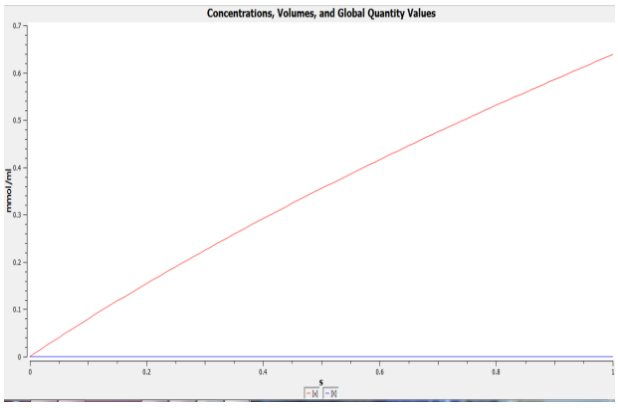

**HLA-DRb5 Plot**

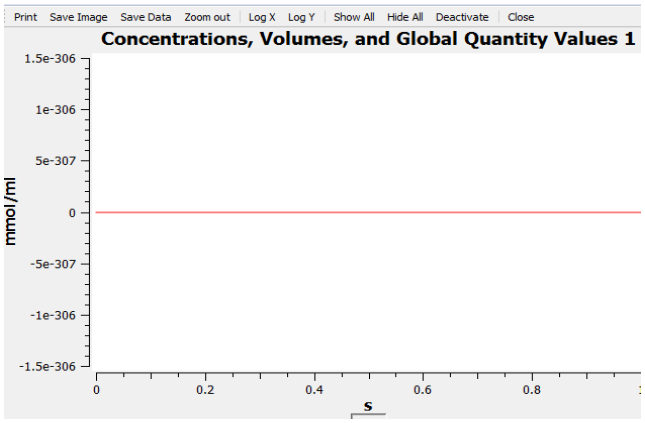
**IGHV3-6**




**FAM212A**

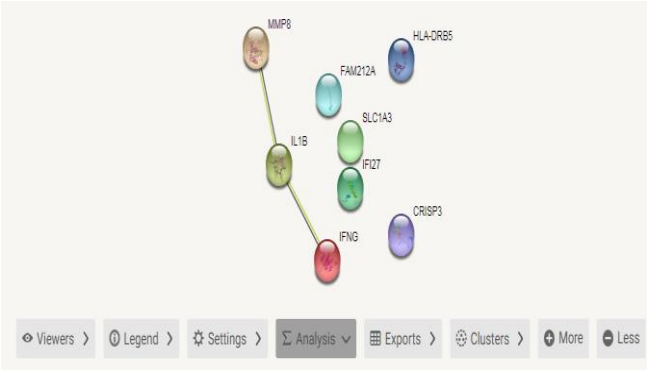


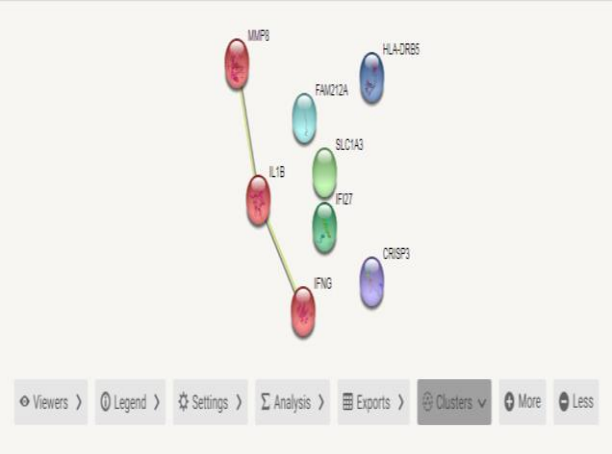



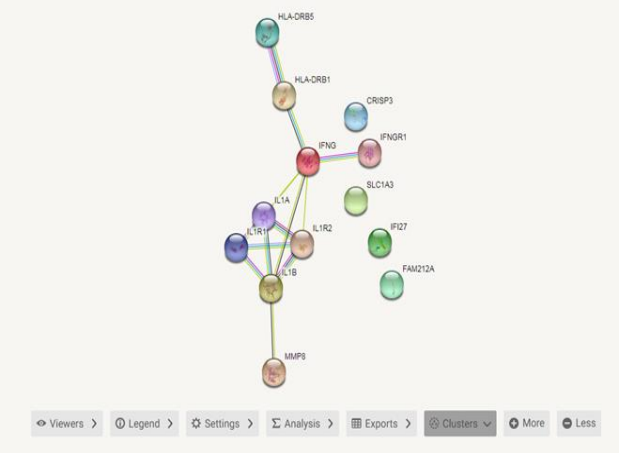




**(TNF)-α ,IFI27, Pld5, Mmp8, Crisp3,IL-10, interleukin (IL) - 1 beta, Slica3,THBS1,IFNM1, Interferon-γ,IL-4**

## String Result

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