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Nephrotic Syndrome

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Declaration of Authorship

I, Rahim KARGAR, declare that this project titled, "Nephrotic Syndrome" and the work presented in it are my own. I confirm that:

- This work was done wholly or mainly while as a project for the course of Introduction to Computational and Systems Biology at this University.
- Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this project is entirely my own work.
- I have acknowledged all main sources of help.

Signed:

Date:

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Abstract

Faculty of Science and Engineering
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for Introduction to Computational and Systems Biology

Nephrotic Syndrome

by Rahim KARGAR

Birth defects involving the kidney and urinary system are often encountered and frequently occur in association with other structural abnormalities. A congenital urinary tract anomaly may provide the first clue to the recognition of multiorgan developmental abnormalities. Nevertheless many renal anomalies remain asymptomatic and undiagnosed. Therefore it is critical, not only for pediatric nephrologists but also for pediatricians in general, to be familiar with the common anomalies involving the kidney and urinary system and the more complex disorders with which they may be associated.

The kidney is a pivotal organ in dysmorphology. Although the number of single malformations involving the kidney is limited, combinations of these malformations in conjunction with anomalies involving other organ systems are found in more than 500 syndromes. In addition, many well-known sequences and associations involve the kidney and urinary tract.

This project discusses common malformations, involving the kidney and urinary tract. As one of these malformations we will give some information about Nephrotic Syndrome.

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List of Tables

To Arezou & Benyamin

Chapter 1

Introduction

1.1 Preliminary

To understand the pathophysiologic basis of structural abnormalities, it is important to be familiar with the meaning of certain terms, for instance *Malformation*, *Deformation*, *Disruption*, *Sequence*, *Syndrome* and *Association*, as they are used in describing malformations and syndromes. Below are just two things.

Malformation refers to a single structural anomaly that arises from an error in organogenesis. Such an error may be due to the failure of cells or tissues to form, to die (programmed cell death), or to induce others. Examples include renal agenesis, horseshoe kidney, and bladder exstrophy.

Syndrome refers to a consistently observed pattern of anomalies found in an individual, whether malformation, deformation, or disruption. Anomalies comprising a syndrome are thought to have a single cause, although in many cases, their causes are still unknown. Examples include Turner syndrome and fetal alcohol syndrome.

1.2 Nephrotic Syndrome (NS)

Since Nephrotic Syndrome is partially unknown, in this section we are going to give some information about it. We start by defining it.

Nephrotic syndrome is a kidney disorder that causes your body to excrete too much protein in your urine. NS is a common chronic glomerular disease in children and is characterized by significant proteinuria ($> 40 mg/m^2/hr$ or a spot urinary protein-to-creatinine ratio of more than $2 mg/mg$) and consequent hypoalbuminemia ($< 3.0\, g/dL$), which in turn causes edema and hyperlipidemia (Eddy, 2003; Wiggins, 2007; Sinha, 2012). Although NS is associated with many types of renal disease, the most common form (90%) in children is primary (idiopathic) NS, which develops in the absence of clinical features of nephritis or associated primary extrarenal disease. Occasionally, childhood NS can be associated with systemic inflammatory or autoimmune disease or can develop as a result of ischemic insult, infections, drugs, toxins, or inherited renal diseases (Eddy, 2003; Wiggins, 2007; Sinha, 2012).

1.2.1 Symptoms

Signs and symptoms of nephrotic syndrome include:

- Severe swelling (edema), particularly around your eyes and in your ankles and feet
- Foamy urine, which may be caused by excess protein in your urine
- Weight gain due to excess fluid retention

- Fatigue
- Loss of appetite

1.2.2 Causes

Nephrotic syndrome is usually caused by damage to the clusters of tiny blood vessels (glomeruli) of your kidneys. The glomeruli filter your blood as it passes through your kidneys, separating things your body needs from those it doesn't. Healthy glomeruli keep blood protein (mainly albumin) which is needed to maintain the right amount of fluid in your body from seeping into your urine. When damaged, glomeruli allow too much blood protein to leave your body, leading to nephrotic syndrome.

1.2.3 Complications

Possible complications of nephrotic syndrome include:

- *Blood clots.* The inability of the glomeruli to filter blood properly can lead to loss of blood proteins that help prevent clotting. This increases your risk of developing a blood clot (thrombus) in your veins.
- *High blood cholesterol and elevated blood triglycerides.* When the level of the protein albumin in your blood falls, your liver makes more albumin. At the same time, your liver releases more cholesterol and triglycerides.
- *Poor nutrition.* Loss of too much blood protein can result in malnutrition. This can lead to weight loss, but it may be masked by swelling. You may also have too few red blood cells (anemia) and low levels of vitamin D and calcium.
- *High blood pressure.* Damage to your glomeruli and the resulting buildup of wastes in your bloodstream (uremia) can raise your blood pressure.
- *Acute kidney failure.* If your kidneys lose their ability to filter blood due to damage to the glomeruli, waste products may build up quickly in your blood. If this happens, you may need emergency dialysis an artificial means of removing extra fluids and waste from your blood typically with an artificial kidney machine (dialyzer).
- *Chronic kidney disease.* Nephrotic syndrome may cause your kidneys to gradually lose their function over time. If kidney function falls low enough, you may require dialysis or a kidney transplant.
- *Infections.* People with nephrotic syndrome have an increased risk of infections.

The above three subsections 1.2.1, 1.2.2 and 1.2.3 are from `https://www.mayoclinic.org/diseases-conditions/nephrotic-syndrome/symptoms-causes/syc-20375608`.

1.2.4 Pathophysiology

The kidney glomerulus filters the blood that arrives at the kidney. It is formed of capillaries with small pores that allow small molecules to pass through that have a molecular weight of less than 40,000 Daltons, but not larger macromolecules such as proteins, see the following web-site:

https://web.archive.org/web/20080908021010/http://escuela.med.puc.cl/paginas/Cursos/tercero/IntegradoTercero/Mec231_38.html.

In NS, the glomeruli are affected by an inflammation or a hyalinization (the formation of a homogenous crystalline material within cells) that allows proteins such as albumin, antithrombin or the immunoglobulins to pass through the cell membrane and appear in urine (Álvarez, 1999). Albumin is the main protein in the blood that is able to maintain an oncotic pressure, which prevents the leakage of fluid into the extracellular medium and the subsequent formation of edemas. As a response to hypoproteinemia the liver commences a compensatory mechanism involving the synthesis of proteins, such as alpha-2 macroglobulin and lipoproteins (Álvarez, 1999). An increase in the latter can cause the hyperlipidemia associated with this syndrome.

1.3 Congenital NS

Congenital NS (CNS), which presents within the first 3 months of life, is commonly associated with causative mutations. Indeed, mutations have been identified in 75-100% of cases of CNS (Wang, 2017). CNS can be classified into two types: primary (hereditary) and secondary (non-hereditary) (Zhang, 2011). Primary CNS is usually related to gene mutations that alter the glomerular filtration barrier. The most common type of primary CNS is the Finnish type (NPHS1 gene mutation). Secondary CNS is usually associated with various types of congenital infections, such as cytomegalovirus (CMV), syphilis, toxoplasmosis, rubella, hepatitis B virus (HBV), and human immunodeficiency virus (HIV) infections.Maternal systemic lupus erythematosus (SLE), maternal steroid/chlorpheniramine use, and neonatal autoantibodies against neutral endopeptidase can also cause secondary CNS (Jalanko, 2009).

Causative mutations appear to largely occur in one of five genes (NPHS1, NPHS2, WT1, LAMB2 and PLCE1). NPHS1, encoding nephrin, is the main gene implicated in CNS, and mutation is responsible for the autosomal recessive Finnish type (CNF), which typically has a severe phenotype with massive proteinuria and rapid progression to ESRD (Kestila, 1998). However, the NPHS1 mutation detection rate remains high amongst non-Finnish cases of CNS (Wang, 2017). Mutations in the NPHS2 gene, encoding podocin, are also responsible for a significant number of CNS cases, and the phenotype varies from the severe CNF presentation to milder disease with onset of proteinuria occurring later than in those with NPHS1 mutations (Benoit, 2010). Mutations in the PLCE1, WT1 and LAMB2 genes have also been detected in patients presenting with isolated CNS.

Chapter 2

Data Set and Network

2.1 Data Set

In this chapter we first present some data set including some genes that are susceptible to be involved in NS from different sources including papers and books. After our investigation, 53 genes are found which play a key role in NS and they are somehow related to NS. These genes are the largest number found to date. For instance one can refer to (Preston, 2019). Tables 2.1 and 2.2 (Table 2.2 is the continuation of Table 2.1) provide more details on this.

2.2 Network

In this section we are going to find a network of interactions between genes. Such networks are modelled mathematically as directed graphs, consisting of nodes standing for all the proteins in the network, and directed edges between them standing for each signal transduction relationship between them. Each edge carries a positive "weight" signifying the relative strength of the corresponding interaction (Kanhaiya, 2018). The desired software is called *NetControl4BioMed* and is based on the concept of target structural control of linear networks. *NetControl4BioMed* generates novel molecular interaction networks by combining pathway data from various public databases starting from the user's query. It then identifies a set of nodes that is enough to control a given, user-defined set of disease-specific essential proteins in the network, i.e., it is able to induce a change in their configuration from any initial state to any final state (Kanhaiya, 2018).

TABLE 2.1: Genes related to the NS

Number	Genes	Protein
1	NPHS1	Nephrin
2	NPHS2	Podocin
3	PLCE1	Phospholipase C epsilon 1
4	CD2AP	CD2-associated protein
5	TRPC6	Transient receptor potential channel C6
6	CRB2	Crumbs family member 2
7	FAT1	FAT atypical cadherin 1
8	WT1	Wilms' tumour protein 1
9	LMX1B	LIM homeobox transcription factor 1β
10	SMARCL1	SMARCA-like protein
11	NUP93	Nuclear pore complex protein 93
12	NUP107	Nuclear pore complex protein 107
13	NUP205	Nuclear pore complex protein 205
14	XPO5	Exportin 5
15	E2F3	E2F transcription factor
16	NXF5	Nuclear RNA export Factor 5
17	PAX2	Paired box protein 2
18	LMNA	Lamin A and C
19	WDR73	WD repeat domain 73
20	ACTN4	α-actinin 4
21	MYH9	Myosin heavy chain 9, non-muscle
22	INF2	Inverted formin 2
23	MYO1E	Myosin 1E
24	MAGI2	Membrane Associated Guanylate Kinase, inverted 2
25	ANLN	Anillin actin binding protein
26	ARHGAP24	Rho GTPase-activating protein 24

TABLE 2.2: (continued) Genes related to the NS

Number	Genes	Protein
27	ARHGDIA	Rho GDP dissociation inhibitor alpha
28	KANK 1/2/4	Kidney ankyrin repeat-containing protein
29	SYNPO	Synaptopodin
30	PTPRO	Protein-tyrosine phosphatase-R O
31	EMP2	Epithelial membrane protein 2
32	APOL1	Apolipoprotein L1
33	CUBN	Cubilin
34	PODXL	Podocalyxin
35	LAMB2	Laminin subunit β2
36	ITGB4	Integrin β4
37	ITGA3	Integrin α3
38	COL4A3/4/5	Type IV collagen α3, α4, α5
39	GPC5	Glypican 5
40	CD151	CD151 antigen
41	COQ2	Coenzyme Q2
42	COQ6	Coenzyme Q6
43	PDSS2	Prenyl-diphosphate synthase subunit 2
44	ADCK4	AarF domain containing kinase 4
45	MTTL1	Mitochondrial tRNA 1
46	SCARB2	Scavenger receptor class B, member 2
47	OCRL1	Oculocerebrorenal syndrome of Lowe
48	ZMPSTE24	Zinc metallopeptidase STE24
49	PMM2	Phosphomannomutase 2
50	ALG1	Asparagine-linked glycosylation 1
51	TTC21B	Tetratricopeptide repeat protein 21B
52	CFH	Complement factor H
53	DGKE	Diacylglycerol kinase epsilon

2.3 Network Analysis

For further investigation and visualization of the network we used the application *NetControl4BioMed*. This is a bioinformatics software tool which generates large protein-protein interaction networks around a given set of genes. Our network is shown in Figure 2.1.

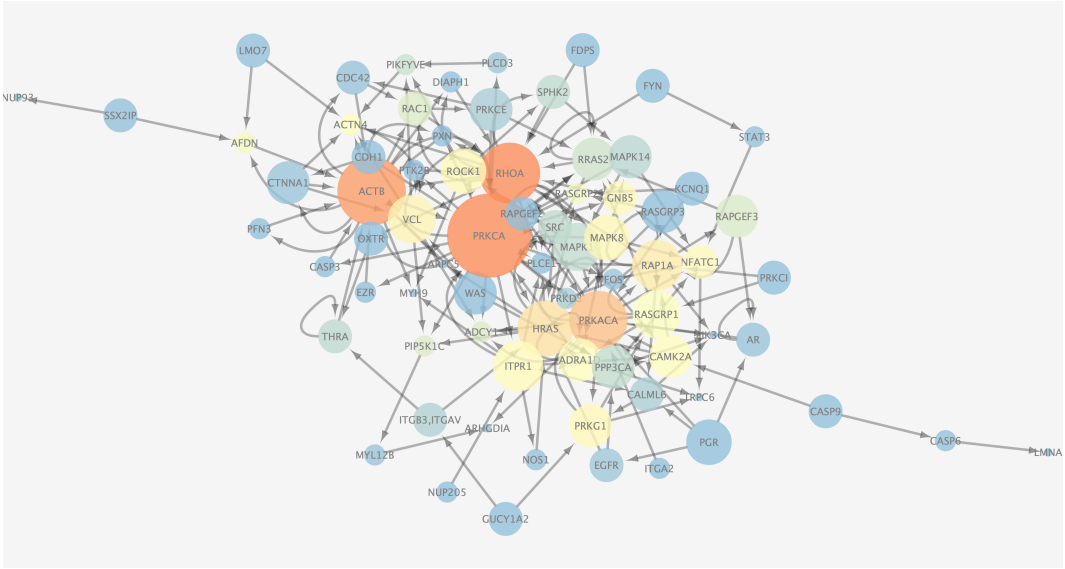

FIGURE 2.1: The protein-protein interaction network between genes Tables 2.1 and 2.2

In our analysis of this network we found that CASP9 is predicted as a very influential node over the genes we found linked to NS. This could be seen as prediction of CASP9 having an important role in NS. This correlates well with some very recent report in (Xu, 2018). It is also interesting that CASP9 is not a "key" node in the network: it has a low outdegree and a low betwenness centrality. The fact that is so influential seems to come form the fact that it is succeeded on the control pathway by CAMK2A, a node that is much "heavier" in the network: it has both a large outdegree and large betwenness centrality. This is also well documented to have a role in NS, see (Park, 2019).

2.4 Discussion

We discussed in this project a kidney disease called Nephrotic Syndrome. Based on our research, we found 53 different genes involved in the disease (Tables 2.1 and 2.2). By using of an application called *NetControl4BioMed*, we find out a protein-protein interaction network associated with these genes (Figure 2.1).

Appendix A

Appendix

A.1 Algorithm

The algorithm of our network is contained in the following table.

RandomSeed	427870062
MaxIteration	10000
MaxIterationNoImprovement	1000
MaxPathLength	20
Repeats	2
Heuristics	A;B;C;D;E;F;G;Z;
CurrentIteration	138
CurrentIterationNoImprovement	137

Bibliography

Álvarez, S.D. (1999). "Complicaciones agudas del síndrome nefrótico". In: *Rev Cubana Pediatr* **71**.4, pp. 245–253. URL: http://scielo.sld.cu/pdf/ped/v71n4/ped10499.pdf.

Benoit G., Machuca E. Antignac C. (2010). "Hereditary nephrotic syndrome: a systematic approach for genetic testing and a review of associated podocyte gene mutations". In: *Pediatr Nephrol* **25**.9, pp. 1621–1632. URL: https://link.springer.com/article/10.1007/s00467-010-1495-0.

Eddy A.A., Symons J.M. (2003). "Nephrotic syndrome in childhood". In: *The Lancet* **362**.9384, pp. 629–639. URL: https://www.sciencedirect.com/science/article/pii/S0140673603141840?via%3Dihub.

Jalanko, H. (2009). "Congenital nephrotic syndrome". In: *Pediatr Nephrol* **24**.11, pp. 2121–2128. URL: https://link.springer.com/article/10.1007%2Fs00467-007-0633-9.

Kanhaiya K., Rogojin V. Kazemi K. Czeizler-E. Petre I. (2018). "NetControl4BioMed: a pipeline for biomedical data acquisition and analysis of network controllability". In: *BMC Bioinformatics* **19**.7, pp. 185–195. URL: https://bmcbioinformatics.biomedcentral.com/track/pdf/10.1186/s12859-018-2177-3.

Kestila M., Lenkkeri U. Mannikko M. Lamerdin-J. McCready P. Putaala H. Ruotsalainen V. Morita T. Nissinen M. Herva R. Kashtan C.E. Peltonen L. Holmberg C. Olsen A. Tryggvason K. (1998). "Positionally Cloned Gene for a Novel Glomerular Protein-Nephrin-Is Mutated in Congenital Nephrotic Syndrome". In: *Mol Cell* **1**.4, pp. 575–582. URL: https://www.sciencedirect.com/science/article/pii/S109727650080057X?via%3Dihub.

Park S.-J., Kim Y. Yang S.-M. Henderson M.J. Yang W. Lindahl M. Urano F. Chen Y.M. (2019). "Discovery of endoplasmic reticulum calcium stabilizers to rescue ER-stressed podocytes in nephrotic syndrome". In: *Proc. Natl. Acad. Sci. USA* **116**.28, pp. 14154–14163. URL: https://www.pnas.org/content/116/28/14154.short?rss=1.

Preston R., Stuart H.M. Lennon R. (2019). "Genetic testing in steroid-resistant nephrotic syndrome: why, who, when and how?" In: *Pediatric Nephrology* **34**.9, pp. 195–210. URL: https://link.springer.com/content/pdf/10.1007/s00467-017-3838-6.

Sinha A., Bagga A. (2012). "Nephrotic syndrome". In: *Indian J Pediatr* **79**.8, pp. 1045–1055. URL: https://link.springer.com/article/10.1007%2Fs12098-012-0776-y.

Wang F., Zhang Y. Mao J. Yu-Z. Yi Z. Yu L. Sun J. Wei X. Ding F. Zhang H. Xiao H. Yao Y. Tan W. Lovric S. Ding J. Hildebrandt F. (2017). "Spectrum of mutations in Chinese children with steroidresistant nephrotic syndrome". In: *Pediatr Nephrol* **32**.7, pp. 1181–1192. URL: https://link.springer.com/article/10.1007%2Fs00467-017-3590-y.

Wiggins, R.C. (2007). "The spectrum of podocytopathies: a unifying view of glomerular diseases". In: *Kidney Int* **71**.12, pp. 1205–1214. URL: https://www.sciencedirect.com/science/article/pii/S0085253815522977.

Xu X., Eales J.M. Akbarov A. et al. (2018). "Molecular insights into genome-wide association studies of chronic kidney disease-defining traits". In: *Nat. Commun.* **9**.4800, pp. 1–12. URL: https://www.nature.com/articles/s41467-018-07260-4.

Zhang H.-W., Ding J. (2011). "Advances on treatment of congenital nephrotic syndrome". In: *J Appl Clin Pediatr* **26**.5, pp. 373–374. URL: http://www.wanfangdata.com.cn/details/detail.do?_type=perio&id=syeklczz201105022.