UNIVERSITY OF MEDICINE AND PHARMACY ”CAROL DAVILA”

BUCHAREST

PEDIATRICS

ELEMENTS OF MOLECULAR BIOLOGY IN IDIOPATIC NEPHROTIC SYNDROME IN CHILDREN

PhD Thesis Summary

PhD Supervisor:

PROF. UNIV. DR. MIHAELA BĂLGRĂDEAN

Student:

IOANA (CÎNDEA) ȚIERANU

2022

Table of contents

I. OVERVIEW AND BACKGROUND

1. IDIOPATHIC NEPHROTIC SYNDROME – Overview

According to KDIGO guidelines dating from 2012, nephrotic syndrome(NS) is defined by the presence of sustained proteinuria (protein to urinary creatinine ratio above 2000mg/g or the presence of more than 3+ proteins on urinary dipstick) and hypoalbuminemia lower than 2.5gr/dl, along with clinical evidence of oedema. [1] Although there are various childhood-onset glomerular diseases with similar clinical and biological changes, idiopathic nephrotic syndrome (INS) represents the most frequent cause. [2] Moreover, in pediatric patients, INS is the most frequently diagnosed of all inherited glomerulopathies. [3]

Despite massive progress in understanding the role of podocytes – alterations at this level being responsible for massive proteinuria – the exact etiology of INS remains enigmatic. There is, however, literature data suggesting implications of immunologic mechanisms in INS debut, as well as relapses, based on observations showing a direct correlation of both debut and relapses to concurrent viral infections of the upper respiratory tract. [4]

There are multiple parameters by which INS can be classified: response to steroid therapy, number of relapses, histological changes in renal biopsy samples, etc. Based on the response to steroid therapy, patients can be classified as steroid sensitive, with complete remission in the first 4 weeks from treatment initiation, and steroid refractory, with persistent nephrotic proteinuria after 6-8 weeks from treatment initiation. According to 2012 KDIGO guideline, complete remission is defined as the absence of proteinuria with a protein to urinary creatinine ration lower than 200mg/g or less than 1+ for proteins on urinary dipstick, for at least 3 consecutive days. [1]. To mention, some of the initial steroid sensitive patients may experience one or more relapses, with 50% of them developing frequent relapses and, ultimately, steroid dependance. [5,6]

2. INTERLEUKIN-13 - Overview

Interleukin-13 (IL-13) is a cytokine with a chain of 132 amino acids, encoded by a gene located of chromosome 5p23-q31, consisting of 6 exons. [7] Actually, there is research interest

related to several single nucleotide polymorphisms(SNPs) for *IL-13* gene, like rs1800925 – located in the promoter region, rs2066960 – located at the level of intron 1, rs1295686- located at the level of intron 3, rs20541 (Arg144Gln) and rs1295685 – both located at the level of intron 4.

Recently, there have been studies on INS patients looking into genetic polymorphisms for various molecules shown to be involved in the alterations of glomerular functions. The aim of these studies was to establish if genetic variability is correlated with disease susceptibility of disease phenotype. [8] Due to its well-known role in altering podocyte functions and, also, to literature data showing overexpression of IL-13 in patients with active INS, the latter was one of the studied molecules for genetic variability. [9,10]

Several SNPs for *IL-13*, like rs20541, have been proven to be implicated in predicting the risk for the development of INS in pediatric population, of specific ethnic groups - the MCD histological variant. [9]

3. TUMOR NECROSIS FACTOR ALPHA (TNF-ALPHA) – Overview

TNF-alpha gene in located in the major histocompatibility complex, on the short arm of chromosome 6, and genetic variations of this locus are directly related to cytokine synthesis. [11] Many SNPs have been identified in the promoter region of this gene (region extremely important for the protein synthesis), these being located based on the transcription initiation site, in the following positions: −1031 (T→C), −863 (C→A), −857 (C→A), −851 (C→T), −419 (G→C), −376 (G→A), −308 (G→A), −238 (G→A), −162 (G→A), și −49 (G→A), part of them being, however, rarely encountered in Caucasian population.

Of those, by far the most studied SNP is the one located in -308 position, with mutation at this level consisting of a change of guanine with adenosine, contributing to the development of two alleles – the common allele with guanine and the mutant allele with adenosine. The latter seems to be associated with an increased production of cytokine, spontaneously, as well as in response to different stimuli in vitro and in vivo. [12]

Till present, there is conflicting literature data regarding the implications of *TNF-alpha* minor allele -308A in INS disease susceptibility. Moreover, studies have generated heterogenous results regarding its influence on INS patients' response to treatment.

4. INTERLEUKIN-4 – Overview

Even though the existing literature data suggests that INS is an immune mediated disease associated to an imbalance of cytokines secreted by T helper 1 and T helper 2 lymphocytes, its pathogenesis remains highly unknown. [13]

Some studies on patients with INS and histological aspects of minimal changes glomerulonephritis have shown an inadequate response of the delayed hypersensibility, thus suggesting a possible role for cellular immunity T helper 1 lymphocyte dependent. [14] Moreover, the role of T helper 2 lymphocyte secreted cytokines in the pathogenesis of INS has been underpinned by several other authors. [15,16–18]

Interleukin-14 (IL-4) represents o pleiotropic glycoprotein identified for the first time in 1982, with its complementary DNA being successfully cloned later, in 1986. [19,20]

The gene encoding IL-14 is very polymorphic, with various structure variations: mononucleotide polymorphisms, insertions, deletions and other types of arrangements like repetitions transpositions etc. Among the most studied SNPs for *IL-4* are -34C/T (rs2070874), -590G/T (rs2243250) and -1098T/G (rs2243248).

Regarding -590C/T SNP – the existing evidence suggests implications in INS prognosis as well as in clinical evolution of the disease. [21] Also, for this SNP there have been identified differences in genotypic as well as allelic frequencies between patients with INS, MCD histological group, and controls. [22]

II. PERSONAL CONTRIBUTIONS

5. THE ROLE OF SINGLE NUCLEOTIDE POLYMORPHISMS OF *IL-13* GENE IN ROMANIAN PEDIATRIC POPULATION WITH IDIOPATHIC NEPHROTIC SYNDROME

5.1 Introduction

Interleukin-13 (IL-13) is a cytokine secreted by activated T helper 2 lymphocytes, being an important regulator of the immune response. [23] Its encoding gene is part of the genetic cluster of Th2 type cytokines located on chromosome 5q23-31 along with *IL-4* and *IL-5* genes, and consists of 6 exons encoding 2 isoforms of the same cytokine, according to NCBI database. [24]

5.2 The link between nephrotic syndrome and atopy

As mentioned above, the mechanisms implicated in INS pathogenesis remain unclear. At least for minimal changes glomerulonephritis (MCD), the most frequent histological variant of the disease, literature data suggest a pivotal role of the immune system. [25] Moreover, numerous studies have reported the existence of a link between this histological variant of INS, elevated serum levels of IgE and predisposition for atopy.[26]

First reports of the association between allergies and INS belong to Fanconi et al., as early as 1951. Their study showed that 43% of NS patients also had signs of "allergic diathesis".[27] Since then, data has flourished regarding the implications of various allergens like pollen, mold or insect bites, as precipitating factors for NS relapses.[28,29,30]

5.3 Genetic polymorphisms of *IL-13* in pediatric population with INS

Lately, there has been intense research on the possible link between the polymorphisms of various gene's encoding different molecules implicated in glomerular functions and the risk for INS development. Specifically, literature data points at glypican-5 (*GPC5*) gene encoding a proteoglycan found on the surface of podocytes, with molecular variations at this level causing podocyte injury and concurrent proteinuria. [31] Other interesting molecules have been macrophage migration inhibitory factor(*MIF*), with its role in adjusting the immunosuppressive effect of systemic steroids [32], neural nitric oxide synthetase (n*NOS*) with a pivotal role in

increased glomerular permeability[33] and *IL-13*, the latter altering podocyte functions and being found to be overexpressed in patients with relapses of INS.[9]

Regarding the IL-13 cytokine, the existing literature data looking into the role of its polymorphisms in disease susceptibility and response to treatment are rather scarce and inconclusive. More specifically, the existing studies on Caucasian and Asian populations, analyzing various gene polymorphisms (e.g. Arg144Gln (rs20541)), *526C/A(rs848), polymorphisms in the 3'UTR region), have generated contradictory results.[10,34]

Thus, the aim of this study was to investigate the implications of single nucleotide polymorphisms of *IL-13* in the risk for INS development, in Romanian pediatric patients with INS. Also, we studied the relationship between these two SNPs with steroid treatment response, as well as with the presence of atopy.

The present study represents the first research project in Romania aiming at the implications of these genetic variations in pediatric idiopathic nephrotic syndrome, the most frequently diagnosed glomerulopathy in childhood.

5.4 Materials and methods

Database development and patients' selection

For the present project, 75 pediatric patients with idiopathic nephrotic syndrome (INS) diagnosed between 2015 and 2018 in the Pediatric Nephrology Department of "Marie Sklodowska Curie" Children's Emergency Hospital, were included in the database.

The INS diagnosis was established following the guidelines criteria proposed by Kidney Disease Improving Global Outcomes.[1] There were 55 male patients, and 20 females. The median age was 4 years and 9 months. The patients were divided into two subgroups: steroid sensitive – patients who achieved complete remission during the first 4 weeks following steroid treatment initiation (N= 62, 46 M/16 F, mean age 5 years and 1 month) and steroid resistant– patients failing to achieve remission after 8 weeks of steroid treatment (N=13, 9 M/4 F, mean age of 4 years and 6 months).

For the control group, 110 blood samples were used. 66 samples were provided by the DNA collection of the Immunology and Physiopathology Department from the Medicine Faculty, "Carol Davila" University of Medicine and Pharmacy from Bucharest, which was initiated in 2006

and consist of genomic DNA extracted from blood samples pertaining to healthy subjects. These patients are listed as possible organ donors in the "Prof. Dr. C. T. Nicolau" National Transfusion Institute database. The rest of the control group, 44 blood samples, were extracted from pediatric patients admitted in the Pediatric Nephrology Department with different acute diseases but without any personal disease history. Every subject from the control group had previously signed an informed consent regarding genetic investigation studies.

Another group of patients was represented by 84 pediatric patients diagnosed with various food allergies (F/M 29/55). These patients were diagnosed with allergic diathesis based on the tests undergone in the Outpatient Immunology Clinic of "Marie Sklodowska Curies" Children's Emergency Hospital.

The present study has been approved by the Hospital's Ethics Committee with written informed consent obtained from all patients prior to blood sampling for genetic studies. (16266/10.06.2015).

5.5 Results and conclusions

Our findings suggest the existence of an inverse association between the presence of the minor allele for the rs1800925 single nucleotide polymorphism and the risk of INS development (p=0,03, OR 0,53). For rs20541 SNP, the allelic and genotypic frequencies were similar between patients and controls, showing no association with INS (p>0,05). The rs20541/rs1800925 CT haplotype was significantly more frequent in controls as compared to INS patients (p=0,02).

Regarding response to steroid treatment, minor allele frequency and carrier status for the minor allele (genotypes CT and TT) were significantly lower in steroid-resistant patients for rs1800925 SNP (p=0,02, OR 0,14, respectively p=0,03, OR 0,14). Following a higher scale study confirmation, this SNP might be considered a predictor for steroid treatment refractoriness.

Moreover, this study did not show any correlation between *IL-13* gene polymorphisms and the risk of food allergy development.

5. THE ROLE OF *TNF-alpha* GENE SINGLE NUCLEOTIDE POLY-MORPHISMS IN ROMANIAN PEDIATRIC POPULATION WITH IDIOPATHIC NEPHROTIC SYNDROME

6.1 Introduction

TNF-alpha is a multifunctional proinflammatory cytokine secreted by macrophages and T lymphocytes.[35] It exhibits various biologic functions with a complex mechanism of action. The protein has a role in infections conferring resistance when facing different pathogens [36],, as well as a role in autoimmune diseases such as rheumatoid arthritis [37], possibly due to different signaling pathways; moreover, TNF-alfa exhibits and anti-tumor effect, especially in combination with Interferon. [36,38]

TNF-alpha gene is located on the short arm of chromosome 6, preceded by *TNF-beta* gene [39] and in strong relationship with the major histocompatibility complex genes. It consists of 4 exons and 3 introns with an approximate 3kb weight. There have been identified a series of polymorphisms in the promoter region of the *TNF-alpha* gene: -1031 (T/C), -863(C/A), -857(C/T), -419(G/C), -308(G/A), -238(G/A) and 162(G/A). -308(G/A) polymorphism, rs1800629 respectively, has been associated with an elevated transcription of TNF protein [328,329], along with the -857(C/T) polymorphisms being responsible for the susceptibility to develop immune-mediated diseases [40]

Towards the role of *TNF-alpha* gene polymorphisms and the susceptibility for INS development, and INS response to immune suppressive medication, literature has generated conflicting results. There is a need for larger cohort studies to evaluate this hypothesis.

The aim of our study was to obtain preliminary data regarding the association between two polymorphisms of the *TNF-alpha* gene (rs1799724 (-857C/T) and rs1800629 (-308G/A)) and the risk of INS development in Romania pediatric population. Moreover, we investigated a possible relationship between these two polymorphisms and response to steroid treatment.

This is the first study of its kind in Romania, investigating the role of these two polymorphisms in INS.

6.2. Materials and methods

Database development and patients' selection

For this study, 229 unrelated Caucasian subjects of Romanian origin were enrolled. Of these, 70 patients were diagnosed with INS (50 males and 20 females), mean age of 9 years old recruited from the Pediatric Nephrology Department of "M.S. Curie" Children's Emergency Hospital in Bucharest. The diagnosis was made according to the guideline proposed by the Kidney Disease Improving Global Outcomes.[1] Patients were further subdivided based on their response to steroid treatment in two subgroups: steroid sensitive – achievement of complete remission during the first 4 weeks of steroid treatment (N=59, 42M/7F, mean age of 8 years old), and steroid resistant, defined as failure to achieve remission after 8 weeks of steroid treatment (N=11, 8M/3F, mean age of 9 years and 11 months old).

The control group consisted of 159 subjects (103M/56F, mean age of 39 years old), without signs or symptoms suggestive for chronic renal disease and without personal history of proteinuria or oedema. The patients are part of the DNA collection of the Immunology and Physiopathology Department from the Medicine Faculty, "Carol Davila" University of Medicine and Pharmacy from Bucharest, which was initiated in 2006 and consist of genomic DNA extracted from blood samples pertaining to healthy subjects. These patients are listed as possible organ donors in the database from "Prof. Dr. C. T. Nicolau" National Transfusion Institute from Bucharest.

The present study has been approved by the Hospital's Ethics Committee with written informed consent obtained from all patients prior to blood sampling for genetic studies. (16266/10.06.2015).

6.3 Results and conclusions

We investigated the association between two polymorphisms of the *TNF-alfa* gene and INS, in Romanian pediatric population. Regarding rs1799724 SNP, even though there was an elevated frequency for the minor allele in the control group, the results did not reach statistical significance (p=0,46, OR=0,818).

Commented [1]:

Also, there were no differences observed between steroid responders and non-responders neither for allelic nor for genotypic frequencies. Moreover, there were no differences between the two patient groups and controls.

7. THE ROLE OF *IL-4* GENE MONONUCLEOTIDE POLYMORPHISMS IN ROMANIAN PEDIATRIC POPULATION WITH IDIOPATHIC NEPHROTIC SYNDROME

7.1 Introduction

Interleukin 4 (IL-4) is a powerful immune regulating cytokine, produced by mastocytes, eosinophils, basophils and T helper 2 lymphocytes (Th2). [41] IL-4 was first identified by Howard and Paul in 1982, showing activity in different immune processes such as cellular immunity through Th2 cells [42] or immunoglobulin E subset transformation into immunoglobulin B. [44] Its implications in host's immune response is of utmost importance in clinical practice, as this cytokine along with its signaling pathways are targets for molecules used in the treatment of allergic diseases.[45]

7.2 *IL-4* genetic polymorphisms in the pediatric population with idiopathic nephrotic syndrome

To date, according to the literature, Th17 and Th2 lymphocytes have a role in nephrotic syndrome pathogenesis, through some of their synthesis cytokines such as IL-4, IL-5, IL-9 and IL-10, which have been demonstrated to promote the development of this disease. [46,47]

A series of mononucleotide polymorphisms of the *IL-4* gene have been described, some of the most studied being the following: rs2243248 (-1098 G/T, upstream variant), rs2243250 (-590C/T, promoter region), rs2070874 (-34C/T; 5'- untranslated region), rs2227284 (G/T, intron located), rs2243268 (A/C, intron located) and rs2234665 (variable number of tandem repeats, VNTR).[48]

By the molecular point of view, there is data suggesting an association between genetic polymorphisms of *IL-4* gene and MCNS in Asian populations, but this has not been replicated in European populations.[9,22,34]

There are also published data regarding a possible association between genetic polymorphisms of some cytokines pertaining to Th2 subtype lymphocytes (IL-4, IL-6), and response to steroid treatment in pediatric population. [21,49,50]

The primary objective of our study was to obtain data regarding the possible association between two mononucleotide polymorphisms of *IL-4* gene (rs2243250/-590C/T

and rs2070874/-34C/T) and disease susceptibility for INS, in pediatric population from Romania. We also investigated the role of this polymorphisms in steroid response of these patients.

The current study is the first study from Romania to address the implications of these mononucleotide polymorphisms in INS.

7.3. Materials and methods

Database development and patients' selection

For this study, 235 unrelated Caucasian subjects of Romanian origin were enrolled. Of these, 75 patients were diagnosed with INS (55 males and 20 females), mean age of 4 years and 9 months old, recruited from the Pediatric Nephrology Department of "M.S. Curie" Children's Emergency Hospital in Bucharest. The diagnosis was made according to the guideline proposed by the Kidney Disease Improving Global Outcomes.[1] Patients were further subdivided based on their response to steroid treatment in two subgroups: steroid sensitive – achievement of complete remission during the first 4 weeks of steroid treatment (N=62, 46M/16F, mean age of 5 years and 1 month old), and steroid resistant, defined as failure to achieve remission after 8 weeks of steroid treatment (N=13, 9M/4F, mean age of 4 years and 6 months old).

The control group consisted of 160 subjects (104M/56F, mean age of 39 years old), without signs or symptoms suggestive for chronic renal disease and without personal history of proteinuria or oedema. The patients are part of the DNA collection of the Immunology and Physiopathology Department from the Medicine Faculty, "Carol Davila" University of Medicine and Pharmacy from Bucharest, which was initiated in 2006 and consist of genomic DNA extracted from blood samples pertaining to healthy subjects. These patients are listed as possible organ donors in the database from "Prof. Dr. C. T. Nicolau" National Transfusion Institute from Bucharest.

The present study has been approved by the Hospital's Ethics Committee with written informed consent obtained from all patients prior to blood sampling for genetic studies. (16266/10.06.2015).

7.4 Results and conclusions

This retrospective study aimed at investigating the possible association between two *IL-4* gene polymorphisms and INS, in Romanian pediatric population.

There were 75 pediatric patients and 160 healthy controls enrolled, the two groups showing the presence of Hardy-Weinberg equilibrium for all mononucleotide polymorphisms studied. The final genotyping rate was 100%.

The results obtained from the control group were compared to the available data for European descend listed in NCBI Database of Short Genetic Variations (dbSNP). Allelic and genotypic frequencies in the control group for the two investigated SNPs were similar with those reported by the HapMap-CEU for the European population.

Regarding the rs2243250 SNP, the frequency of the minor allele T was 12.84% in the INS group and 17.5% in the control group. Thus, even though the frequency of the minor allele T was more elevated in the control group, the difference failed to reach statistical significance. (p=0,18, OR=0,684). Regarding the second studied SNP (rs2070874), there were no significant differences between groups in terms of allelic or genotypic frequencies.

Moreover, the comparison between steroid sensitive and steroid resistant INS groups did not show any significant differences for neither allelic nor genotypic frequency. Finally, no differences were identified between either of the two groups and the control group.

The level of linkage disequilibrium in the studied population was estimated based on the r^2 value, with a calculated level of 0.72. Two haplotype variant had frequencies of over 5% in the control group: rs2243250/rs2070874 CC - 81% and TT - 16%. No associations with disease susceptibility were found with haplotype analysis.

III. GENERAL CONLUSIONS AND PERSONAL CONTRIBUTIONS

As a result of these three case-control studies, where we aimed at establishing possible correlations between T helper 2 cytokines nucleotide polymorphisms and INS in pediatric population, we can conclude the following:

THE ROLE OF *IL-13* GENE MONONUCLEOTIDIC POLYMORPHISMS IN ROMANIAN PEDIATRIC POPULATION WITH IDIOPATHIC NEPHROTIC SYNDROME

1. This is the first study to investigate mononucleotide polymorphisms for *IL-13* gene in a group of patients with INS from Romania.

2. For rs1800925 SNP of *IL-13* gene, we observed an increased frequency of the minor allele T in the control group, as compared to the INS group. We can postulate that the presence of the minor allele T might be considered a protective factor against the development of INS, in Romanian pediatric population.

3. For rs20541 SNP, the frequency of the minor allele T was similar between the 2 groups, thus confirming by statistical means the absence of any association.

4. Haplotype analysis showed that rs20541/rs1800925 CT might represent a protective factor, being significantly more frequent in the control group than in the INS group. CC haplotype was more frequent in the diseased group but failed to reach statistical significance.

5. rs1800925 SNP minor allele was found to be significantly less frequent in the steroid resistant INS group, suggesting that the major allele might be associated to lack of response to steroid treatment.

6. No association was found between the two SNPs analyzed and good response to steroid treatment. There were no significant differences between the steroid sensitive INS patients and the control group regarding the allelic and genotypic frequencies for these two studied SNPs.

7. No correlation was identified between the investigated SNPs and the risk for food allergies.

THE ROLE OF *TNF-alpha* GENE MONONUCLEOTIDE POLYMORPHISMS IN ROMANIAN PEDIATRIC POPULATION WITH IDIOPATHIC NEPHROTIC SYNDROME

1. To our knowledge, this is the first study to address the implications of *TNF-alpha gene* polymorphisms in the development and response to treatment of INS, in Caucasian populations, and, particularly, the Romanian pediatric population.

2. The rs1799724 SNP minor allele T was more frequent in the control group but the difference to the INS group failed to reach statistical significance.

3. There were no differences in terms of allelic or genotypic frequencies for the same SNP able to differentiate the two INS treatment response groups (steroid-sensitive and steroid-refractory).
4. Moreover, there were no significant differences between the two INS groups and controls for the same SNP.

THE ROLE OF *IL-4* GENE MONONUCLEOTIDE POLYMORPHISMS IN ROMANIAN PEDIATRIC POPULATION WITH IDIOPATHIC NEPHROTIC SYNDROME

1. This is the first study in Romania to investigate the link between the mononucleotide polymorphisms of this gene and disease susceptibility for INS.

2. For rs2243250 SNP, we found an increased frequency of the minor allele T in the control group, without reaching the statistical significance threshold.

3. There were no differences in terms of allelic and genotypic frequencies for the same studied SNP between steroid sensitive and steroid resistant groups.

4. Moreover, we found no differences between any of the diseased groups and controls regarding the allelic and genotypic frequencies of the same SNP.

5. For -34C/T polymorphism, we did not find any statistically significant differences between the studied groups in terms of allelic and genotypic frequency. Moreover, our results showed no influence of the SNP on steroid treatment response.

All our results need to be carefully interpreted, as each population's genetic background is different. Therefore, our results are difficult to apply outside the studied population. Also, there is a major limitation in our study related to the small number of patients enrolled, as this could influence our results, and might contribute to incorrect conclusions. Finally, we consider necessary the reproduction of our results on larger cohorts of patients to clearly determine if these genetic polymorphisms do have a role in INS disease susceptibility and response to steroid treatment.

References

1. Kidney Disease Improving Global Outcomes. KDIGO Clinical practice guideline for glomerulonephritis. Kidnet Int Suppl. 2012;2:1–274.

2. Noone DG, Iijima K, Parekh R. Idiopathic nephrotic syndrome in children. Lancet. 2018.

3. Dossier C, Lapidus N, Bayer F, Sellier-Leclerc AL, Boyer O, de Pontual L, et al. Epidemiology of idiopathic nephrotic syndrome in children: endemic or epidemic? Pediatr Nephrol. 2016;

4. Yap HK, Han EJ, Heng CK, Gong WK. Risk factors for steroid dependency in children with idiopathic nephrotic syndrome. Pediatr Nephrol. 2001;16:1049–52.

5. Koskimies O, Vilska J, Rapola J, Hallman N. Long-term outcome of primary nephrotic syndrome. Arch Dis Child. 1982;

6. Tarshish P, Tobin JN, Bernstein J, Edelmann Jr. CM. Prognostic significance of the early course of minimal change nephrotic syndrome: report of the International Study of Kidney Disease in Children. J Am Soc Nephrol. 1997;

7. McKenzie AN, Culpepper JA, de Waal Malefyt R, Briere F, Punnonen J, Aversa G, et al. Interleukin 13, a T-cell-derived cytokine that regulates human monocyte and B-cell function. Proc Natl Acad Sci U S A. 1993;90:3735–9.

8. Suvanto M, Jahnukainen T, Kestilä M, Jalanko H. Single Nucleotide Polymorphisms in Pediatric Idiopathic Nephrotic Syndrome. Int J Nephrol. 2016;

9. Acharya B, Shirakawa T, Pungky A, Damanik P, Massi MN, Miyata M, et al. Polymorphism of the interleukin-4, interleukin-13, and signal transducer and activator of transcription 6 genes in Indonesian children with minimal change nephrotic syndrome. Am J Nephrol. 2005

10. Wei CL, Cheung W, Heng CK, Arty N, Chong SS, Lee BW, et al. Interleukin-13 genetic polymorphisms in Singapore Chinese children correlate with long-term outcome of minimal-change disease. Nephrol Dial Transplant; 2005 [cited 2022 Apr 19];20:728–34. Available from: https://pubmed.ncbi.nlm.nih.gov/15728267/

11. Tsukamoto K, Ohta N, Shirai Y, Emi M. A highly polymorphic CA repeat marker at the

human tumor necrosis factor alpha (TNFAα) locus. J Hum Genet. 1998;

12. Mira JP, Cariou A, Grall F, Delclaux C, Losser MR, Heshmati F, et al. Association of TNF2, a TNF-α promoter polymorphism, with septic shock susceptibility and mortality: A multicenter study. J Am Med Assoc. 1999;282:561–8.

13. MATHIESON PW. Cytokine polymorphisms and nephrotic syndrome. Clin Sci. 2003;

14. Fodor P, Saitúa MT, Rodriguez E, González B, Schlesinger L. T-Cell Dysfunction in Minimal-Change Nephrotic Syndrome of Childhood. Am J Dis Child. 1982;

15. Yap HK, Cheung W, Murugasu B, Sim SK, Seah CC, Jordan SC. Th1 and Th2 cytokine mRNA profiles in childhood nephrotic syndrome: evidence for increased IL-13 mRNA expression in relapse. J Am Soc Nephrol. 1999;

16. Kimata H, Fujimoto M, Furusho K. Involvement of interleukin (IL)-13, but not IL-4, in spontaneous IgE and IgG4 production in nephrotic syndrome. Eur J Immunol. 1995;25:1497–501.

17. Neuhaus TJ, Wadhwa M, Callard R, Barratt TM. Increased IL-2, IL-4 and interferon-gamma (IFN-gamma) in steroid sensitive nephrotic syndrome. Clin Exp Immunol. 1995;100:475–9.

18. Punnonen J, Aversa G, Cocks BG, McKenzie AN, Menon S, Zurawski G, et al. Interleukin 13 induces interleukin 4-independent IgG4 and IgE synthesis and CD23 expression by human B cells. Proc Natl Acad Sci U S A. 1993;

19. Lee F, Yokota T, Otsuka T et al. Isolation and characterization of a mouse interleukin cDNA clone that expresses B-cell stimulatory factor 1 activities and T-cell- and mast-cell-stimulating activities. Proc Natl Acad Sci U S A. 1986;83(7):2061–5.

20. Noma Y, Sideras P, Naito T et al. Cloning of cDNA encoding the murine IgG1 induction factor by a novel strategy using SP6 promoter. 1986;319(6055):640-646. Nature. 1986;319(6055):640–6.

21. Jafar T, Agrawal S, Mahdi a a, Sharma RK, Awasthi S, Agarwal GG. Cytokine gene polymorphism in idiopathic nephrotic syndrome children. Indian J Clin Biochem. 2011;26:296–302.

22. Kobayashi Y, Arakawa H, Suzuki M, Takizawa T, Tokuyama K, Morikawa A. Polymorphisms of interleukin-4-related genes in Japanese children with minimal change nephrotic syndrome. Am J Kidney Dis. 2003;

23. Rengarajan J, Szabo SJ GL. Transcriptional regulation of Th1/Th2 polarization. Immunol Today. 2000;21:479–83.

24. Kent WJ, Sugnet CW, Furey TS, Roskin KM, Pringle TH, Zahler AM HD. The human genome browser at UCSC. Genome Res. 2002;12:996–1006.

25. Shalhoub RJ. PATHOGENESIS OF LIPOID NEPHROSIS: A DISORDER OF T-CELL FUNCTION. Lancet. 1974;

26. Schulte-Wisserman H, Gortz W, Straub E. IgE in patients with glomerulonephritis and minimal-change nephrotic syndrome. Eur J Pediatr. 1979;131:105–11.

27. FANCONI G, KOUSMINE C FKW. Prognosis of the nephrosis syndrome. Helv Paediatr Acta. 1951;6:219-24.

28. Reeves WG, Cameron JS, Johansson SG, Ogg CS, Peters DK, Weller RO. Seasonal nephrotic syndrome. Description and immunological findings. Clin Allergy. 1975;

29. Wittig HJ, Goldman AS. NEPHROTIC SYNDROME ASSOCIATED WITH INHALED ALLERGENS. Lancet. 1970;

30. RYTAND DA. Onset of the nephrotic syndrome during a reaction to bee sting. Stanford Med Bull. 1955;13:224–33.

31. Okamoto K, Tokunaga K, Doi K, Fujita T, Suzuki H, Katoh T, Watanabe T, Nishida N, Mabuchi A, Takahashi A, Kubo M, Maeda S, Nakamura Y NE. Common variation in GPC5 is associated with acquired nephrotic syndrome. Nat Genet. 2011;43:459–63.

32. M. Vivarelli, L.E.D"Urbano GS. Association of the macrophage migration inhibitory factor −173*C allele with childhood nephrotic syndrome. Pediatr Nephrol. 2008;23:743–8.

33. Alasehirli B, Balat A, Barlas O KA. Nitric oxide synthase gene polymorphisms in children with minimal change nephrotic syndrome. Pediatr Int. 2009;51:75–8.

34. Tenbrock K, Schubert A, Stapenhorst L, Kemper MJ, Gellermann J, Timmermann K, et al.

Type I IgE receptor, interleukin 4 receptor and interleukin 13 polymorphisms in children with nephrotic syndrome. Clin Sci. 2002;102:507–12.

35. Weissbach A, Garty BZ, Lagovsky I, Krause I, Davidovits M. Serum Tumor Necrosis Factor-Alpha Levels in Children with Nephrotic Syndrome: A Pilot Study. Isr Med Assoc J [Internet]. Israel; 2017 [cited 2022 Apr 20];19:30–3. Available from: https://pubmed.ncbi.nlm.nih.gov/28457111/

36. Fiers W. Tumor necrosis factor. Characterization at the molecular, cellular and in vivo level. FEBS Lett. England; 1991;285:199–212.

37. Beutler BA. The role of tumor necrosis factor in health and disease. J Rheumatol Suppl [Internet]. Canada; 1999 [cited 2022 Apr 20];57:16–21. Available from: https://pubmed.ncbi.nlm.nih.gov/10328138/

38. Jafar T, Agrawal S, Mahdi AA, Sharma RK, Awasthi S, Agarwal GG, et al. Does cytokine gene polymorphism affect steroid responses in idiopathic nephrotic syndrome? J Biol Chem [Internet]. United States; 2017;19:18324–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29218062%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC5706754

39. Spriggs DR, Deutsch S, Kufe DW. Genomic structure, induction, and production of TNF-alpha. Immunol Ser. 1992;56:3–34.

40. Xiao M, Bai S, Chen J, Xie L, Li Y, Li Y, et al. Correlation of TNF-α and IL-10 gene polymorphisms with primary nephrotic syndrome. Exp Ther Med. 2020;20:87.

41. Gadani SP, Cronk JC, Norris GT, Kipnis J. IL-4 in the brain: a cytokine to remember. J Immunol [Internet]. J Immunol; 2012 [cited 2022 Apr 20];189:4213–9. Available from: https://pubmed.ncbi.nlm.nih.gov/23087426/

42. Howard M, Farrar J, Hilfiker M, Johnson B, Takatsu K, Hamaoka T, et al. Identification of a T cell-derived B cell growth factor distinct from interleukin 2. J Exp Med [Internet]. J Exp Med; 1982 [cited 2022 Apr 20];155:914–23. Available from: https://pubmed.ncbi.nlm.nih.gov/6977612/

43. Seder RA, Paul WE, Davis MM, Fazekas de St. Groth B. The presence of interleukin 4

during in vitro priming determines the lymphokine-producing potential of CD4+ T cells from T cell receptor transgenic mice. J Exp Med [Internet]. J Exp Med; 1992 [cited 2022 Apr 20];176:1091–8. Available from: https://pubmed.ncbi.nlm.nih.gov/1328464/

44. Geha RS, Jabara HH, Brodeur SR. The regulation of immunoglobulin E class-switch recombination. Nat Rev Immunol [Internet]. Nat Rev Immunol; 2003 [cited 2022 Apr 20];3:721–32. Available from: https://pubmed.ncbi.nlm.nih.gov/12949496/

45. Junttila IS. Tuning the Cytokine Responses: An Update on Interleukin (IL)-4 and IL-13 Receptor Complexes. Front Immunol [Internet]. Front Immunol; 2018 [cited 2022 Apr 20];9. Available from: https://pubmed.ncbi.nlm.nih.gov/29930549/

46. Stachowski J, Krynicki T, Barth C, Runowski D, Lewandowska-Stachowiak M, Warzywoda A, Bortkiewicz E, Dobosz M MJ. Does the changed Th1/Th2 activity in children with the assessment of body water in children with nephrotic syndrome: initial results. Pol Merkur Lek. 2000;8:222–4.

47. Wang L, Li Q, Wang L, Li C, Yang H, Wang X TH. The role of Th17/IL-17 in the pathogenesis of primary nephrotic syndrome in children. Kidney Blood Press Res. 2013;37(4-5):332-45. Kidney Blood Press Res. 2013;37:332–45.

48. Anovazzi G, Medeiros MC, Pigossi SC, Finoti LS, Souza Moreira TM, Mayer MPA, et al. Functionality and opposite roles of two interleukin 4 haplotypes in immune cells. Genes Immun [Internet]. Genes Immun; 2017 [cited 2022 Apr 20];18:33–41. Available from: https://pubmed.ncbi.nlm.nih.gov/28053321/

49. Tripathi G, Jafar T, Mandal K, Mahdi A a, Awasthi S, Sharma RK, et al. Does cytokine gene polymorphism affect steroid responses in idiopathic nephrotic syndrome? Indian J Med Sci. 2008;62:383–91.

50. Hanan A. Madani HMB and HR. Association of cytokine genes polymorphisms and the response to corticosteroid therapy in children with idiopathic nephrotic syndrome: A pilot study in Egypt. Int Res J Med Med Sci. 2014;2:84–90.

THE LIST OF PUBLISHED PAPERS GENERATED BY THIS RESEARCH PROJECT

1. **Tieranu I**, Tieranu CG, Dutescu MI, Berghea CE, Balgradean M, Popa OM. Genetic Variants of Interleukin-4 in Romanian Patients with Idiopathic Nephrotic Syndrome. Medicina (Kaunas). 2022 Feb 10;58(2):265. Medicina | Free Full-Text | Genetic Variants of Interleukin-4 in Romanian Patients with Idiopathic Nephrotic Syndrome (mdpi.com)

2. **Tieranu I**, Dutescu MI, Bara C, Tieranu CG, Balgradean M, Popa OM. Preliminary Study Regarding the Association between Tumor Necrosis Factor Alpha Gene Polymorphisms and Childhood Idiopathic Nephrotic Syndrome in Romanian Pediatric Patients. Maedica (Bucur). 2017 Sep;12(3):164-168. Preliminary study regarding the association between tumor necrosis factor alpha gene polymorphisms and childhood idiopathic nephrotic syndrome in Romanian pediatric patients – MÆDICA – a Journal of Clinical Medicine (maedica.ro)