Genetic Analysis of Steroid Resistant Nephrotic Syndrome (SRNS) in South Indian children

Introduction

The term “Nephrotic Syndrome” describes the clinical state characterized by the presence of proteinuria, hypoalbuminemia, and edema. An English physician Richard Bright first demonstrated that edema and proteinuria were dependent on change in the kidney in 1827, and for the next 80 years, nephrotic syndrome (NS) was known as “Bright’s disease” \(^1,2\). In 1905 Friedrich von Muller further delineated kidney diseases into “nephritis” and “nephrosis,” and finally in 1929 Henry Christian included the phrase “nephrotic syndrome” in his writing\(^3\). Nephrotic syndrome is a condition frequently characterized by the following: (Fig.1)

- very high levels of protein in the urine
- low levels of protein in the blood
- swelling, especially around the eyes, feet, and hands
- high cholesterol in the blood

Kidney’s tiny blood-filtering units are called glomerular filtration barrier where from urine is made. Healthy kidney (glomerulai) keeps protein in the blood, which helps the blood soak up water from tissues, but kidneys with damaged filters may show inability to restrict protein in the urine, resulting in edema\(^4\). Nephrotic syndrome (NS) is a common kidney defect in children, the first sign of a disease that damages the glomeruli\(^5\).

According to the clinical relevant definitions, it requires the presence of edema, massive proteinuria (>40 mg/m\(^2\)/hr or a urine protein/creatinine ratio >2.0 mg/mg), and hypoalbuminemia (<2.5 g/dl) \(^5,6,7\). Most of the children with NS respond to corticosteroid treatment. Based on the responses to treatment they are divided into – urinary protein excretion <4 mg/m\(^2\)/h; steroid dependent nephrotic syndrome (SDNS) which is occurrence of 2 consecutive relapses during the tapering of steroid therapy or within 2 weeks of its
Figure 1. Overview of protein/fluid leak in NS children

- Histochemical changes in filters (Nephron) leaking protein into the urine
- Fluid leak out of the blood vessel
- Edema
- Urine dipstick used to detect protein leak in urine
cessation. Steroid-resistant nephrotic syndrome (SRNS) which is failure to achieve remission after 4 weeks of daily therapy with oral prednisolone at a dose of 2 mg/kg/day\textsuperscript{8,9}.

Age of initial presentation has an important impact on the disease distribution and in the response to steroids, though most of the children 75-80% are SSNS and of 15-20% are SRNS. If they do not respond to the treatment, they run the risk of developing end stage renal disease (ESRD)\textsuperscript{10,11}.

**Genetic condition in children**

Pediatric hospital admission reveals that more than 35% cases have genetic condition and it is a most important contribution\textsuperscript{12}. The largest part of death in hospitalized children was deeply associated with genetic disorder\textsuperscript{12}. In most of the cases it is not clearly identified and recorded. Non-infectious chronic diseases in children have genetic component. Genetic disorder can present in children at an earlier age; some of the most obvious and severe diseases begin in childhood. Major categories of genetic condition in children include single nucleotide polymorphism (SNP’s), single gene deficiency, chromosome defects, and multifactorial conditions. In person to person, single-gene disorders are rare, but commonly they represent a main contribution to childhood disease. Single-gene forms are likely to occur when mutations have a reflective effect on the function of the gene product such as protein defects; in these special effects is contained loss or gain of the function. The phenotypes associated with single-gene disorders can be inconsistent and adapted and then make use of other genes or the environment. Single-gene disorders may occur familially or sporadically, as seen with increased frequency in specific populations (e.g.) NPHS1 mutation has been mostly reported in Finnish population. In the case of infrequent single-gene disorders, the pediatrician will work directly with the specialists and they will focus on arriving at correct diagnosis, so as to enable counseling the family about natural history and management of the disease/disorder as well as risk, and implementation of a management and treatment plan.
Renal related syndrome and association/identified genes in congenital cases

The average number of nephron varies from individual to individual, and it ranges from 3,00,000 to 1 million in each kidney\textsuperscript{13}. Genetic, environmental and other factors such as nutrition during pregnancy can influence the glomerular numbers\textsuperscript{14}.

<table>
<thead>
<tr>
<th>Syndromes</th>
<th>Gene defects</th>
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<tbody>
<tr>
<td>Ochoa (Urofacial) syndrome</td>
<td>Chr 10q23-24</td>
</tr>
<tr>
<td>Ellis van Crefeld</td>
<td>EVC, EVC2</td>
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<tr>
<td>Frasier,Denys-Drash,WAGAR, idiopathic NS</td>
<td>WT1</td>
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<tr>
<td>Finnish type NS</td>
<td>NPHS1</td>
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<tr>
<td>Nail-patella-syndrome</td>
<td>LMX1B</td>
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<tr>
<td>Pierson syndrome</td>
<td>LAMB2</td>
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<tr>
<td>Steroid- resistant NS</td>
<td>NPHS2</td>
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<tr>
<td>Oligomeganephronia</td>
<td>PAX2</td>
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Table 1. List of renal related syndrome

Genetic linkage studies have identified several genes involved (Table 1) in the development of nephrotic syndrome and contributed to the understanding of the pathophysiology of glomerular proteinuria and/or focal segmental glomerulosclerosis. The mutation in podocyte genes (NPHS1, NPHS2, CD2AP, PLCE1, ACTN4, TRPC6 and INF2), has been associated with different forms of nonsyndromic SRNS. Mutation in NPHS1 is responsible for most of the cases of congenital nephrotic syndrome (CNS)\textsuperscript{15}, and the mutation in NPHS2 is responsible for most of the early onset SRNS cases.

Histopathology of kidney damage in Nephrotic Syndrome

Ethnic origin may play/affect the role of the histological modification and the response to immunosuppressive treatment\textsuperscript{16-18}. Three different histological variants of NS (MCNS, FSGS and DMP) are seen in children; these different patterns are in different age groups\textsuperscript{19}. Minimal change nephrotic syndrome (MCNS) most often responds to corticosteroids, whereas some patients with MCNS are resistant to corticosteroids, while most of them are in remission\textsuperscript{20}. A significant proportion of patients with focal (only some
of the glomeruli are involved) segmental (only part of an entire glomerulus involved) sclerosis (FSGS) respond to corticosteroids, most of them are resistant. Histologically, FSGS has been classified according to olympia criteria. They are collapsing variant, tip lesion variant, cellular variant, perihilar variant and also not otherwise specified. Based on etiology, FSGS is classified into three categories: idiopathic, genetic, and secondary due to medication, injury or drug abuse. Genetic forms of FSGS are classified in the syndromic and non-syndromic forms. In syndromic forms WT1 (Frasier, Denys-Drash syndrome), COL4A3, A4, A5 (Alport syndrome, GLA (Fabry syndrome), LMX1B (nail-patella syndrome) are important and non syndromic NS are NPHS1, NPHS2, ACTN4, CD2AP, and PLCE1.

Development of NS is characterized by numerous morphological changes in podocytes. Podocyte damage such as minimal change disease (MCNS), and the injury minimal to foot process effacement, and podocyte numbers always maintain the same in MCNS. A more severe form of podocyte injury occurs, leading to podocyte detachment resulting in the segmental scar characteristic of FSGS. Finally, podocyte injury may lead to either low/high rates of proliferations such as diffuse mesangial sclerosis (DMS) or collapsing glomerulopathy.

Early evidences are independent of this disease and characterized by alteration in molecular composition of slit diaphragm (SD) without visible changes in morphology, more visibly, by reorganization of the foot process (FP) structure with fusion of filtration slits and apical displacement of the SD. Advanced a studies in this field have clearly demonstrated that the detachment of the podocyte from the glomerular basement membrane (GBM) results in the leakage of protein towards the GBM at the site of detachment. Based on this finding podocytes appear to form a major segment of the kidney’s filtration barrier. These types of information are very useful in judging the fundamental morphologies of the basic disease mechanism in different types of patients.

Molecular and cell biology of the podocyte

Glomerular capillaries are composed of three components: endothelial cell layer, extracellular glomerular basement membrane (GBM), and a glomerular epithelial cell (podocyte) layer with distal. Glomerular development is usually divided into four stages:
Figure 2. Molecular mechanism and its linkage to proteinuria
renal vesicle, S shaped body, capillary loop, and maturing glomeruli. Podocyte is responsible for continuous cleaning of the filter. The podocyte is a very impressive cell type; its location, its architecture, and its functions are most important. The foot processes of bordering podocytes shaped interdigitate, leaving between them meandering filtration slits that are bridged by an extra cellular structure, known as the slit diaphragm. The filtration slits have a constant width of ~30-40 nm and are bridged by the slit diaphragm\textsuperscript{27,28}. The rectangular pores have the approximate size of an albumin molecule. The filtration barrier is permeable by water and small solutes, but to a larger extent, the size selectivity of the filtration barrier for proteins is represented by the slit diaphragms of podocytes. The slit-diaphragm is evocative of a tight junction with differentiated structure and functions. It presents an electron dense zipper-like structure composed of the extra-cellular components nephrin, nephrin homolog-1, Pcadherin, and FAT connected by other specialized structures (i.e., podocin, CD2AP) to the main cell body\textsuperscript{29-36}.

Podocyte causes

Podocyte number also is correlated with disease progression in human disease and it is a critical determinant for the development of glomerulosclerosis and that it leads to progressive renal failure\textsuperscript{37} (Fig.3). Kim et al., proposed that physiological aging process in rats leads to podocyte loss, and it is directly linked to the glomerulosclerosis\textsuperscript{38}. A single injection of puromycin aminonucleoside (PAN), a podocyte toxin, causes a marked decrease in podocyte number in rats, while repeated injection of PAN greater than before, causes complete loss of podocyte and the sections developed glomerulosclerosis\textsuperscript{38}.

With regard to decreased cell number due to apoptosis, the study by Schiffer et al., showed that podocytes undergo apoptosis in glomerular disease, and the apoptotic cells are flushed out in the urine, making it technically difficult to detect these cells\textsuperscript{39}. Moreover, apoptosis has recently been shown in podocyte with human glomerular disease. It is increased in TGF-β transgenic mice, which leads to a decrease in podocyte number and glomerulosclerosis\textsuperscript{39}. Follow-up studies have demonstrated that TGF-β induced podocyte apoptosis is mediated by S mad pathways and its effectiveness in the absence of the cyclin dependent kinase-inhibitors p21 and p27.
Figure 3. Causes of podocyte injury
When podocytes become stressed, irrespective of the causative stimulus, they undergo foot process effacement and loss of slit diaphragms—two key steps leading to proteinuria. Thus, proteinuria is the unifying denominator of a broad spectrum of podocytopathies.

**Epidemiology**

NS can occur at any age but most common between the ages of 2-8 years. Age at initial presentation has an important impact on the disease distribution frequency. The annual incidence of NS in most countries is estimated to range from 2 to 7 new cases per 1,00,000 children. It seems to affect boys more often than girls. The annual incidence of NS in US and European children were 2 and 2.6; 3.4 and 16.9 for Afro-Caribbean and Asian children per 100,000 respectively and the cumulative prevalence was about 16 per 1,00,000. The incidence was lower in white children (1.9 per 1,00,000) than in non-white children (2.8 per 1,00,000) although the numbers of non-white children were small and the incidence was higher in lower socio-economic groups. In particular, Hispanic and Black patients are more commonly irrespinsive to NS than white patients. During the first year of life, congenital and infantile genetic disorders, infections are much more common than MCNS and FSGS.

In our center, the statistical data collected between 2000-2011 years revealed that 3669 children were diagnosed for nephrotic syndrome in pediatric nephrology outpatient department. Of these 2086 patients were males and 1583 were females. Of these NS cases, renal biopsy was done on 678 cases, 434 were boys; 244 were girls (Fig.4).

**Immunological pathogen intervention and abnormality**

In the past, the theory of NS link to dysregulation of the immune system had existed. However, NS responsiveness (corticosteroids, alkylating agents, calcineruim inhibitors, mycophenolate mofetil) inhibitors of T lymphocyte function were proposed. The chief complication of NS is infection, followed by thromboembolic events. Increased predisposition to infections occurs due to loss of immunoglobulins, complement and properdin, altered T cell functions, immunosuppressive therapy and presence of edema. Of the severe infections, peritonitis has an incidence of 2-6 per cent. Other common
Figure 4. Year wise NS new cases statistics in ICH & HC
infections are cellulitis, pneumonias and upper respiratory tract viral infections, induction of remission of NS following infections with measles and malaria, disease known to depress cell-mediated immunity. Only a specific immunological intervention studies are available for nephrotic syndrome. For example, hepatitis B & C and cryoglobulinemia may cause the nephrotic syndrome such as hydatid disease; omission of medication causing immune mediated nephrotic syndrome. Studies involving transgenic mice expressing recombinant retrovirus MPSV-neo and the early protein of Simian Virus-40 confirmed the viral involvement in the development of FSGS. FSGS is considered idiopathic if no secondary causes (HIV infection, sickle cell disease and reflux nephropathy) are identified. The FSGS is characterized by segmental glomerular capillary obliteration with increased deposition of mesangial matrix and intracapillary hyaline, with or without focal adhesions of the capillary tufts to Bowman’s capsule.

Pathogens shape the genetic structure of human populations. The pathogenesis of FSGS is unclear, although the frequency of FSGS has increased considerably during the last 20 years. FSGS frequently affects in African Americans to inconsistent level. Genetic predisposition to develop FSGS in Blacks with viral infections was reported earlier. FSGS may be associated with viral infections such as Parvovirus B19, SV40 and HIV. In this connection increased shedding of virus in kidney could also result in the loss of renal epithelial cells or immune cells in the urine consequent to renal inflammation.

Medical complications of NS are also notable, long term sequelae of NS and its treatment, especially effects on bone growth, and the cardiovascular system. The risk of steroid-induced osteoporosis has significant long-term implications. A prospective study from India has showed that 22 of 100 patients with NS had features suggestive of low bone mass. Factors predictive of low bone mass were older age at onset, low calcium intake and the cumulative steroid dosage.