1.0 Introduction

Kidneys are the most frequent examined organs, as they play an essential role in filtration and removal of metabolic waste components from tissues and blood (Sadowski et al., 2015). Glomerular machinery usually does not allow the passage of larger plasma protein molecules and other cellular elements (Kanwar and Jakubowski, 1984). But, if the glomerular filtration machinery gets disturbed, it allows to passage of large plasma proteins molecules (i.e. specially albumin) into the urine, causing nephrotic syndrome (NS) (Spitzer 1985). NS is generally characterized as a group of symptoms, where patient suffers from rigorous proteinuria, hypoalbuminai, edema and dyslipidemia (Kim et al. 2005; Korbet 2002). Annular incidence of NS is nearly 15-20 cases per 100,000 children, making this situation one of the often diagnosed kidney diseases in childhood while its gradual forms can lead to end-stage renal disease (ESRD).

The incidence of NS has increased by 2- to 4-fold, but the principle pathogenesis for this increase is still unsolved (D'Agati 2008). Literature strongly support genetic causes of NS among affected children showing highly heterogeneous disorder (Ramensky et al. 2002). Therefore, it is important to know about genetic components of underlying cause. A rising number of mutated genes have been recognized that can lead to inherited forms of idiopathic NS. These genes helps in guiding different structural proteins or enzymes that work in harmony to manage the glomerular membrane permeability and take part in various signaling events of regulating podocyte enlargement, segregation, and communications among cell-cell, cells-matrix interaction (Hickson et al. 2009; Zenker et al. 2009). This study was undertaken to carry out molecular screening of targeted genes polymorphisms (TRPC6 and NPHS2) associated with NS in Indian children.
1.1 The Human Kidney

The humans kidneys are bean-shaped organs, situated at the rear of the abdominal cavity in the retroperitoneal space at a slightly oblique angle. The right kidney is somewhat lower and smaller than the left kidney; it is more towards middle due to unevenness within the abdominal cavity, cause by the position of the liver (Glodny et al., 2009). The parenchyma of the kidneys is structurally separated into two main regions, the outer renal cortex and the inner renal medulla (Emamian et al., 1993). Abhorrently, these structures forms 8-18 cone shaped conformation of renal lobes, each containing renal cortex adjoining a portion of medulla called as renal pyramids. Each kidney ejects urine into the ureter which finally drains into the bladder (Schumann et al., 1988).

1.2 Function of Kidneys

The kidneys play multiple essential regulatory functions, such as removal of metabolic waste from blood, regulation of body fluids and maintaining electrolyte balance, pH homeostasis and blood pressure (Kanwar and Jakubowski, 1984; Sadowski et al., 2015). They also account for reabsorption of water glucose, amino acid and production of some important hormones (i.e. calcitriol and erythropoietin) and enzymes (renin). The filtration of these body fluids by glomerular capillary commence in human embryo between the 9 to 12 weeks of intrauterine life span (Spitzer 1985). Every day in healthy individuals, glomerular ultrafiltrate results in about 1 to 2 liters of urine formation (Kriz & Kaissling 1985, Tisher & Madsen 1991). The glomerular filtration barrier machinery controlling glomerular ultrafiltration has been point of great importance in research field since the last few decades (Sadowski et al., 2015). From reports it was known that the trans-capillary movement of plasma proteins and
water molecules is synchronized by a number of physical factors (Kanwar and Jakubowski, 1984). These factors include glomerular flow rate, the charge, size and conformation of molecules being filtered, capillary hydrostatic and oncotic pressure, chemical and physical properties of glomerular system (Kanwar and Jakubowski, 1984).

All these aspect are very essential in sustaining glomerular permeability balance and homeostasis to prevent the further escape of plasma proteins into the urinary space. Nephron is the primary filtration unit of the kidneys; it is made up of glomerulus and its tubular system (Fig. 1). Glomerular filtration unit is composed up of: fenestrated endothelial cells, glomerular basement membrane (GBM), and visceral epithelial cells (i.e. Podocytes) with intervening slit diaphragm (SD). It is essential to preserve the integrity of these structural elements for the continuation of normal ultrafiltration (Kanwar and Jakubowski, 1984).

![Fig. 1 Basic structure of nephron, its anatomy and physiology (Kurts et al., 2013)](image-url)
1.3 Glomerular filtration unit

1.3.1 Structure and function of glomerular basement membrane

The GBM is an extracellular matrix sandwiched between the endothelium and the epithelial processes (Fig. 2). It is the chief filtration barrier which limits the movement of macromolecules in accordance to their size, charge and conformation (Bohrer et al., 1978; Brenner et al., 1978; Kanwar and Jakubowski, 1984; Remuzzi and Remuzzi, 1994). In humans the width of GMB is around 250-300nm and contains an inner most layer with an electron dense material and the lamina. Either sides of lamina lay electron lucid areas termed as lamina rara interna and externa (Kanwar and Jakubowski, 1984). GMB is made up of collagen type IV, these components are important for its structure stabilization and filtration function (Miner, 1999). Nidogen cross links and stabilizes the compressed associations of laminin and collagen type IV which plays an important role in size selection and sieving properties of the glomerular filtration unit (Miner, 1999).

![Glomerular filtration unit diagram](image)

**Fig. 2** Arrangement of the glomerular filtration barrier: Glomerular filtration barrier between blood and the urinary space, forms the sieve through which the primary urine is formed (arrows). Here, LRE, stands for lamina rara externa; LD, stands for lamina densa; LRI, stands for lamina rara interna (Kanwar and Jakubowski, 1984).
1.3.2 Structure and function of epithelial cells and slit diaphragms

The middle structure of the epithelial cell lies in the urinary space. The cytoplasmic expansions of these cells affix to GMB forms a sequence of inter digitizing foot processes also known as podocytes. The glomerular podocytes are highly coated with negatively charged protein podocalyxin, as well as other proteins that provide a supplementary electrostatic barrier for the passage of proteins transversely the capillary wall (Kerjaschki et al., 1984). Additionally the SD, which is a customized tight junction between two neighboring podocytes, grants the final size selection barricade to the loss of protein molecules. The structure of SD is ladder type or zipper type, made up of pores with a size of 40x140 Å, these pores and are separated by inner filament and cross bridges (Rodewald and Karnovsky, 1974; Abrass, 1997). In the year 1974, Rodewald and Karnovsky made a hypothesis according to which the pore size of SD is somewhat smaller than the size of albumin (Rodewald and Karnovsky, 1974). The molecular nature of the SD has still been a mystery, but since last two years, noteworthy expansion has been made in accepting its structure and function in regulating glomerular permeability.

1.4 Proteinuria

Proteinuria in healthy individuals is generally below 150 mg/day, but sometimes as elevated as 300 mg/day is believed to be within the average range. When the unification of glomerular filtration machinery is disturbed, higher levels of protein molecules pass into the urinary space causing proteinuria. Here urinary proteins basically include albumin, immunoglobulins and other kinds of plasma and tissue (Dennis and Robinson 1985).
Depending upon its pathogenesis, proteinuria commonly is divided into five main classes: (1) glomerular, (2) tubular, (3) overflow, (4) secretory and (5) histuria proteinuria. Out of all these five types, glomerular proteinuria is the common one (Dennis and Robinsson 1985).

**Glomerular proteinuria:** Glomerular proteinuria can be either selective or non-selective, where principal urinary protein is albumin; it is a reporting sign for many renal disorders in the early times which may in turn endorse the development of kidney diseases (Dennis and Robinsson 1985).

**Tubular proteinuria:** Tubular proteinuria leads to tubular dysfunction and causes re-absorption of mainly microglobulin and globulins (Maack et al. 1985).

**Overflow proteinuria:** The elevated levels of plasma proteins are found in urine due to failure of GMB in re-absorption capacity which may lead to overflow proteinuria (Dennis and Robinsson 1985).

**Secretory and Histuria proteinuria:** Proteinuria is named to as secretory or histuria, when the origin of urinary proteins is from nearby tissues or organs through excretion and secretion (Dennis and Robinsson 1985).

### 1.4.1 Glomerular transformation in Proteinuria

The data generated from clinical and experimental models reported alterations in both cellular and extra-cellular components of the glomerular capillary systems which can lead to proteinuria (Kriz et al., 1990) (Fig. 3). Altered podocytes usually develops common morphological changes that comprise fusion of foot process, decline in the entire length of SD pore junctions, dislocation of the junctions apart from the GBM, and formation of vacuoles in podocyte cytoplasm. All these alteration directly or indirectly led to
insufficiency or loss in podocyte functions that initiate the ultimate pathologic progression of glomerulosclerosis (Fries et al., 1989; Kriz et al., 1990; Rennke, 1994; Pagtalunan et al., 1997).

Alterations in GMB many times do not show much visible changes, but in few cases it may turn thick due to abnormal regulation of synthesis or degradation. A deviation in the mesangium includes elevated cell-proliferation along with consequent matrix deposition and deposition of proteins or antibody that escorts to glomerular capillary blockage. In such conditions interstitial extension and tubular degeneration seems to be chief mechanisms in glomerular injury (Kashgarian and Sterzel, 1992; Couser and Johnson, 1994). The findings related to the pathogenesis of proteinuria typically relay on the idea that GBM, podocytes and SD pore junctions are chronological obstruction in the protein filtration mechanism and each of them is necessary for regular glomerular function.

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**Fig. 3** Pathophysiology of nephrotic disorder-Increased glomerular permeability causing leakage of plasma protein in urine, Hypoalbuminemia is the cause of main clinical features (Mauya and Kumar, 2016)
1.5 Nephrotic syndrome

During the 20th century attempts were made by several researchers to discriminate nephrosis (i.e. kidney disease distinguished by exudation and proliferation) from nephritis (i.e. nephritis). It was noticed that nephrosis is not a single disease, but a group of related diseases that causes proteinuria. The word “nephrosis” was replaced by “nephrotic syndrome” (NS) (Arneil, 1971b; Arneil, 1971c). Clinically NS features develop into rigorous proteinuria, hypoalbuminemia, edema and hypercholesterol conditions. These circumstances are closely related to foremost structural and morphological changes in glomerular epithelial cells (i.e. podocytes) (Smoyer and Mundel, 1998). Glomerulus is the chief target of numerous innate and acquired glomerular dysfunctions which can be further distinguished by NS (protein secretion per day is greater than 3.5 g). Endothelial cells possess many outlets known as “fenestrate”, size of around 65 to 95 nm in diameter, which forms a substantial barrier for passageway of macromolecules from plasma into the renal tubule. During NS, thickening of the foot process occurs in a particular area but the remaining of the cell generally remains conserved (Smoyer and Mundel, 1998). Moreover, the electron microscopy information leads to the recognition of negative charged molecules in the GMB, which prevent the passage of anionic macromolecules like albumin (Dantal et al., 1994).

1.5.1 Classification of nephrotic syndrome

NS can be classified as primary and secondary NS; the earlier is due to primary or “idiopathic” glomerular (90% prevalence), while the secondary NS is coupled with definite etiological actions or a complexity of some other diseases (Fig. 4). Since the
occurrence of clinical symptoms is secondary NS, has quite inadequate diagnostic value as it can differ drastically between various forms of the disease. During primary NS, size of around 60-280 KDa plasma proteins are lost that makes remarkable changes in plasma protein level (Caulfield and Farquhar, 1974; Klahr, 1990). The total ontonic pressure and plasma protein level decides the secondary effects of NS, where plasma protein level goes up to 800g/l causing extension in plasma volume and swift development to ESRD (Arneil, 1971b; Arneil, 1971c).

![Types of Nephrotic syndrome](image)

**Fig. 4** General classification of nephrotic syndrome (Arneil, 1971b; Arneil, 1971c)

On the basis of their etiology, NS can be divided into, (1) Acquired: caused due to metabolic of environment factors such as toxins or infection and (2) Genetics: includes hereditary and familial renal diseases) (Arneil, 1971e). It is obvious that these groups may have considerable overlap with each other. In various cases, the actual cause of the disease is still less noticeable. But in the past few years there is substantial improvement
Introduction and review of literature

characterizing renal biopsies in the classification of NS. Though in some way clinically available tools sometimes lacks in accurateness in identification (Peten and Striker, 1994).

1.6 Idiopathic nephrotic syndrome

In accordance to histological abrasions, primary NS can be differentiated into several subgroups such as Minimal Change nephropathy, Focal segmental glomerulosclerosis, Mesangial proliferative glomerulonephritis and Membranous glomerulonephritis. Each of these complications has been explained in detail below along their biopsy sample image showing possible alteration (Fig. 5).

A: Minimal Change nephropathy; No such glomerular changes are found
B: Focal segmental glomerulosclerosis
C: Mesangial proliferative glomerulonephritis; representing mesangial hypercellularity
D: Membranous glomerulonephritis; with mild thickening of capillary loop

Fig. 5 Different biopsy image of idiopathic nephrotic syndrome observed under light microscope (Eddy and Symons, 2003; Swaminathan et al., 2006)
1.6.1 Minimal Change nephropathy

Minimal change nephropathy (MCN) is one of the frequent root cause of NS in children, with an prevalence period from 3-5 years, counting for nearly 70-80% of childhood NS cases (Savin, 1993; Eddy and Symons, 2003). This syndrome is also termed as minimal change lesion or minimal change disease (MCD). Individuals with MCD represent characteristically features like edema, selective proteinuria and normal glomerular filtration rate (GFR) (Savin, 1993). The major portion of glomeruli appears normal, with slight enhancement in cellular and mesangial area. Moreover there is extension of epithelial cells, presence of lipid droplets in the proximal tubules and the glomeruli is diploid of immunoglobulins and complements (Glassock, 1991). Results from electron microscopy showed the effacement and renunciation of the epithelial podocytes in addition to destruction of the SD membrane-pore complex. Factors responsible for MCD includes: (1) circulatory proteins causing charge neutralization of GBM molecules, (2) glomerular membrane proteins, (3) immunogens, and (4) overproduction of reactive oxygen species (Kanwar and Farquhar, 1979; Savin, 1993; Couser and Johnson, 1994). These findings are validated by several in vivo studies, which is broadly used to investigate the pathogenesis of MCD (Kanwar and Farquhar, 1979; Couser and Johnson, 1994).

1.6.2 Focal segmental glomerulosclerosis

Focal segmental glomerulosclerosis (FSGS), like MCD, stands for sinister beginning of NS. It’s prevalence is observed both in children and adults, accounting for 7-20% of the primary NS, respectively. Here, a focal stand for damage in some of the filters of glomeruli that leads to sclerosis abrasion, typically those in the profound, juxtamedullary cortex region due to the greater perfusion pressure (Glassock, 1991; Stokes et al., 2006;
D'Agati, 2008b). The general clinical characteristics presented by electron microscopy are podocyte effacement and disconnection of foot processes from GMB, specifically in case of heavy proteinuria (Glassock, 1991). Additionally there exists deposition of IgM and C3 in glomeruli. Patients with FSGS have different responses to steroid treatment. There are chances for reoccurrence of FSGS even after renal transplantations, but plasma exchange treatment have shown positive response in reduce or vanishing of proteinuria. These findings strongly suggest the role of circulating factors accountable for glomerular damage in patients with this entity (Savin, 1993). Whereas some certain types of FSGS, presented obvious role of genetic factors in the pathogenesis of FSGS (Eddy and Schnaper, 1998).

1.6.3 Mesangial proliferative glomerulonephritis

On the basis of components deposited into mesangium, mesangial proliferative glomerulonephritis has been divided: non-IgA glomerulonephritis and IgA nephropathy (Glassock et al. 1991a). “Non-IgA glomerulonephritis” is comparatively unusual type of NS. It is distinguish by hematuria and non-selective proteinuria. Distinct results of light microscopy are accumulation of matrix, higher cellularity and IgM and C3 sedimentation are more observed in the mesangium (Glassock, 1991). The complete pathogenesis of non-IgA glomerulonephritis is still a mystery, but on bases of the observation of IgM and C3 decompositions, circulating immune complexes appears to play an effective role. IgA nephropathy, is one of the well known type of NS. Here mesangium consist diffused depositions of granular IgA molecules (Nolin and Courteau, 1999). Evidences signify that IgA nephropathy is a type of immune complex glomerulonephritis. The pathogenesis of IgA nephropathy comprises effects of reactive oxygen species (ROS), IL-6, viruses and
genetic factors (Glassock, 1991).

1.6.4 Membranous glomerulonephritis

In adults membranous glomerulonephritis (MGN) is the foremost source of primary NS (Shankland, 2000). In this condition there is deposition of immune complexes below epithelium. The GMB gets thicken and mesangial matrix elevates, without any noteworthy proliferation of endo- or epithelial cells (Ambrus and Sridhar, 1997). The finding of Immunofluorescence reveals presence of IgG and C3 in capillary loops (Glassock, 1991). The pathogenesis of human MGN is not yet clearly known.

1.7 Childhood nephrotic syndrome

Today, NS is recognized as one of the most common chronic illnesses for appointment to a pediatric nephrologist for assessment, the critical commencement frequently causes delayed diagnosis. The group of features that exemplify NS develops from primary alteration of the premselectivity glomerular barrier, which is no longer able to restrict the urinary loss of protein loss of 50 to 60 mg/kg per day or greater (Range of nephrotic proteinuria includes; urinary protein to creatinine ratio higher than 0·25 g protein per mmol creatinine or >2·0 mg protein per mg creatinine (Arneil, 1971b; Arneil, 1971d). The proteinuria in childhood NS is selective, chiefly composed by albumin. The distinct origin of the proteinuria is exclusively in the glomerulus without any associated tubular dysfunction. Though NS might be connected with number of renal diseases, the most common form of childhood NS is idiopathic as MCD OR FSGS (Eddy and Symons, 2003). A third discrete type, MGN, is rare in children. Other causes of NS can be subdivided into two main classes: rare genetic disorders, and secondary diseases in association with drugs,
infections, or neoplasia (Arneil, 1971a).

1.7.1 A child with nephrotic syndrome has these signs

The universal sign and manifestation of NS are swelling, weight gain, fatigue, blood clots, and infections where as some patients may develop acute renal failure (Arneil, 1971a). Generally the toilet bowel shows frothy appearance due to the increased excretion of protein into the urine. The total protein level in the blood reduces which results in swelling conditions. The protein in the blood prompts the flow of liquid in the bloodstream, due to low protein level this fluid leaks out of into tissues, causing edema (Fig. 6) (Gulati et al., 2003a; Gulati et al., 2003b). In the initial stage the swelling is found in the nearby regions of legs and eyes, in due course of time this swelling is observed in other body parts too along with rapid weight gain (Leonard et al., 2004). Very less number of patient’s are found to have weight loss and this may be due to malnutrition or an principal circumstances, such as badly controlled diabetes mellitus, a chronic viral infection, or the presence of other syndromic conditions (Arneil, 1971a).

Slowly NS develops in kidney dysfunction, with no or less symptoms at early stage but conversely kidney dysfunction continues to worsen finally developing ESRD symptoms, with shortness of breath, weakness and easy fatigability (from anemia) and loss of appetite (Ichikawa et al., 1983; Vande Walle and Donckerwolcke, 2001). The concentration of lipids especially cholesterol and/or triglycerides can become greatly elevated in patients causing increased risk of coronary artery disease (Vande Walle and Donckerwolcke, 2001; Joshi and Mistry, 2016). Patients with NS are at greater threat of blood clots in the veins or arteries which travel through lungs and leads to dangerous and fatal stage. Patients with
severe NS are at increased danger for infections, even though the reasons for this are not well understood (Gulati et al., 2002).

Fig. 6 Symptoms in child with nephrotic syndrome (http://www.slideshare.net/munivenkatesh420/nephrotic-syndrome-43851461)

1.7.2 Diagnosis of nephrotic syndrome

A urine analysis along with microscopic examination is suggested for reorganization hematuria and cellular casts, which should precipitate evaluation for proteinuria. High contains of waste products in blood sample have a direct indication of kidney damage. Normal kidney generally removes creatinine and urea nitrogen from the blood. First morning Up/c will establish the degree of proteinuria in NS patients (Fliser et al., 1999; Lucisano et al., 2015). Complement 3 and antinuclear antibody screen for proteinuric diseases associated with hypocomplementemia, including membrano proliferative glomerulonephritis and systemic lupus erythematosus, it require further investigation with laboratory tests and kidney biopsy. Simple diagnosis for NS includes urine visualization test, here foamy appearance of urine is more than normal because of the quantity and quantity of protein in it. Diagnosis may also require a kidney biopsy. Kidney biopsy of children over the age of 12-14 is recommended because of the frequency of diagnoses other
than MCD. It can either be FSGS as the maximum of kidney diseases in patients undergo biopsy for proteinuria in this region (Gulati et al., 2002).

1.7.3 Treatment and management

Patients who show positive signs and symptoms of intense attack are supposed to be treated with an intensive care and love. Current studies confirmed the effectiveness of intravenous theophylline in dropping the period and intense leaky phase of an acute NS. Different vasopressors drugs (Cattran, 2001). Now a day’s treatment with corticosteroids in response to inflammation has stopped or minimized as it is believed that steroids may be damaging to patients who face more frequent attacks and even the affect of steroid course in subsequent episodes is uncertain. Many patients who go through more rigorous attacks require mechanical ventilation because of flash pulmonary edema (Gulati et al., 2003a; Gulati et al., 2003b). The main goals of cure are to reduce symptoms, avoid complications, and hinder ESRD. Some commonly used treatments are enlisted below to control NS.

a. Use of ACE inhibitors i.e. Angiotensin-converting enzyme, to diminish proteinuria by maintaining blood pressure at or below 120/80 mmHg to improve response. The suggested dose is unclear; the actual dosage varies from patient to patient. Keep blood pressure at or below 130/80 mmHg to delay kidney damage (Sorof et al., 1998).

b. Treatment with corticosteroids remains different among adults and children and is more clearly proved that children respond well compare to grownups, in some patient’s it is beneficial while others do not respond at all. Previous studies show that patients with minor rigorous glomerular changes responded well to steroids treatment. It is recommended that family physicians must consult with nephrologists whether treatment
with corticosteroids is sensible, on the contrary the indecisive benefits and chance of adverse effects. Use of alkylating agents has few less proof for improving disease condition, but may be considered for patients who do not respond to corticosteroids (Sorof et al., 1998).

c. Studies are going on to inspect the benefits and problems of lipid-lowering treatments in NS. A number of confirmations suggested an enlarged hazard of atherogenesis or myocardial infarction in patients with NS, perhaps connected to increased lipid levels. Treating high cholesterol levels and decrease the risk of heart and blood vessel problems medication with triglycerides are usually needed (Brater, 1998).

d. Along with all these therapies doctors recommended few antibiotic and anticoagulating treatments deepening upon patients response to NS. A low-salt and low-protein diet may help with swelling in the hands and legs and control adverse effects of proteuria (Rybak, 1985; Singh et al., 1992).

1.8 Epidemiology status of childhood nephrotic syndrome

The occurrence of NS has been growing worldwide in nearly all racial and age groups with the annual incidence of 2-7 per 100,000 children younger than 18 years of age (McEnery and Strife, 1982; Hogg et al., 2000; D'Agati et al., 2011). Half of the NS presenting high levels of proteinuria has reached towards ESRD within the age of 3 to 8 years where the chances of recurrence are 20-25% even after kidney transplantation (KT) (Wiggins, 2007; D'Agati, 2008b; Kiffel et al., 2011). The peak occurrence of NS is at the age of 1 to 5 years, in 95% cases the condition is primary (idiopathic) (Fig. 7). Age onset at initial presentation has a major impact on the disease distribution frequency, 70-75% of MCD
patients are younger than 5 years; 20–30% of NS patients with MCD later develop FSGS at a median age of 6 years (McEnery and Strife, 1982; Gbadegesin et al., 2007; Kiffel et al., 2011). Over the past few years, the allover prevalence of childhood idiopathic NS remain stable, but the characterized histological patterns is changing.

![Fig. 7 a: Incidence of nephrotic syndrome cases, in number per million populations. Here, MPGN: Membranoproliferative glomerulonephritis, FSGS: Focal segmental glomerulosclerosis, MesPGN: Mesangial proliferative glomerulonephritis, MCNS: minimal-change nephropathy, MN: Membranous nephropathy (Swaminathan et al., 2006). b: Incidence of NS frequency (%) in different Asian countries (Olowu et al., 2010; http://bestpractice.bmj.com/best-practice/monograph/939/basics/epidemiology.html).](image)

The number of inherited forms of childhood NS (i.e. specially FSGS) are increasingly reported, even after amendment for changes in renal biopsy practices. In US, occurrence of NS is 3-4 cases per 100,000 children per year (McEnery and Strife, 1982). Increasingly this has been gone up to 16 cases per 100,000 children. The conventional studies showed that yearly occurrence rates of FSGS in African Americans were considerably higher than Caucasians (0.4 to 1.9 cases per annum, respectively), with high risk of FSGS in black persons (50%) than that of white (35%) (D'Agati, 2008b). There is 1-2 folds increase in the occurrence of FSGS in Asian population. The male-female ratio for NS is 1.2:1, it seems to affect more among boys than girls of adolescent age groups (McEnery and Strife, 1982;
1.9 Pathogenesis of childhood nephrotic syndrome

In course of NS the glomeruli is unable to filter back albumin or other immunoglobulins back into blood, rather these molecules pass through the membrane and are found in urine. Albumin is the major blood protein that regulates plasma ontonic pressure (Gulati et al., 1999; Kari, 2002; Asinobi et al., 2015). This causes increase in hepatic lipoprotein and transcapillary water level that later on leads to hyperlipidemia and edema conditions linked with NS. Literature explains the role of T-cells in up-regulation of circulating factors (i.e. soluble form of the urokinase-type plasminogen activator receptor (uPAR) and cardiotrophin like cytokines of immune cells beside the injury to podocytes due to oxidative stress) or down-regulation of inhibitory factors in reaction to unrevealed immunogens and cytokines (Appel et al., 1985; Gulati et al., 1999; Kari, 2002; Asinobi et al., 2015). Few potential mechanisms for changes of NS have been suggested by numerous studies in animal models of NS that have indirectly linked to oxidant injury of podocytes. The actual mechanism by which this glomerular membrane gets damaged in primary and secondary form of NS is undefined, but proof robustly relates the importance of genetic factors (i.e. 10%) (Fig. 8) (D'Agati, 2008b). A rising number of mutated genes have been recognized that can lead to inherited forms of idiopathic NS. These genes helps in guiding different structural proteins or enzymes that work in harmony to manage the glomerular membrane permeability and take part in various signaling events by regulating podocyte enlargement, segregation, communications among cell-cell and cells-matrix interactions (Pardon et al., 2006; Zenker et al., 2009). Proteinuria results from the damage caused by
these transformations in glomerular filtration barrier and in this event, podocytes require their specific epithelial cell markers such as fibroblast specific protein, nephrin, desmin, actin, collagen, and fibronectin (D’Agati, 2008a). The findings of these new podocyte proteins and their mutation study have shed light on the pathogenesis of proteinuria linked with NS.

**Fig. 8** Pathogenesis of nephrotic syndrome, here the combined role of cytokines and circulating mediators seems to play role in NS, Moreover the genetic facts are also involved in the pathogenesis of NS, which can leads to ESRD (to be published by Joshi et al.)

1.10 Pathophysiology conditions involved in Nephrotic syndrome
In NS, the pathophysiology of normal glomerular filtration function is strongly interrupted, resulting in severe-range proteinuria and hypoalbumina conditions. A diverse metabolic consequence of proteinuria includes infection, hypocalcemia, bone abnormalities, hypercoagulability and hypovolemia.

**Infection:** During infections patients are more susceptible to Varicella infection along with *Streptococcus pneumoniae, Haemophilus influenzae, Escherichia coli*. The most common infectious complications are bacterial sepsis, cellulitis, pneumonia, and peritonitis (Arneil, 1971a; Mittal et al., 1999).

**Hypocalcemia:** NS patients are very frequently affected by hypocalcemia conditions caused by low serum albumin level; on the other hand low bone density and abnormal bone histology are also reported. Loss of vitamin D binding proteins through urine leads to reduction in intestinal calcium absorption among NS patients (Tessitore et al., 1984).

**Bone abnormalities:** It is possible that either long duration of this syndrome or its treatments are the significant risk factors for bone disease in NS patients (Mittal et al., 1999).

**Hypercoagulability and Hypovolemia:** Venous thrombosis and pulmonary embolism are eminent complications of NS, in these cases urinary loss of anticoagulant proteins, like antithrombin III and plasminogen occurs, beside synchronized raise in clotting factors, particularly factors I, VII, VIII, and X leads to conditions such as Hypercoagulability (Sorof et al., 1998). A report by Mahmoodi et al confirmed the increase in venous thromboembolism (VTE) and arterial thrombotic events together with coronary and cerebrovascular ones with 10 to 20 times greater effect in NS compare to normal ones. Acute renal malfunction may point to a fundamental glomerulonephritis, however it is
more frequent causes of hypovolemia or sepsis. Hyper tension is a final result of all these consequences in context with reduced kidney function which may develop in patients with chronic ESRD (Brater, 1998; Sorof et al., 1998).

1.11 Molecular expression profile of glomeruli

Normal development of glomeruli can be achieved by distinctive changes in gene expression level that precedes the morphological changes. A continuous progress has been observed in resolving the role of cellular and molecular events taking place in the early stages of NS (Bard et al., 1994; Horster et al., 1999; Kuure et al., 2000). From the available information regarding pathogenesis of NS, it is clear that only small number of regulatory genes have been identified to be involved in childhood NS. Moreover, the data collected so far gives only a superficial insight to how NS is mediated at the genetic level. Some of the glomerular factors important in cellular growth developmental and differentiation include; (1) transcription factors, (2) growth factor and (3) extracellular matrix components.

**Transcription factors:** *In situ* hybridization studies in mice have discovered the expression of number of homeobox (Hox) and paired box containing genes. Expression of these genes seems to be associated to with the multiple morphogenetic programs guiding kidney organogenesis (Reeve et al., 1985; Bard et al., 1994). The Hox genes code for a definite protein which has a DNA-binding domain and their products regulates transcription of other genes (Bard et al., 1994).

**Growth factors:** Along with transcription factors and other nuclear proteins, different secreted growth factors are involved in maintaining controlled cell division and development (Green and Smith, 1991).
**Extracellular matrix components:** At the time of renal development, a significant change occurs in the composition of extracellular matrix. Extracellular matrix is comprised of matrix components (such as collagen type IV, laminin and heparansulfate proteoglycan), integrins (α/β) and cell adhesion molecules (i.e. cadherins). Cadherins, participate in cell-recognition and cell-sorting during development. Their presence and absence takes major decision in tissue differentiation and segment specific morphogenesis events of kidney (Goto et al., 1998). Reiser et al. reveled localization of P-cadherin into the podocyte SD and speculated its central structural role in creating glomerular permeability barrier (Reiser et al., 2000).

**1.12 Regulation and deviation in Human Genome**

Hans Winkler introduced the word “genome”. It is a Greek word, where “genesis” means creation and “soma” means body, this now clarifies an organism’s heredity information (Lederberg, 2001). DNA is located in cell nucleus and is arranged in 23 pairs of chromosomes in human body. These chromosomes consist of chromatins; DNA folded with histone and non-histone proteins, which play an important role in regulatory processes (Moraes et al., 1991). The double helical structure of DNA is made up of adenine (A), guanine (G), thymine (T) and cytosine (C), where pair of complementary bases is bounded by hydrogen bonds. Ultimately, they code the information for the transcription of ribonucleic acid (RNA) which in the process of translation forms proteins. This flow of genetic information was termed as the “Central Dogma of life” in molecular biology by Francis Crick in 1958. In total, the human genome is made up of around 3,200 megabases, 25,000 protein encoding genes, which correspond to only 1 to 2% of its size (Lander et al.,
2001; Venter et al., 2001). The majority of the region is made up of non-coding portion, serving no specific biological functions, leaving behind the regulatory elements (i.e. promoters or enhancers) (Claverie, 2005).

Every individual is comprised of different phenotypic variability; to allow such variations the synthesis of a functional gene product is necessary. Chromatin play a crucial role in the regulation of gene expression; it is highly structured and can be compressed to variable degrees. This has a great impact on the accessibility of the DNA for the transcription machinery (Berger, 2007). Chromatin remodeling is not only responsible in transcription regulation but is also essential for DNA repair processes. Moreover histone proteins can undergo various post-translational modifications (PTMs) like glycation, phosphorylation, sumoylation and methylation. These PTMs either directly or indirectly acts on chromatin structure causing alterations in electrostatic charges which serves as a signal for chromatin remodeling complexes and other chromatin-binding proteins (Berger, 2007; Greer and Shi, 2012). Different PTMs act as activators or repressors in transcription process.

1.12.1 Single Nucleotide Polymorphisms

Genetic variations leading to genetic differences among individuals are created by recombination and mutations. Mutations are main cause for natural selection and provide diversity among species. They often harm normal biological functions and are dangerous for the individual (Eyre-Walker and Keightley, 2007). Mutations takes place due to multiple reasons such as: (1) errors in recombination or chromosome segregation during meiosis, (2) blunder in DNA replication, (3) mobile DNA elements (i.e. transposomes), (4) mutagenic agents causing DNA damage, etc. Mutations present in the germ line cells are
the one that pass on to the next generation, where as the somatic mutations can be the origin for a diverse diseases like cancer (Yates and Campbell, 2012). Classification of mutation is based on its size, biochemical nature and its functional effect. Novel biological marker, named single nucleotide polymorphisms (SNPs), have recently appeared on the picture in dominating the molecular genetics field for human and animal genome studies enlightening polymorphisms at the DNA level (Cooper and Krawczak, 1989). SNPs are biallelic co-dominant markers with only a single alter base present in a DNA sequence, with a population frequency of ≥1%. The probable chance of SNPs to occur is hardly one in every 1000 to 2000 base pairs, currently released SNP data projected total 37,530,471 SNPs in the human genome (dbSNP web query for build 144: Jun 08, 2015).

SNPs can either be synonymous; change in codon that code for same amino acid), non-synonymous (nsSNP): change in codon that code for different amino acid or nonsense: coding for a translation stop (Fig. 9) (Eyre-Walker and Keightley, 2007). nsSNPs can also be called as missense mutations can be benign or deleterious to the protein function (i.e. subsequent protein product ultimately results in a structural or functional change in the protein product whose consequences may be minor or major phenotypic change accounting for the pathology of disease.) e.g. when there is exchange of amino acid for a functionally conserved residue. Around 50-60% of induced mutations in concern with the inherited genetic disorders are due to nsSNPs (Wang et al., 1998). Including coding regions, SNPs also have an effect on gene regulatory elements like promoters, enhancers, transcription factor binding sites or splice junctions, thus possibly manipulating regulatory mechanisms (Haraksingh and Snyder, 2013). Deletions and insertions (InDels) are another type of variations with a length of few base pairs (bp), approximate +<-5bp. InDels have the
capacity to alter reading frame of coding sequences, thus seems to be potentially more damaging (Bhangale et al., 2005).

Fig. 9 Commonly predicted special types of single nucleotide polymorphisms responsible for several types of genetic diseases (Eyre-Walker and Keightley, 2007).

Quick improvements in high throughput sequencing technologies have greatly advanced the study of human genome and several other large scale projects endeavor to find human genetic variations. The 1000 Genomes Project (1000 Genomes Project Consortium et al., 2012), identified approximately 3.6 million SNPs and 344,000 InDels per individual, which corresponds to more than 0.1% of the entire genome (Bhangale et al., 2005). All together, these data reveal a high degree of genome variability within the human beings; moreover the human genome sequence can also be a used as case-control studies using sequencing techniques in many diseases. The progressively more comprehensive classification of human genetic mutations will optimistically lead to a broader
understanding of health, growth and disease conditions.

1.13 Techniques used in identification of disease associated genes

1.13.1 Traditional methods used for SNP detection

The hunt for disease associated genes began with the linkage analyses studies amid affected families. Various methods like PCR-restriction fragment length polymorphisms (RFLPs), denaturing high performance liquid chromatography (dHPLC) or direct sequencing have been used frequently used for the identification of SNPs (Kwok and Chen, 2003; Kwok and Duan, 2003). PCR-RFLP, detects the SNPs that can causes differences in enzymatic cleavage sites flanked between two alleles. Thus DNA fragments of uneven lengths are formed by restriction digestion enzymes which can be detection using simple agarose gel electrophoresis (Botstein et al., 1980). SNP detection using dHPLC method uses mixing of two or more chromosomes by providing partial denaturation condition, these chromosomes forms duplexes upon renaturation. In presence of SNPs in the DNA sequences leads to heteroduplexes formations, which retains for the short time less than the original homoduplexes (Frueh and Noyer-Weidner, 2003). This method is less specific as we can only detect the presence but not the exact position and nature of SNP, which can be detected using direct sequencing in the next step. Frederick Sanger in 1977, for the first time brings in a method for direct sequencing, which became the most popular one in the following years (Sanger et al., 1977). It is based on the principle of integration of 2',3'-dideoxynucleotides triphosphate (ddNTPs) into the DNA sequence, that behave as particular chain terminating inhibitors of DNA polymerases. Reactions are performed in a cycle sequencing manner that includes denaturation, primer
annealing and primer extension steps. Integration of fluorescently labeled ddNTPs at every cycle leads to termination. SNPs in the DNA sequence by visualizing digital data generated through electrophoretic end product separations and laser excitations (Sanger et al., 1992).

1.13.2 Rapid and advanced method used for SNP detection

In 2005, the next-generation sequencing (NGS) technologies were introduced for the first time, then after they evolved speedily and released new commercially available platforms (Margulies et al., 2005; Shendure et al., 2005). The costing of NGS have been drastically reduced, less than $0.1 per megabase in 2013, thus making it much more cost effective, judge against to Sanger sequencing ($500 per megabase) (Shendure and Ji, 2008; Shendure and Lieberman Aiden, 2012). In compare to microarrays, NGS technologies are independent from DNA hybridization using specific probes. This helps us to enable the detection of novel mutations at a single-base substitution without a priori sequence information. Clustered amplicons generated for sequencing can be achieved by using emulsion PCR, in situ polonies or bridge PCR etc. PCR amplicons created from individual library are then clustered on planner surface or on micron-scale beads. NGS is based on the principal of sequencing-by-synthesis approach, though all the NGS platforms differ in their sequencing chemistry. Here the DNA dense array is enzymatic sequence repeating in combination with image based data collection (Shendure and Ji, 2008; Shendure and Lieberman Aiden, 2012).
Next-generation DNA sequencing

Polymerases or ligases enable the incorporation of nucleotides where the fluorescently labeled nucleotides or bioluminescence emitted by luciferase helps in sequencing image generation (Shendure and Ji, 2008). Figure 10; demonstrate the basic workflow of NGS.

Fig. 10 Diagram representing workflow of next generation sequencing (http://www.slideshare.net/ueb52/introduction-to-next-generation-sequencing-v2)
Introduction and review of literature

Based upon their workflow and individual chemistry, the NGS platforms have their own strengths and limitations in terms of through-output, read lengths, and error rates. For the sequencing of genomic DNA, the whole genome sequencing is not a feasible approach for many studies due to its high costs; in such cases whole exome sequencing and targeted re-sequencing provides valuable alternatives to the users. In whole exon sequencing almost all protein-coding regions are targeted that provide a large coverage (Shendure and Ji, 2008; Shendure and Lieberman Aiden, 2012). In contrast when we already have possible knowledge about candidate genes and disease related pathways, the targeted re-sequencing of specific regions seems to be more promising option. Many applications which were not possible few years ago got enabled after the advancement in NGS technologies as for example: (1) the genomes of a large range of species are fully sequenced now, (2) refinement of phylogenetic trees and (3) the more precise understanding of model organisms. Large scale projects were accomplished using NGS approach like the 1000 Genomes Project and the Exome Sequencing Project (ESP) of the National Heart, Lung, and Blood Institute (NHLBI) (Tennessen et al., 2012; Fu et al., 2013). These projects were set with the aim to form directory of genomic diversity of large number of individuals belonging to different ethnic backgrounds. They also included patients suffering from several diseases facilitating wider understanding of genomic variation related to human health and disease pathogenesis. Additionally, NGS technologies can also be used in different fields such as expression analysis and the determination of protein-DNA interactions. Thus NGS allow us more and more in biomedical research studies and enhance our understanding in evolution, individual development and disease conditions (Shendure and Lieberman Aiden, 2012).
1.13.3 Informative Databases of submitted SNPs

There are more than 1000 databases of human genetic variation, but we are exposed to only a few of them (Hirakawa et al., 2002). These datasets can be divided into two classes as (1) frequent genetic variation (2) infrequent genetic variation (Hirakawa et al., 2002). NCBI-dbSNP mutation database is one of the largest and frequently used dataset showing full-fledged exponential growth after the Human Genome project. Appropriate information related to mapping of notoriously known variant into human genome, identification of novel SNPs around and inside gene, assay designing for specific variant measurement and estimating the validity that they truly exists along with their allelic frequency in various populations can be generated using this database (Hirakawa et al., 2002; Rajeevan et al., 2003).

International HapMap and dbSNP project are noteworthy datasets providing essential information related to SNPs. It fulfills joints information regarding allelic frequency and linkage disequilibrium patterns, observed among the global population and thus make it available as a proper platform of knowledge for major genetic associated projects (Rajeevan et al., 2003). Few additional datasets designed to characterize variants present among or around human populations, which includes; (1) Japanese SNP database (JSNP), (2) ThaiSNP database, the Taiwan-Han Chinese SNP database, (3) CEPH genotype database but majority of them wholly depends on dbSNP and HapMap for their SNP information. There is high contribution of these databases like calculating percent frequency of SNP in given population obtaining their power calculations which helps to analyze SNP data generated after sequencing (Rajeevan et al., 2003; Yu et al., 2008).
1.13.4 Software used for detecting deleterious SNPs

Today, playing with bioinformatics tools has become more comfortable without any help of bioinformatician to guide them. These software also offer resources to hunt for an beginning of SNP genetics by conducting wet lab programs and developing our understanding on way to apply SNP tools and databases (Yu et al., 2008). Various tools such as SIFT, PolyPhen, PANTHER, SNP&go etc, are now a day’s widely use in the detection of deleterious SNPs. As, these SNP tools and software keeps on posting updated information, which may help user to correlate data obtained through sequencing studies or in the discovery of new mutation (Amigo et al., 2008; Yu et al., 2008). SNP software’s are constructed in a way that they carry out genetic study related to project plan assortment by organizing available genetic information. Genome Analysis Tool Kit (GATK) is one of the well known tool use to analyze data generated from NGS. Tools such as SAS (Statistical Analysis System) have been created to perform statistical analysis of data by calculating linkage disequilibrium patterns and haplotypes (Amigo et al., 2008; Yu et al., 2008).

1.14 Genetic aspects of nephrotic syndrome

Since, last few years, studies of familial NS have lead to the detection of various podocyte genes, associated with proteinuria. These findings have transferred the spotlight from GBM to podocytes in pathogenesis of pediatric NS (Wiggins, 2007; Kiffel et al., 2011). In brief, podocytes are specialized epithelia cells with several foot processes that are connected with each other through SD (Zenker et al., 2009). All the identified genetic defects affects gene transcription and assembly of podocyte structure together with actin based cytoskeleton, and adhesion complexes (Table 1) (Hinkes et al., 2007).
<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Size and location</th>
<th>Protein</th>
<th>Mutational study</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPHS1</td>
<td>26 kb Chr.19q13.1</td>
<td>Nephrin</td>
<td>Overall 37 different NPHS1 mutations are identified in 44 unrelated patients, 58% of these mutations were missense, where as others included splice-site and nonsense mutations</td>
<td>(Beltcheva et al., 2001; Schoeb et al., 2010)</td>
</tr>
<tr>
<td>NPHS2</td>
<td>25 kb Chr. 1q25.2</td>
<td>Podocin</td>
<td>Overall 10-30% of sporadic FSGS were found to have mutations in NPHS2 gene in different parts of the world</td>
<td>(Boute et al., 2000; Reiterova et al., 2012)</td>
</tr>
<tr>
<td>TRPC6</td>
<td>42 kb Chr.11q22.1</td>
<td>Transient receptor potential channel-6 (TRPC6)</td>
<td>Missense mutations of TRPC6 gene (i.e. H218L, P112Q, N125S, E897K, M132T, R895L, Q889K and R895C,) were reported to cause increase in Ca²⁺ current amplitudes in NS patients, probably its showing pathogenic effect</td>
<td>(Montell, 2005; Reiser et al., 2005)</td>
</tr>
<tr>
<td>WT1</td>
<td>48 kb Chr.11q13</td>
<td>Wilms tumor 1 (WT1)</td>
<td>A number of sequencing studies relate WT1 mutations as a cause of NS or relate with urogenital malformations, majority of these variants are characteristically heterozygous, germline or de novo in nature.</td>
<td>(Gessler et al., 1990; Kaltenis et al., 2004)</td>
</tr>
<tr>
<td>CD2AP</td>
<td>149 kb Chr. 6p12</td>
<td>CD2-associated protein</td>
<td>Among African Americans, heterozygous forms of SNPs are reported to causes CD2AP splicing that leads to FSGS conditions. In recent studies, homozygous mutation in CD2AP resulted in premature stop codon to some extent forming a truncated protein. This protein down-regulated CD2AP expression by lymphocytes and the binding with F-actin.</td>
<td>(Shih et al., 2001; Gigante et al., 2009)</td>
</tr>
<tr>
<td>ACTN4</td>
<td>83kb Chr.19q13</td>
<td>Actinin alpha 4</td>
<td>ACTN4 mutations have been associated with the autosomal dominant form of NS. In different in vivo experiments, up-regulation of actinin alpha-4 in presence of mutations has been reported, leading to proteinuria.</td>
<td>(Mathis et al., 1998; Michaud et al., 2003)</td>
</tr>
</tbody>
</table>
Cloning techniques were used in the identification of various genes such as \textit{NPHS1, NPHS2, TRPC6, WTI} and \textit{CD2AP} which were involved in podocytes damage and were further confirmed by knockout or transgenic models (Caridi et al., 2001; Rood et al., 2012). Additional information can also be obtained by querying the On-line Mendelian Inheritance in Man (OMIM) database web sites (www.ncbi.nih.gov). The inheritance patterns associated with the different NS genes can be either autonomic dominant or recessive. The SNP data generated using dbSNP in different podocyte genes is graphically presented in Figure 11.

\textbf{Fig. 11} SNP data of different podocyte gene generated using NCBI-dbSNP and plotted graphically using Consurf software, the maximum area is covered by \textit{TRPC6} gene (i.e. area in sky blue colour) (to be published by Joshi et al.).

\subsection*{1.15 Susceptible gene in nephrotic syndrome}

\subsubsection*{1.15.1 TRPC6 gene}

- \textbf{Chromosome location-11} (Fig. 12)
- \textbf{Size-42 kb}
Fig. 12 Cytogenetic location of TRPC6 gene on chromosome 11 (http://www.genecards.org/cgi-bin/carddisp.pl?gene=TRPC6)

The TRPC6 (transient receptor potential channel-6) gene is made up of 13 exons that are located on chromosome 11, having 931 amino acids encoding for the short transient receptor potential channel protein with a size of 106325Da (Dietrich et al., 2005). TRPC6 protein is a part of transient receptor potential (TRP) family of cation-selective ion channels. TRP subfamily (TRPC1- TRPC7) is expressed in many tissues that regulate intracellular Ca$^{2+}$ concentration via G protein- coupled receptors and receptor tyrosine kinases (Bach, 2001; Winn et al., 2005b). Expression of this ion channel in podocyte cells and have been recognized as a constituent of the SD. On the basis of their primary conformation, TRP proteins are differentiated into six subclasses; TRPC, TRPV, TRPM, TRPP, TRPML, and TRPA. Further analysis showed that TRPC is subdivided into different consecutive proteins, TRPC1, TRPC4, and TRPC5 or TRPC3, TRPC6, and TRPC7, respectively, that could particularly work together in network form homo-tetramers and hetero-tetramers which can interact with several other proteins (Bach, 2001; Winn et al., 2005b). Activation or modulation of TRPC proteins have been reported to be stimulated by receptor mediated phospholipase C. TRPC are involved in varied biological mechanisms such as cellular growth, maintaining ion homeostasis, PLC-dependent Ca$^{2+}$ entry into cells, etc. Ca$^{2+}$ is a secondary messenger that influences many of these cellular
functions (Boulay et al., 1999; Gudermann, 2005; Reiser et al., 2005).

Mutations in TRPC6 gene have been currently found to be associated with to NS. Mutations in this gene were found with autosomal dominant NS (Hofstra et al., 2013). Findings demonstrated 12 different mutations with childhood NS and 4 with late onset of sporadic cases (age of 15-55 years, with a few exception at 1-9 years of age) resulting towards unpredictable rate of development to ESRD (Winn et al., 2005a). In the New Zealand population, nsSNP in exon 2 of TRPC6 gene, causing a proline to glutamine substitution, P112Q was found to be responsible for the disease in all the affected individuals. Through in vitro studies it was proved that, p.P112Q was also associated with exaggerated calcium signaling (Obeidová et al.; Hofstra et al., 2013). If similar results of increase in calcium signaling are observed in vivo, this mutation could lead to a gain-of-function and elevated levels of cellular calcium influx, which may further interrupt glomerular cell functions (Obeidová et al.; Obeidova et al., 2012). TRPC6 gene mutations usually cause a late onset of disease; it may be possible that these variations produce minor intracellular changes that guide to irreversible cell injury. Recently in 2015 two SNP’s, p.N157T and p.A404V showed damaging effect to protein stability by altering glycation and ligand binding sites using in silico based approach (Joshi et al., 2015).

Almost all the reported mutations were missense, except two p.K874X and 89fsX8 mutations. Eight of these missense mutations (i.e. p.H218L, p.P112Q, p.N125S, p.E897K, p.M132T, p.R895L, p.Q889K and p.R895C) were gain-of-function that cause increase in Ca$^{2+}$ current amplitudes where as the rest may probably showing pathogenic effect on the basis of biochemical and biophysical variations. Majority of TRPC6 mutations were dispersed all throughout N and C terminal cytosolic domains while no mutation has been
observed in transmembrane domains. In European and African families, 6 families were recognized having autosomal dominant FSGS with a distinct missense variant (Gudermann, 2005; Reiser et al., 2005). Thus a controlled cellular regulation of Ca\textsuperscript{2+} homeostasis by TRPC6 is expected for to normal podocyte function. SNPs in this gene might work as modifiers of proteinuria. Number researchers persuasively showed that TRPC6 activity at the SD is important for proper maintenance of podocyte structure and functions. In context to the earlier identified NS genes which play a role in podocyte cytoskeleton structure or function, TRPC6 is the first calcium-permeable channel gene that has been concerned in NS pathogenesis (Obeidová et al.; Obeidova et al., 2012).

1.15.2 NPHS2 gene

- **Chromosome location-1** (Fig. 13)
- **Size-25kb**

![Fig. 13 Cytogenetic location of NPHS2 gene on chromosome 1 (http://www.genecards.org/cgi-bin/carddisp.pl?gene=NPHS2&keywords=nphs2)](http://www.genecards.org/cgi-bin/carddisp.pl?gene=NPHS2&keywords=nphs2)

The NPHS2 gene is located on chromosome region 1q25-q31, made of 383 amino acid residues that encodes for podocin. Podocin is a hairpin like integral membrane protein with approx 42 kD, made up of one transmembrane domain and a C-terminal cytoplasmic tail. The 3-prime untranslated portion posses polyadenylation signals that are placed upstream of the poly(A) tail (Boute et al., 2000; Reiterova et al., 2012). Comparison of datasets till
now demonstrated that the podocin contains an extensively analogous portion between its central region and proteins of the band-7-stomatin family, unlike any other known protein. Within glomeruli the RNA expression of podocin is arrested to the podocytes, but in the developing kidney its expression occurs in time-dependent manner without any signal detected in the earlier stages. This expression enhances future in podocytes with the lower segment of the S-body (Kawachi et al., 2003; Roselli et al., 2004). Podocin is an essential raft-associated component of the podocyte foot- processes, located in the insertion of the SD (Fig. 1). It precisely organizes and regulates glomerular membrane structure and interacts with \textit{NPHS1, CD2AP, TRPC6} and various other genes (Reiterova et al., 2012). Podocin facilitates membrane transport of nephrin and directs podocytes intracellular signaling pathways (Kawachi et al., 2003; Roselli et al., 2004).

Mutations in \textit{NPHS2} gene were formerly identified in the children with early NS. Boute et al. in 2000 identified \textit{NPHS2} gene as causative agent for early onset autosomal recessive steroid resistance form of NS. Overall 6.4-30\% of cases were found to have mutations in \textit{NPHS2} gene in different parts of the world (Boute et al., 2000; Reiterova et al., 2012). From the time when the \textit{NPHS2} gene got identified, various researchers in Europe, North America and Middle East confirmed \textit{NPHS2} gene mutation taking place in 10-30\% of children with sporadic NS (Caridi et al., 2001). In Initial reports suggested recessive form of \textit{NPHS2} mutation in children between 3 months to 5 years of age source, but the current data presented its association with a wide range of clinical spectrum in a much larger cohort of patients leading towards ESRD from all over the world (Boute et al., 2000; Frishberg et al., 2002; Karle et al., 2002; Tsukaguchi et al., 2002; Kawachi et al., 2003). One of the study presented, 9 out of 30 families having \textit{NPHS2} gene mutations showing
autosomal recessive inheritance pattern with delayed onset of NS (Tsukaguchi et al., 2002). Podocin polymorphism p.R229Q is one of most frequently reported one with marginally higher frequency of around 5% in SRNS as compared to healthy individuals (Ruf et al., 2004). So far more than 50 podocin mutations have been account in NPHS2 gene and these reported mutations determine every kind of alteration including missense, nonsense, and deletion (Caridi et al., 2001; Ruf et al., 2004; Weber et al., 2004). Thus podocin protein is spinning out to be a foremost contributor to the genetic trouble of NS.
2.0 Aims and Objectives

2.1 Aim of the present study

The primary cause of a disease is necessary for accepting its mechanisms, categorization, prediction, and dealing with its consequences. Recently, the etiologies of NS have been revealed due to gene defects. Now a day’s podocyte genes are of special interest to combinatorial polymorphisms, their structural and functional effects, and general susceptibility to NS. We hypothesize that genetic makers may modulate the protein structural and functional stability, thus increasing the risk progression of NS. Though these gene polymorphisms are clearly established as causative agents for NS among European and Asian countries, but there are very few studies reported among Indian children. In this context, this study is aim to characterize targeted genes (*TRPC6* and *NPHS2*) polymorphisms in NS patients and their effect at transcript expression of concerned proteins. In the present study we tried to associate *in silico* analysis with case-control analysis using NGS to validate the role of SNPs in Indian children.

2.2 Objectives

- To identify deleterious SNPs using various bioinformatics tools in targeted genes (*TRPC6* and *NPHS2*).
- To study polymorphism of targeted genes (*TRPC6* and *NPHS2*) using next generation sequencing analysis and their association with nephrotic syndrome in Indian children.
- To check fold expression of targeted genes at transcript level using RT-qPCR