

Minimal Change Nephrotic Syndrome—A Complex Genetic Disorder

W K Gong,*^{MBBS, M Med (Paed), MRCP (UK)}, W Cheung,**^{BA}, H K Yap,***^{FAMS, MD, FRCPCH}

Abstract

Introduction: Minimal change nephrotic syndrome (MCNS) is the most common primary nephrotic syndrome in childhood. While the pathogenesis of this disease is still unknown, there is considerable evidence that it is an immune disease. This role of genetic susceptibility in this disease is the subject of this review. **Methods:** Reported studies addressing potential genetic factors in MCNS were reviewed. These factors included human leukocyte antigen (HLA) associations, genes involved in the renin-angiotensin system and cytokines. **Results:** Several authors have reported the presence of familial clustering, human leukocyte antigen (HLA) associations, and association with asthma and atopy, suggesting a genetic susceptibility to the disease. Moreover, recent studies on the role of the renin angiotensin system, cytokines and their respective receptors on the severity and clinical course of the disease, have lent further support to the immunogenetic basis of this disease. **Conclusion:** Knowledge of the genetic basis of MCNS may have important therapeutic implications in this disease, in particular, the role of cytokines and their respective receptors, including the influence of environmental factors on their expression.

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Key words: Angiotensin converting enzyme gene, Cytokines, Flt-1, Gene polymorphism, Human leukocyte antigen

Introduction

Minimal change nephrotic syndrome (MCNS) is the most common cause of childhood nephrotic syndrome.¹ It is characterised by the presence of gross proteinuria, hypoalbuminaemia, oedema and hyperlipidaemia. The incidence of primary nephrotic syndrome in childhood occurs at an annual rate of 2 to 3 new cases per 100,000 population under the age of 18 years.²

The pathogenesis of MCNS remains unknown, and poses one of the most puzzling enigmas in nephrology. The close association between altered immune responsiveness and the occurrence of MCNS led Shalhoub to propose that the immunological and renal abnormalities in this condition have a common pathogenesis.³ In addition, there may be an inherited predisposition for the disease. Familial cases in monozygotic twins have been reported.^{4,5} Subsequently, a European survey found that 3.3% of all nephrotic children, excluding those with congenital nephrotic syndrome, had a positive family history.^{6,7} This incidence was higher than that predicted from the frequency of MCNS. The age at onset, renal morphology on biopsy, and response to treatment were very consistent within the families. As such, a combination of immune and genetic factors may contribute to the pathogenesis of this disease (Fig. 1).

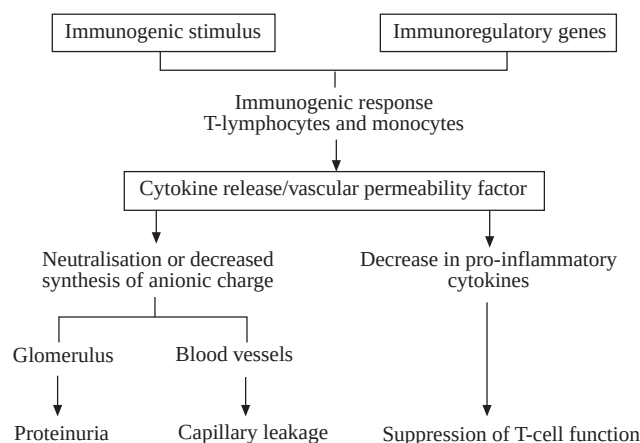


Fig. 1. Proposed immunopathogenetic mechanism in minimal change nephrotic syndrome (MCNS).

Human Leukocyte Antigen (HLA) Genes

One of the most important genetic elements that controls an individual's immune response to foreign antigens resides within the human leukocyte antigen (HLA) region of human chromosome 6. The HLA region consists of 3 classes of genes, class I (HLA-A, B, C), class II (HLA-DR,

* Assistant Professor

** Graduate Student

*** Professor

Department of Paediatrics

National University of Singapore

Address for Reprints: Dr Hui-Kim Yap, Department of Paediatrics, National University of Singapore, 5 Lower Kent Ridge Road, Singapore 119074.

DP, DQ), and class III genes that control the synthesis of the complement components C2, C4 and Factor B.

Of the three classes of HLA antigens, data from HLA class II are most relevant. HLA class II data strongly indicate that the susceptibility or resistance genes for the development of MCNS lie in this region of the major histocompatibility complex (MHC). Several studies have shown the association of MCNS with a variety of single HLA antigens including HLA-B8,⁸⁻¹⁰ B44,^{8,11} DR3¹² and DR8.¹³ Of the class II antigens, most studies have shown an increase of HLA-DR7 frequency in MCNS.¹⁴⁻²⁰ In addition, HLA extended haplotypes have been found in association with MCNS. Lagueruela et al²¹ have demonstrated that HLA-DQW2 and two extended haplotypes, (HLA-A1, B8, DR3, DRW52, SCO1) and (HLA-B44, DR7, DRW53, FC31), occur with significantly increased frequency in children with MCNS. In a study of 64 Singapore Chinese children with steroid-responsive nephrotic syndrome (SRNS) using sequence specific oligonucleotide probes for typing both class I and II HLA loci, we have demonstrated a significantly higher frequency of HLA-A11 allele in children with SRNS (relative risk, RR = 3.01, $P < 0.02$).²²

HLA class I and class II genes code for glycoprotein molecules whose main function is to bind self and foreign peptides to form complexes that are recognised by antigen-specific T-lymphocytes. Antigens associated with class I molecules are recognised by CD8+ cytotoxic T-lymphocytes, whereas class II-associated antigens are recognised by CD4+ helper T-cells. Thus, T-lymphocyte recognition of these foreign peptides is dependent upon the T-cell receptor binding not only to the peptide but also to self-HLA molecules. T-cell activation may then proceed, which leads to the appropriate immune response. What remains to be discovered in MCNS are the means by which HLA antigens and T-cell receptor interactions mediate the immune response and, in particular, what role the T-cell plays in the pathogenesis of MCNS.

Genes and the Renin-Angiotensin System

Genes encoding components of the renin-angiotensin system present attractive candidates for studies of renal disease. Angiotensin-converting enzyme (ACE) is an important component within the renin-angiotensin system, activating angiotensin I to angiotensin II, and inactivating bradykinin.²³ The plasma circulating ACE level is under genetic control and is related to the insertion allele and deletion allele (ID) polymorphism in intron 16 of the ACE gene. Those with the II genotype have the lowest serum ACE levels, DD subjects have the highest, and those with the ID genotype have intermediate levels. Angiotensin II has been shown to induce vasoconstriction, sodium retention, and renal haemodynamic abnormalities, effects

that result in intraglomerular hypertension and glomerular hyperfiltration.²⁴ Recently, it has been shown that angiotensin II modulates podocyte function directly via an angiotensin subtype 1 (AT1) receptor.²⁵ Elevated serum ACE activity in experimental models of nephrotic rats has been reported.^{26,27} Inhibition of ACE activity has been shown to reduce albuminuria in nephrotic rats.²⁸

Several reports indicate a possible relationship between ACE gene I/D polymorphism and renal disease.²⁹⁻³³ The DD genotype is associated with poor outcome in a variety of settings. In Korean patients,³² the DD genotype was found to be more frequent in primary nephrotic syndrome due to focal segmental glomerulosclerosis (FSGS) compared to MCNS. Patients with DD genotype had an earlier age of onset of clinical symptoms, were less responsive to corticosteroids, and had a higher incidence of chronic renal failure in this study.³² This was further confirmed by Frishberg et al,³³ who showed that patients with the II genotype were significantly less likely to have progressive renal disease than those with the ID and DD genotypes. In our study of 53 children with SRNS, we found a significantly lower frequency of the II genotype (22.6%) as compared to 92 normal controls (46.7%) ($P = 0.0063$).³⁴ The relationship between ACE genotype frequencies and progressive disease in FSGS could possibly be explained by the effect of angiotensin II on mesangial matrix production via stimulation of transforming growth factor- β (TGF- β) synthesis.³⁵ The role of ACE polymorphisms in MCNS is less clear, however, angiotensin II has been shown to modulate cellular functions of podocytes.²⁵ How this translates to regulation of the filtration slit structure and hence glomerular filtration coefficient remains to be shown.³⁶

Other components of the renin-angiotensin system (RAS) could also modulate progression of renal diseases. Polymorphism of angiotensinogen gene has also been associated with essential hypertension.^{37,38} In addition, polymorphism of angiotensin II type I receptor (ATR) gene has been associated with hypertension, independent of ACE.³⁹ These observations are of particular relevance in renal disease as the RAS is now firmly established as an important factor in progression of renal damage and development of end-stage renal disease.

Cytokine Genes

Cytokines are humoral immunomodulatory proteins or glycoproteins which control or modulate the activity of target cells by binding to specific cytokine receptor ligands, initiating signal transduction and second messenger pathways resulting in gene activation, mitotic division, growth, differentiation or apoptosis.⁴⁰⁻⁴⁴ Both CD4+ and CD8+ T-cells produce a wide range of cytokines. There are

3 subsets of CD4⁺ T-cells. Th1 cells secrete the pro-inflammatory cytokines interleukin-2 (IL-2), interferon- γ (IFN- γ) and tumour necrosis factor- α (TNF- α), while Th2 cells produce the anti-inflammatory cytokines IL-4, IL-5, IL-6, IL-10 and IL-13. Th0 cells exhibit an unrestricted cytokine profile.⁴⁵ There are two subsets of CD8⁺ T-cells (Tc1 and Tc2). CD8⁺ Tc1 cell secretes IFN- γ but not IL-4, and is restricted to MHC class I, while Tc2 cell secretes IFN- γ , IL-4, IL-5, IL-10 but not IL-6, and is restricted to MHC class II.⁴⁶

Cytokine and cytokine receptor genes are generally highly conserved in terms of exon sequences.^{47,48} Conservative (silent) mutations may influence protein expression by altering mRNA splicing, mRNA stability and levels of gene transcription. Polymorphism within the 5'- and 3'-regulatory sequences or introns of genes may affect transcription process by altering the structure of transcription factor binding sites within gene promoters or the structures of enhancers and silencers within introns. In addition, they may alter binding sites within the nuclear matrix for architectural transcription factors, which modulate promoter geometry.⁴⁹

There is strong evidence that proteinuria, which is the hallmark of MCNS, is mediated by cytokines.⁵⁰ Table I outlines the different cytokines and cytokine receptors that have been implicated in MCNS. The cytokines include those produced by monocytes/macrophages, i.e. monokines (IL-1 α , IL-1 β , IL-8, IL-12, IL-15, TNF- α),⁵¹⁻⁵⁹ Th1 cells (IL-2, IFN- γ),^{51,57,58} and Th2 cells (IL-4, IL-6, IL-10, IL-13).^{55,60-62} Cytokine receptors that have been studied in MCNS include IL-1R α , IL-2R and IL-4R.^{52,60,63} The mechanism by which cytokines may cause selective albuminuria through abolition of charge selectivity of the glomerular filter could involve direct neutralisation of charge by a cationic molecule, inhibition of synthesis of glucosaminoglycans (GAG) by a cytokine, or via a cytokine-mediated enhanced turnover of GAG in glomerular basement membrane.

Although data on the role of Th1, Th2 cytokines and monokines in the pathogenesis of MCNS are often conflicting, however, on the whole, there is convincing evidence that the balance between Th1 and Th2 cytokines may be intimately linked to the pathogenesis of MCNS. The close relationship between atopy and MCNS, as demonstrated by our group and other workers, suggests a common immune pathway.⁶⁴⁻⁶⁸ Stimuli such as allergens may activate common immune mechanisms, which subsequently result in proteinuria in children with MCNS. In allergic diseases, the balance is tipped in favour of Th2 cytokines,^{69,70} with IL-4 and IL-13 as the important cytokines. IL-4 and IL-13 are located at close proximity on chromosome 5q31. In addition, they share a common

TABLE I: CYTOKINES AND CYTOKINE RECEPTORS IN MINIMAL CHANGE NEPHROTIC SYNDROME

TH1 cytokines and receptors	Findings	Authors
IL-2	Increase in relapse No change	Hulton et al, 1994 ⁶⁰ Yap et al, 1999 ⁶¹
IFN- γ	Increase in relapse No change	Neuhaus et al, 1995 ⁵⁴ Yap et al, 1999 ⁶¹
IL-2R	Increase in relapse	Hulton et al, 1994 ⁶⁰
TH2 cytokines and receptors		
IL-4	Increase in relapse No change	Hulton et al, 1994 ⁶⁰ Yap et al, 1999 ⁶¹
IL-4R and Fc ϵ RII	Increase in relapse	Cho et al, 1999 ⁶³
IL-6	Decrease in relapse	Liu et al, 1995 ⁵⁵
IL-10	Decrease in relapse	Matsumoto, 1995 ⁶²
IL-13	Increase in relapse	Yap et al, 1999 ⁶¹
Monokines and receptors		
IL-1 α	Decrease in relapse Increase in relapse No change	Matsumoto, 1989 ⁵¹ Saxena et al, 1993 ⁵² Neuhaus et al, 1995 ⁵⁴
IL-1 β	Increase in relapse Decrease in relapse	Saxena et al, 1993 ⁵² Liu et al, 1995 ⁵⁵
IL-1R α	Decrease in relapse	Saxena et al, 1993 ⁵²
IL-8	Increase in relapse No change Decrease in relapse	Garin et al, 1994 ⁵⁶ Neuhaus et al, 1995 ⁵⁴ Yap et al, 1998 ⁵⁷
IL-12	Increase in relapse Decrease in relapse	Matsumoto et al, 1999 ⁵⁸ Yap et al, 1998 ⁵⁷
IL-15	Increase in relapse	Matsumoto et al, 1999 ⁵⁹
TNF- α	Increase in relapse	Bustos et al, 1994 ⁵³

receptor subunit. IL-4, IL-4 receptor- α (IL-4R α) chain and IL-13 functional polymorphisms have been reported in association with allergic asthma.⁷¹⁻⁷⁵ However, in the one reported study in children with MCNS, there was no association between IL-4 and IL-4R polymorphisms and susceptibility to disease.⁷⁶ Further genetic studies in the regulation of these cytokines and their actions, in particular the cytokine gene and cytokine receptor polymorphisms, may uncover some clue to the pathogenesis of this disease.

Vascular permeability factor (VPF) was one of the earliest lymphokine implicated in the pathogenesis of proteinuria in MCNS. It is released by concanavalin A-stimulated lymphocytes and was first described by Sobel et al.⁷⁷ VPF is 50,000 times more potent than histamine in inducing vascular permeability and a powerful promoter of angiogenesis.⁷⁸ The mechanism by which VPF induces vascular permeability is not known. Recent evidence has shown that the glomerular epithelial cells are the major intrarenal source of VPF,^{79,80} and a possible role for VPF is the maintenance of the endothelial cell fenestrations within the glomerulus.^{81,82} VPF activity is regulated through inhibition by a soluble form of its major receptor, Flt-1.⁸³ Flt-1 has seven extracellular domains, a transmembrane

portion and an intracellular kinase domain. A dinucleotide repeat has been found in the 3' non-coding region of Flt-1^{83,84} and has been shown to be polymorphic.^{85,86} Dinucleotide repeats in the 3' untranslated region of the Flt-1 gene may play a role in gene expression by influencing mRNA stability.⁸⁷

VPF mRNA expression in peripheral blood mononuclear cells has been shown to be increased in MCNS.⁸⁸ Abnormalities in Flt-1 expression may cause dysregulation of VPF in maintenance of endothelial cell fenestration thus altering the filtration function of the glomerulus, leading to proteinuria. Unfortunately, there was no association between the dinucleotide repeat polymorphism within the Flt-1 gene and MCNS in a predominantly Caucasoid population in the United Kingdom.⁸⁹ As about 80% of individuals in the United Kingdom are homozygous for the most common allele, the polymorphism is unlikely to be informative in this population studied.

Conclusion

There is abundant evidence that immunogenic stimuli interacting with immunoregulatory genes form the basis for the immunopathogenesis of MCNS. Knowledge of the genetic basis of MCNS may have important therapeutic implications in this disease, in particular, the role of cytokines and their respective receptors, including the influence of environmental factors on their expression. The current approach in many association studies is to examine polymorphisms of potential candidate cytokine genes. However, with the increasing availability of genome-wide markers, including single nucleotide polymorphisms, the power of association studies will be greatly increased.

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