GENETICS OF IgA NEPHROPATHY

KHALIQ S. AND ARA G.
Department of Human Genetics and Molecular Biology, University of Health Sciences, Lahore – Pakistan

ABSTRACT
Immunoglobulin A nephropathy (IgAN) is a form of primary glomerulonephritis that causes kidney disease in young adults characterized by prominent polymeric IgA deposition within the glomerular mesangium, leading to mesangial proliferation and sclerosis. IgAN is considered an autoimmune disease partly caused by an abnormal O-glycosylation of the IgA1 molecule. Because of the deficiency of B1,3 galactose in the hinge-region of IgA1 molecule it has an increased tendency of self-aggregation and/or the increased binding capacity for circulating glycoproteins. The knowledge about IgAN indicates that at least four events contribute in the development of IgA nephropathy i.e. aberrant glycosylation of IgA1, synthesis of anti-galactose – deficient IgA1 antibodies, binding of the galactose – deficient IgA1 by the anti-glycan/glycopeptides antibodies to form immune complexes (ICs), and accumulation of these complexes in the glomerular mesangium to initiate renal injury. IgAN is considered to be a complex disorder and like other complex diseases it also does not obey single gene Mendelian inheritance pattern. In addition to genetic factors, environmental stimuli and various inflammatory mediators are also considered to play role in IgAN pathogenesis. Variable disease prevalence in European, Asian and African cohorts indicate the role of susceptibility genes of variable frequencies in these populations. Familial IgAN has also been reported all over the world. After family studies three genetic loci IGAN1, IGAN2 and IGAN3 on chromosomes 6q22–23, 4q26–31 and 17q12–22 respectively have been reported to be associated with IgAN. Based upon their functional involvement, several genes like core-1, 2, 3-galactosyltransferase (C1GALT1 and its specific molecular chaperone b1, 3-GalNAc a2, 6-sialyltransferase (ST6GALNAC2) transferrin receptor (TFRC) immunoglobulin A, Fcα receptor (CD89) and toll – like receptor 4 (TLR4) become promising candidate genes for IgAN. SNP analysis from the candidate genes suggested that polymorphisms in C1GALT1 and TLR4 genes might influence the risk to develop IgAN. Further genetic association studies revealed more candidate genes i.e. HLA (human leucocyte antigen), renin–angiotensin system-related genes (RAS), complement factor immunoglobulins and cytokines, T-cell receptor alpha or beta chain genes are related to the predisposition and progression of the IgAN disease.

Key words: Immunoglobulin A nephropathy (IgAN), glomerulonephritis, Henoch – Schönlein nephritis (HSN), Genetic association of IgAN.

INTRODUCTION
Kidney Function and Physiology
The kidneys are important to maintain the homeostatic mechanisms of the human body. The reduced renal function correlates with increased morbidity and mortality. Major functions of the kidneys include filtration, reabsorption and excretion. The kidneys are bean shaped paired structures (Cheuck, 2013) that are microscopically divided into two parts the cortex and medulla. The functional unit of the kidney is nephron and each nephron consists of glomerulus, proximal tubules, loop of henle, distal tubule and collecting duct. The glomerulus is formed of specialized capillary network that acts as a selective filtration barrier for blood passing through its capillaries. Toxic, anoxic, or immunological factors contribute in the loss of nephrons that may initially injure the glomerulus, the tubule or both together. Glomerular diseases (glomerulonephritis) are defined as the inflammation of the glomeruli that could either be due to primary glomerular disease or systemic disease. The common form of primary glomerulonephritis is IgA nephropathy (IgAN) and the systemic form is Henoch – Schönlein nephritis (HSN). IgA nephropathy is characterized by prominent polymeric IgA deposition within the glomerular mesangium, leading to mesangial proliferation and sclerosis (Amico, 2000).

Immunoglobulin Structure
Human immunoglobulins are classified into five classes, IgG, IgA, IgM, IgE, and IgD on the bases of structure and functions. These are differentiated on the
bases of heavy chain found in the molecule. IgA is secreted by plasma cells in the mucosa and by bone marrow cells. The polymeric IgA molecules are produced by mucosal immune system and are transported to mucosal fluid. IgA has two subclasses IgA1 and IgA2. As mentioned earlier IgA1 type immunoglobulin deposition is the main feature of IgA nephropathy. The heavy chains of IgA1 contain a distinctive hinge region between the 1st and 2nd constant region domains. The distinctive hinge segment is the site of attachment for three to five O-linked glycan chains. O-glycan chains consists of two residues, N-acetylgalactosamine (GalNAc) and beta 1, 3-linked galactose. Both residues may be sialylated. On normal serum IgA1, the composition of two carbohydrate residues of O-linked glycans is variable. Mostly circulating IgA1 from IgA nephropathy (IgAN) patients is galactose deficient and contains terminally sialylated N-acetylgalactosamine (GalNAc) (Figure 1).

**Incidence of IgAN**

The incidence of IgAN is high in males as compared to females, the reported male: female ratios range from 2:1 in Japan to 6:1 in the United States and northern Europe (Jennette, et al, 1985). The incidence of this disorder varied in different countries as shown in figure 2. In Pakistan the prevalence of IgA nephropathy was reported to be 13% and 21% from two centers of different provinces (Muzaffar, et al, 2003).

**Pathogenesis of IgAN**

IgAN is considered as a complex disease. The true pathogenesis of IgA nephropathy yet has not been clearly defined. However IgAN is considered an autoim-
immune disease partly caused by an abnormal O-glycosylation of the IgA1 molecule. Genetic factors certainly influence the pathogenesis of IgA nephropathy (Hsu, et al, 2000). About 75% patients with IgA nephropathy have galactose-deficient IgA1 and its serum level is more than 90% in patients as compared to normal controls. It was observed in various ethnic groups that the level of aberrantly glycosylated galactose-deficient IgA1 in serum is a heritable trait and 30% to 40% first degree relatives of the IgAN patients also had elevated levels of galactose-deficient IgA1. Contrary to this it has been observed that relatives of IgAN patients with normal galactose-deficient IgA1 levels had levels that cannot be differentiated from controls. It specifies that only IgA1 glycosylation abnormalities are not sufficient to produce the IgAN phenotype.

As these differences always cannot be explained therefore, other factors like environmental stimuli and various inflammatory mediators like dysregulation of innate immunity, abnormalities in cellular immunity

**Fig. 2:** A geographic variation in the prevalence of IgA Nephropathy, white arrow indicates the prevalence in Pakistan.

**Fig. 3:** A proposed multistep model of IgA nephropathy, demonstrating the interaction of genetics, environmental factors, and both innate and acquired immunity. (Adapted from Clin. J. Am. Soc. Nephrol. 2014; 9: 617–625).
are also considered to play role in the pathogenesis of IgAN as shown in figure 3 (Barratt et al., 2007, Gesualdo, et al, 1990 and Schena, et al, 2002). There has been a possibility that the combination of all above mentioned factors may result in impaired elimination of mucosal antigens, prolonged antigen exposure to B cells, hyperactivity of T helper cell (Lai, et al, 1994) and defective suppressor T cell function (Sakai, et al, 1979). In another study by Suzuki, et al, (2009) suggested some additional immune factors like IgG that might be associated with deposition. Through molecular analysis of IgG autoantibodies specific for abnormally glycosylated IgA1 molecules, a specific amino acid substitution has been identified in the variable region of the IgG1 heavy chain.

It was observed that these mutated IgG1 molecules were more frequently found in the IgAN patients then in normal controls. They have suggested that the randomly occurring mutation due to an exposure to some viral or bacterial antigens enhances the binding of IgG1 with galactose – deficient IgA1 molecules. They have designated the mutation event as a “second hit” that predisposes to disease development of sporadic type in individuals with genetically elevated galactose–deficient IgA1 levels (Suzuki, et al, 2009). Genetic approaches have greatly enhanced our understanding for many other diseases through analysis of families with multiple affected individuals and large scale association studies. IgA nephropathy is a multi factorial disease. Most cases of IgA nephropathy are sporadic while familial cases are also reported. Studies have shown that IgAN does not follow the basic Mendelian segregation pattern, although an increased risk of the disease was observed in close family members of probands (Schena, et al, 2002). Family studies of IgAN revealed that familial IgAN could be transmitted under a dominant mode with incomplete penetrance (Scolari, et al, 2003; Gharavi, et al, 2000).

IgAN Genetics

A report on genome–wide linkage analysis of 24 Italian and 66 American families with IgAN identified a genetic locus, on chromosome 6q22 – 23 designated as IGAN1 (Gharavi, et al, 2000). Another study on a group of Italian IgAN families showed that the majority of families did not map to IGAN1 locus. These results gave evidence confirming the genetic heterogeneity in familial IgAN. Later two more loci, IGAN2 and IGAN3 on chromosome 4q26 – 31 and 17q12 – 22, respectively were reported by European IgA consortium (Bisceglia, et al, 2006). Although it is not considered as a true hereditary disease but the increasing evidence strengthens the involvement of genetic components in the pathogenesis and variation of clinical presentations of IgAN (Ramírez, et al, 2000). To date, no single disease causing gene has been reported underlying these linkage intervals. Researchers have given many reasons of this failure that include the spectrum of clinical presentations that overlap with the related similar diseases, presence of locus heterogeneity and contribution of genetic variants from non-coding regions. Unavailability of families especially with multiple affected individuals and requirement of kidney biopsy for diagnosing IgAN are the major drawbacks in family studies.

In the last two decades, several genetic association studies involving a collection of sporadic cases and a group of unrelated controls were performed in various laboratories and they revealed that many candidate genes i.e. PIGR (polymeric immunoglobulin receptor; Obara, et al, 2003), TRAC (Li, et al, 2009), FCAR (fc fragment of IgA, receptor; Tsuge, et al, 2001), HLA – DRA (Akiyama, et al, 2002), IFNG (Masutani, et al, 2003), EDNI (Maixnerova, et al, 2007), FCGR 3b – 2 (Xu, et al, 2007), CD14 (monocyte differentiation antigen CD14; Yoon, et al, 2003), TNFa (tumour necrosis factor a; Tughluri, et al, 2003), TGFb1 (transforming growth factor – b1, Lim et al, 2005), SELE (E-Eselectin gene; Takashi, et al, 2002), ACE (Draman, et al, 2008), AGT (Bantis, et al, 2004), VEGFA (Chow, et al, 2006) and MUC20 (Li, et al, 2006), are related to the predisposition and progression of IgAN (Hsu, et al, 2000). However, majority of these studies were severely underpowered since they were performed in a small case – control population and few SNP markers that increase the possibility of negative, inconclusive findings. Further one can not deny the drawback of association studies as often its hard to interpret the results that can easily yield false – positive results.

In a case – control association study, genes that could be involved in the development of IgAN were investigated in Italian patients with IgAN. For the study six candidate genes, core-1-b, 3-galactosyl transferase (C1GALT1, chr. 7p13-14) and its specific molecular chaperone b1, 3-GT (C1GALT1C1, chr. Xq24), GalNAc a2, 6-sialyltransferase (ST6GALNAC2, chr. 17q25.1) transferrin receptor 1 (TFRC CD71, chr. 3q29) immunoglobulin A Fcα receptor (CD89, chr. 19q13.4) and toll – like receptor 4 (TLR4, chr. 9q33.1) were selected based upon their possible functional involvement or their location within the IGAN1, IGAN2 and IGAN3 loci. Some of these genes are involved in the glycosylation pathway. SNP analysis of these candidate genes suggested that polymorphisms in C1GALT1 and TLR4 genes might influence the risk to develop IgAN and proteinuria, respectively. Studies also have reported few risk haplotypes for IGAN of ST6GALNAC2 and CIGALT1 genes. Recently Foo et al., (2015) have discovered novel associations at 3q27.3 (ST6GAL1), 11p11.2 (ACCS) and 8q22.3 (KLFL10) loci.

Similarly genes for HLA (human leukocyte antigen), T-cell receptor alpha or beta chain, rennin – angiotensin system – related genes (RAS) and several
inflammatory factors or cytokine genes (Hsu et al., 2000) were also studied for their role in IgA nephropathy. It has been shown that most of these genes are involved in the progression rather than the pathogenesis of IgAN (Feehally, 2005, Hsu, et al, 2000). IgA nephropathy patients of white European ancestry showed strong association with locus for HLA DQ. Recently a genome wide association study (GWAS) using 23,465 microsatellite (MS) markers identified three genes (HLA, TSPAN8, PTPRR) related to IgAN in a Japanese population (Saka et al., 2015).

Along with HLA antigens, complement factor H (CFH) immunoglobulins, cytokines and the TCRs are also considered as one of the strong candidate genes involved in immune regulation in IgAN patients (Barrat, et al, 2007). IgA deposits are associated with components of complement system. IgA deposits have a strong capacity to activate the alternative complement pathway. Activation of local complement results in cell injury, which induces the inflammation leading to disease progression. In vivo studies have revealed that combined deletion of CFH and its related genes (CFHR), confer a reduced risk of IgAN indicating that these genes control the activation of combined alternative complement pathway. Another association of transforming growth factor-β1 (TGFβ1), an important cytokine gene, with IgAN is reported. TGF – β1 plays a vital role in the pathophysiology and progress of glomerulonephritides. Two single nucleotide polymorphisms (SNPs), C-509T (promoter region) and T869C (Leu10-Pro), have been associated with the transcriptional activity of the gene for TGF – β1 and protein levels in plasma. It has been shown that the frequencies of genotypes producing high TGF – β1 protein were higher in IgAN patients as compared to normal population and patients with genotypes producing high TGF – β1 plasma levels had poor renal survival (Lim, et al, 2005).

Polymeric immunoglobulin receptors (PIGR) expressed on several glandular epithelia. Obara, et al, 2003) in a Japanese case-control study reported a significant association between IgAN and 6 SNPs from Polymeric immunoglobulin receptors (PIGR) gene. They also demonstrated that biopsy specimens from IgAN patients were positively stained by antibody against the secretory component of PIGR. However, it is stated that these genetic associations had not generally been observed in any other population therefore, could not be proved to be involved in IgA nephropathy causation or progression (Maxwell and Wang, 2006).

Various immune mediators like tumor necrosis factor α (TNFα) have been shown to play a role in the inflammatory process in IgAN. TNFα is produced during the early stage of the inflammatory process by generating a cascade of other mediators and cytokines, including interferon gamma (IFNγ), IL-6, IL-8 and IL-10. TNFα is predominantly produced by macrophages, the dominant infiltrating cells in the kidney of IgAN patients. It has been reported that the degree of hypercellularity is correlated with the degree of irreversible glomerular injury. The plasma levels and urinary excretion of TNFα are shown to be elevated in patients with IgAN. A – 308 polymorphism in the promoter region of the TNFα gene has been associated with the increased TNFα production. It has been demonstrated that TNF gene polymorphisms influence the occurrence or initiation of the disease, but do not play a significant role in the progression of IgAN.

One of the key pathogenic mechanisms in various types of glomerulonephritis is accumulation of leukocytes within the glomerulus and interstitium of the kidney (Adler and Brady 1999). Adhesion molecules that are involved in these interactions are selectins, integrins, and certain proteins belonging to the immunoglobulin supergene family. To date, three selectins, E, L and P have been characterized, predominantly expressed in cytokine – activated endothelium, circulating leukocytes, and in activated endothelial cells and platelets respectively. Marked increase in selectins levels have been reported in biopsy samples from patients with IgAN (Chaudhury et al., 1996). In a case control association study, using SNPs found in the selectin gene cluster (1q24 – 25) it has been shown that these SNPs are significantly associated with IgAN in Japanese patients (Takei, et al, 2002).

It has been demonstrated in various studies that at least four events contribute in the development of IgA nephropathy that include, aberrant glycosylation of IgA1, synthesis of anti-galactose – deficient IgA1 antibodies, binding of the galactose – deficient IgA1 by the anti-glycan / glycopeptides antibodies to form immune complexes (ICs), and accumulation of these complexes in the glomerular mesangium to initiate glomerular injury.

The pathogenic importance of ICs is well accepted. However, factors that effect the formation and composition of these ICs and the mechanisms leading to cell activation and glomerular damage are still not clear. Molecular mechanisms of autoantibody-immune complex induced organ injury and their clearance involve two main components, receptors for the Fc region of IgG (FcRs) and the complement system. Studies have been conducted to associate the polymorphisms within the gene for FCGR and complement factor gene (CFH). Their data revealed genetic associations with disease the phenotype and highlighted the potentially pathogenic roles of complement factor and Fc receptor gene polymorphisms in IgAN (Xu-jie Zhou, et al, 2013).

Although IgAN is considered as humoral immune-mediated disease due to the IgA deposition and their elevated level in the blood, contribution of cellular immunity cannot be denied. T cell receptors (TCR) play an important role in immunoglobulin synthesis and recognition of major histocompatibility complex
(MHC) bound antigens presented by B cell. From different studies it has been concluded that gene for TCR is one of the candidate genes involved in immune regulation in IgAN patients and its polymorphisms have been associated with IgA nephropathy. SNPs (single nucleotide polymorphisms) are used in genetic association studies to find out relationship between polymorphic alleles in the candidate gene and the disease status. Polymorphisms in genes for TCR constant beta and alpha chains have been linked to the progression of IgA nephropathy in Japanese and Chinese Han people, respectively (Li; Xue, et al, 2009). An SNP in the promoter region, −560 C/T of TCRα chain has been associated with the clinical presentation of IgAN. Some studies have reported that the high proteinuria is associated with CT genotype of TCRα − 560 SNP and few studies have reported that the incidence rate of proteinuria varied significantly as TT genotype confers low proteinuria and high in cases with CT genotype. However, results of a similar study on Pakistani patients by our group showed no statistical difference in genotype distribution between IgAN patients and control subjects indicating that TCRα promoter polymorphism − 560 C/T does not confer susceptibility to IgA nephropathy in this set of patients, which might be because of less number of samples that were analyzed (unpublished data). All three genotypes of TCRα − 560 (CT, TT and CC) were associated with high proteinuria. In addition CT and TT genotype correlated with gross hematuria, microscopic hematuria and hypertension and CC genotype showed none of the above clinical features except proteinuria.

It is concluded that IgAN is an autoimmune disease partly caused by an abnormal O-glycosylation of the IgA1 molecule, caused by aberrant glycosylation of IgA1, production of anti-galactose − deficient IgA1 antibodies, formation of immune complexes, and accumulation of these complexes in the glomerular mesangium leading to renal injury. Various studies have associated genetic and environmental factors to play role in the pathogenesis and progression of the disease, however most of the studies are underpowered. Therefore, we suggest for establishing the association for a genetic marker, analysis should be done on a large number of samples from ethnically diverse populations. The identification and validation of such genetic markers for the IgAN would provide the clinician a vital tool “Biomarker” to facilitate the diagnosis, and more importantly would offer screening test for the assessment of individuals at risk of developing IgAN.

Author Participation
SK conceived the idea of project and prepared the manuscript, GA participated in literature review and helped in manuscript preparation

REFERENCES