

Genomics and Disease Progression in IgA Nephritis

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Abstract

Apart from clinical, histological and biochemical indices, genomics are now being employed to unravel the pathogenetic mechanisms in the disease progression of IgA nephritis (IgAN). The results of angiotensin converting enzyme (ACE) gene polymorphism have been controversial. Those patients with the DD genotype seem to have a poorer prognosis. However, with high dose angiotensin receptor blocker (ARB) therapy, the ACE gene polymorphism status of a patient may no longer be a matter for concern as those with the DD genotype would also respond favourably to high dose ARB therapy. Association studies with gene sequencing and haplotypes have suggested that multiple genes are involved in the pathogenesis of IgAN. Some workers have reported a synergistic effect in the combined analysis of AGT-M235T and ACE I/D polymorphism. With the use of deoxyribo nucleic acid (DNA) microarray, tens of thousands of gene expressions genome-wide can be examined together simultaneously. A locus of familial IgAN has been described with strong evidence of linkage to IgAN1 on chromosome 6q22-23. Two other loci were reported at 4q26-31 and 17q12-22. DNA microarray techniques could also help in the identification of specific pathogenic genes that are up- or down-regulated and this may allow genome wide analyses of these genes and their role in the pathogenesis and progression of IgAN. Recently, using genome-wide association studies (GWAS) more loci for disease susceptibility for IgAN have been identified at 17p13, 8p23, 22q12, 1q32 and 6p21.

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Introduction

IgA nephritis (IgAN) is the most common primary glomerulonephritis occurring worldwide but of unknown pathogenesis. It is common among Asians and Europeans but uncommon among Africans.¹ Both sporadic and familial IgAN have been reported. Efforts to map disease susceptibility genes have not been successful. There are 2 approaches for the genetic studies for IgAN, one is through a linkage-based approach; the other is through candidate gene association studies. Using genome wide analysis of linkage in 30 multiplex IgA kindreds, Gharavi et al² have demonstrated linkage of IgAN to 6q22-23 under a dominant model of transmission with incomplete penetrance. This is the first time an IgAN locus on a chromosome has been identified and is the preliminary step towards the identification of a disease susceptibility gene for IgAN.

Some European investigators have constituted a European IgAN Consortium³ where data from linkage analysis studies, family association studies and case control association studies have been collected. The Consortium has to date identified 2 more loci for IgAN located on chromosomes 4q26-31 and 17q12-22.⁴ This is in addition to the gene from Gharavi's group.²

Disease Progression

In 1985, we studied a cohort of 151 patients with IgA nephritis after a mean follow-up period of 5 years and found that 84% of the patients had stable renal function, 5% of them had slow deterioration with renal impairment and 11% of them had progressed to end-stage renal failure (ESRF). The bad prognostic indices were proteinuria >1 gm/day,

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hypertension, crescents on renal biopsy, glomerulosclerosis and medial hyperplasia of blood vessels. The cumulative renal survival was 89% at 5 years.⁵

Fifteen years later, in 2000, we ascertained that 53 out of 151 (35%) patients had developed ESRF.⁶ The indices analysed by univariate analysis were clinical, biochemical and histological. Patients who developed ESRF had a higher incidence of hypertension, abnormal serum creatinine, higher urinary protein, non selective proteinuria; segmental and global sclerosis, crescents, medial hyperplasia of blood vessels and tubular atrophy.

However, using multivariate analysis by the regression model of Cox, we found that only hypertension, serum creatinine, protein selectivity, segmental and global glomerulosclerosis and crescents were significant. The 20-year cumulative renal survival of the 151 patients was 65%. We concluded from this study that IgA nephritis is not a benign disease. It has a cumulative renal survival of 89% after 5 years, 75% after 10 years and 65% after 20 years.

In this commentary, we shall report on the involvement of genomics to elucidate the pathogenesis and disease progression of IgA nephritis, focusing mainly on the local scene over the past decade.

Gene Polymorphism

Individuals are prone to certain diseases because of disease susceptibility genes. In many instances, such genes are present at birth, while in other situations, a particular set of genes could be modified by environmental factors, predisposing the individual to development of a certain disease.

The renin angiotensin system (RAS) which regulates salt and blood pressure within our body comprises genes which have several forms of polymorphism. One such polymorphism is the deletion/insertion polymorphism of the angiotensin converting enzyme (ACE) gene. This deletion polymorphism is associated with raised serum and cellular ACE levels and may be associated with hypertension, left ventricular hypertrophy and ischaemic heart disease. However, this association varies depending on the genetic make-up and environmental factors affecting individuals.^{7,8} Angiotensin II is a most powerful vasoconstrictor within the body. It promotes hypertrophy and collagen accumulation within the heart leading to ischaemic heart disease and heart failure. Within the kidney, it causes mesangial cell proliferation, matrix expansion leading to progressive renal injury with eventual renal failure.

IgA nephritis is one disease where the role of renal injury related to the RAS is well documented. The 3 ACE genotypes have been invoked in the progression of IgA nephritis:

1. homozygote for deletion allele (DD)
2. heterozygote (ID)

3. homozygote for insertion allele (II)

Individual antiproteinuric response to ACEI/ATRA therapy varies depending on ACE gene polymorphism as those with the DD genotype respond better to the antiproteinuric efficacy of ACEI/ATRA^{9,10} therapy. Yoshida⁹ in a study examining the role of the deletion polymorphism of the ACE gene in the progression and therapeutic responsiveness of IgA nephropathy using the ACEI lisinopril reported that the ACEI lisinopril significantly decreased proteinuria in the DD genotype patients but not in the II or ID genotype. Similar findings have also been reported by Moriyama.¹¹

The DD genotype was more frequently associated with declining creatinine clearance and had 4-fold risk of progression to renal failure compared to those with ID or II genotype. The increased risk of renal failure with the ACE DD genotype was a risk factor independent of the risk factor of hypertension. It was found that the ACE DD genotype was significantly higher in frequency among patients with declining renal function even in the absence of hypertension.¹² These authors¹³ further found that patients with glomerular hypertrophy at the time of biopsy with the DD genotype (7/9) developed loss of renal function 10 years later, compared to patients (0/4) with no glomerular hypertrophy. Also, patients with the DD or ID genotype had more glomerulosclerosis compared to those with II genotype.

Among studies conducted in the West, the ACE DD genotype was also associated with progressive decline of renal function in IgA nephritis,^{12,13} indicating that the ACE I/D genotype crosses geographical and racial boundaries.

However, with respect to the influence of ACE gene ID polymorphism on the response to ACEI/ARB therapy, we have recently reported that¹⁴ with high dose ARB, irrespective of the ACE gene polymorphism, whether it is DD, ID or II, patients will still respond with more effective reduction of proteinuria and earlier recovery of renal impairment with regression of glomerulosclerosis. We concluded that in these days of high dose ARB usage, the ACE gene ID polymorphism status of a patient may no longer be a matter for concern as patients will respond to therapy as long as they are adequately treated with ACEI/ARB therapy. In addition, high dose ARB therapy confers the additional benefit of early improvement in renal function in some patients. In today's terms, as far as IgAN is concerned, the ACE gene polymorphism may no longer be relevant and might be redundant as it does not appear to feature at all in the genomics of IgAN.

At least 3 gene polymorphisms in the RAS have been associated with IgA nephritis. These are the ACE gene, the angiotensinogen (AGT) gene and the angiotensin II type 1 receptor (ATR) gene. Several studies conducted on

Caucasian and Japanese had reported conflicting reports. Our earlier studies¹⁵ on genetic polymorphism in these 3 genes in 118 Chinese patients with IgA nephritis compared to 94 healthy Chinese controls have been reported. Genotyping was performed with DNA from peripheral leukocytes, polymerase chain reaction (PCR) amplification of the polymorphic sequence, restriction enzyme digestion and separation and identification of DNA fragment. The results of genetic studies were correlated with clinical and renal biopsy data at entry to study and final status. Among the controls, genotype distributions were in Hardy-Weinburg equilibrium. Comparing all IgA nephritic patients with controls, the AGT and the ATR genotype distributions were similar but when we examined the ACE genotype we found that IgA nephritic patients had significant increase in the ACE DD genotype ($P < 0.05$). Patients with the ACE DD genotype were supposed to have higher renin levels and higher incidence of cardiovascular disorders compared to those with the ID or the II genotype. Comparing patients with ESRF, there was no difference in any of the 3 gene polymorphism (i.e. ACE, AGT and ATR gene).

But in contrast, there were significant differences in higher male prevalence ($P < 0.05$), increased serum creatinine at presentation ($P < 0.05$), more glomerular sclerosis ($P < 0.01$) and higher tubulointerstitial lesion score ($P < 0.001$) in those with the DD genotype. Among the ACE, AGT and ATR gene polymorphism, only the DD genotype may predispose the individual to IgA nephritis in our Chinese population¹⁵ and these are patients with a poorer prognosis as they are predisposed to ESRF.

Gene Sequencing

Apart from studying genetic polymorphism of the ACE gene, other methods include studying deoxyribo nucleic acid (DNA) sequencing of the gene and haplotypes. The DNA sequence of any 2 individuals is 99% identical. But it is the variation in the remaining 1% which may decide whether one individual is at greater risk of developing a particular disease compared to another. The sites in the DNA sequence where an individual differs at a single DNA base is called single nucleotide polymorphisms (SNPs). Sets of nearby SNP on the same chromosome can be inherited as a block. Such a block is referred to as a haplotype. The development of a haplotype map will be a useful means to finding genetic variations which may relate to certain diseases.

Arising from our sequencing studies,¹⁶ we were able to document certain haplotype patterns in IgA nephritis which could be correlated with certain data as to why certain patients with IgA nephritis do worse than others or respond better to ACE inhibitors (ACEI) or angiotensin II receptor antagonists (ATRA). In this respect, our preliminary studies

have so far identified 2 areas of polymorphism among patients with the ACE DD gene who respond to ACEI/ATRA therapy in our study. This has been reported in our work on ACE gene sequence and nucleotide variants in IgA nephropathy.¹⁶

Association studies with SNPs have been contradictory. We found that the study of haplotypes (blocks of more than 1 SNP on the same chromosome) were more helpful. With gene sequencing, all SNPs can be found for construction of haplotypes. Narita¹⁷ had earlier reported that certain haplotypes (blocks of more than 1 SNP inherited together on the same chromosome) of the angiotensinogen gene can influence therapeutic efficacy of renin-angiotensin system blockade in IgAN. It seemed possible that more than 1 SNP of a haplotype acting in synergy may be a better predictor than the individual SNP.¹⁸ Nucleotide sequencing of a gene may reveal all available SNPs for the construction of haplotypes. In our study, we determined the entire genomic sequence of the ACE gene (about 24,000 base pairs) in 24 Chinese subjects (20 patients with IgAN and 4 healthy controls). All identified nucleotide variants and haplotype constructs were tested for predicting disposition to IgA nephropathy and for prognosticating disease progression to ESRF.

The frequency of SNPs is high in the human genome and it is not cost effective to genotype all SNPs.¹⁹ From a block of common (absolute or closely linked) SNPs, a single haplotype-tagging SNP (htSNP) maybe selected for genotyping without loss of power. Our findings with the ACE gene were similar. Two of the 6 haplotype constructs from 5 significant-variants in our study had predictive value for risk or low risk of ESRF (haplotype 3 and 5). However the predictive power of the individual component SNPs were quite similar. Thus a single SNP may be selected for genotyping without loss of power for prediction. Again, the obvious reason for such redundancy was that these variants were highly linked. Between each of the 4 variants and the Alu variant, the correlation of genotype was highly significant, all r values > 0.9 and $P < 0.001$. Keavney et al²⁰ reported similar findings.

Hence a reasonable proposition for future development is finding a haplotype of non-redundant SNPs from many genes genomewide.²¹ In this respect, Bantis et al²² reported synergistic effect in the combined analysis of AGT-M235T and ACE I/D polymorphism. Yoon et al²³ also reported interdependent effects of ACE and PAT-AH polymorphisms on the progression of IgAN. We conclude from our studies together with that of others that multiple interacting genes may be involved in the pathogenesis of IgAN.

DNA and RNA Microarray

Using traditional methods, researchers usually work on one gene in one experiment which makes throughput very

limited. In recent years with the advent of “DNA microarray” it is now possible to monitor the whole genome on a single chip (genome chip). With this, researchers can now have the whole picture of the interaction among thousands of genes at the same time.

The term array refers to an orderly arrangement of the samples. Basically it provides a medium for matching known and unknown DNA samples based on base pairing rules on microplates or standard blotting membranes. Microarrays are usually less than 200 microns in diameter and the array contains thousands of spots. The DNA chips are fashioned by robotics on glass or nylon substrates. Probes with known identities are used to determine complementary binding. The array is exposed to labelled sample DNA prepared from patient's tissue or cells, thus allowing massively parallel hybridisation and detection of gene expression after probe scanning and data analysis. An experiment with a single DNA chip can provide researchers with information on thousands of gene simultaneously, a dramatic increase in throughput. Much research have been focussed on gene expression with regard to the development of diabetic nephropathy so as to find genes that could be involved in the process.^{24,25} In these studies, experimental models for diabetes and cell cultures stimulated with high glucose concentrations were used. Gene expression profiles have also been obtained in normal human renal cortex,²⁶ in patients with IgA nephritis²⁷ and in those with congenital nephrotic syndrome.²⁸

DNA microarrays could serve as a research tool in unravelling the pathogenesis of IgA nephritis. Patients with IgA nephritis could have one or more genes which could be related to disease susceptibility. Some of these genes could be associated with disease progression. Large scale gene expression profiling has been used in diagnostic tumor specimens from patients with diffuse large B-cell lymphoma.²⁹ A small subset of genes was differentially expressed between patients with a favourable prognosis and those with a poorer prognosis. In another study on breast cancer, a subset of marker genes could predict high risk of relapse and occurrence of distant metastases.³⁰

In nephrology, microarray might prove as useful as in oncology. There may be shared gene expression among patients with different types of kidney disease and even within these groups there could be those with differentially expressed gene profiles in terms of disease progression or even response to drug therapy. Currently, one limitation of the microarray technique in nephrology is that relatively large amounts of RNA, at least 10 µg are needed for hybridization. In a kidney biopsy, the amount of renal tissue is often limited as the specimens have to be processed for light microscopy, immunofluorescence as well as electron microscopy. One solution to the problem of limited amount of tissue from a single biopsy specimen is to pool the RNA

from multiple biopsies.

A recent study by Adler³¹ quantitates the mRNA in renal biopsy specimens of patients with type I diabetic nephropathy comparing with those from type II diabetic nephropathy. It was found that the glomerular mRNA levels of Type IV collagen are 2 fold higher in patients with microalbuminuria compared to those who were normalbuminuric. There was no significant difference in the degree of mesangial expansion in the 2 groups. The mRNA could be used as a prognostic tool and correlated with the change in renal function and degree of proteinuria but a major disadvantage is the necessity for repeat renal biopsies to obtain glomerular mRNA in the follow-up studies as a renal biopsy is an invasive procedure not without risks. In this respect, some researchers have resorted to measurements of mRNA in the urine and blood^{23,24} in renal transplant patients to diagnose renal allograft rejection.

We conducted a study of the genome-wide expression profiles of the circulating leukocytes from patients with IgA nephritis,³² comparing them with non-IgA nephritic patients and healthy controls, using Affymetrix high-density gene chip array technology. IgA nephritis is the commonest primary glomerulonephritis seen in Singapore and is an important cause of end-stage renal failure. This study may help determine which gene(s) is up or down regulated in IgA nephritis compared to patient and healthy controls. Such information may help in understanding the pathogenesis of the disease and identify the genes related to disease susceptibility and disease progression. Our paper on urotensin 2 and retinoic acid receptor alpha (RARA) gene expression in IgA nephropathy illustrates how microarray techniques can be employed.³²

DNA microarray technique allows tens of thousands of gene expressions genome-wide to be examined together simultaneously. This advance in gene expression profiling has given rise to immense potential for the characterisation and prognostication of disease, and for analysing the complex biological processes involved which would allow a better understanding of the molecular basis of diseases.^{33,34}

Microarray technology together with the aid of bioinformatics softwares is now widely employed for rapid investigation into the complex biology in health and in disease.³⁵⁻³⁸ In IgA nephropathy, Preston et al³⁹ had shown that gene expression profiles of circulating leukocytes correlate with renal disease activity in IgA nephropathy by combining microarray technology with clustering statistics. Genome-wide scan technique has also been applied in a mouse model of IgAN with rapid results in the identification of a susceptibility locus in chromosome 10.⁴⁰

In our study,³² we analysed the genome-wide gene expressions in 7 patients with IgAN patients, 6 patients with minimal change disease as patient controls and another 7 normal subjects as normal controls by using the GeneChip

Human Genome U1³³ Plus 2.0 Arrays from Affymetrix. After this, individual specific gene expression of interest was confirmed using Taqman real-time PCR method.

The data suggested that urotensin 2 (URT2) is a marker of vascular disease in IgAN as patients with raised blood pressure (BP) (systolic BP and diastolic BP) tend to have higher levels of URT2.

URT2 levels were low in patients with minimal change nephrotic syndrome in remission. This was consistent with the findings of Balat et al.⁴¹ We found that diastolic BP and proteinuria were also correlated with URT2 in minimal change disease. In addition, our data also showed that patients with IgAN had down-regulation of RARA expression.

Subsequently, we discovered that another colleague Shaier et al⁴² using Thy-GN rats with mesangial proliferative glomerulonephritis (GN) (an animal model for IgAN) and these nephritic animals respond to RARA agonist therapy with reduction of proteinuria and normalisation of BP. Hence there may be a role for clinical trials with RARA specific retinoids in patients with IgAN since IgAN is also a mesangial proliferative GN like Thy-GN.

Thousands of genes remain to be explored. Furthermore, with the gradual identification of many untitled and hypothetical proteins, the list of identified genes may be repeatedly visited for more information. Assayed simultaneously on the same microarrays, these genes are linked in expression, be it up- or down-regulation. Such linkage information on gene-gene interactions genome-wide may be integrated into a panoramic view of disease pathways to explain the origin and development of IgAN. The present preliminary analysis of genome-wide gene expressions has implicated URT2 up-regulation and RARA down-regulation in the pathogenesis of IgA nephropathy. These may be relevant targets for further research and perhaps development of new drug therapy.

Genome-wide Expression and the 10K GeneChip

With the development of newer techniques in genomics, less labour intensive and therefore much more efficient methods for genotyping became available, one of which was the availability of the gene chip which could screen 10,000 SNPs in one go simultaneously.⁴³

Genotyping for 10,204 SNPs genome wide was done with the GeneChip Human Mapping 10K Microarray (Affymetrix) to screen for susceptibility genes in IgA nephropathy.⁴³ Thirty patients with IgAN and 30 normal subjects were screened and analysed for differences in genotype frequency, allele frequency and heterozygosity reduction. The results showed that among the most significantly associated SNPs, 48 SNPs were found mapping directly to the intron of 42 genes that localised in 13 somatic

chromosomes and chromosome X. Genotype distribution of these SNPs did not deviate from the Hardy-Weinberg equilibrium in normal subjects.

A locus for familial IgAN called “IgAN1” on chromosome 6q22-23 had been described in human² and in mice.⁴⁴ The European IgAN Consortium³ in a genome-wide scanning of 22 multiplex families identified 2 regions with the strongest evidence of linkage, 4q26-31 and 17q12-22.⁴ However, no identification of any causal gene has been made. Nevertheless, there is great potential in such genetic studies that might reveal new insights in the aetiology and mechanism of pathogenesis. New data in this area may suggest more specific approaches in seeking preventive measures and better treatment for IgAN. The GeneChip Human Mapping 10K Xba 142 Array genotypes 10,204 SNPs on a single array allows rapid, accurate, cost-effective and whole-genome scan for susceptibility genes in the genetic study of diseases. Using this chip, we screened 30 patients with biopsy proven IgAN and 30 normal subjects for differences in their genetic composition.

There are few genetic studies that search for susceptibility genes in IgAN using high-throughput SNP technologies. A locus for familial IgAN has been described with strong evidence of linkage to “IgAN1” on chromosome 6q22-23.² Two other loci were reported at 4q26-31 and 17q12-22.⁴ However, none of these reports identified the gene responsible. Study conducted in a large Lebanese family with 38 members,⁴⁵ found no evidence of linkage to the loci, 2q36, 4q22-31 and 6q22-23. In our study, 6 SNPs were mapped to chromosome 6. The SNP-pair (Affymetrix Prob Set-ID: SNP_A -1510472 / SNP_A-1519262) mapped to Triadin (TRDN) at 6q22.31 which is within the IgAN1 region of 6q22-23. There were 3 SNPs mapping to chromosome 2 but none to the region of 2q36. No associated SNP mapped to chromosomes 4 and 17. Thus besides the IgAN1 locus, association with other reported loci have not been detected here. However, we only screened 10,204 SNPs or about 0.3% of the estimated 3 million SNPs in the whole human genome of 3 billion base pairs. Therefore it is very likely that the chromosome regions of these known loci may not be covered by the SNPs included in the GeneChip Human Mapping 10K Array.

At the end of our search we found among the patients with IgA nephritis 48 SNPs which mapped directly to the intron of 42 genes which were significantly different from normal healthy controls. This offers an avenue for further investigation of the 42 genes which may be associated with IgA nephritis. Future studies may show that some of these associated genes may play a role in the pathogenesis of IgA nephritis, the commonest form of glomerulonephritis of unknown etiology with an elusive cure. This is a pilot study on a small group of patients.⁴³ What is needed is a larger cohort of patients with much funding to study a

wider array of genes. A large consortium may have to be engaged for this.

In this respect, it would appear that we may already have some answers from the recent findings of two large studies on the genomics of IgAN using genomic wide association studies (GWAS).^{46,47} Yu et al⁴⁶ have reported in a GWAS involving 1434 patients of Chinese Han ancestry with IgAN and a normal control group 4270 subjects the presence of 3 associations, the first 17p13 (rs3803800), the second 8p23 (rs2738048) which implicated the gene encoding tumour necrosis factor (TNFSF13) and α -defensin as susceptibility gene and 2 other sites at the major histocompatibility complex (MHC) region, rs660895 and rs2523946. They also confirmed a previously reported association at 22q12 (rs12537)⁴⁷ at the MHC. These genetic polymorphisms are involved in immunity and inflammation and can influence both susceptibility to and clinical manifestations of IgAN. Elevated serum α -defensin at 1 to 3 time normal concentrations have been observed in type 1 diabetic nephropathy⁴⁸ and lupus nephritis.⁴⁹

Gharavi et al,⁴⁷ using GWAS studied 1194 cases with IgAN and 902 controls, all of Chinese Han ancestry comparing them with European cohorts comprising 1950 cases with IgAN and 1920 controls. They identified 3 independent loci within 6p21 in the MHC region as well as a common deletion of CFHR1 and CFHR3 at chromosome 1q32 and a locus at chromosome 22q12. All 5 loci surpassed genome-wide significance for association between 1.59×10^{-26} and 4.84×10^{-9} . To determine the cumulative risk conferred by these loci, Gharavi et al⁴⁷ computed a genetic risk score and this risk score collectively explained 5% to 7% of the variation in disease risk in the Chinese cohorts and 4% in the European cohort. The frequency of protective alleles were lower among the Chinese when compared with the Europeans, thus explaining the higher prevalence of IgAN among the Chinese. The frequency of risk alleles were highest amongst Asians, intermediate among Europeans and lowest among Africans.⁴⁷

Conclusion

This commentary aims to focus on certain areas of genomics is meant to elucidate some aspects of the pathogenesis of IgAN.

Towards this shared goal, the European IgAN Consortium, set up since October 2000,³ would be better poised to unravel the genomics and pathogenesis of IgAN. This Biobank Consortium³ with its huge depository of DNA and other tissue samples coupled with the clinical data of IgAN patients and their kindreds would possess a sizable database and together with advanced genomic technologies and international IgAN expertise would have the necessary arsenal for the task ahead. For the next generation, it is

envisaged that GWAS which allow examination of larger cohorts thereby increasing the power of the studies with larger throughput will pave the way for advancing our knowledge of the genomics of IgAN.

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