Controversies concerning the importance of genetic polymorphism in IgA nephropathy

Luc Frimat and Michèle Kessler

Department of Nephrology, University Hospital Nancy, France

In a recent letter to *Nature Genetics*, Gharavi and colleagues reported a study of 30 multiplex kindreds, demonstrating the linkage of IgA nephropathy (IgAN) to 6q22-23 [1]. This finding is a considerable step towards a comprehensive explanation of this form of chronic glomerulonephritis. As previously suggested, genetic studies of familial IgAN provide the best way to identify IgAN genes [2]. Following this approach, the number of publications on genetic polymorphism underlying IgAN grew rapidly in the 1990s. For example, in 1996, in this journal, significant results of the first studies showing an association between a genetic polymorphism of the renin-angiotensin system (RAS) and IgAN were met with great enthusiasm [3]. A few years later, reports of negative findings with respect to this association tempered this initial impression [2,4,5]. Some authors were even unable to confirm their own results [5,6]. We propose here a reappraisal of the background implications and methodological foundations of existing research into IgAN, which might be an important source of conflicting results.

IgAN as a complex, multifactorial disorder

The IgAN phenotype does not exhibit classic Mendelian recessive or dominant inheritance attributable to a single gene locus [7]. This is a common feature, which IgAN shares with cardiovascular disease,
cancer, type 2 diabetes and Alzheimer’s disease [8], although the incidence of IgAN is much lower in absolute terms. IgAN is characterized by high levels of genetic complexity [7,8]. For instance, some individuals who inherit a predisposing allele might not manifest IgAN (incomplete penetrance), whereas others who inherit no predisposing allele might develop the disease as a result of an environmental cause. Furthermore, mutations in any one of several genes (locus heterogeneity) may possibly result in identical phenotypes. Consequently, there is no way to distinguish between different genetic causes of phenotypically identical cases of IgAN unless the genes can be mapped. Of importance, is the possibly common occurrence of alleles associated with increased susceptibility to IgAN in the general population, and the fact that a given gene might proportionally contribute only minimally to the total genetic/environmental variance. Two main methods have been used to depict potential genetic traits in complex diseases. Most of the genetic studies reported to date on IgAN have been association studies, i.e. case-control studies, searching for a disease-allele correlation in a given population [7]. The alternative method is linkage analysis, focusing on concordant inheritance in familial IgAN.

Defining IgA nephropathy

The numerous problems encountered in genetic research in kidney disease are related above all to difficult issues of nosology [9]. IgA is unique among glomerular diseases in being defined by immunofluorescence description, i.e. mesangial deposits of IgA, rather than by light microscopy. However, biopsy specimens are largely inadequate to clearly establish a limit between various diseases. I-Hong Hsu et al. reminded us of the problem, demonstrating how difficult it is to unravel the genetic situation of the Zuni Indians, a highly consanguinous community in the US, who have genuine IgAN with consistent deposits of IgM and IgG in 94 and 45% of cases, respectively [2,7]. Moreover, renal biopsies are not always obtained in uremic patients, so the definitive diagnosis of IgAN is not validated in each instance. Even in Gharavi’s report, five of the 17 ESRD patients did not have biopsy-proven IgAN [1].

Late onset of clinical symptoms is typical for IgAN, thus hampering diagnosis in the early stages of the disease [2,10–12]. Light microscopy findings range from minimal changes to extracapillary crescents. Primary IgAN can appear either as macroscopic haematuria or microscopic haematuria/proteinuria, and less frequently as nephrotic syndrome. The description of IgAN before, during or after another disease is often reported and clouds the clinical reports. For example, the concurrent appearance of IgAN and minimal change disease points out the lack of mutually exclusive diagnostic criteria [9]. The discovery of IgAN with terminal glomerulosclerosis in a diabetic patient also illustrates the problem of the lack of independence between disease and risk factor assessments [9]. Finally, glomerular lesions in Henoch–Schönlein purpura are often indistinguishable from IgAN and each disease can occur in a multiplex kindred. In their study, Gharavi et al. did not discuss this matter [1].

To get around this clinical diversity, geneticists have suggested restricting the patient population, so work can be done on a trait with a pattern that is closer to Mendelian inheritance, and thus a theoretically more homogeneous genotype [7]. For instance, the clinical phenotype could be limited to patients with bouts of macroscopic haematuria. One could also hypothesize that patients with early-onset IgAN might be genetically more homogeneous than those with late-onset disease. This approach has been applied to study polymorphisms of the mannose-binding lectin gene in a sub-group of patients <18 years old at disease onset [13]. Another solution is to consider only patients with very severe disease, i.e. rapidly progressive IgAN; however, these cases are very rare.

Identifying genes that initiate IgAN or promote kidney failure

The clinical puzzle is muddled further by physiological considerations, because the diagnosis of IgAN does not imply a common pathogenetic mechanism [2,10]. There are, however, three physiological sequences relatively characteristic of IgAN. The first concerns the processes initiating mesangial IgA deposits. This condition probably occurs more often than one might imagine. Actually, unquestionable IgA deposits are frequently discovered in systematic autopsies of subjects who died without a renal history [14]. This is the hidden part of the ‘iceberg’. The second sequence concerns only a small portion of these subjects, those with pathological IgA deposits generating mesangial cell damage and activation. The third sequence is not specific to IgAN, since the processes subsequently promoting kidney failure are likely to be generic (with few differences), with analogous events in other types of chronic glomerulonephritis [9,10].

This context hinders the construction of realistic hypotheses. Yet, suitable association studies should investigate associations that make biological sense and alleles that affect the gene product in a physiologically meaningful way [8]. The study design should, at the very least, clearly distinguish between initiators likely to be specific for IgAN and general promoters of kidney failure. Logically, early initiators should be studied in comparison with a healthy control group. One study devoted to a candidate gene for control of IgA1 production is an illustrative example [15]; comparison with a control group produced a negative result, but a concurrent comparison considering the same gene as a promoter was positive. Another example is the apparently significant role
of polymorphism in the functional promoter region of the FcαR gene, demonstrated by comparing 90 patients with IgAN, 50 with other glomerulonephritides and 83 healthy controls, without taking progression into account [16].

Under relevant functional assumptions, it is undeniable that the renin-angiotensin system plays a role in promoting kidney failure [2,3,10]. If an association between a gene and IgAN could be found, it would be consistent since the bias of heterogeneity of pathogenesis is avoided in the more homogeneous IgAN group [7]. Generalization to other progressive nephropathies would be realistic. Unfortunately, association studies have produced conflicting results about genetic polymorphisms of the RAS in IgAN [2–6]. Many explanations of failure to replicate initial results can be given: small sample size, artefacts, random events, and initial false-positive results [8]. The main problem is the choice of a control group [7], cognate with the methodological difficulties encountered in assessing the progression of nephropathies [4,9]. The end point should be unambiguous, clinically relevant and definitive. Among the three largest RAS polymorphism-IgAN association studies (all three showing negative results), two used end-stage renal failure as the end point [4,5]. Another possibility is an underestimation of the DD genotype due to competing cardiovascular risk [2]. In any case, if an association between the ACE gene D allele and progression of IgAN exists, it has a very weak effect.

Since association studies bring forward limited inferences, linkage analyses of familial IgAN might be a more appropriate approach [2]. This offers the advantage of searching for genes without any prior biological assumptions. Gharavi et al. [1] first sought linkages between IgAN and all candidate genes (exclusively initiators) suggested by biological abnormalities. Surprisingly, no significant linkage could be found. The linkage of IgAN to locus 6q22-23 could only be demonstrated with genome-wide analysis using a dominant model with incomplete penetrance and locus heterogeneity [1]. Now the challenge is to identify the efficient genes of this locus and their effects. Further research is therefore needed to explain the phenotypic variations among 6q22-23 carriers and to correlate genotypes more closely with outcomes. One other unanswered question is why this study failed to find a linkage with genes implicated in the pathogenesis of IgAN.

Correlations, interactions and causation

Finally, no matter how we choose to study IgAN, it is more than likely that we will become entrenched in circular arguments. The Human Genome Project has recently provided us with a complete catalogue of all human genes [8], so positional cloning will now come down to systematic evaluation of candidate genes [7]. Gold standard tests for human genes should combine association studies and transgenic studies demonstrating that gene addition or gene knockout in animals produce a phenotypic effect [7]. The challenge would be to gather an adequate sample size, e.g. 1000 cases and as many controls [8]. This would allow an accurate estimate of correlations and gene–gene interactions or gene–environment interactions (for instance medications) [17], with small \( P \) values, i.e. \( < 10^{-6} \) [8]. Systematic collection of data on risk factors for IgAN and renal injury would also be most helpful in building conceptual models for causation [9].

However, IgAN results from the interplay of environment, behaviour and common, low-penetration genes with additive effects. Many combinations could all lead to the same pathogenic effect. This complexity casts doubt on whether accurate prediction will ever be possible [18]. In IgAN, the attributable risk of each susceptibility-conferring genotype will be very weak. So testing patients for specific genotype would not be as efficient as thought at the beginning of genetic research [3]. Fortunately, we may be more optimistic for treatment and prevention, since genomics will undoubtedly provide us with new options for clinical management.

References

1. Gharavi AG, Yan Y, Scolari F et al. IgA nephropathy, the most common cause of glomerulonephritis, is linked to 6q22-23. Nat Genet 2000; 26: 354–357
7. Landers ES, Schork NJ. Genetic dissection of complex traits. Science 1994; 265: 2037–2048
8. Todd JA. Interpretation of results from genetic studies of multifactorial diseases. Lancet 1999; 354 [Suppl. 1]: 15–16
13. Piruli D, Boniotti M, Vatta L et al. Polymorphisms in the promoter region and at codon 54 of the MBL2 gene are not


