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IgA nephropathy—the case for a genetic basis becomes stronger

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Primary IgA nephropathy (IgAN) is a common glomerular disease with a complex genetic architecture. Ethnic differences in susceptibility to IgAN, as well as inter-individual variation in the disease course and prognosis strongly argue for the crucial role of genetic factors in its pathogenesis. For example, IgAN occurs with greatest frequency in Chinese and Japanese populations but is relatively rare in individuals of African descent [1]. A high frequency of IgAN has also been reported in biopsies of Zuni and Manitoba Native Americans and Australian aborigines [2–6]. Familial aggregation of IgAN was first reported in the 1970s, and multiple large series of familial cases have provided further evidence for genetic contribution. Two European studies demonstrated that 4–10% of patients with IgAN had a family history of kidney disease [7,8]. In other studies, urinary abnormalities were detected in over 20% of asymptomatic first degree relatives of IgAN patients [9]. Several extended kindreds with IgAN have also been reported throughout the world, including the United States [10], France [11], Italy [12], Canada [13], Australia [5] and Lebanon [14]. In all reported families, segregation of IgAN is consistent with autosomal dominant transmission with incomplete penetrance, although more complex genetic models are also compatible with the observed pedigrees. The incomplete penetrance is likely explained by the requirement of additional environmental or genetic factors for clinical manifestation of disease.

To date, three genome-wide linkage studies of familial IgAN have been reported, but no causal gene has yet been identified [13,15,16]. Unfortunately, numerous difficulties

hinder linkage studies of familial IgAN. Foremost, familial forms are under-recognized, since the associated urinary abnormalities are often mild or intermittent in affected family members. Most prohibitive is the lack of a reliable non-invasive screening test for IgAN. Currently, the unaffected status is difficult to confirm because renal biopsy carries too many risks to be performed on all family members. As a result, linkage studies are often limited by the use of less accurate phenotypes (such as microscopic haematuria) or affected-only analyses. Both of these approaches result in decreased power to detect linkage. Additionally, microscopic haematuria is relatively common in the general population, thus phenotype misclassification can occur rather easily in the absence of kidney biopsy. Finally, considering significant variability in the clinico-pathologic features of IgAN, it is likely that this diagnosis encompasses several disease subsets. Presently, there is no good way to distinguish such subsets, although the recently proposed Oxford classification of IgAN, as well as ongoing research in the area of IgAN biomarker discovery may offer some help in this respect [17]. From the statistical perspective, heterogeneity is of primary concern when many small pedigrees are analysed jointly. In this situation, increasing the sample size of a linkage study by inclusion of new families may, paradoxically, lead to decreased power. One way to circumvent this issue is to study large multiplex families; however, this approach poses multiple computational challenges, and large families are more difficult to ascertain. Similarly, large genealogies reported from Kentucky or Italy, while clearly demonstrating genetic contribution to the IgAN, are not tractable to linkage methods. Many candidate genes have been suggested, but nearly all published genetic association studies suffer from small sample size, inadequate correction for population stratification and lack of independent replication [18]. In addition, many of the studies focus on IgAN progression rather than causality. Most importantly, however, our limited understanding of the disease pathogenesis restricts the choices of plausible candidate genes. Hence, further progress in the genetics of IgAN will require application of genome-wide association studies in large cases and controls cohorts.

Despite the above challenges, rapid progress in the field of genomics combined with recent advances in the IgAN biomarker research is likely to accelerate gene-identification efforts. Particularly, the development of a reliable IgAN biomarker would enable diagnosis without the requirement of kidney biopsy and greatly facilitate family screening in linkage studies. In this area, the most promising are studies of the glycosylation defects of IgA1, a subclass of IgA present in serum and mesangial deposits of IgAN patients. In healthy individuals, the hinge region of IgA1 contains up to 6 O-linked glycans, composed of *N*-acetyl-galactosamine (GalNAc) with beta-1,3-linked galactose, both of which can be sialylated. In IgAN patients, a significant portion of the circulating IgA1 is galactose-deficient (Gd-IgA1). Galactose-deficient O-linked glycans of IgA1 are potentially antigenic and may promote formation of immune complexes when recognized by naturally occurring anti-glycan antibodies in the circulation [19]. Subsequent mesangial deposition of immune complexes containing Gd-IgA1 is likely responsible for glomerular injury. Interestingly, galactose deficiency appears to be specific to IgA1 molecules, and it is absent in other glycoproteins, such as IgD, in the same patients [20]. Therefore, it is most likely that the IgA1 glycosylation defect arises secondary to aberrant immunoregulation or disruption within the specific subpopulation of B-cells that produce IgA1. The molecular mechanisms involved in the development and regulation of IgA1-secreting cells remain to be elucidated.

Recently, a reliable lectin-based ELISA assay for determination of serum Gd-IgA1 has been developed. This assay utilizes a naturally occurring lectin derived from *Helix aspersa* snail to detect galactose-deficient O-linked glycans in the circulation. In a Caucasian population from the southeastern USA, this test had 90% specificity and 76% sensitivity to diagnose sporadic IgAN [21]. Although this assay will need to be standardized and validated in more diverse populations, it appears to be extremely promising as a new non-invasive diagnostic test for IgAN. Moreover, this assay facilitates genetic studies that seek to identify genes involved in defective glycosylation of IgA1.

The first strong evidence that IgA1 glycosylation defects may be inherited came from a study examining serum Gd-IgA1 levels in a large cohort of IgAN cases and their relatives [22]. The heritability or the proportion of variation in Gd-IgA1 that is attributable to genetic factors was highly significant and estimated at 54%. A clear cosegregation of high Gd-IgA1 levels and IgAN phenotype was observed in the pedigrees with familial disease. Moreover, Gd-IgA1 levels were significantly higher among relatives at risk for IgAN under autosomal dominant transmission compared to controls or individuals who married into the families. Similar observations have recently been reported in a smaller cohort of Chinese patients with sporadic IgAN [23], as well as in a group of paediatric cases of IgAN with Henoch–Schönlein purpura (R. Wyatt, unpublished observations). Interestingly, among individuals with sporadic IgAN, two distinct subgroups have emerged. The index cases with high Gd-IgA1 (majority of cases) had relatives with predominantly high Gd-IgA1. However, the index cases with low Gd-IgA1 levels had relatives with levels that were indistinguishable from healthy controls. In addition, there were many relatives of IgAN patients who had high Gd-IgA1, yet they lacked any signs of kidney disease. Taken together, it appears that Gd-IgA1 is neither required nor sufficient to cause the clinical disease. Rather, it should be considered as one of the quantitative risk factors for IgAN, a situation that may be analogous to a serum cholesterol level in the risk of coronary artery disease. Additional cofactors including other genetic or environmental triggers are likely required for full disease manifestation. For example, a recent study suggests that the individual's propensity to develop anti-glycan antibodies that promote immune complex formation with Gd-IgA1 may represent one of such cofactors [24].

The above data suggest that stratification by Gd-IgA1 may be used to define subpopulations of IgAN patients with distinct disease pathogenesis. This information can be used to reduce heterogeneity in genetic studies, thus enhance their power to detect novel susceptibility loci. The Gd-IgA1 level can also be used as a quantitative endophenotype in linkage and genetic association studies. Quantitative

endophenotypes are frequently preferred in the genetic studies of a complex disease, since they may be a closer reflection of a specific underlying pathogenic process. Furthermore, analysis of quantitative traits typically provides greater power compared to discrete trait. A similar approach has been successful in genetic studies of serum LDL to map the genes responsible for dyslipidaemia [25,26] or serum IgE levels to map novel susceptibility loci for asthma [27,28]. Lastly, joint genetic analyses of Gd-IgA1 and IgAN phenotypes may enable identification of mediators and co-factors in the pathway leading to renal injury. Integration of quantitative phenotype–genotype correlations and genome-wide gene expression profiles of IgA1-secreting cells may also be helpful in elucidating pathogenic pathways. The declining cost of high throughput genotyping, improved quality of gene expression microarrays and the existence of robust statistical methods for the analysis of genome-wide data will facilitate these types of studies.

In summary, serum level of Gd-IgA1 defines a new endophenotype that is highly inherited and closely linked to IgAN pathogenesis. Quantitative genetic studies of Gd-IgA1 are likely to be more powerful than linkage studies base on clinical phenotypes. In addition, profiling cases based on Gd-IgA1 level may help to identify case subgroups with distinct disease aetiologies, thus reducing heterogeneity in linkage and genetic association studies of IgAN. In the subset of IgAN cases with high levels of Gd-IgA1, the inciting genetic defect is likely to reside within the complex immunoregulatory pathways that control IgA1 synthesis and its post-translational modification. Finally, in the face of phenotypic complexity of IgAN, large cohorts and considerable international collaboration will be needed to perform adequately powered genome-wide studies of Gd-IgA1 and IgAN.

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