Genetic studies of IgA nephropathy: past, present, and future

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Abstract Immunoglobulin A nephropathy (IgAN) is the most common form of primary glomerulonephritis worldwide and an important cause of kidney disease in young adults. Highly variable clinical presentation and outcome of IgAN suggest that this diagnosis may encompass multiple subsets of disease that are not distinguishable by currently available clinical tools. Marked differences in disease prevalence between individuals of European, Asian, and African ancestry suggest the existence of susceptibility genes that are present at variable frequencies in these populations. Familial forms of IgAN have also been reported throughout the world but are probably underrecognized because associated urinary abnormalities are often intermittent in affected family members. Of the many pathogenic mechanisms reported, defects in IgA1 glycosylation that lead to formation of immune complexes have been consistently demonstrated. Recent data indicates that these IgA1 glycosylation defects are inherited and constitute a heritable risk factor for IgAN. Because of the complex genetic architecture of IgAN, the efforts to map disease susceptibility genes have been difficult, and no causative mutations have yet been identified. Linkage-based approaches have been hindered by disease heterogeneity and lack of a reliable noninvasive diagnostic test for screening family members at risk of IgAN. Many candidate-gene association studies have been published, but most suffer from small sample size and methodological problems, and none of the results have been convincingly validated. New genomic approaches, including genome-wide association studies currently under way, offer promising tools for elucidating the genetic basis of IgAN.

Keywords IgA nephropathy · Genetics · Hereditary disease · IgA1 glycosylation · Genome-wide association study

Introduction

Primary immunoglobulin A nephropathy (IgAN) is a complex trait [1] and a significant cause of renal insufficiency in young adults [2–5]. Complex diseases refer to disorders with a genetic basis that do not obey single-gene Mendelian inheritance patterns. They are typically determined by the
action of multiple genes and environmental factors acting independently or through more complex gene–gene and gene–environment interactions. The environmental risk factors for IgAN remain poorly defined. Observational studies associate male gender and mucosal infections with increased risk of primary IgAN, but the causal mechanisms underlying these observations are not clear. The arguments in support of genetic factors include profound differences in prevalence among different ethnicities, familial clustering of IgAN, and interindividual variation in disease course and prognosis. For instance, Asians (Chinese and Japanese) have a relatively high prevalence of IgAN compared with Caucasians, whereas the disease is infrequently diagnosed in individuals of African ancestry [6]. High frequency of IgAN has also been reported in biopsy series for Native Americans and Oceania [7–11]. Extended kindreds with familial IgAN have been reported throughout the world, including the USA [12], France [13], Canada [14], Italy [15], Australia [10], and Lebanon [16]. Familial disease accounts for 10–15% of all cases in regions such as northern Italy, France, or eastern Kentucky in the USA, where thorough surveys of relatives have been performed [13, 17–20].

Recognition of familial disease has many clinical implications, particularly for selecting donors for transplantation. In most reported families, segregation of IgAN is consistent with autosomal dominant transmission with incomplete penetrance (not all obligate carriers develop the disease), though more complex genetic models cannot be excluded. The incomplete penetrance is likely explained by the requirement of additional genetic or environmental factors for clinical manifestation of the disease and is also consistent with a complex disease model. Gene-mapping studies of traits with complex determination are difficult, and thus far, no single mutation has been conclusively demonstrated to cause IgAN. In this review, we concentrate on the approaches to genetic studies of IgAN; we summarize the studies of the last 10–15 years, review the most recent work, and attempt to project future directions in the field.

What do we know about genetics of IgAN?

Until now, two basic approaches have been used in genetic studies of IgAN: linkage studies and candidate-gene association studies. Linkage studies involve recruiting families with multiple affected individuals. In a typical whole-genome linkage scan, up to 400 microsatellites, or equivalently ~10,000 single nucleotide polymorphism (SNP) markers, equally spaced across the genome, are typed in families to interrogate marker cosegregation with a disease phenotype. The advantage of genome-wide linkage studies is that they do not require a priori assumptions about disease pathogenesis. Unfortunately, these studies are very sensitive to phenotype misspecification, and their power is limited to detecting rare genetic variants with a relatively large effect on the risk of disease. The LOD score (logarithm of the ratio of odds) is used to determine whether a given genomic locus is linked with a disease trait. An LOD score of 3 indicates 1,000:1 odds that linkage between the marker and the disease locus exists and is generally accepted as significant in genome-wide linkage studies.

Linkage studies of IgAN are faced with multiple challenges. Familial forms of IgAN are frequently under-recognized because the associated urinary abnormalities in affected family members are often mild or intermittent. Moreover, once familial disease is documented, systematic screening by renal biopsy cannot be justified among asymptomatic at-risk relatives, necessitating reliance on less accurate phenotypes, such as microscopic hematuria, to diagnose affection. Additionally, IgAN has been observed to co-occur in families with thin basement membrane disease (TBMD), an autosomal dominant disease caused by heterozygous mutations in the collagen type IV genes (COL4A3/COL4A4) [21]. Short of kidney biopsy or direct sequencing of the very large collagen genes, TBMD cannot be reliably excluded among relatives of IgAN patients. Finally, because urinary abnormalities may manifest intermittently, one also cannot unequivocally classify at-risk relatives as unaffected, necessitating affected-only linkage analysis. The inability to classify affected and unaffected individuals accurately is commonly encountered in linkage studies of complex traits, leading to decreased study power. Increasing sample size by including additional families is also not necessarily helpful in these situations because the diagnosis of IgAN likely encompasses several disease subsets, such that expansion to larger sample size can paradoxically reduce analytic power due to increased heterogeneity [22–25].

To date, three genome-wide linkage studies of familial IgAN have been reported [14, 26, 27]. Families in these studies have all been ascertained via at least two cases with biopsy-documented IgAN, with additional family members diagnosed as affected based on clinical evidence (renal failure or multiple documentation of hematuria/proteinuria). In the first study, 30 families with two or more affected members were examined [26]; multipoint linkage analysis under the assumption of genetic heterogeneity yielded a peak LOD score of 5.6 on chromosome 6q22–23 (locus named IGAN1), with 60% of families linked. The remainder of families linked to chromosome 3p24–23 with a suggestive LOD of 2.8. This study demonstrated IgAN is genetically heterogeneous but argued for the existence of a single locus with a major effect in some families. Another genome-wide linkage study involved 22 families that replicated linkage to chromosome 6q22–23 (nominal p = 0.01 at IGAN1 locus) but also detected two suggestive signals on chromosome 4q26–31 (LOD 1.8) and 17q12–22 (LOD 2.6) [27]. The most
recent linkage scan was based on a uniquely large pedigree with 14 affected relatives (two individuals with biopsy-defined diagnosis, and 12 with hematuria/proteinuria on urine dipstick) [14]. Linkage to chromosome 2q36 was detected with a maximal multipoint LOD of 3.47. Most linkage intervals reported did not contain obvious candidate genes, but the 2q36 locus encompasses the \( \text{COL4A3} \) and \( \text{COL4A4} \), which are mutated in TBMD. Together with the high penetrance of hematuria, this finding suggests that affected individuals in the 2q36-linked family may belong to an IgAN subtype that overlaps with TBMD. To date, none of the genes underlying these linkage loci has been identified. The underlying reasons are numerous, including the phenotyping difficulties discussed above; the presence of locus heterogeneity, which limits the ability to precisely map the disease interval and find additional linked families to refine loci; or contribution from noncoding susceptibility alleles (e.g. point mutations or structural genomic variants within intronic or promoter regions), which usually escape detection if mutational screening is confined to exonic regions. It is expected that the availability of inexpensive Next-Gen sequencing will enable comprehensive interrogation of linkage intervals, facilitating identification of disease-risk alleles.

Genetic association studies typically involve a collection of sporadic cases and a group of unrelated controls. Association studies are predicated on the premise that human populations share susceptibility alleles inherited from remote ancestors and that these alleles were not purified out because they individually confer a small excess risk of disease, resulting in a relatively high frequency in human populations (common variant/common disease hypothesis). Consistent with this starting premise, association studies are limited to detecting common disease-contributing genetic variants (i.e. population frequency typically >5%). The association approach can thus identify variants with moderate to relatively small effects and, compared with linkage scans, may be less sensitive to locus heterogeneity or phenotype misspecification. On the other hand, these studies are very sensitive to population stratification, i.e. undetected population mismatches between cases and controls that create spurious associations. As with linkage studies, association studies can now be carried out on a genome-wide scale [Genome-Wide Association Studies (GWAS)], providing an unbiased examination of the genome and the ability to detect and correct for population stratification. In addition, current standards necessitate replication of findings in independent cohorts to declare true associations.

In contrast to the GWAS approach, candidate-gene association studies examine polymorphisms in only specific genes that are selected based on a priori assumptions about their involvement in the disease pathogenesis, and they are highly sensitive to population stratification, multiple testing, and reporting bias. As a result, most candidate-gene association studies in the literature have not been replicated [28].

Not surprisingly, candidate-gene studies for IgAN have also been largely unrevealing. Many candidates have been proposed, but most were studied in the context of IgAN progression rather than causality. In addition, in part due to our lack of knowledge about disease pathogenesis, most candidates were predicated on sparse a priori evidence for involvement in IgAN. Over the last 15 years, there were 123 candidate-gene association studies for IgAN published in the English literature and indexed on PubMed (Fig. 1, listed in the Supplemental Table 1S). Of these, 39 (31%) studies examined genetic polymorphisms in association with susceptibility to IgAN, 40 (32%) examined an association with disease severity, progression, or complications, and 44 (35%) examined both susceptibility and risk of progression. Approximately one third of all studies involved polymorphisms in the renin–angiotensin system (RAS). The quality of most studies was astoundingly poor. In general, they were severely underpowered, thus negative findings were almost universally inconclusive. Overall, the average size of case–control cohorts per study was 182 cases (range 23–916) and 171 controls (range 21–816), although a recent trend for increasing size of IgAN cohorts is notable (Fig. 1c, d). The lower average number of controls is a reflection of poor emphasis on the proper assembly of control groups. Many studies used ad hoc controls derived from unscreened blood donors who were poorly matched to the cases in terms of ancestry and geography. The potential impact of confounding by population stratification was ignored by a majority of studies, including very recent ones, despite the fact that the tools for quantification of this problem have been developed. Additionally, many studies tested several hypotheses (either multiple polymorphisms, multiple phenotypes, or multiple genetic models) and did not adequately correct for multiple testing. A very few studies performed permutation testing to derive empiric \( p \) values in the face of multiple, nonindependent tests. Other major problems included inadequate or variable SNP coverage of candidate genomic areas, with several studies examining only a single polymorphism. Thus far, only one group attempted to survey the entire genome, albeit in a severely underpowered cohort and with inadequate coverage of \~80,000 SNPs [29, 30]. The results have not been replicated, and because these efforts do not pass current standards for genome-wide association studies, they remain inconclusive and difficult to interpret. Moreover, 77% of all published candidate-gene studies reported positive findings, an observation that is likely explained by a combination of high rate of false positives and a strong publication bias. Another silent problem in the literature relates to the fact that same patient cohorts are being tested for new polymorphisms without accounting for their use in prior publications. Most findings were not reproduced in other populations. None of the above problems is unique to the field of IgAN [31], and for these reasons, new general
guidelines aimed at improving the design and execution of genetic association studies have recently been formulated (please refer to the STROBE [32] and STREGA [33] statements for more detailed discussion of these issues).

New approaches and ongoing studies: genetics of IgA1 glycosylation abnormalities

The requirement for a kidney biopsy for diagnosing IgAN is a major obstacle for family studies and a limiting step in the assembly of large case groups for genetic association studies. Serum IgA levels, though elevated in a significant portion of IgAN patients, lack the sensitivity and specificity required for a clinically useful diagnostic test. Fortunately, recent studies of glycosylation abnormalities of IgA1 offer prospects for a more reliable diagnostic biomarker for IgAN. In humans, IgA1 represents one of the two structurally and functionally distinct subclasses of IgA. Unlike IgA2, IgM, and IgG, IgA1 has heavy chains that contain a unique hinge-region segment between the first and second constant-region domains, which is the site of attachment of three to five O-linked glycan chains. O-glycans on circulatory IgA1 consist of N-acetylgalactosamine (GalNAc) with a \(\beta1,3\)-linked galactose; both residues may be sialylated. Carbohydrate composition of O-linked glycans on normal serum IgA1 is variable. Prevailing forms include the galactose-GalNAc disaccharide and its mono- and disialylated forms. Galactose-deficient variants with terminal GalNAc or sialylated GalNAc are more common in IgAN patients. These aberrantly glycosylated galactose-deficient forms predominate in glomerular immunodeposits and circulating complexes in IgAN [34–36].

O-linked glycans on circulating IgA1 are synthesized in a step-wise manner, beginning with attachment of GalNAc to serine or threonine of the hinge region catalyzed by GalNAc transferases (Fig. 2). The O-glycan chain is then extended by attachment of galactose followed by addition of sialic acid residues to the GalNAc or galactose or both. Addition of galactose is catalyzed by Core-1-beta-1, 3-galactosyltransferase (C1GalT1), and its stability is mediated by a molecular chaperone, Cosmc. The glycan structure is completed by the alpha-2,6-sialyltransferase II (ST6GalNAcII) and alpha-2,3-sialyltransferases (ST3Gal) that attach sialic acid to the GalNAc and galactose residues, respectively. Alternatively, ST6GalNAcII can add sialic acid to terminal GalNAc, a step that blocks any subsequent modifications and thus is a terminal step of O-glycan synthesis [37, 38].
Delineation of the IgA1 glycosylation pathway provided new logical candidates for genetic association studies. Genetic variations in genes encoding C1GalT1 (C1GALT1, chr. 7p13-14), Cosmc (C1GALT1C1, chr. Xq24), and ST6GalNAcII (ST6GALNAC2, chr. 17q25.1) were recently examined in a large cohort of 670 Chinese IgAN cases and 494 controls [39, 40], as well as in a smaller Italian study [41]. These studies identified risk haplotypes in ST6GALNAC2 and C1GALT1 and suggest a genetic interaction between these haplotypes. Similar to all other candidate studies, these results are preliminary and require validation.

Critically, recent studies have shown that IgA1-producing cells are the source of elevated serum galactose-deficient IgA1 levels in patients with IgAN [42]. Aberrantly glycosylated IgA1 can be detected within these cells, and serum levels of galactose-deficient IgA1 correlated extremely well with levels of this immunoglobulin in the supernatant of cultured IgA1-producing cells isolated from peripheral blood of the same individual. These data indicate that aberrant glycosylation is not a secondary phenomenon attributable to modification of IgA1 during formation of immune complexes but, rather, reflect a specific defect originating in IgA1-producing cells.

Additional studies did not reveal any specific defects in individual IgA1 glycosylation enzymes but found that each enzymatic step in the glycosylation pathway is shifted toward increased production of galactose-deficient IgA1, with elevated content of sialic acid on GalNAc [42]. Moreover, expression and enzymatic activity of C1GalT1 appear to be dissociated from O-glycosylation in IgAN [43], and aberrant glycosylation affects solely IgA1 and not other glycoproteins with O-linked glycans, such as IgD [44]. Taken together, these observations isolate the IgA1 glycosylation defect to a specific cell type and indicate that the primary disturbance likely originates in an upstream regulatory pathway(s) of these cells rather than in the specific glycosylation enzymes.

Recently, a reliable lectin-based enzyme-linked immunosorbent assay (ELISA) for determining serum galactose-deficient IgA1 has been developed. It utilizes a naturally occurring HAA lectin isolated from the Helix aspersa snail to detect circulating galactose-deficient O-linked glycans. In a large cohort of Caucasians from the southeastern USA, this test had 90% specificity and 76% sensitivity for diagnosing sporadic IgAN [45]. Similarly, ELISA-determined serum galactose-deficient IgA1 was elevated in 77% of pediatric...
patients with IgAN [46]. This simple and inexpensive serum ELISA holds much promise for providing the first noninvasive screening test for IgAN.

Our group used this assay to investigate the inheritance of galactose-deficient IgA1 in familial and sporadic forms of IgAN [47]. A high serum galactose-deficient IgA1 level was present in the majority of index cases, as well as among their parents (39%), siblings (28%), and children (30%), providing further support for a major dominant effect. Levels in spouses were indistinguishable from controls, ruling out an environmental effect. Heritability of galactose-deficient IgA1 (the proportion of a trait’s variation explained by inherited factors) was therefore statistically significant and estimated at 54%. Segregation analysis of galactose-deficient IgA1 suggested inheritance of a major dominant gene with an additional polygenic component. We further examined galactose-deficient IgA1 levels in relatives after stratification for galactose-deficient IgA1 levels in the index case. Among relatives of IgAN patients with high galactose-deficient IgA1 values, 33% of individuals also had high values. In contrast, relatives of IgAN patients with normal galactose-deficient IgA1 levels had levels that were indistinguishable from controls. These data strongly argue that galactose-deficient IgA1 values can identify distinct subpopulations among IgAN patients, which may differ in the underlying disease pathogenesis. Inheritance of galactose-deficient IgA1 has been confirmed in Chinese patients with familial and sporadic adult IgAN [48, 49] and recently extended to pediatric IgAN and Henoch-Schönlein purpura with nephritis (HSPN) [50].

Thus, aberrant IgA1 glycosylation is a common inherited defect that provides a unifying link in the pathogenesis of HSPN, and familial and sporadic IgAN among many populations across the globe. Furthermore, these data demonstrate that an elevated serum galactose-deficient IgA1 level is antecedent to disease but, because most family members with elevated levels are asymptomatic, IgA1 glycosylation abnormalities are not sufficient to produce IgAN, and additional cofactors must trigger formation of immune complexes.

A recent study by Suzuki et al. offered some insights into the additional cofactors required to form immune complexes and their deposition in glomeruli [51]. Molecular characterization of IgG autoantibodies that recognize abnormally glycosylated IgA1 molecules (specifically, galactose-deficient GalNAc-containing epitopes in the hinge region of IgA1) revealed a specific amino-acid substitution in the variable region of the IgG1 heavy chain that is more frequent in IgAN cases compared with controls. This substitution greatly enhances IgG1 binding to the galactose-deficient IgA1 molecules. It is likely that this substitution arises as a somatic mutation in IgG1-producing cells, which is then positively selected in the course of immune response to yet unidentified antigens. The triggering antigens may include viral or bacterial pathogens (supported by frequent synpharyngitic disease exacerbation) or possibly by ingested food epitopes (supported by clinical overlap of IgAN with celiac disease). These important observations established antiglycan IgG1 antibodies as at least one additional risk factor, or a “second hit”, which predisposes to disease development.

These data enable formulation of a working hypothesis on the pathogenesis of IgAN (Fig. 3). Analogous to elevated serum cholesterol level, which in conjunction with additional risk factors (such as smoking or hypertension) leads to the development of coronary heart disease, elevated galactose-deficient IgA1 levels constitute a genetic risk factor that, in combination with a second independent hit (the production of antiglycan antibodies), leads to formation of circulating immune complexes that deposit in the glomerulus, producing inflammation and kidney damage. This simple model can potentially explain familial or sporadic occurrence of IgAN. Whereas in the majority of cases elevated galactose-deficient IgA1 appears to be an inherited risk factor [47], one can surmise that the propensity to produce antiglycan antibodies results from an independent, stochastic process such as a somatic mutation or an environmental insult (e.g. a viral infection). Thus, the occurrence of this second hit in an individual with genetically elevated galactose-deficient IgA1 levels would result in sporadic IgAN. One can also hypothesize that in some cases, the propensity to produce antiglycan antibodies is by itself genetically determined, such that cosegregation of risk alleles for galactose-deficient IgA1 and antiglycan antibody production in the same family would produce the pattern of familial IgAN. Because the two mutations would be independently inherited, only a small number of family members would carry both risk alleles, resulting in the pattern of variable penetrance and small number of affected individuals typical of familial IgAN. The relative prevalence of such risk alleles among different populations could also explain geographic variation in IgAN prevalence. Most importantly, the pathogenesis model depicted in Fig. 3 predicts that interventions that decrease levels of galactose-deficient IgA1 or anti-glycan antibodies would reduce formation of immune complexes and positively impact the course of IgAN. Thus, identification of genes and pathways that specifically affect each side of the equation would provide targets for therapeutic intervention in IgAN. Such interventions would be analogous to administration of cholesterol-lowering drugs to reduce the risk of coronary heart disease.

Future outlook: genomic approaches to IgA nephropathy

The availability of assays for circulating galactose-deficient IgA1 and antiglycan antibody production will likely
accelerate gene-mapping studies. For example, profiling serum galactose-deficient IgA1 levels can now be used to reduce heterogeneity in linkage scans for IgAN. Additionally, galactose-deficient IgA1 can be used more directly, as an endophenotype (i.e., a measurable intermediate component of the causal pathway between genotype and disease) in genetic linkage studies of IgAN. Quantitative endophenotypes are frequently preferred in genetic studies of a complex disease, because they may more closely reflect specific pathogenic processes and provide more statistical power to detect genotypic correlations. Moreover, statistical methods used in quantitative linkage analysis are less sensitive to a trait’s heterogeneity. In parallel to linkage approaches, quantitative endophenotypes can also be used to enhance genetic association studies. This is best exemplified by quantitative studies of serum IgE levels that identified novel susceptibility loci for asthma using both genome-wide linkage and association approaches [52, 53]. Quantitative gene-mapping studies of serum galactose-deficient IgA1 levels in IgAN are underway.

The field of complex disease genetics is also evolving rapidly, and a new generation of genetic studies is soon to emerge for IgAN. First, rapid and cost-effective screening of the human genome with >1 million polymorphisms is now possible, enabling efficient execution of high-resolution genome-wide genetic association studies. In a GWAS design, a dense map of SNPs is surveyed for association with a trait of interest. Typically, large and well-phenotyped case–control groups (>1,000 individuals per group) are required to discover genetic variants, and independent cohorts are needed to replicate findings [54]. The main issues facing GWAS approaches include the analytical challenge of multiple hypotheses testing and accounting for population stratification. Both of these issues have been addressed in the genetics community, with rigorous standards now widely accepted [55, 56]. GWAS is also inherently limited to detecting susceptibility alleles that are relatively common. To date, most GWAS-discovered variants reside in noncoding segments of the genome and impart a small effect on disease, presumably via a regulatory role on expression of neighboring genes (odds ratios 1.15–1.35). Variants with even smaller effects can be detected with larger sample sizes [57]. Small effects of common susceptibility variants are hypothesized to be the consequence of long-standing purifying selection against alleles that produce major alterations in the encoded protein and may impair reproductive fitness. There are, however,
exceptions to this rule in which common alleles impart relatively large effects on disease risk. Two examples are diseases of very late onset in which reproductive fitness is not impaired (e.g. common allele ε4 at APOE locus with large effect on the risk of Alzheimer’s disease [58]), and diseases in which recent changes in the environment may have resulted in alleles that were once neutral or favored now becoming disease contributing. Many diseases of the immune system may fit into the latter category, including type 1 diabetes mellitus, macular degeneration, systemic lupus erythematosus (SLE), inflammatory bowel disease, and celiac disease. In each case, common variants had moderate to large effect sizes, facilitating their detection by GWAS [59–64]. IgAN is also likely to fall into this category; thus, the GWAS design for IgAN may represent a powerful approach.

In addition to GWAS, several other genomic approaches are likely to be integrated for the purpose of gene identification (Table 1). These include analyses of structural rearrangements, genome-wide expression, and deep-sequencing data. Copy-number variants (CNVs) have been recognized as an important source of genetic variation in humans [65]. Comprehensive surveys of small insertions, deletions, and segmental duplications across the genome can be efficiently accomplished with modern SNP genotyping platforms [66]. This is relevant to IgAN because structural variants have been implicated in several immune-mediated traits, such as psoriasis or SLE [67–69]. We anticipate that genome-wide CNV analyses are likely to appear alongside the first GWAS for IgAN. Genome-wide expression profiling is another approach that can be helpful in dissecting the genetic basis of IgAN. Recent data suggest that tissue-specific patterns of gene expression are highly inherited, and expression quantitative trait loci (eQTL) mapping has been used to define loci that control transcription efficiency [70]. The combination of gene mapping and gene-expression profiling provides a powerful tool for understanding complex traits, as these techniques generate independent information that allows reciprocal prioritization of candidate genes. Such information can be integrated with the GWAS approach to better define functional defects responsible for disease susceptibility [71]. Moreover, novel integrative genomic approaches enable joint analysis of genetic, gene-expression, and biochemical phenotypes to derive pathways and molecular networks driving disease pathogenesis [72]. Application of these methods in humans is challenging because most tissues of interest cannot be readily accessed, and sampled specimens (such as kidney) are composed of heterogeneous cell types with distinct gene-expression profiles. However, this approach would be ideally suited for genetic studies of IgAN because expression studies can be performed in IgA1-secreting cells, enabling interrogation a single cell type and the specific biochemical phenotype of defective IgA1 glycosylation [42]. Lastly, high-throughput sequencing technology is evolving rapidly, and cost-effective whole-genome deep sequencing is now becoming feasible. Direct sequence analysis offers hope of detecting important rare genetic variants that contribute to the pathogenesis of common complex diseases [73–76]. The recent feasibility of exome sequencing (sequencing of all the exons of the genome in one shot) now also enables detection of mutations underlying oligogenic traits [77, 78]. This approach is quite promising, as variants identified via GWAS collectively account for a small fraction of the heritability of phenotypes studied, suggesting major contributions from rare sequence variants to disease pathogenesis. General approach and statistical tools for this approach are still under development, but this type of study is expected to provide a new wave of findings in the genetic determination of complex traits.

**Summary and clinical implications**

Similar to other immune-mediated disorders, IgAN is a genetically complex trait. Although there is a clear contribution of genetic factors to IgAN susceptibility, specific genes have not yet been identified. Gene-mapping

| Table 1 Genetic approaches to studies of immunoglobulin A nephropathy (IgAN) in humans |
| --- |
| **Family-based approaches:** |
| 1. Family aggregation studies (numerous reports) |
| 2. Traditional whole-genome linkage scans (3 reported) |
| 3. Quantitative whole-genome linkage scans for Gd-IgA1 and related phenotypes (in progress) |
| **Population-based approaches:** |
| 1. Candidate gene associations for susceptibility and progression of IgAN (over 120 reported) |
| 2. Genome-wide case–control association studies of IgAN (in progress) |
| 3. Genome-wide quantitative association studies of Gd-IgA1 and related phenotypes (in progress) |
| **Anticipated future approaches:** |
| 1. Studies of copy number variants (CNVs) in IgAN |
| 2. Population-based and family-based studies of genome-wide gene expression in IgAN |
| 3. Integrative genomics approaches to derive disturbed molecular networks in IgAN |
| 4. Whole-genome sequencing to discover rare variants in IgAN |

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studies for complex traits are challenging, but successful discovery of IgAN susceptibility genes will have major impact and far-reaching implications worldwide. Such findings may open doors to novel targeted therapeutic approaches. Clinical applications may involve genetic screening and diagnosis, improved risk stratification, or selection of suitable kidney transplant donors among related individuals based on genetic testing. Discovery of IgAN biomarkers combined with the availability of novel genomic technology are likely to transform the approach of genetic studies of IgAN. However, it is now clear that much larger cohorts of patients will be required than have been previously studied. Referral of patients for genetic research studies and systematic biobanking of blood, serum, and kidney tissue will be critical for execution of such studies. International and interdisciplinary collaborations will likely be required for successful identification of specific genetic causes of IgAN.

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Conflicts of Interest  None

Questions

(Answers appear following the reference list)

1. Which is true about familial forms of IgAN?
   a. Familial forms of IgAN have only been observed in genetically isolated populations
   b. Patients with familial IgAN should never be transplanted because of high risk of recurrence
   c. Patients with familial IgAN can be identified based on their characteristic presenting symptoms
   d. Most familial IgAN displays autosomal dominant inheritance
   e. b and d

2. Which is true about glycosylation defects of IgA1 in IgAN?
   a. It is associated with a generalized defect in glycosylation of most circulating immunoglobulins
   b. The abnormally glycosylated IgA1 has a higher propensity for immune complex formation and deposition in mesangium
   c. Elevated levels of galactose-deficient IgA1 correlate with symptoms and prognosis in IgAN
   d. Elevated levels of galactose-deficient IgA1 are observed in large proportion of family members of IgAN patients
   e. b and d

3. Which is true about genetic studies of IgAN?
   a. Consistent association of IgAN with cytokine haplotypes have been identified
   b. Multiple different genes can cause familial IgAN because different families demonstrate linkage to different segments of the genome
   c. Glycosylation defects in IgAN are caused by mutations in the C1GALT1 (beta-1,3 galatosyltransferase gene)
   d. Genome-wide association studies will be able to detect rare genes with small effect that contribute to IgAN
   e. b and d

4. Which is true about serum levels of galactose-deficient IgA1:
   a. Elevated serum level of galactose-deficient IgA1 is sufficient to make the diagnosis of IgAN
   b. Normal serum level of galactose-deficient IgA1 is sufficient to exclude the diagnosis of IgAN
   c. Elevated serum level of galactose-deficient IgA1 is required for the development and progression of IgAN
   d. In the populations studied to date, inherited factors are estimated to account for approximately 50% of the total variation in galactose-deficient IgA1 levels
   e. None of the above

5. Which is NOT true about genetic studies of IgAN:
   a. Genetic heterogeneity decreases power of linkage studies of familial IgAN
   b. The results of genetic association studies may be biased if cases and controls are derived from heterogenous populations
   c. Most candidate gene associations in sporadic IgAN have not been replicated
   d. Galactose-deficient IgA1 level represents a promising endophenotype for genetic linkage and association studies
   e. Several common copy-number polymorphisms have been consistently associated with IgAN

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Answers:
1. d
2. c
3. b
4. d
5. e