



# What Genetics Tells Us About the Pathogenesis of IgA Nephropathy: The Role of Immune Factors and Infection

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Immunoglobulin A nephropathy (IgAN) is the most common type of primary glomerulonephritis, which is characterized by IgA1-containing immune-deposits in the glomerular mesangium. The epidemiologic observations of familial clustering as well as ethnic and regional discrepancies indicate a genetic component to IgAN. Large, international, genome-wide association studies have identified several susceptibility genes and loci for IgAN, many of which have been implicated in immune regulation and are shared with other autoimmune diseases. Notably, increasing numbers of genes involved in mucosal immunity have been detected; such genes may impact the susceptibility and progression of IgAN through interaction with environmental stimuli (especially infection). Here, we discuss the innate and adaptive immune mechanisms that drive protective immunity against pathogens. Our goal is to provide a representative overview of the synergistic roles between genetic predisposition and infection in IgAN pathogenesis. We anticipate that these results will provide potential therapeutic agents and advances in precision medicine.

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KEYWORDS: genetic predisposition; genome-wide association study; IgA nephropathy; mucosal infection

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Immunoglobulin A nephropathy (IgAN) is the most common type of primary glomerulonephritis. IgAN is characterized by the deposition of IgA, in particular galactose-deficient IgA1 (Gd-IgA1), in the mesangial area of glomeruli.<sup>1</sup> A wide spectrum of clinical and pathologic features has been observed, and ≈20% to 40% of IgAN patients progress to end-stage renal disease in ≈20 years.<sup>2</sup> The exact pathogenesis of IgAN remains unclear; that is, specific recommended approaches to the diagnosis and therapy of IgAN are lacking.

Familial aggregation of IgAN<sup>3,4</sup> suggests a genetic component to its pathogenesis. Furthermore, regional registries of kidney biopsy procedures and findings show a marked geographic variation in the prevalence of IgAN in patients; prevalence is modest in the United States (10%–20% of primary glomerulonephritis), higher in some European countries (20%–30%) and

highest in developed countries in Asia (40%–50%).<sup>5</sup> With technological advances in genotyping, genetic studies, in particular hypothesis-free genome-wide association studies (GWASs),<sup>6–10</sup> have documented significant associations of IgAN with several single-nucleotide polymorphisms within or near immune-related genes such as major histocompatibility (*MHC*) loci, thereby highlighting the immune component of this disease. Interestingly, the enhanced IgA response conferred by GWAS-confirmed risk alleles such as *ITGAM-ITGAX* is probably protective against helminth infection. This finding suggests that an increased incidence of IgAN in some geographic areas may represent an untoward consequence of protective adaptation to mucosal invasion by local pathogens. In addition, the notion of shared genetic components in immune-related diseases, such as inflammatory bowel disease (IBD) and IgAN, has been studied widely.<sup>11–17</sup> Using increasing amounts of data on the role of infection in autoimmune diseases,<sup>18,19</sup> Couser and Johnson<sup>20</sup> summarized the triggering role of infection in the pathogenesis of various glomerulonephritic conditions, including IgAN. Further research into the role of immune factors and infection in IgAN will be useful for monitoring disease development and providing insight into personalized treatment options.

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In this review, we summarize the genetic discoveries in IgAN, analyze the role of infection in its pathogenesis, discuss pleiotropic effects of susceptibility variants, modify the model of IgAN pathogenesis, and outline the clinical implications of, and future directions for, genetic studies.

## Advanced Genetic Discoveries

### Infection Pressures

Mainly, 2 genetic approaches, including pedigree-based linkage study and sporadic patients-based association study, have been applied in IgAN. Up to now, 4 kindreds have been reported for IgAN.<sup>21–24</sup> Although the large linkage studies have yielded genetic signals at 6q22–23 (locus named IgAN1),<sup>21</sup> 4q26–31 (locus named IgAN2),<sup>22</sup> 17q12–22 (locus named IgAN3),<sup>22</sup> and 2q36<sup>23</sup> for familial IgAN. Using a large Lebanese-Druze kindred (5 generations and 16 affected individuals), Karnib *et al.*<sup>24</sup> found no evidence of linkage to the known IgAN loci on chromosomes 6q22–23, 2q36, and 4q22–31. Furthermore, no likely candidate genes in these loci have yet to be identified (Supplementary Table S1). The elusive results of linkage studies indicate the complex genetic background of IgAN, and for complex trait conditions, such as IgAN, accuracy in classifying affected and unaffected individuals is crucial. GWAS ushered in an era of rapid advancement in the establishment of genetic-susceptibility determinants for complex traits. To date, 5 GWASs have been applied to the IgAN phenotype.<sup>6–10</sup> More importantly, a recent GWAS with IgAN used increasingly larger sample sizes and, consequently, more susceptibility loci were found. If this trend continues, we can expect yet more loci to be discovered. In fact, IgAN GWAS have already identified several novel pathways of pathogenic disease. The loci that have shown significant association across multiple GWASs are involved mainly in the following pathways: adaptive immunity (*MHC*), complement system (*CFH*, *CFHR3-1*, and *ITGAM-ITGAX*), mucosal innate immunity (*DEFA*, *CARD9*, *VAV3*, *ODF1-KLF10*, and *UBR5*), and regulation of mucosal IgA production (*TNFSF13*, *HORMAD2*, and *ST6GALI*) (Table 1). Notably, the susceptible loci involved in adaptive immunity and the complement system have been replicated and confirmed in functional studies, thereby validating the role of immunity and complement activation in IgAN pathogenesis. Further support is provided by the fact that several of the loci detected are shared with other autoimmune diseases, such as systemic lupus erythematosus, rheumatoid arthritis, and multiple sclerosis (discussed in detail in the Pleiotropic Effects of Susceptibility Variants section). Some of the loci identified recently (e.g., *ODF1-KLF10*)

have not yet been replicated in other populations, but they have been suggested to have novel pathways in IgAN. Interestingly, increasing numbers of loci associated with mucosal innate immune responses and regulation of IgA production have been detected. Combined with increasing data on the potential role of infection in IgAN pathogenesis,<sup>20</sup> searching for the infectious agents (e.g., using microbiome studies) to explain IgAN pathogenesis would be beneficial.

Mucosal tissue (especially mucosal tissue in the gastrointestinal tract) constitutes a physical barrier against the “outside” and is, therefore, regarded as a large “immune organ.” The “Bermuda triangle” of the host’s immune system, commensal microorganisms (also known as microbiota) and pathogens, is involved in the dynamic environment of the intestinal mucosa. By interaction with trillions of microbiota, the host immune system provides a barrier to inhibit the growth and dissemination of pathogens.<sup>25</sup> Large international GWASs have identified several susceptibility loci involved in this process and associated with susceptibility to IgAN. We discuss in detail, from a genetic viewpoint, the innate and adaptive immune mechanisms that drive protective mucosal immunity against pathogens (Figure 1).

### Genes Associated With Innate Immune Responses

During infection, the host detects pathogens through binding of pathogen-associated molecular patterns by pattern-recognition receptors such as toll-like receptors and cytosolic nucleotide-binding oligomerization domain-like receptors. Then, the host initiates the first line of defense, innate immunity, which includes induction of cytokine expression, secretion of antimicrobial proteins, and recruitment or activation of cellular mediators (i.e., neutrophils), to defend against infection.

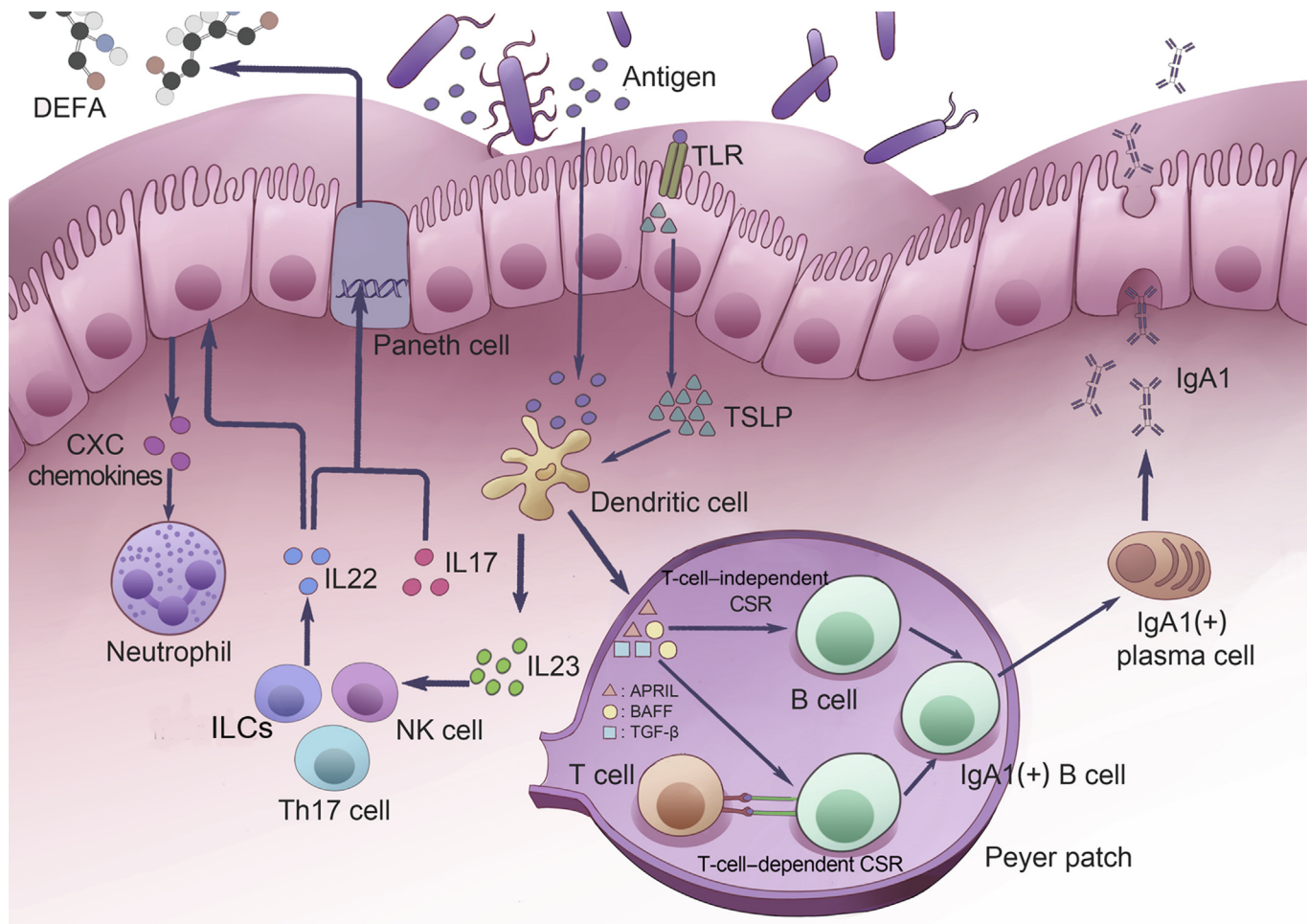
The *DEFA* gene cluster encodes proteins exhibiting antimicrobial activity, which are important effector molecules in innate and adaptive immunity. In humans, there are 2 families of defensins:  $\alpha$  and  $\beta$ .  $\alpha$ -Defensins are expressed mainly in neutrophils and the paneth cells of the intestine, which are involved in maintenance of the intestinal mucosal barrier or regulation of the mucosal immune response.<sup>26</sup> Expression of human  $\alpha$ -defensin 5 and human  $\alpha$ -defensin 6 in mice confers resistance to oral infection with *Salmonella* species.<sup>27,28</sup> In accordance with these findings, low total copy number variations of the *DEFA* locus, including *DEFA1A3*, *DEFA3*, and a noncoding deletion variant, are associated strongly with susceptibility to and progression of IgAN.<sup>29</sup> *DEFA1A3* copy number variations could explain the associative effect of the reported single-nucleotide

**Table 1.** The IgA nephropathy susceptibility loci detected by genome-wide association studies

Chr	SNPs independently associated with IgAN	Risk allele	OR	P value	RAFs <sup>a</sup> (Americans–Europeans–Asians)	Genes in the region	Gene functions
1q32	rs6677604	G	1.47	$2.96 \times 10^{-10}$	0.65–0.82–0.94	<i>CFH, CFHR3, CFHR1, CFHR4, CFHR2, CFHR5</i>	<i>CFH</i> cluster genes encode factor H-related peptides involved in the activity of the alternative complement pathway.
1p13	rs17019602	G	1.17	$6.80 \times 10^{-9}$	0.21–0.22–0.19	<i>VAV3</i>	<i>VAV3</i> encodes guanine nucleotide exchange factors essential for regulation of mucosal immunity and IgA production.
3q27	rs7634389	C	1.13	$7.27 \times 10^{-10}$	0.22–0.39–0.44	<i>ST6GAL1</i>	<i>ST6GAL1</i> encodes ST6 betagalactosamide alpha-2,6-sialyltransferase, a member of glycosyltransferase family involved in the generation of the cell-surface carbohydrate determinants and differentiation antigens.
6p21	rs2523946	C	1.21	$1.74 \times 10^{-11}$	0.42–0.53–0.56	<i>HLA-A</i>	MHC class II molecules are critical for antigen presentation and adaptive immunity.
6p21	rs9275596	T	1.59	$1.59 \times 10^{-26}$	0.65–0.69–0.83	<i>HLA-DQA1, HLA-DQB1, HLA-DRB1</i>	
6p21	rs660895	G	1.34	$4.13 \times 10^{-20}$	0.11–0.23–0.21		
6p21	rs1794275	A	1.30	$3.43 \times 10^{-13}$	0.27–0.23–0.17		
6p21	rs7763262	C	1.41	$1.80 \times 10^{-38}$	0.66–0.72–0.68		
6p21	rs1883414	C	1.30	$4.84 \times 10^{-9}$	0.83–0.68–0.79	<i>HLA-DPA1, HLA-DPB1, HLA-DPB2</i>	They belong to MHC class II molecules with limited related studies yet.
6p21	rs9357155	G	1.53	$2.11 \times 10^{-12}$	0.95–0.85–0.85	<i>TAP1, TAP2, PSMB8, PSMB9</i>	<i>PAMB8</i> and <i>PSMB9</i> are interferon-induced genes that mediate intestinal NF-κB activation in IBD.
8p23	rs2738048	G	1.26	$3.18 \times 10^{-14}$	0.20–0.34–0.35	<i>DEFA1, DEFA3, DEFA4, DEFA5, DEFA6</i>	<i>DEFA</i> cluster genes encode α-defensins that protect against microbial pathogens in innate immunity
8p23	rs10086568	A	1.16	$1.00 \times 10^{-9}$	0.42–0.33–0.24		
8p23	rs12716641	T	1.15	$9.53 \times 10^{-9}$	0.73–0.54–0.78		
8p23	rs9314614	C	1.13	$4.25 \times 10^{-9}$	0.20–0.48–0.40		
8q22	rs2033562	C	1.13	$1.41 \times 10^{-9}$	0.38–0.63–0.44	<i>ODF1, KLF10, UBR5</i>	<i>KLF10</i> encodes a transcriptional repressor involved in the transforming growth factor-β signaling pathway. <i>UBR5</i> encodes an E3 ubiquitin ligase that has a role in IL-17 production in T cells and inflammatory response in the small intestine by interacting with the deubiquitinase DUBA.
9q34	rs4077515	T	1.16	$1.20 \times 10^{-9}$	0.29–0.41–0.31	<i>CARD9</i>	<i>CARD9</i> is involved in activation of the NF-κB pathway, mediates intestinal repair, T helper 17 responses, and controls bacterial infection.
11p11	rs2074038	T	1.14	$3.93 \times 10^{-9}$	0.02–0.11–0.28	<i>ACCS, PHACS, EXT2</i>	<i>ACCS</i> encodes α1-aminocyclopropane-1-carboxylate synthase homolog, which interacts with the protein encoded by <i>FBF1</i> (Fas [TNFRSF6] binding factor 1) involved in polarization of epithelial cells, assembly of the apical junction complex, and ciliogenesis.
16p11	rs11574637	T	1.32	$8.10 \times 10^{-13}$	0.71–0.80–1.00	<i>ITGAM, ITGAX</i>	<i>ITGAM</i> and <i>ITGAX</i> encode integrins αM and αX, which have roles in formation of leukocyte-specific complement receptor 3 and 4 by combining with the integrin β2 chain.
16p11	rs7190997	C	1.22	$2.26 \times 10^{-19}$	0.50–0.54–0.74		
17p13	rs3803800	A	1.21	$9.40 \times 10^{-11}$	0.29–0.78–0.67	<i>TNFSF13, MPDU1</i>	<i>TNFSF13</i> encodes APRIL induced by intestinal bacteria, which is involved in CD40-independent IgA class switching.
22q12	rs2412971	G	1.25	$1.86 \times 10^{-9}$	0.29–0.55–0.69	<i>HORMAD2, MTMR3, LIF, OSM</i>	LIF and OSM are IL-6-related cytokines implicated in mucosal immunity.
22q12	rs12537	T	1.28	$1.17 \times 10^{-11}$	0.40–0.34–0.20		

APRIL, a proliferation-inducing ligand; Chr, chromosome; DUBA, deubiquitinating enzyme; IBD, inflammatory bowel disease; IgAN, IgA nephropathy; IL, interleukin; NF-κB, nuclear factor κB; OR, odds ratio; RAFs, risk allele frequencies; SNP, single nucleotide polymorphism.

<sup>a</sup>Risk allelic frequencies expressed based on 1000 G data. Notably, all risk alleles with larger effect sizes (ORs: 1.3–1.5) are most frequent in Asians and least frequent in Africans.



**Figure 1.** The innate and adaptive immune mechanisms that drive protective mucosal immunity against pathogens. During infection, the host initiates innate and adaptive immune responses to defend against pathogens. In 1 context, on pathogen recognition through toll-like receptors (TLRs), dendritic cells in the mucosa produce proinflammatory cytokines such as interleukin (IL)-23. T helper 17 (Th17) cells, nature killer (NK) cells, and innate lymphoid cells (ILCs) express the IL-23 receptor. The combination of IL-23 and its receptor triggers production of the downstream cytokines IL-17 and IL-22, which then induce production of antimicrobial peptides such as defensins. In addition, IL-22 induces epithelial-cell production of CXC chemokines, which recruit neutrophils to the infection site. In another context, immune component-primed B cells migrate to the mucosal lamina propria, where they mature into IgA-secreting plasma cells. The release of thymic stromal lymphopoietin (TSLP) after “sensing” bacteria through TLRs could increase the production of the tumor necrosis factor ligand superfamily member 13 (APRIL), the tumor necrosis factor ligand superfamily member 13B (BAFF), and the transforming growth factor  $\beta$  (TGF- $\beta$ ) by dendritic cells, which result in increasing IgA1 production through IgA class switching recombination (CSR).

polymorphism rs2738048 by GWAS of IgAN. Expression of the protein products of *DEFA1A3*, human neutrophil peptides 1 to 3 (HNP1–3), is increased in the sera and urine samples of IgAN patients; however, neutrophils from healthy individuals produce more HNP1–3 than do those from IgAN patients if stimulated by lipopolysaccharide. Those results suggest expressional or functional defects of HNP1–3 in IgAN patients. In this way, the copy number variations of the *DEFA* locus could be associated with the susceptibility and progression of IgAN by influencing the expression and activities of HNP1–3 proteins. However, the correlations of HNP1–3 expression and protein levels with the copy numbers of *DEFA1A3*<sup>30–32</sup> still need validation in the future.

Another gene, *UBR5* at the *ODF1-KLF10* locus, encodes an E3 ubiquitin ligase that has a role in interleukin (IL)-17 production in T cells and the inflammatory response in the small intestine by interacting with deubiquitinating enzyme A.<sup>33</sup> In addition, several loci involved in nuclear factor KB activation that may create a proinflammatory state through up-regulation of intestinal inflammation have been identified. For example, *PSMB8/PSMB9* and *CARD9* show *cis*-expression quantitative trait locus effects and have been reported to be involved in the inflammatory response in IBD.<sup>12,34,35</sup> *PSMB8* and *PSMB9* are interferon-induced subunits of the immune-proteasome that mediate intestinal nuclear factor KB activation. *PSMB8* is up-regulated in active IBD lesions,<sup>36</sup> whereas

treatment with a PSMB8 inhibitor (bortezomib) attenuates experimental colitis.<sup>37</sup> The loci mentioned have not been validated in populations other than Chinese, but those results suggest the role of the T helper (Th17) pathway in IgAN pathogenesis.

Similarly, CARD9 is a proinflammatory molecule that activates nuclear factor KB<sup>38</sup> and mediates intestinal repair and the Th17 response, which in turn controls bacterial infection after intestinal epithelial injury in mice.<sup>39</sup> The IL-23/Th17 cell axis has been studied extensively with respect to the intestinal cytokine response to pathogenic bacteria.<sup>25</sup> On pathogen recognition through toll-like receptors and nucleotide-binding oligomerization domain-like receptors, dendritic cells and other mononuclear immune cells in the mucosa produce proinflammatory cytokines such as IL-23, which are important regulators of the mucosal host response.<sup>40,41</sup> Various cell types, including Th17 cells, natural killer T cells,  $\gamma\delta$  T cells, and innate lymphoid cells, express the IL-23 receptor.<sup>42</sup> The combination of IL-23 and its receptor triggers production of the downstream cytokines IL-17 and IL-22, which are highly induced in mucosal infection. Then, IL-22 induces epithelial-cell production of CXC chemokines, which recruit neutrophils to the infection site. In addition to chemokines, IL-17 and IL-22 induce the production of antimicrobial peptides such as defensins, which in turn modulate the microbial composition of the intestinal lumen.

Taken together, these GWAS loci are prominent examples of innate immune responses that keep the gut-mucosa environment stable and free of pathogens. Next, we describe the regulation of IgA production by which the host can recognize specific harmful bacteria.

### Genes Associated With Regulation of IgA Production

The interaction of mucosal pathogens and IgA immunity has a central role in IgAN. Several GWAS loci detected to date represent an inherited propensity for abnormal mucosal production of IgA against microbial antigens. All 5 GWAS<sup>6–10</sup> concerning IgAN have identified loci of genome-wide significance within the *MHC* region, which is critical for antigen presentation and adaptive immunity. Also, *TNFSF13* has been identified; this gene encodes for a proliferation-inducing ligand (APRIL)—a tumor necrosis factor-ligand associated with the response to mucosal infection and with IgA production in gut-associated mucosal lymphoid tissue.<sup>43</sup> The risk variant in this locus is associated with an increased level of IgA in serum,<sup>44,45</sup> whereas inactivation of *TNFSF13* in mice produces a significant decrease in serum levels of IgA and a reduced serum IgA antibody response to mucosal immunization.<sup>46</sup> The mechanism by which APRIL may

affect Gd-IgA1 production is not well understood, but there is evidence for a role of enhanced activation of the Janus kinase/signal transducer and activator of transcription pathway.<sup>45,47</sup> Mice transgenic for B cell-activating factor (BAFF), a homologous protein of APRIL, develop commensal flora-dependent IgAN through breakdown in the normal barrier between mucosal and peripheral compartments.<sup>48</sup>

In addition to elucidation of the roles of *MHC* and *TNFSF13*, another signal in the GWAS of IgAN resides in rs2412971 in *HORMAD2* on Chr 22q12.2. This association extends across a large linkage disequilibrium segment that encompasses genes including *LIF* and *OSM*. These 2 genes encode cytokines expressed in mucosal tissues, where they exert immune-regulatory effects,<sup>49</sup> and are associated with increased serum levels of IgA.<sup>7</sup> *LIF* and *OSM* are IL-6-related cytokines, and increased levels of IL-6 have been detected in some IgAN patients.<sup>50</sup> *In vitro* studies in IgA1-secreting cells taken from IgAN patients have shown that IL-6-family cytokines could enhance Gd-IgA1 production.<sup>45</sup>

Taken together, these lines of genetic evidence for genetic effects suggest a role for interactions between the host and intestinal pathogens in causing IgAN. Furthermore, most of the loci involved have shown relatively high risk allele frequencies in Asians compared with Africans (Table 1) that parallel the observed patterns of disease incidence. These genetic findings also suggest a potential role for local selective pressures, which may have increased the risk allele frequency in Asians systematically through multilocus adaptation.

### Triggering Role of Infection

Consistent with the genetic findings suggesting a role for infection pressures in IgAN, several studies have focused on the role of infection in IgAN pathogenesis.<sup>20,51</sup> We did not find a specific antigen that might be associated with IgAN frequently or characteristically. However, current evidence suggests a common triggering role for particular pathogens, chronic exposure to mucosal infections, or microbiotic dysbiosis.<sup>52</sup>

### Role of Mucosal Infection

The role of infection in IgAN has been accepted but not understood fully; the initiating factors remain elusive. Various infection sites have been observed to be associated with IgAN: respiratory tract, gastrointestinal tract, urinary tract, and periodontal tissue. Though without convincing uniformity, various agents derived from hemolytic *streptococcus*, *Helicobacter pylori*, human hepatitis B virus, cytomegalovirus, Epstein-Barr virus, and adenoviruses have been detected in the mesangial cells of IgAN patients.<sup>53–55</sup> Therefore, it has been postulated that infection has a triggering role through a common (rather than an organism-specific) pathway.

Using a high-throughput multiplexed sequencing approach, *Prevotella*, *Fusobacterium*, *Sphingomonas*, and *Treponema* have been detected as the dominant flora in the tonsils of IgAN patients. However, no bacterial genus has been seen significantly more frequently in patients with IgAN versus those with recurrent tonsillitis. Nevertheless, a different pattern of bacteria occurs in the tonsillar crypts of these 2 groups of patients compared with healthy control subjects with tonsillar hyperplasia.<sup>56</sup> The above-mentioned “common-trigger” hypothesis is supported by the fact that food-derived antigens (e.g., soybean proteins, casein, and milk-whey proteins) are also found occasionally as mesangial deposits in some IgAN patients.<sup>57</sup> The aberrant host response to these enteric pathogens has a role in IgAN development.

The manner in which pathogens trigger an abnormal mucosal immune response is attributed mainly to 2 mechanisms. First, pathogens amplify inflammation responses such as the expression of chemokines that promote recruitment of inflammatory cells to induce kidney damage. By undergoing whole-genome screening of kidney tissue from IgAN patients with gross hematuria, the potential role of the C-X3-C motif receptor 1/fractalkine axis in the exacerbation of gross hematuria has been validated.<sup>58</sup> Second, the poorly glycosylated structures of Gd-IgA1 and the bacterial Tn antigen indicate an autoimmune response in IgAN patients through molecular mimicry, activation of preexisting autoreactive T and B cells, epitope conformational changes, epitope spreading, or antigen-antibody complementarity.<sup>20</sup>

### Role of Chronic Exposure to Pathogens and Dysbiosis

The recurrence and subsequent disappearance of IgA deposition in a subclinical IgAN donor kidney after renal transplantation<sup>43</sup> suggests sustained stimulation of IgA1 regulation. Chronic exposure to mucosal infection or intestinal dysbiosis has been reported to have an important role in IgAN by producing a continuous challenge to the mucosal immune system, leading to IgA hyperproduction. Periodontitis, chronic tonsillitis, and *H. pylori* infection have been reported to be associated with IgAN.<sup>55,59</sup> Therefore, tonsillectomy or eradication of *H. pylori* should reduce mucosal infections and, consequently, reduce the stimulus for the release of abnormal IgA. However, results have been inconclusive, possibly because the tonsils represent only a small part of lymphoid tissue compared with gut-associated lymphoid tissue.

An increasing number of studies suggest a key role of intestinal dysbiosis in triggering IgAN.<sup>51</sup> Similar to the above-mentioned bacterial pattern in the tonsils of

IgAN patients, the salivary and intestinal microbiomes of IgAN patients also differ significantly from those of healthy control subjects; this phenomenon may have an important role in IgAN pathogenesis.<sup>60,61</sup> Sustained stimulation by mucosal pathogens may cause a breakdown in the normal mucosal barrier and thereby increase translocation, absorption, and circulation of living bacteria and bacterial components such as bacterial lipopolysaccharide (a phospholipid that constitutes the outer membrane of most Gram-negative bacteria<sup>62</sup>). However, due to small sample sizes and multiple issues with testing, none of the existing IgAN microbiome studies have provided conclusive evidence. Microbiome composition is determined by several other factors (e.g., diet, lifestyle, medicines, travel, and population) rather than the disease itself. Hence, carrying out longitudinal analyses of IgAN patients developing infection-related manifestations (e.g., gross hematuria) in a cohort study would help to identify the causal role of commensal microbiomes in IgAN pathogenesis.

### Pleiotropic Effects of Susceptibility Variants

Recent studies have largely supported the notion of shared genetics in immune-related diseases, including IgAN. By searching the Phenotype–Genotype Integrator database ([www.ncbi.nlm.nih.gov/gap/phegeni/](http://www.ncbi.nlm.nih.gov/gap/phegeni/)), which merges the National Human Genome Research Institute’s GWAS catalog data with several databases, several GWAS loci identified in IgAN patients were found to be shared with various immune-inflammation related diseases such as systemic lupus erythematosus, rheumatoid arthritis, and IBD (Supplementary Figure S1). Many of the shared loci are located in the human leukocyte antigen (HLA) region, confirming the presence of an immune abnormality in IgAN. Notably, the non-*HLA* loci associated with IgAN, such as the *CARD9* and *HORMAD2* loci for IBD, are involved mainly in mucosal innate immunity and regulation of IgA production.<sup>12,13</sup> The role of mucosal immunity in IgAN has been confirmed by a gene-set analysis of 582 non-*HLA* single-nucleotide polymorphisms reported previously to be associated with autoimmune or inflammatory traits in IgAN. The results show that 87 of 582 signals are associated with the risk to IgAN ( $P < 0.05$ ). These signals are involved mainly in the Kyoto Encyclopedia of Genes and Genomes pathways “Intestinal Immune Network for IgA Production” ( $P < 1.0 \times 10^{-16}$ ) and “Leishmania Infection.”<sup>9</sup> To explore the interrenal mechanism of IgAN, we undertook a shared gene analysis for IgAN and the most common secondary nephritis, lupus nephritis,<sup>63,64</sup> and detected several genes: *CFH*, *RGS1*, *LYST*, *PXK*, *HLA-DRA*, *HLA-DRB1*, *BLK*, *RASGRP1*, and *UBE2L3*.

Interestingly, 8 of 9 of the shared loci have opposing effects in IgAN versus lupus nephritis. Nevertheless, these different association directions of the same alleles support the notion of pleiotropy (effect of a single gene on multiple phenotypes). In accordance with this notion, a contrary prevalence of IgAN and lupus nephritis has been observed among different populations. Thus, future replication studies and functional assays for these genes will be of particular interest for determination of the exact underlying mechanism of pleiotropy in IgAN and lupus nephritis.

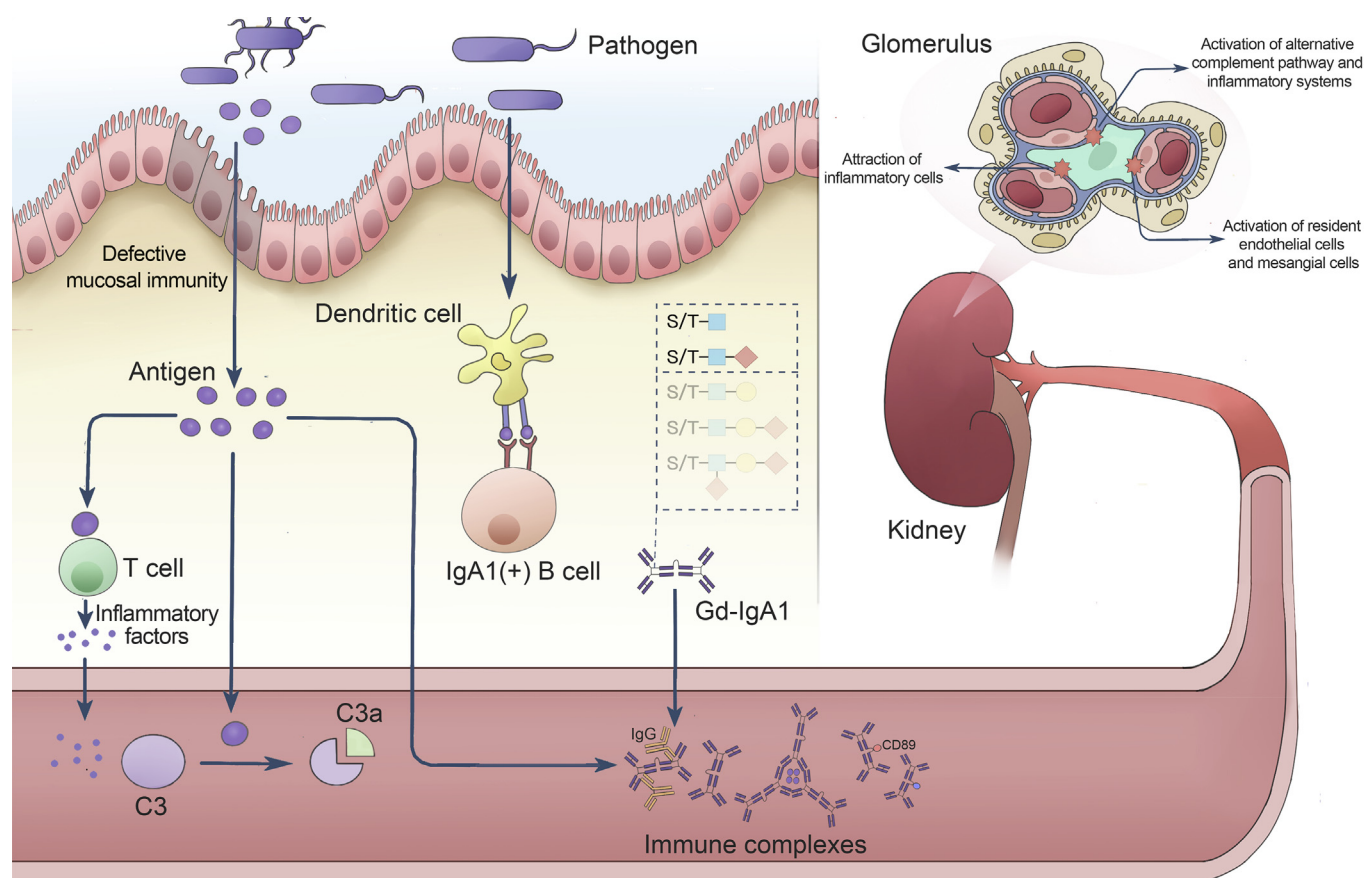
### Modification of the Model of IgAN Pathogenesis

Combining the lines of evidence with the findings from above-mentioned GWASs, IgAN may be regarded as an aberrant immune response to etiologic agents in genetically predisposed individuals. In this context, the genetic predispositions of IgAN are regarded as “first hits,” whereas the etiologic events that trigger the disease are “second hits”<sup>20</sup> (Figure 2). That is,

further in-depth studies, including meta-genomic studies, would be helpful to uncover abnormal microbiota patterns in IgAN patients.

### Triggering Role of Infection

IgA is the main effective molecule in immunologic homeostasis between commensal flora in the gut and the local immunological environment.<sup>65,66</sup> Systemic B cells produce mostly IgA1, whereas mucosal B cells produce IgA1 and IgA2. The subclass IgA1, which has a unique hinge region between the first and second constant-region domains of the heavy chains, is the predominant form in the circulating or mesangial immune complexes of IgAN patients;<sup>67</sup> this finding suggests a triggering role in mucosal infection. However, how Gd-IgA1 enters the circulatory system of IgAN patients and is deposited subsequently in the mesangial area is not known. One hypothesis is migmigration of mucosal IgA1-secreting cells to the bone marrow,<sup>68,69</sup> yet no such “homing” molecule has been identified



**Figure 2.** Proposed scheme of production of IgA1-containing immune complexes in the pathogenesis of IgA nephropathy. In individuals genetically predisposed to IgA nephropathy, an abnormal response to microbiota with defective mucosal immunity could lead to increased alimentary antigens, triggering mucosa-associated lymphoid tissue activation and subclinical intestinal inflammation. Antigens reach mucosa-associated lymphoid tissue and activate dendritic cells and T cells, leading to the secretion of inflammatory factors, the activation of the complement system (mostly through the alternative and lectin pathways), and the synthesis of galactose-deficient IgA1 (Gd-IgA1) and anti-Gd-IgA1 IgG autoantibodies. Several circulating macromolecular forms of IgA1, such as Gd-IgA1-CD89, Gd-IgA1-IgG, and Gd-IgA1 autoaggregates, deposit in subendothelial and mesangial areas, leading to local activation of the complement system, the attraction of inflammatory cells, and the activation of resident endothelial and mesangial cells.

clearly. Another hypothesis refers to the “spillover” of IgA1 from mucosal sites; this is stimulated by pathogens affecting the class switching and production of IgA. Tonsillar epithelial cells release thymic stromal lymphopoietin after toll-like receptors “sense” the presence of bacteria; thymic stromal lymphopoietin, in turn, increases production of BAFF, APRIL, and transforming growth factor  $\beta$ 1 by activating dendritic cells, resulting in increased production of IgA through IgA class-switching recombination.<sup>70</sup>

### Production of Unique Antigliyan Antibodies

It is well established that the underlying mechanisms for autoimmunity induced by infection are dependent on detection of the poorly glycosylated structures of Gd-IgA1 and Tn antigens expressed commonly by many bacterial pathogens.<sup>71</sup> In genetically predisposed individuals, an infection with a pathogen expressing the Tn antigen may induce increased levels of Gd-IgA1 antibodies, which in turn can react with Gd-IgA1 via molecular mimicry. This hypothesis is supported by the fact that higher levels of circulatory IgA1-containing immune complexes have been observed during disease activity.<sup>72–74</sup> The characteristics of Gd-IgA1 antibodies have been studied using Epstein–Barr virus–immortalized Ig-producing cells from IgAN patients and healthy control subjects. Although there is overlap of Gd-IgA1 levels between patients and control subjects, in IgAN patients an A  $\rightarrow$  S substitution in the complementarity-determining region 3 of the variable region of the heavy chain of the IgG antibody against Gd-IgA1 has been detected. This, in turn, enhances the ability of IgG to bind to Gd-IgA1 in IgAN patients compared with healthy control subjects. IgG antibodies against Gd-IgA1 appear to be predominantly glycan-specific, so it is possible that there are additional types of antibodies generated by the epitope-spreading process described in many autoimmune diseases.<sup>75</sup> Indeed, as reported previously, IgG and IgA1 can bind to Gd-IgA1 in patients with IgAN.<sup>76</sup> Further study is needed to determine the role of Gd-IgA1 antibodies in IgAN pathogenesis.

### Formation and Deposition of Immune Complexes

In IgAN patients, deposition of IgA1-IgG immune complexes in the mesangial area of glomeruli has been reported to stimulate proliferation of mesangial cells and increase secretion of cytokines, chemokines, and extracellular-matrix proteins, whereas uncomplexed Gd-IgA1 does not seem to affect cellular proliferation.<sup>74,77</sup> However, the literature addressing the frequency and degree of IgG deposition in IgAN patients typically suggests the presence of IgG codeposits in  $\approx$  60% of biopsies.<sup>78,79</sup> This finding is consistent with our mass-spectrometry results in IgAN-biopsy tissue

(unpublished data). Factors that may promote the formation of circulating Gd-IgA1-containing immune complexes in IgAN patients are of particular interest.

Several potential signaling molecules, including cluster of differentiation (CD) 89,<sup>80</sup> transglutaminase 2,<sup>81</sup> and CD71,<sup>82–84</sup> have been implicated in the formation or deposition of immune complexes in mesangial cells, though determination of the precise mechanism requires additional investigation. Several hypotheses have attempted to use this evidence to explain the deposition of immune complexes: Gd-IgA1 aggregation, Gd-IgA1-soluble CD89 (IgA fragment crystallizable [Fc]-specific receptor) complexes, complexes of Gd-IgA1 with environmental or microbial antigens, complexes of Gd-IgA1 with IgG or IgA, and direct binding of Gd-IgA1 to extracellular-matrix proteins in the glomerular mesangium.<sup>85–88</sup> These Gd-IgA1-containing immune complexes are deposited in the mesangial cells of IgAN patients, causing a subsequent immune response that includes activation of complement and amplification of inflammatory responses.

### Activation of Complement or Inflammatory System

Circulating complement C3 is the most commonly codeposited molecule with IgA1, affecting  $\approx$  90% of patients with IgAN.<sup>89</sup> Serologic complement activation and increased CFH levels in urine have been identified in IgAN patients,<sup>90,91</sup> suggesting the important role of complement activation in the disease. The genomic region 1q32 contains *CFH* and the *CFH*-related protein genes (*CFHR3*, *CFHR1*, *CFHR4*, *CFHR2*, and *CFHR5*), which are arranged in tandem within the regulators of complement activation gene cluster.<sup>92</sup> This region, identified by GWAS, has rs6677604 as the top single-nucleotide polymorphisms and *CFHR3-1* deletion (*CFHR3-1 $\Delta$* ) as the top signal for copy number variations. Interestingly, the rs6677604-A allele highly tags the variant *CFHR3-1 $\Delta$* , which is associated with higher serum levels of CFH and lower levels of C3a. Because *CFHR3-1* proteins are competitive antagonists of CFH, the variant *CFHR3-1 $\Delta$*  reportedly leads to absence of *CFHR3-1*, and is associated with higher CFH levels.<sup>93</sup> The CFH protein is a well-known inhibitor of complement activation and has various functions, including competition with factor B to hinder the formation of C3 convertase, facilitation of the dissociation of C3 convertase, and support of proteolytic cleavage of C3b by factor I.<sup>94,95</sup> Subsequently, higher CFH levels result in the robust complement inhibition represented by higher circulating levels of C3 and lower circulating levels of C3a.<sup>96</sup> Furthermore, by sequencing all exons, their intronic flanking regions, and the untranslated regions of *CFHR5*, rare variants in *CFHR5* have been



detected that may also contribute to a genetic susceptibility to IgAN.<sup>97</sup>

In addition, the intrarenal inflammatory reaction also has an important role in kidney injury. Immune complexes have been reported to stimulate mesangial proliferation and local production of cytokines and chemokines, such as IL-6 and transforming growth factor  $\beta$ , that can enhance the inflammatory response by recruiting leukocytes and promoting glomerular and tubulointerstitial fibrosis.<sup>98</sup>

### Clinical Implications

The 2012 Kidney Disease Improving Global Outcomes (KDIGO) guidelines emphasize use of angiotensin-converting enzyme inhibitors and angiotensin II receptor-blockers in patients with IgAN to control blood pressure and proteinuria. However, IgAN is a complex disease. Its pathogenesis is known only partially, and a disease-targeted treatment is not available. GWASs have identified several genes involved in the mucosal immune response and adaptive immunity, stimulating investigation into targeted treatments more specific to the pathogenic process in IgAN.<sup>99</sup> Ongoing clinical trials are expected to refine these therapeutic strategies (Figure 3).

In the context of abnormal mucosal responses in IgAN patients, detection of microbiota dysbiosis could lead to new supportive therapeutic strategies, such as the use of probiotics (e.g., lactobacilli and bifidobacteria) to restore some microbial gaps and use of fecal microbiota transplantation, which has been used in the treatment of various diseases (e.g., obesity as well as autoimmune and neurologic disorders). Promising agents to address increasing levels of circulating IgA by targeting immunosuppression to sites of mucosal B-cell induction include blisibimod (BAFF inhibitor) and atacicept (humanized recombinant TACI-IgGFc fusion protein with anti-APRIL and anti-BAFF activity); these may provide more specific therapy than what is available currently. The Effect of Nefecon in Patients With Primary IgA Nephropathy at Risk of Developing End-Stage Renal Disease (NEFIGAN) trial (ClinicalTrials.gov; NCT01738035) was designed to evaluate the safety and efficacy of Nefecon, an oral formulation that releases the glucocorticosteroid budesonide in the lower ileum and ascending colon. The drug reduces proteinuria significantly compared with placebo at 9 months (by 25%–30%), though the long-term outcomes on proteinuria or decline in the glomerular filtration rate need to be evaluated.<sup>100</sup>

In the context of aberrant autoimmune immunity, production of Gd-IgA1 autoantibodies suggests the possible benefits of immunosuppression. Results of a large, international, multicenter, randomized controlled

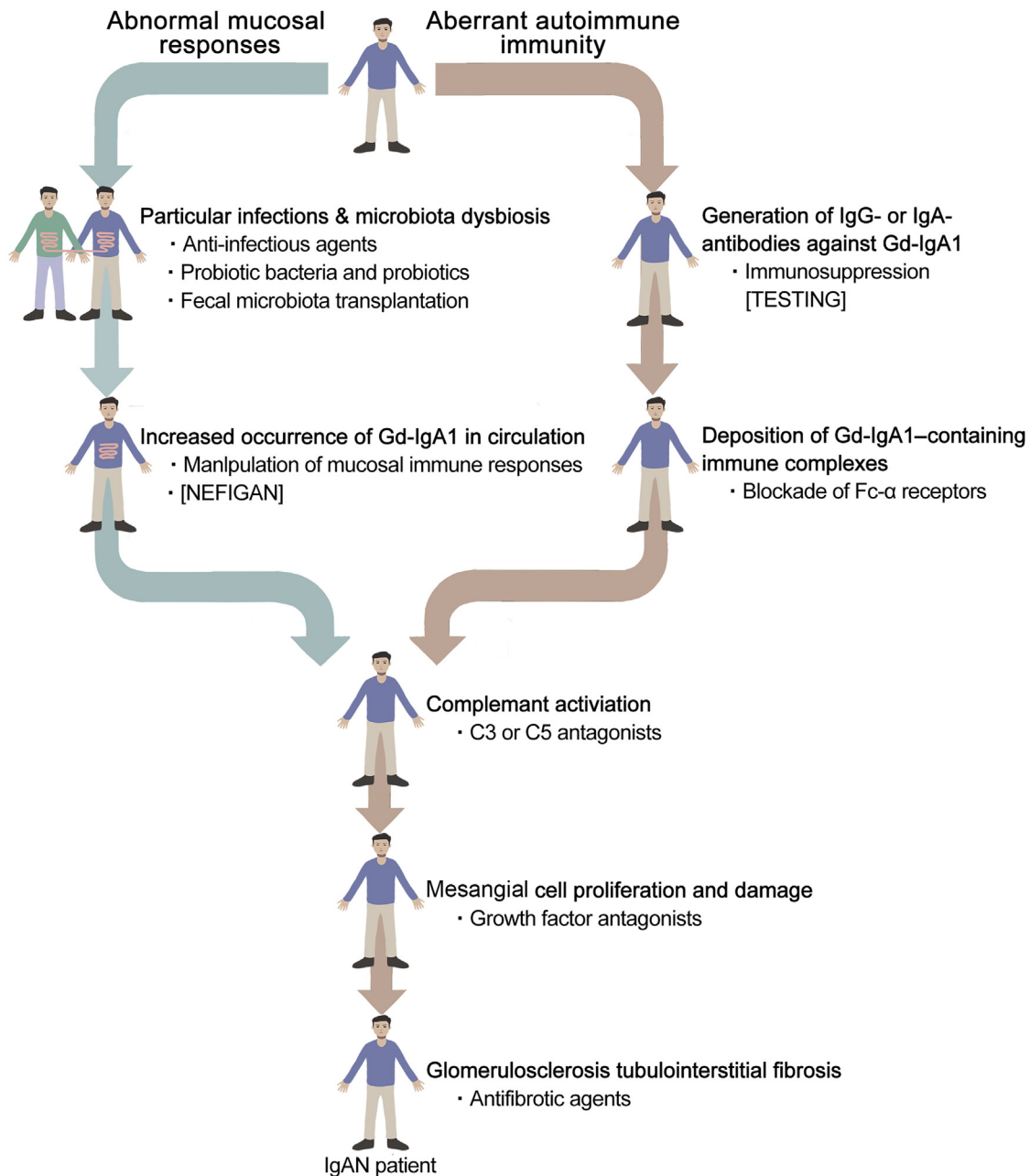
trial, the Therapeutic Evaluation of STeroids in IgA Nephropathy Global (TESTING) study, show that immunosuppressive therapy could increase the ratio of patients in clinical remission. However, this benefit has a price in more serious adverse events in the immunosuppression group. Ongoing long-term follow-up will help to further define the balance of risks and benefits. Anti-B-cell therapies directed against CD20, BAFF (blisibimod and atacicept), B-cell receptors, and proteasomes (bortezomib) may provide alternatives to the traditional regimens of systemic immunosuppression that are associated with significant side effects.

Together, the deposition of Gd-IgA1-containing immune complexes, complement activation, and secretion of inflammatory cytokines constitute a common pathway leading to renal damage. This may drive treatments that employ complement inhibitors (C3 or C5 antagonists), growth-factor antagonists, and antifibrotic agents in selected patients. Findings from GWASs are expected to produce therapeutic treatments and, more generally, advance the goals of precision medicine.

### Future Directions

The phenotype-genotype interplay of disease, especially for complex diseases such as IgAN, is controlled by multiple genes, by the structure-pattern formation of cytotogenesis, and by morphogenesis, which is involved in cell-signal transduction, the gene regulation network, and cell-lineage mapping. The disease phenotype can be regarded as the functional result of genetic variance (Supplementary Figure S2). Modification of the classification of the disease phenotype and use of cutting-edge technology to maximize and analyze data thoroughly has great potential to revolutionize understanding of disease and, ultimately, offer insight into novel preventative or therapeutic strategies.

First, detection of additional associated loci is critical for uncovering the pathogenesis of IgAN. Genetic studies have provided intriguing clues for its pathogenesis but the risk loci discovered to date explain  $\leq 10\%$  of the disease risk. The missing loci might be uncovered by carrying out GWASs with larger sample sizes, using meta-analyses based on imputation, and performing shared genetic analyses of IgAN and autoimmune- or inflammation-related diseases. Genome sequencing and system genetics can also be used to identify these missing loci. Second, for identified loci, interpretation of their functions using bioinformatics methods, including *in silico* analyses of public databases, pathway analyses, and functional assays, is of great importance. Third, perhaps the most important consideration is that IgAN is characterized



**Figure 3.** Clinical implications based on the pathogenesis of IgA nephropathy. Fc- $\alpha$ , fragment crystallizable  $\alpha$ ; Gd-IgA1, galactose-deficient IgA1; IgAN, IgA nephropathy; NEFIGAN, Effect of Nefecon in Patients With Primary IgA Nephropathy at Risk of Developing End-Stage Renal Disease; TESTING, Therapeutic Evaluation of STeroids in IgA Nephropathy Global study.

by a wide spectrum of pathologic and clinical manifestations. The appropriateness of considering IgAN to be a unique single entity or aggregation of distinct conditions is controversial. A better phenotypic characterization that can help to predict therapeutic and prognostic implications is needed. For example, performing a genome analysis on all patients who have IgA pathologic diagnosis (familial IgAN, idiopathic nephrotic syndrome with IgA pathology, or IgA deposition secondary to a systemic illness) or have IgA deposit in the kidney from various etiologies would be useful to search for the shared pathogenic link between

these disorders. Another possibility is to carry out genetic studies according to different clinical, pathologic, and prognostic phenotypes<sup>8,101,102</sup> or treatment responses to identify the specific genes involved in these molecular events.

### Summary

Studies have highlighted numerous potential candidate genes for IgAN pathogenesis. Many more genes are likely to be identified in the coming years, but the molecular pathways implicated by the known genes yield insights into the important role of immunity and

infection in IgAN pathogenesis. Interacting with genetic predisposition, etiologic factors may trigger an abnormal immune response, which in turn has an important role in the onset and progression of IgAN. Together with complex etiologic factors, the genetic network involved in IgAN pathogenesis may shape the heterogeneous landscape of this disease. Future work in integrating genetic, clinical, and bioinformatic datasets seems a promising contribution to individualized diagnosis and therapy for IgAN patients.

## DISCLOSURE

All the authors declared no competing interests.

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## SUPPLEMENTARY MATERIAL

**Figure S1.** Pleiotropic effects of IgA nephrology genome-wide association study loci among immune-related diseases.

**Figure S2.** Future directions of genetic studies in IgA nephropathy.

**Table S1.** The IgA nephropathy susceptibility loci detected by pedigree-based linkage studies.

Supplementary material is linked to the online version of the paper at [www.kireports.org](http://www.kireports.org).

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