

The Pathophysiology of IgA Nephropathy

Hitoshi Suzuki,^{*,‡} Krzysztof Kiryluk,[†] Jan Novak,[‡] Zina Moldoveanu,[‡] Andrew B. Herr,[¶] Matthew B. Renfrow,[§] Robert J. Wyatt,^{**} Francesco Scolari,^{††} Jiri Mestecky,^{‡||} Ali G. Gharavi,[†] and Bruce A. Julian^{‡||}

*Department of Internal Medicine, Division of Nephrology, Juntendo University Faculty of Medicine, Tokyo, Japan;

†Department of Medicine, Columbia University, College of Physicians and Surgeons, New York, New York; Departments

of ‡Microbiology, §Biochemistry and Molecular Genetics, and ¶Medicine, University of Alabama at Birmingham,

Birmingham, Alabama; ¶Department of Molecular Genetics, Biochemistry and Microbiology, University of Cincinnati

College of Medicine, Cincinnati, Ohio; **Department of Pediatrics, University of Tennessee Health Sciences Center and

the Children's Foundation Research Center at the Le Bonheur Children's Hospital, Memphis, Tennessee; and ††Second

Division of Nephrology, Montichiari Hospital, University of Brescia, Montichiari, Italy

ABSTRACT

Here we discuss recent advances in understanding the biochemical, immunologic, and genetic pathogenesis of IgA nephropathy, the most common primary glomerulonephritis. Current data indicate that at least four processes contribute to development of IgA nephropathy. Patients with IgA nephropathy often have a genetically determined increase in circulating levels of IgA1 with galactose-deficient O-glycans in the hinge-region (Hit 1). This glycosylation aberrancy is, however, not sufficient to induce renal injury. Synthesis and binding of antibodies directed against galactose-deficient IgA1 are required for formation of immune complexes that accumulate in the glomerular mesangium (Hits 2 and 3). These immune complexes activate mesangial cells, inducing proliferation and secretion of extracellular matrix, cytokines, and chemokines, which result in renal injury (Hit 4). Recent genome-wide association studies identify five distinct susceptibility loci—in the MHC on chromosome 6p21, the complement factor H locus on chromosome 1q32, and in a cluster of genes on chromosome 22q22—that potentially influence these processes and contain candidate mediators of disease. The significant variation in prevalence of risk alleles among different populations may also explain some of the sizable geographic variation in disease prevalence. Elucidation of the pathogenesis of IgA nephropathy provides an opportunity to develop disease-specific therapies.

J Am Soc Nephrol 22: 1795–1803, 2011. doi: 10.1681/ASN.2011050464

IgA nephropathy (IgAN) was described histologically for the first time in 1968 by Berger and Hinglais as *les dépôts intercapillaires d'IgA-IgG* (intercapillary deposits of IgA-IgG).¹ Over the ensuing decades, this renal disease has been recognized as the most common primary glomerulonephritis and has been shown to arise from a systemic process wherein the kidneys are damaged as innocent bystanders. The latter point is best illustrated by the experience with renal transplanta-

tion. IgAN frequently recurs in allografts; in contrast, kidneys from donors with subclinical IgAN are clear of IgA deposits shortly after transplantation into recipients with non-IgAN renal diseases.² The glomerular IgA eluted from tissue specimens from patients with IgAN is exclusively of the IgA1 subclass, predominantly in the polymeric form, and, most importantly, glycosylated aberrantly. Specifically, this aberrant IgA1 exhibits galactose deficiency

in the O-linked glycans in the hinge region of the heavy chain. Blood levels of a similarly aberrantly glycosylated IgA1 are higher in patients with IgAN than in healthy controls or patients with other kidney diseases. However, as we discuss here, a high circulating load of galactose-deficient IgA1 alone does not induce the renal injury. Rather, several sequential processes or hits are necessary for the clinical expression of IgAN.

PATHOGENESIS OF IgAN

Four processes come together to induce renal injury that culminates in IgAN: aberrant glycosylation of IgA1, synthesis of antibodies directed against galactose-deficient IgA1, binding of the galactose-deficient IgA1 by the anti-glycan/glycopeptide antibodies to form immune complexes, and accumulation of these complexes in the glomerular mesangium to initiate renal injury. We recently performed a genome-

Published online ahead of print. Publication date available at www.jasn.org.

H.S. and K.K. contributed equally to this work.

Correspondence: Dr. Bruce A. Julian, Division of Nephrology, Department of Medicine, THH 643, 1530 Third Avenue South, Birmingham, AL 35294. Phone: 205-934-9045; Fax: 205-934-7742; E-mail: bjulian@uab.edu

wide association study (GWAS) that identified five susceptibility loci for IgAN and provided molecular candidates for these processes.³

Hit 1: Hereditary Increase in Galactose-Deficient Circulating IgA1

As is the case for other immunoglobulins, IgA1 is glycosylated. An altered pattern of its glycosylation has been recognized as a potentially pathogenic abnormality in IgAN for nearly 20 years.⁴ The key feature is the deficiency of galactose in the hinge region of the IgA1 heavy chains. The hinge region of IgA1 extends by 13 amino acids longer than the hinge region of IgA2 and is found only in humans and higher primates (Figure 1A).⁵ It can carry up to six relatively short and simple sugar chains, called *O*-linked glycans, each attached by glycosidic linkage to an oxygen atom of a serine or threonine. These glycans are synthesized in stepwise fashion and include up to six different forms (Figure 1B). Patients with IgAN have increased circulating levels of IgA1 with abbreviated glycans composed of *N*-acetylgalactosamine (GalNAc), with or without sialic acid, that are devoid of a galactose moiety, as in Figure 1B structures I and II. A GalNAc-specific lectin, *Helix aspersa* agglutinin, is frequently used in an ELISA to measure the amount of galactose-deficient IgA1 (IgA1 with hinge-region *O*-glycan structures I and II in Figure 1B) after treatment of the IgA1 with neuraminidase to remove terminal sialic acid.⁶ Recent high-resolution mass spectrometry studies demonstrate significant heterogeneity in the composition and attachment sites of these glycans.^{7–12} Determination of the precise structural abnormality in IgAN is complicated by the multiplicity of *O*-glycosylation sites and combinatorial possibilities of glycan variants. However, examination of IgA1-producing cells shows that aberrant *O*-glycosylation in IgAN is driven by combined abnormalities in expression or activity of the glycosyltransferases involved in the sequential post-translational modification of IgA1

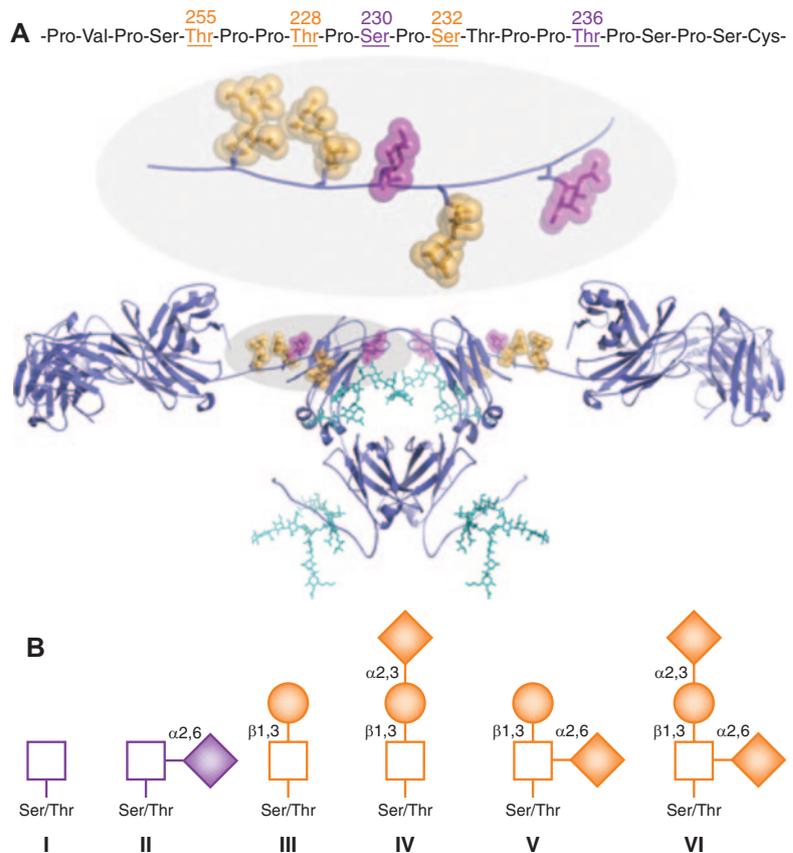


Figure 1. Human IgA1: hinge-region amino acid sequence (A) and possible glycan variants (B). (A) IgA1 contains up to six *O*-glycans per hinge region: five major sites are shown (in orange or magenta) and the sixth site is Thr233.⁹ Novel approaches using IgA-specific bacterial proteases and lectin binding, and, more recently, high-resolution mass spectrometry with electron capture and electron transfer dissociation, have been used to determine *O*-glycan heterogeneity, the sites of glycosylation, and the microheterogeneity at the individual sites.^{6,7,9,45,46} The model of intact IgA1 was generated from published crystal and solution structures of IgA1.^{47,48} *N*- and *O*-glycans were modeled using the GlyProt server and related databases (<http://www.glycosciences.de>), based on observed IgA1 glycoforms.^{9,49} For clarity, the *O*-glycans are shown with transparent spheres for each atom, and are colored orange for GalNAc-galactose residues and magenta for GalNAc; the illustrated *O*-glycan distribution was taken from a study by Takahashi *et al.*⁹ (B) The variants of *O*-glycans on circulatory IgA1. Galactose-deficient glycans present in elevated amounts in patients with IgAN are represented by structures I and II in magenta.^{6,19} Galactosylated variants are in orange as structures III to VI. The largest *O*-glycan on circulatory IgA1 is a GalNAc-galactose with two sialic acids, *i.e.*, tetrasaccharide, structure VI. IgA1 with GalNAc and sialylated GalNAc (structures I and II in magenta) is present at elevated serum levels in patients with IgAN due to the changes in expression and activity of specific glycosyltransferases, ST6GalNAcII, and C1GalT1.⁵⁰ The stability of C1GalT1 during translation is controlled by Cosmc, a foldase.⁵¹ Structure I is generated by a GalNAc-transferase⁵²; structure II, by ST6GalNAcII⁵³; structure III, from structure I by C1GalT1⁵⁴; and structures IV to VI, by sialyltransferases. Symbols: rectangle, GalNAc; circle, galactose; diamond, sialic acid.

(Figure 1), implicating complex regulatory defects.¹³

In patients with IgAN, the predominant sites where the cells secreting galactose-de-

icient IgA1 originate from and reside remain uncertain. Circulatory IgA1 is produced mainly in the bone marrow, whereas aberrantly glycosylated IgA1 may be syn-

thesized in response to a mucosal infection, and thus abnormalities in the mucosal response to common microbial or food antigens may be involved in production of galactose-deficient IgA1.¹⁴ For example, blood levels of galactose-deficient IgA1 directed against mucosal pathogens are increased in patients with IgAN compared with those of healthy controls.¹⁴ This process may include dysregulated innate immune responses through Toll-like receptors.¹⁵

Serum levels of galactose-deficient IgA1 are above the 90th percentile for healthy controls in as many as 70 to 80% of IgAN patients.⁶ Furthermore, 40 to 50% of first-degree relatives of IgAN patients have elevated levels comparable to that of patients, demonstrating significant heritability of this trait.^{16,17} The heritability of galactose-deficient IgA1 is observed in diverse racial groups and is not explained by variation in serum IgA levels, suggesting that distinct genetic mechanisms influence IgA1 glycosylation and production. These data also indicate that aberrant IgA1 glycosylation precedes clinically overt disease and constitutes an inherited risk factor for the development of IgAN. Moreover, because most persons with elevated levels of galactose-deficient IgA1 do not exhibit clinical signs of renal injury, this hereditary defect is insufficient to cause IgAN, implicating additional pathogenic hits as described in this article.¹⁶

GWAS data have identified a major locus on chromosome 22q12.2 influencing susceptibility to IgAN.³ This locus is also associated with variation in serum IgA levels and has been previously associated with risk of inflammatory bowel disease,¹⁸ further implicating this interval in the regulation of mucosal inflammation. Two cytokine genes within the associated region, *LIF* and *OSM*, are excellent positional candidates, as both are expressed in B cells and may participate in the regulation of mucosal immunity. It is not yet known if this locus also influences aberrant IgA glycosylation. Further studies, including resequencing of this locus and evaluation of its effect on serum levels of galactose-deficient IgA1, will clarify causal variants and their role in the synthesis of IgA1.

Hit 2: Circulating Antibodies Directed against Galactose-Deficient IgA1

Aberrantly glycosylated IgA1 in the blood of patients with IgAN is found nearly exclusively within immune complexes bound to IgG or IgA1 antibodies. We have recently shown that these IgG antibodies recognize GalNAc-containing epitopes on the galactose-deficient hinge region O-glycans of IgA1, defining an autoimmune component to IgAN.¹⁹ Furthermore, these IgG autoantibodies exhibit unique features in the complementarity-determining region 3 (CDR3) of the variable region of their heavy chains.¹³ Specifically, the third position in CDR3 is typically serine in patients with IgAN, a feature necessary for efficient binding of the IgG to galactose-deficient IgA1. Importantly, serum levels of IgG antibodies specific for galactose-deficient IgA1 correlated with disease severity, as assessed by the magnitude of proteinuria. It is not known whether the CDR3 serine substitution originates from somatic mutations that arise during maturation of the antibody-producing cells or from inherited germline mutations. These antibodies are also present in sera of healthy individuals, albeit at lower levels. One can postulate that these antibodies are produced in response to bacterial or viral cell-surface GalNAc-containing glycoconjugates on commensal or infectious microorganisms and then cross-react with galactose-deficient O-linked glycans on IgA1. A predominant IgA1 autoantibody response¹⁹ directed against galactose-deficient IgA1 may explain why some patients have IgA1 as the sole Ig isotype in the glomeruli. It is to be noted, however, that the presence of IgG in the renal biopsy specimens also correlates with mesangial and endocapillary cellularity.²⁰

The strongest signals in the recent GWAS for IgAN were localized within the MHC complex (encoding polypeptides that present antigens to T cells),^{3,21} a region highly associated with risk for many autoimmune disorders. Based on careful conditional analyses, we identified three independent

susceptibility loci within the MHC complex.³ The strongest genetic effect was observed for the MHC-II locus containing the *HLA-DQB1*, *DQA1*, and *DRB1* genes. This effect appeared to be conveyed by a highly protective haplotype *DRB1*1501-DQA1*0102-DQB1*0602*. Specifically, the *DQB1*0602* allele reduced the odds of disease by over 50% per copy. This is a relatively common classical HLA allele, present in 10 to 20% of Europeans and 2 to 10% of Asians.²² The second independent genetic effect is from a region encompassing two genes encoding transporters associated with antigen processing (*TAP1* and *TAP2*) and two genes encoding components of the immunoproteasome (*PSMB8* and *PSMB9*). These molecules process antigens in the cytosol and transport them into the endoplasmic reticulum for delivery to the cell surface in association with MHC-I molecules. It is not yet clear which of these four genes is involved in the susceptibility to IgAN. Finally, the third locus of association is on chromosome 6p21 and encodes MHC-II molecules *DPA1*, *DPB1*, and *DPB2*, and thus also relates to the process of antigen presentation. Taken together, these new genetic findings strongly implicate adaptive immunity in the pathogenesis of IgAN, and define the genetic context required for the recognition of galactose-deficient IgA1 as an antigen and for generation of pathogenic anti-glycan antibodies.

Hit 3: Formation of Pathogenic IgA1-Containing Immune Complexes

It is generally agreed that, in IgAN, the mesangial cells represent the primary target of pathogenic deposits formed by circulating immune complexes (Figure 2, solid lines) or by lanthanic deposits of aberrantly glycosylated IgA1, followed by binding of newly generated anti-glycan antibodies to form immune complexes *in situ* (Figure 2, broken lines).²³ The presence of circulating IgA1-containing immune complexes is not unique to patients with IgAN. Such complexes can be detected in persons without apparent renal disease, includ-

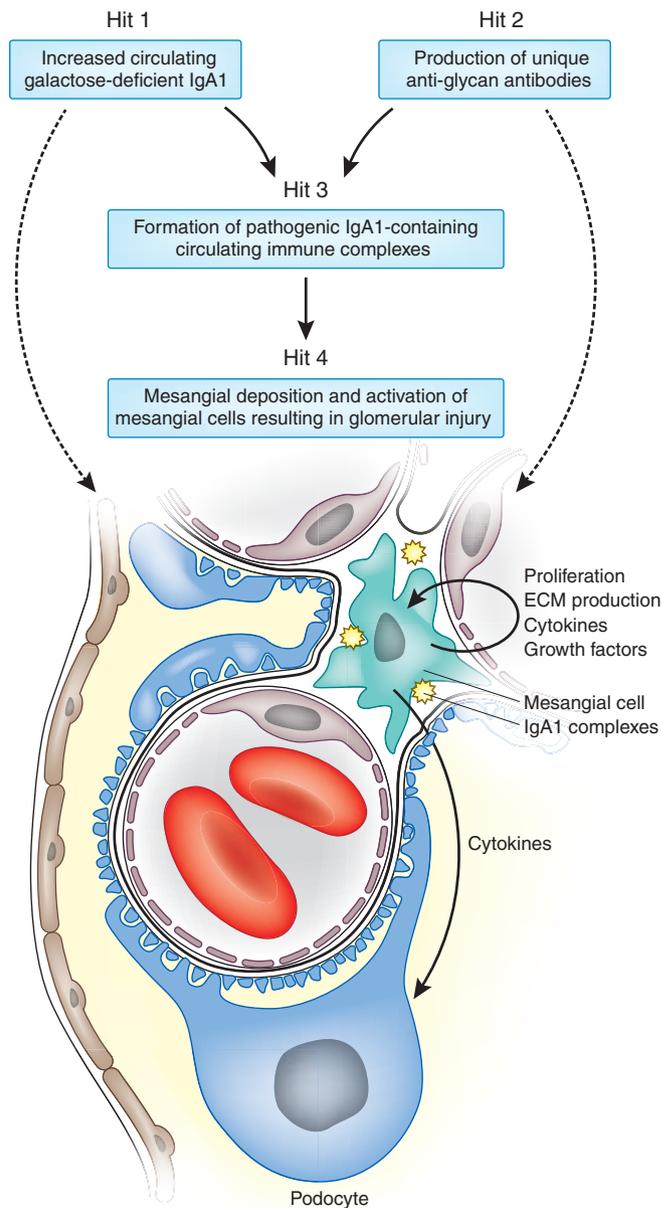


Figure 2. Proposed pathways involved in the pathogenesis of IgAN: multi-hit mechanism. Hit 1: Production of galactose-deficient IgA1 by a subpopulation of IgA1-secreting cells. IgA1 production may be affected by the IgAN-associated locus on chromosome 22q12.2.³ Hit 2: Formation of anti-glycan antibodies with specific characteristics of the variable region of the heavy chain that recognize galactose-deficient IgA1. Hit 3: Formation of immune complexes from autoantigen (galactose-deficient IgA1) and O-glycan-specific antibodies. Hits 2 and 3 may be regulated by the three MHC loci on chromosome 6p21 associated with risk of IgAN.³ Hit 4: Deposition of pathogenic immune complexes in the mesangium, activation of mesangial cells, and induction of glomerular injury. Hits 3 and 4 may be affected by genotype at the complement factor H locus on chromosome 1q32 that regulates the alternative complement cascade.³ The first pathway assumes formation of immune complexes in the circulation and their subsequent mesangial deposition (solid lines).^{13,19,55,56} An alternative theory proposes that some of the aberrantly glycosylated IgA1 molecules are in the mesangium as lanthanoid deposits (left broken line) and are later bound by newly generated anti-glycan antibodies to form immune complexes *in situ* (right broken line) that activate mesangial cells.²³ ECM, extracellular matrix.

ing healthy individuals and patients with Henoch-Schoenlein purpura without nephritis.^{19,24,25} The complexes in patients with Henoch-Schoenlein purpura without nephritis consist of IgA, but not IgG, and are of smaller mass than the complexes found in patients with IgAN. As these persons do not develop overt renal disease, it can be assumed that these IgA complexes are not nephritogenic. In contrast, patients with Henoch-Schoenlein purpura with nephritis have larger circulating immune complexes containing IgA and IgG.²⁴ By analogy with other human diseases caused by immune complexes, it is likely that, in IgAN, the molecular proportion of antigens (galactose-deficient IgA1) and antibodies (IgG or IgA1) determines the size of the formed immune complexes and, consequently, their rate of removal from the circulation, as well as biologic activity. The pathogenic circulating IgA1-IgG immune complexes in patients with IgAN are relatively large (>800 kD) and thus may be excluded from entry into the hepatic space of Disse to reach the asialoglycoprotein receptor (ASGP-R) on hepatocytes, the normal catabolic pathway for circulatory IgA1. As a result, these immune complexes enter the renal circulation. Due to the unique location of the mesangium between the fenestrated endothelial lining of the capillaries and the glomerular basement membrane, the mesangium is prone to deposition of immune complexes. While it is not completely understood what determines the entry of circulating immune complexes into the mesangium, the factors involved likely include the size of immune complexes, their amount, and local hemodynamic factors.²⁶ The biologic activity of large circulating immune complexes with galactose-deficient IgA1 increases in IgAN patients during episodes of macroscopic hematuria.²⁷ However, it is not known whether this increase in activity is due to greater production of galactose-deficient IgA1, anti-glycan antibodies, or other undefined factors influencing the formation of these complexes and/or their composi-

tion.^{28,29} MHC risk alleles may participate in this step by influencing the efficiency of antigen presentation, recognition, and processing, and subsequent activation of autoreactive B cells.

Hit 4: Mesangial Deposition of IgA1-Containing Immune Complexes, Cell Activation, and Initiation of Glomerular Injury

The pathogenetic importance of immune complexes has been shown by *in vitro* studies. The glomerular injury of IgAN histologically manifests as proliferation of mesangial cells and expansion of extracellular-matrix components. The detailed mechanisms of activation of mesangial cells remain to be elucidated. Nonetheless, cultured human mesangial cells provide a convenient model for evaluating the biologic activities of IgA1-containing complexes. Immune complexes from patients with IgAN containing galactose-deficient IgA1 bind to the cells more efficiently than do uncomplexed IgA1 or immune complexes from healthy controls. Complexes with galactose-deficient IgA1 induce cultured human mesangial cells to proliferate, secrete extracellular matrix components, and release humoral factors such as TNF α , IL-6, and TGF β . These factors can, in turn, alter podocyte gene expression and glomerular permeability.^{30,31} In contrast, uncomplexed galactose-deficient IgA1 or relatively small immune complexes (<800 kD) have no stimulatory effect on cellular proliferation.

The cellular receptors on mesangial cells involved in the binding of IgA1 are not well characterized. IgA1-containing immune complexes display a high affinity for the extracellular-matrix components fibronectin and type IV collagen in the mesangium, and preferentially bind and activate mesangial cells. None of the well-known IgA receptors (CD89, polymeric Ig receptor, ASGP-R) and complement receptors (CR 1–3) have been confirmed on human mesangial cells.^{32,33} However, transferrin receptor (CD71), which is expressed on the surface of proliferating human mesangial cells, can bind polymeric IgA1.³⁴ Moreover, CD71 on human mesangial cells effectively

binds immune complexes containing galactose-deficient IgA1, leading to enhanced expression of CD71.^{35,36} This binding creates a positive feedback loop, causing overexpression of CD71 on proliferating mesangial cells. However, it is not known whether CD71 is the only receptor that binds IgA1-containing immune complexes or whether it has a direct pathogenic role in IgAN.

Activation of the complement system in glomeruli augments the inflammatory cascade and potentiates tissue injury in IgAN. The immune complexes with IgA1 can activate complement *via* the alternative or lectin pathway. The pattern of glycosylation of IgA1 and the molecular mass of IgA1-containing immune complexes are also important factors in the ability of IgA1 to activate the alternative complement pathway.³⁷ Accordingly, renal biopsy specimens have usually detectable C3, while the components of the classical pathway, such as C1q, are typically absent. Our recent GWAS identified a major IgAN susceptibility locus within the complement factor H gene (CFH) cluster on chromosome 1q32. Products of *CFH* and its neighboring *CFHR* (*CFH-related*) genes participate in the modulation of the alternative pathway by binding C3a and C5a convertases. Mutations in *CFH* lead to uncontrolled activation of the alternative pathway and cause inherited forms of membranoproliferative glomerulonephritis type II, a disease pathologically distinct from IgAN. However, carriers of a common deletion encompassing the neighboring *CFHR1* and *CFHR3* genes had an approximately 30% decreased risk of developing IgAN. The risk was almost 60% lower in the rare individuals who carry two copies of this deletion.³ The role of *CFHR1* and *CFHR3* proteins in the regulation of complement cascade is currently under active investigation. Based on early experimental data, however, *CFHR1* and *CFHR3* compete with *CFH* for binding to C3b, the key activator of the terminal portion of the complement pathway.³⁸ Therefore, a relative loss of *CFHR1* and *CFHR3* may enhance the inhibitory action of *CFH* and thus convey protection against local inflammation.

These mechanistic issues have important clinical and therapeutic implications because subclinical findings consistent with IgAN are common in the general population. Necropsy series found glomerular IgA deposits in 2% and 4% of individuals in Singapore and Germany, respectively.^{39,40} Even more striking, a study in Japan showed that 16% of 510 renal allografts at engraftment were affected, of which 19 (3.7%) had a mesangioproliferative nephritis.⁴¹ To date, the glycosylation pattern of this lanthanitic glomerular IgA has not been examined to determine if it differs from that found in IgAN patients. Such an analysis would clarify whether the IgA deposits are clinically silent because they have a different composition that renders them relatively inert or because there is an intrinsic hyporesponsiveness of the kidney in which they are deposited.

POTENTIAL NEW DIAGNOSTIC AND PROGNOSTIC MARKERS

In Blood

Based on the central role of galactose-deficient IgA1 in the pathogenesis, Moldoveanu *et al.*⁶ investigated the value of serum levels of this protein as a diagnostic test. By receiver operating characteristic (ROC) curve analysis, the serum level of galactose-deficient IgA1 that provided a 0.77 sensitivity had a specificity of 0.90 to distinguish IgAN patients from healthy controls, while a level with a specificity of 1.00 had a sensitivity of 0.44.⁶ Other groups have replicated these findings.^{4,42} Importantly, the serum level of galactose-deficient IgA1 may be significantly elevated long before the diagnosis of IgAN (Olson S. *et al.*, unpublished observation).

IgG specific for galactose-deficient IgA1 represents another potential biomarker, as serum levels of this antibody are significantly elevated in IgAN patients, and the levels correlate with proteinuria. ROC curve analysis indicates that when the specificity of the level of serum IgG antibody directed against galactose-deficient IgA1 reached 0.95, the corresponding sensitivity was 0.88.¹³

Table 1. Summary of the four hits involved in the pathogenesis of IgAN

Hit	Pathogenic Process	Putative Environmental Factors Involved	Putative Genetic Factors Involved	Potential Clinical Biomarkers	Potential Novel Therapeutic Approaches
1	Hereditary increase in circulating galactose-deficient IgA1	Potential role of mucosal exposure to infectious or dietary antigens	Strong evidence for high heritability of serum galactose-deficient IgA1 level Potential role of chromosome 22q12.2	Serum galactose-deficient IgA1 level (HAA-based ELISA)	Suppression of synthesis of galactose-deficient IgA1 Enzymatic boost of galactose transfer to IgA1 hinge-region O-glycans Suppression of sialylation of galactose-deficient O-glycans
2	Circulating antibody directed against galactose-deficient IgA1	Potential role of mucosal exposure to infectious or dietary antigens	Potential role of three MHC-II loci in antigen presentation and humoral response to galactose-deficient IgA1 O-glycans	Serum anti-glycan antibodies (dot-blot assay)	Alteration of processing and presentation of galactose-deficient IgA1 O-glycopeptides Specific B-cell depletion therapy
3	Formation of pathogenic IgA1-containing immune complexes	Unknown	Unknown	Circulating and/or urinary immune complexes	Competitive blockade of immune complex formation by non-cross-linking anti-glycan antibodies or specific glycopeptides
4	Mesangial deposition of IgA1-containing immune complexes, cell activation and initiation of glomerular injury	Unknown	Protective effect of common deletion in <i>CFHR1</i> and <i>CFHR3</i>	Circulating and/or urinary complement degradation products, or novel markers of glomerular injury	Suppression of the alternative complement pathway Targeted <i>CFHR1/3</i> depletion Blocking mesangial cell signaling induced by nephritogenic IgA1-containing immune complexes

HAA: *Helix aspersa* agglutinin, a lectin specific for terminal GalNAc

In Urine

Urinary proteomics holds promise for development of noninvasive tests for IgAN. A subset of mesangial immune complexes apparently enters the urinary space. Aberrantly galactosylated IgA1 within immune complexes has been found in the urine of patients with IgAN but not in patients with non-IgAN proteinuric glomerular diseases.⁴³ It is also possible to develop a diagnostic test without a detailed knowledge of the pathogenesis, based on analysis of the urinary peptidome. In a preliminary study, Julian *et al.*⁴⁴ found that analysis of urine samples by capillary electrophoresis, coupled online with mass spectrometry, distinguished patients with primary IgAN from patients with IgA-immune-complex renal disease due to cirrhosis, even if clinical proteinuria was absent.

Genetic

The GWAS of sporadic IgAN identified five novel genetic variants with relatively strong protective effects against IgAN.³ While these variants are all common, their frequencies vary significantly across different continental populations and closely parallel the prevalence rates of IgAN. For example, African populations, which have the lowest reported prevalence of IgAN, carry the most protective alleles, while Asians, who have the highest reported prevalence, have significantly fewer protective variants. The ROC analysis for a genetic risk score based on these five alleles is estimated in the range of 0.60 to 0.63, with the risk score explaining up to 7% of disease variation among Asian and Caucasian populations. Identification of additional genetic susceptibility loci in follow-up GWAS studies of Caucasian populations will likely improve the predictive power of the genetic risk score. Additionally, a composite risk score based on a combined assessment of circulating, urinary, and genetic disease biomarkers holds promise to ultimately provide a tool for the noninvasive diagnosis of IgAN.

POTENTIAL APPROACHES FOR DISEASE-SPECIFIC THERAPY

The pathogenesis model (Figure 2) provides an opportunity to design and test

rational therapies for IgAN (Table 1). This model predicts that the generation of nephritogenic immune complexes composed of galactose-deficient IgA1 and anti-glycan autoantibodies initiates disease. Therefore, interventions that can reduce generation of galactose-deficient IgA1 or anti-glycan antibodies, or block the interaction between these two components to form nephritogenic immune complexes, may prove effective. This result can be achieved by enhancing enzymatic activity of glycosyltransferases for synthesis of galactose-replete hinge-region *O*-glycans to reduce the availability of aberrantly glycosylated IgA1 for formation of nephritogenic immune complexes. Alternatively, generation of non-cross-linking, monovalent reagents (single-chain antibodies) with high affinity for GalNAc on galactose-deficient IgA1 could theoretically prevent binding of anti-glycan antibodies. Lastly, blocking the antibodies with a glycopeptide may be another strategy to prevent formation of immune complexes (Table 1).

Similarly, interventions aimed at reducing immune complex deposition and the downstream inflammatory signals may prove beneficial. The genetic studies identify the alternative complement pathway as a prime candidate for intervention, and predict that targeted depletion of CFHR1 and/or CFHR3 would be tolerated and prove protective. Moreover, blocking of specific signaling pathways induced in mesangial cells by pathogenic IgA1-containing complexes can be theoretically accomplished by protein-kinase inhibitors, a class of drugs that is frequently used in the treatment of some types of cancer.

CONCLUSIONS

IgAN is an autoimmune renal disease arising from consequences of increased circulating levels of IgA1 with galactose-deficient hinge-region *O*-glycans. However, this glycosylation aberrancy alone is not sufficient to induce nephritis. For the clinical manifestation of renal injury, several additional hits are required, including synthesis of circulating antibody

directed against the aberrantly glycosylated *O*-linked hinge-region glycans to form immune complexes, accumulation of the complexes in the mesangium, and activation of mesangial cells. Genetic factors apparently influence the expression of these hit mechanisms. Elucidation of the pathogenesis of IgAN provides an opportunity to develop a disease-specific therapy that heretofore has been missing.

DISCLOSURES

Supported in part by grants DK082753, DK078244, DK083663, DK075868, DK080301, DK077279, DK071802, and K23-DK090207 from the National Institutes of Health and a grant from the IgA Nephropathy Foundation of America.

REFERENCES

- Berger J, Hinglais N: Les dépôts intercapillaires d'IgA-IgG (Intercapillary deposits of IgA-IgG). *J Urol Nephrol* 74: 694–695, 1968
- Silva FG, Chander P, Pirani CL, Hardy MA: Disappearance of glomerular mesangial IgA deposits after renal allograft transplantation. *Transplantation* 33: 241–246, 1982
- Gharavi AG, Kiryluk K, Choi M, Li Y, Hou P, Xie J, Sanna-Cherchi S, Men CJ, Julian BA, Wyatt RJ, Novak J, He JC, Wang H, Lv J, Zhu L, Wang W, Wang Z, Yasuno K, Gunel M, Mane S, Umlauf S, Tikhonova I, Beerman I, Savoldi S, Magistroni R, Ghiggeri GM, Bordini M, Lugani F, Ravani P, Ponticelli C, Allegri L, Boscutti G, Frasca G, Amore A, Peruzzi L, Coppo R, Izzi C, Viola BF, Prati E, Salvadori M, Mignani R, Gesualdo L, Bertinetto F, Mesiano P, Amoroso A, Scolari F, Chen N, Zhang H, Lifton RP: Genome-wide association study identifies susceptibility loci for IgA nephropathy. *Nat Genet* 43: 321–327, 2011
- Coppo R, Feehally J, Glasscock RJ: IgA nephropathy at two score and one. *Kidney Int* 77: 181–186, 2010
- Mestecky J, Moro I, Kerr MA, Woof JM: Mucosal immunoglobulins. In: *Mucosal Immunology*, 3rd ed, edited by Mestecky J, Bienenstock J, Lamm ME, Mayer L, McGhee JR, Strober W, Amsterdam, Elsevier Academic Press, 2005, pp 153–181
- Moldoveanu Z, Wyatt RJ, Lee J, Tomana M, Julian BA, Mestecky J, Huang WQ, Anreddy S, Hall S, Hastings MC, Lau KK, Cook WJ, Novak J: Patients with IgA nephropathy have increased serum galactose-deficient IgA1 levels. *Kidney Int* 71: 1148–1154, 2007
- Renfrow MB, Cooper HJ, Tomana M, Kulhavy R, Hiki Y, Toma K, Emmett MR,

- Mestecky J, Marshall AG, Novak J: Determination of aberrant O-glycosylation in the IgA1 hinge region by electron capture dissociation Fourier transform-ion cyclotron resonance mass spectrometry. *J Biol Chem* 280: 19136–19145, 2005
8. Renfrow MB, MacKay CL, Chalmers MJ, Julian BA, Mestecky J, Kilian M, Poulsen K, Emmett MR, Marshall AG, Novak J: Analysis of O-glycan heterogeneity in IgA1 myeloma proteins by Fourier transform ion cyclotron resonance mass spectrometry: Implications for IgA nephropathy. *Anal Bioanal Chem* 389: 1397–1407, 2007
 9. Takahashi K, Wall SB, Suzuki H, Smith AD, Hall S, Poulsen K, Kilian M, Mobley JA, Julian BA, Mestecky J, Novak J, Renfrow MB: Clustered O-glycans of IgA1: Defining macro- and micro-heterogeneity by use of electron capture/transfer dissociation. *Mol Cell Proteomics* 9: 2545–2557, 2010
 10. Wada Y, Dell A, Haslam SM, Tissot B, Canis K, Azadi P, Backstrom M, Costello CE, Hansson GC, Hiki Y, Ishihara M, Ito H, Kakehi K, Karlsson N, Hayes CE, Kato K, Kawasaki N, Khoo KH, Kobayashi K, Kolarich D, Kondo A, Lebrilla C, Nakano M, Narimatsu H, Novak J, Novotny MV, Ohno E, Packer NH, Palaima E, Renfrow MB, Tajiri M, Thomsson KA, Yagi H, Yu SY, Taniguchi N: Comparison of methods for profiling O-glycosylation: Human Proteome Organisation Human Disease Glycomics/Proteome Initiative multi-institutional study of IgA1. *Mol Cell Proteomics* 9: 719–727, 2010
 11. Wada Y, Tajiri M, Ohshima S: Quantitation of saccharide compositions of O-glycans by mass spectrometry of glycopeptides and its application to rheumatoid arthritis. *J Proteome Res* 9: 1367–1373, 2010
 12. Odani H, Yamamoto K, Iwayama S, Iwase H, Takasaki A, Takahashi K, Fujita Y, Sugiyama S, Hiki Y: Evaluation of the specific structures of IgA1 hinge glycopeptide in 30 IgA nephropathy patients by mass spectrometry. *J Nephrol* 23: 70–76, 2010
 13. Suzuki H, Fun R, Zhang Z, Brown R, Hall S, Julian BA, Chatham WW, Suzuki Y, Wyatt RJ, Moldoveanu Z, Lee JY, Robinson J, Tomana M, Tomino Y, Mestecky J, Novak J: Aberrantly glycosylated IgA1 in IgA nephropathy patients is recognized by IgG antibodies with restricted heterogeneity. *J Clin Invest* 119: 1668–1677, 2009
 14. Smith AC, Molyneux K, Feehally J, Barratt J: O-glycosylation of serum IgA1 antibodies against mucosal and systemic antigens in IgA nephropathy. *J Am Soc Nephrol* 17: 3520–3528, 2006
 15. Suzuki H, Suzuki Y, Narita I, Aizawa M, Kihara M, Yamanaka T, Kanou T, Tsukaguchi H, Novak J, Horikoshi S, Tomino Y: Toll-like receptor 9 affects severity of IgA nephropathy. *J Am Soc Nephrol* 19: 2384–2395, 2008
 16. Gharavi AG, Moldoveanu Z, Wyatt RJ, Barker CV, Woodford SY, Lifton RP, Mestecky J, Novak J, Julian BA: Aberrant IgA1 glycosylation is inherited in familial and sporadic IgA nephropathy. *J Am Soc Nephrol* 19: 1008–1014, 2008
 17. Kiryluk K, Julian BA, Wyatt RJ, Scolari F, Zhang H, Novak J, Gharavi AG: Genetic studies of IgA nephropathy: Past, present, and future. *Pediatr Nephrol* 25: 2257–2268, 2010
 18. Imielinski M, Baldassano RN, Griffiths A, Russell RK, Annese V, Dubinsky M, Kugathasan S, Bradfield JP, Walters TD, Sleiman P, Kim CE, Muise A, Wang K, Glessner JT, Saeed S, Zhang H, Frackelton EC, Hou C, Flory JH, Otieno G, Chiavacci RM, Grundmeier R, Castro M, Latiano A, Dallapiccola B, Stempak J, Abrams DJ, Taylor K, McGovern D, Silber G, Wrobel I, Quiros A, Barrett JC, Hansoul S, Nicolae DL, Cho JH, Duerr RH, Rioux JD, Brant SR, Silverberg MS, Taylor KD, Barmuda MM, Bitton A, Dassopoulos T, Datta LW, Green T, Griffiths AM, Kistner EO, Murtha MT, Regueiro MD, Rotter JI, Schumm LP, Steinhardt AH, Targan SR, Xavier RJ, Libioulle C, Sandor C, Lathrop M, Belaiche J, Dewit O, Gut I, Heath S, Laukens D, Mni M, Rutgeerts P, Van Gossum A, Zelenika D, Franchimont D, Hugot JP, de Vos M, Vermeire S, Louis E, Cardon LR, Anderson CA, Drummond H, Nimmo E, Ahmad T, Prescott NJ, Onnie CM, Fisher SA, Marchini J, Ghorji J, Bumpstead S, Gwilliam R, Tremelling M, Delukas P, Mansfield J, Jewell D, Satsangi J, Mathew CG, Parkes M, Georges M, Daly MJ, Heyman MB, Ferry GD, Kirschner B, Lee J, Essers J, Grand R, Stephens M, Levine A, Piccoli D, Van Limbergen J, Cucchiara S, Monos DS, Guthery SL, Denson L, Wilson DC, Grant SF, Daly M, Hakonarson H: Common variants at five new loci associated with early-onset inflammatory bowel disease. *Nat Genet* 41: 1335–1340, 2009
 19. Tomana M, Novak J, Julian BA, Matousov K, Konecny K, Mestecky J: Circulating immune complexes in IgA nephropathy consist of IgA1 with galactose-deficient hinge region and antigenic antibodies. *J Clin Invest* 104: 73–81, 1999
 20. Bellur SS, Troyanov S, Cook HT, Roberts IS: Immunostaining findings in IgA nephropathy: Correlation with histology and clinical outcome in the Oxford classification patient cohort. *Nephrol Dial Transplant* 26: 2533–2536, 2011
 21. Feehally J, Farrall M, Boland A, Gale DP, Gut I, Heath S, Kumar A, Peden JF, Maxwell PH, Morris DL, Padmanabhan S, Vyse TJ, Zawadzka A, Rees AJ, Lathrop M, Ratcliffe PJ: HLA has strongest association with IgA nephropathy in genome-wide analysis. *J Am Soc Nephrol* 21: 1791–1797, 2010
 22. Solberg OD, Mack SJ, Lancaster AK, Single RM, Tsai Y, Sanchez-Mazas A, Thomson G: Balancing selection and heterogeneity across the classical human leukocyte antigen loci: A meta-analytic review of 497 population studies. *Hum Immunol* 69: 443–464, 2008
 23. Glasscock RJ: The pathogenesis of IgA nephropathy. *Curr Opin Nephrol Hypertens* 20: 153–160, 2011
 24. Levinsky RJ, Barratt TM: IgA immune complexes in Henoch-Schönlein purpura. *Lancet* 2: 1100–1103, 1979
 25. Hall RP, Lawley TJ, Heck JA, Katz SI: IgA-containing circulating immune complexes in dermatitis herpetiformis, Henoch-Schönlein purpura, systemic lupus erythematosus and other diseases. *Clin Exp Immunol* 40: 431–437, 1980
 26. Sterzel RB, Lovett DH, Stein HD, Kashgarian M: The mesangium and glomerulonephritis. *Klin Wochenschr* 60: 1077–1094, 1982
 27. Novak J, Tomana M, Matousov K, Brown R, Hall S, Novak L, Julian BA, Wyatt RJ, Mestecky J: IgA1-containing immune complexes in IgA nephropathy differentially affect proliferation of mesangial cells. *Kidney Int* 67: 504–513, 2005
 28. Novak J, Moldoveanu Z, Renfrow MB, Yanagihara T, Suzuki H, Raska M, Hall S, Brown R, Huang WQ, Goepfert A, Kilian M, Poulsen K, Tomana M, Wyatt RJ, Julian BA, Mestecky J: IgA nephropathy and Henoch-Schoenlein purpura nephritis: Aberrant glycosylation of IgA1, formation of IgA1-containing immune complexes, and activation of mesangial cells. *Contrib Nephrol* 157: 134–138, 2007
 29. Novak J, Mestecky J: IgA Immune-complex. In: *Recent Advances in IgA Nephropathy*, edited by Lai KN, Hong Kong, Imperial College Press and the World Scientific Publisher, 2009, pp 177–191
 30. Lai KN, Leung JC, Chan LY, Saleem MA, Mathieson PW, Lai FM, Tang SC: Activation of podocytes by mesangial-derived TNF- α : Glomerulo-podocytic communication in IgA nephropathy. *Am J Physiol Renal Physiol* 294: F945–F955, 2008
 31. Lai KN, Leung JC, Chan LY, Saleem MA, Mathieson PW, Tam KY, Xiao J, Lai FM, Tang SC: Podocyte injury induced by mesangial-derived cytokines in IgA nephropathy. *Nephrol Dial Transplant* 24: 62–72, 2009
 32. Leung JCK, Tsang AWL, Chan DTM, Lai KN: Absence of CD89, polymeric immunoglobulin receptor, and asialoglycoprotein receptor on human mesangial cells. *J Am Soc Nephrol* 11: 241–249, 2000
 33. Novak J, Vu HL, Novak L, Julian BA, Mestecky J, Tomana M: Interactions of human mesangial cells with IgA and IgA-containing circulating immune complexes. *Kidney Int* 62: 465–475, 2002
 34. Moura IC, Centelles MN, Arcos-Fajardo M, Malheiros DM, Collawn JF, Cooper MD, Monteiro RC: Identification of the transferrin receptor as a novel immunoglobulin (Ig)A1 receptor and its enhanced expression on

- mesangial cells in IgA nephropathy. *J Exp Med* 194: 417–425, 2001
35. Moura IC, Arcos-Fajardo M, Sadaka C, Leroy V, Benhamou M, Novak J, Vrtovnik F, Haddad E, Chintalacharuvu KR, Monteiro RC: Glycosylation and size of IgA1 are essential for interaction with mesangial transferrin receptor in IgA nephropathy. *J Am Soc Nephrol* 15: 622–634, 2004
 36. Moura IC, Arcos-Fajardo M, Gdoura A, Leroy V, Sadaka C, Mahlaoui N, Lepelletier Y, Vrtovnik F, Haddad E, Benhamou M, Monteiro RC: Engagement of transferrin receptor by polymeric IgA1: Evidence for a positive feedback loop involving increased receptor expression and mesangial cell proliferation in IgA nephropathy. *J Am Soc Nephrol* 16: 2667–2676, 2005
 37. Zhang W, Lachmann PJ: Glycosylation of IgA is required for optimal activation of the alternative complement pathway by immune complexes. *Immunology* 81: 137–141, 1994
 38. Fritsche LG, Lauer N, Hartmann A, Stippa S, Keilhauer CN, Oppermann M, Pandey MK, Kohl J, Zipfel PF, Weber BH, Skerka C: An imbalance of human complement regulatory proteins CFHR1, CFHR3 and factor H influences risk for age-related macular degeneration (AMD). *Hum Mol Genet* 19: 4694–4704, 2010
 39. Waldherr R, Rambousek M, Duncker WD, Ritz E: Frequency of mesangial IgA deposits in non-selected autopsy series. *Nephrol Dial Transplant* 4: 943–946, 1989
 40. Varis J, Rantala I, Pasternack A, Oksa H, Jantti M, Pauun ES, Pirhonen R: Immunoglobulin and complement deposition in glomeruli of 756 subjects who had committed suicide or met with a violent death. *J Clin Pathol* 46: 607–610, 1993
 41. Suzuki K, Honda K, Tanabe K, Toma H, Nihei H, Yamaguchi Y: Incidence of latent mesangial IgA deposition in renal allograft donors in Japan. *Kidney Int* 63: 2286–2294, 2003
 42. Shimozato S, Hiki Y, Odani H, Takahashi K, Yamamoto K, Sugiyama S: Serum undergalactosylated IgA1 is increased in Japanese patients with IgA nephropathy. *Nephrol Dial Transplant* 23: 1931–1939, 2008
 43. Matousovich K, Novak J, Tomana M, Kulhavy R, Julian BA, Mestecky J: IgA1-containing immune complexes in the urine of IgA nephropathy patients. *Nephrol Dial Transplant* 21: 2478–2484, 2006
 44. Julian BA, Wittke S, Novak J, Good DM, Coon JJ, Kellmann M, Zürgbig P, Schiffer E, Haubitz M, Moldoveanu Z, Calcatera SM, Wyatt RJ, Sykora J, Sladkova E, Hes O, Mischak H, McGuire BM: Electrophoretic methods for analysis of urinary polypeptides in IgA-associated renal diseases. *Electrophoresis* 28: 4469–4483, 2007
 45. Gomes MM, Suzuki H, Brooks MT, Tomana M, Moldoveanu Z, Mestecky J, Julian BA, Novak J, Herr AB: Recognition of galactose-deficient O-glycans in the hinge region of IgA1 by N-acetylgalactosamine-specific snail lectins: A comparative binding study. *Biochemistry* 49: 5671–5682, 2010
 46. Moore JS, Kulhavy R, Tomana M, Moldoveanu Z, Suzuki H, Brown R, Hall S, Killian M, Poulsen K, Mestecky J, Julian BA, Novak J: Reactivities of N-acetylgalactosamine-specific lectins with human IgA1 proteins. *Mol Immunol* 44: 2598–2604, 2007
 47. Boehm MK, Woof JM, Kerr MA, Perkins SJ: The Fab and Fc fragments of IgA1 exhibit a different arrangement from that in IgG: A study by X-ray and neutron solution scattering and homology modelling. *J Mol Biol* 286: 1421–1447, 1999
 48. Herr AB, Ballister ER, Bjorkman PJ: Insights into IgA-mediated immune responses from the crystal structures of human FcαRI and its complex with IgA1-Fc. *Nature* 423: 614–620, 2003
 49. Gomes MM, Wall SB, Takahashi K, Novak J, Renfrow MB, Herr AB: Analysis of IgA1 N-glycosylation and its contribution to FcαRI binding. *Biochemistry* 47: 11285–11299, 2008
 50. Suzuki H, Moldoveanu Z, Hall S, Brown R, Vu HL, Novak L, Julian BA, Tomana M, Wyatt RJ, Edberg JE, Alarcón GS, Kimberly RP, Tomino Y, Mestecky J, Novak J: IgA1-secreting cell lines from patients with IgA nephropathy produce aberrantly glycosylated IgA1. *J Clin Invest* 118: 629–639, 2008
 51. Ju T, Cummings RD: Protein glycosylation: Chaperone mutation in Tn syndrome. *Nature* 437: 1252, 2005
 52. Iwasaki H, Zhang Y, Tachibana K, Gotoh M, Kikuchi N, Kwon YD, Togayachi A, Kudo T, Kubota T, Narimatsu H: Initiation of O-glycan synthesis in IgA1 hinge region is determined by a single enzyme, UDP-N-acetyl-α-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 2. *J Biol Chem* 278: 5613–5621, 2003
 53. Raska M, Moldoveanu Z, Suzuki H, Brown R, Kulhavy R, Hall S, Vu HL, Carlsson F, Lindahl G, Tomana M, Julian BA, Wyatt RJ, Mestecky J, Novak J: Identification and characterization of CMP-NeuAc:GalNAc-IgA1 α2,6-sialyltransferase in IgA1-producing cells. *J Mol Biol* 369: 69–78, 2007
 54. Ju T, Brewer K, D'Souza A, Cummings RD, Canfield WM: Cloning and expression of human core 1 β1,3-galactosyltransferase. *J Biol Chem* 277: 178–186, 2002
 55. Julian BA, Novak J: IgA nephropathy: An update. *Current Opin Nephrol Hypertens* 13: 171–179, 2004
 56. Mestecky J, Tomana M, Moldoveanu Z, Julian BA, Suzuki H, Matousovich K, Renfrow MB, Novak L, Wyatt RJ, Novak J: The role of aberrant glycosylation of IgA1 molecules in the pathogenesis of IgA nephropathy. *Kidney Blood Pres Res* 31: 29–37, 2008