Anna Popova

ORCID 0000-0002-3916-4040

The Role of B-Cell Differentiation and Gut Microbiome in the Pathogenesis and Progression of IgA Nephropathy

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Supervisors of the Doctoral Thesis:

Dr. med., Associate Professor **Viktorija Kuzema,**
Rīga Stradiņš University, Latvia

Dr. med., Professor **Juta Kroiča,**
Rīga Stradiņš University, Latvia

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Abstract

Immunoglobulin A nephropathy (IgAN) is the most prevalent primary glomerular disease in numerous countries and continues to be a significant contributor to chronic kidney disease and end-stage kidney disease (ESKD). The clinical course can vary, but on average 30–40 % of patients reach ESKD within 20–30 years of diagnosis, and the disease may recur in the kidney graft. As the prognosis of this disease varies depending on its clinical presentation, the International IgAN Prediction Tool (IIgANPT) was developed in 2019 to integrate clinicopathological prognostic factors at the time of diagnosis and generate an individualised risk of disease progression. According to existing evidence, IgAN is believed to result from a combination of various pathogenic factors rather than a singular cause.

This study was conducted to evaluate the risk of disease progression in IgAN patients and to analyse B-cell differentiation and the gut microbiome as potential factors in the pathogenesis of the disease.

During a median follow-up of 18 months, 14.7 % of IgAN patients progressed to ESKD, corresponding to an incidence rate of 0.11 episodes per patient-year. Gender, MEST-T score and increased diastolic blood pressure were identified as significant risk factors for reduced kidney survival.

We carried out flow cytometry analysis of peripheral blood B cells in IgAN patients and healthy controls (HC) to investigate how B cells are activated to produce pathogenic IgA. Expansion of naïve and reduction in memory B cells was seen in IgAN patients with an increased frequency of IgA-expressing B cells that lacked the classical memory marker CD27, but were $CD21^{+}$. IgAN patients furthermore had an expanded population of IgA^{+} antibody-secreting cells, which correlated with serum IgA levels. Both IgA^{+} plasmabalsts and $CD27^{-}$ B cells co-expressed galactose-deficient IgA1 (Gd-IgA1). Implicating dysregulation at mucosal surfaces as the driver of such B cell differentiation, we found a correlation between lipopolysaccharide in the serum and $IgA^{+}CD27^{-}$ B cell frequency.

Metagenomic analysis and functional profiling of the gut microbiome were performed in IgAN patients categorised either as progressors (defined by an estimated glomerular filtration rate decline > 5 ml/min/1.73 m²/year), or non-progressors, and HC. Compared to HC, IgAN patients showed a reduced abundance of butyrate-producing bacteria. In addition, pathways related to nucleotide and nucleoside biosynthesis were more pronounced in IgAN patients, suggesting underlying immune activation and inflammation. Variations in these metabolic pathways were, in part, explained by Gd-IgA1 levels. Notably, the progressor group exhibited functional microbial changes linked to the stabilisation of bacterial cell membranes.

Keywords: immunoglobulin A nephropathy; kidney survival; B cell differentiation; microbiome characteristics.

Anotācija

Imūnglobulīna A nefropātijas progresijas risks: B šūnu diferenciācijas un zarnu mikrobioma nozīme slimības attīstībā

Imunglobulīna A nefropātija (IgAN) ir biežākais no primārajiem glomerulonefrītiem visā pasaulē, un tā joprojām ir nozīmīgs hroniskas nieru slimības un terminālas nieru mazspējas iemesls. Klīniskā gaita var būt variabla, bet vidēji 30–40 % pacientu attīstās termināla nieru mazspēja 20–30 gadu laikā pēc diagnozes uzstādīšanas, kā arī slimība var recidivēt nieres transplantātā. Tā kā šīs slimības prognoze atšķiras atkarībā no tās klīniskām izpausmēm, 2019. gadā tika izstrādāts Starptautiskais IgAN prognozes kalkulators (IIgANPT), lai integrētu klīniskos un histoloģiskos datus individuālas prognozes noteikšanai diagnozes apstiprināšanas brīdī. Atbilstoši pašreizējiem pierādījumiem uzskata, ka IgAN attīstībā iesaistīti vairāki faktori, nevis tikai viens konkrēts cēlonis.

Šis pētījums tika veikts, lai novērtētu slimības progresijas risku pacientiem ar IgAN un analizētu B šūnu diferenciāciju un zarnu mikrobioma līdzsvaru kā iespējamos faktorus slimības patoģenēzē.

IgAN pacientu novērošanas periodā, kura mediāna bija 18 mēneši, 14.7 % pacientu nieru funkcija pasliktinājās līdz terminālai nieru mazspējai, kas atbilst incidencei 0,11 epizodes uz pacientgadu. Dzimums, MEST-T rādītājs un paaugstināts diastoliskais asinsspiediens tika identificēti kā nozīmīgi riska faktori samazinātai nieru dzīvildzei.

Lai izpētītu B šūnu aktivācijas mehānismus patogēnās IgA sintēzē, IgAN pacientiem un veseliem indivīdiem (HC) tika veikta perifēro B šūnu subtipu analīze, izmantojot plūsmas citometriju. IgAN pacientiem tika novērots naivo B šūnu skaita pieaugums un atmiņas B šūnu skaita samazinājums. Turklāt tika konstatēts paaugstināts IgA-ekspresējošo B šūnu skaits, kurām nebija klasiskā atmiņas marķiera CD27, bet bija pozitīva CD21 ekspresija. Tāpat pacientiem bija palielināta IgA+ antivielas izdalošo šūnu populācija, kas korelēja ar paaugstinātu IgA līmeni serumā. Gan IgA^{+} plazmablasti, gan $CD27^{-}$ B šūnas ekspresēja galaktozes nepietiekamo IgA1 (Gd-IgA1). Tika novērota korelācija starp lipopolisaharīdu līmeni serumā un $IgA^{+}CD27^{-}$ B šūnu biežumu, kas norāda uz iespējamu B šūnu diferenciācijas saistību ar zarnu gļotādas barjeras traucējumiem.

Pacientiem ar IgAN un kontroles grupai tika veikta metagenomiskā analīze un zarnu mikrobioma funkcionālā profilēšana. IgAN pacienti tika iedalīti pacientu grupā ar progresējošu slimības gaitu (definēti kā pacienti ar aprēķinātā glomerulārās filtrācijas ātruma samazinājumu > 5 ml/min/1,73 m²/gadā) un pacientu grupā ar neprogresējošu slimības gaitu. Salīdzinājumā

ar HC, IgAN pacientiem tika novērota samazināta sviestskābi producējošo baktēriju izplatība. Turklāt IgAN pacientiem novērota pastiprināta aktivitāte vielmaiņas ceļos, kas saistīti ar nukleotīdu un nukleozīdu biosintēzi, liecinot par iespējamu imūnās sistēmas aktivāciju un iekaisuma reakciju. Pacientu grupā ar progresējošu slimības gaitu tika novērotas funkcionālas mikrobioma izmaiņas, kas saistītas ar baktēriju šūnu membrānu stabilizāciju. Daļa šo metabolo ceļu izmaiņu korelēja ar paaugstinātu Gd-IgA1 līmeni.

Atslēgvārdi: imūnglobulīna A nefropātija; nieru dzīvildze; B šūnu diferenciācija; mikrobioma raksturojums.

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Abbreviations used in the Thesis

ACEI	angiotensin-converting enzyme inhibitor
ANCA	antineutrophil cytoplasmic antibodies
APRIL	anti-A proliferation-inducing ligand
ARB	angiotensin II receptor blocker
ASC	antibody-secreting cells
BAFF	B-cell activating factor
BMI	body mass index
C	crescents
C1GalT1	core 1 β1,3-galactosyltransferase
CFHR3,1-del	deletion in FH-related genes 1 and 3
CICs	circulating immune complexes
CKD	chronic kidney disease
DN	double negative
DNA	deoxyribonucleic acid
E	endocapillary hypercellularity
EBV	Epstein-Barr virus
EDTA	ethylenediaminetetraacetic acid
eGFR	estimated glomerular filtration rate
EMA	European Medicine Agency
ESKD	end-stage kidney disease
ET1	endothelin 1
FDA	Food and Drug Administration
FDR	false discovery rate
FH	factor H
FI	factor I
Gal	galactose
GalNAc	N-acetylgalactosamine
GalNAc-T2	UDP-N-acetylgalactosaminyltransferase 2
GALT	gut-associated lymphoid tissue
Gd-IgA1	galactose-deficient IgA1
HC	healthy controls
IBD	inflammatory bowel disease
ICC	intraclass correlation coefficient
IgAN	immunoglobulin A nephropathy
IgAV	IgA vasculitis

IIgANPT	International IgAN Prediction Tool
KDIGO	Kidney Disease: Improving Global Outcomes
LPS	serum lipopolysaccharide
M	mesangial hypercellularity
MALT	mucosa-associated lymphoid tissue
MBL	mannose-binding lectin
MCD	minimal change disease
mIgA	monomeric IgA
miRNA	microRNA
MMF	mycophenolate mofetil
NeuAc	N-acetylneuraminic acid
PBMC	peripheral blood mononuclear cell
PBS	phosphate-buffered solution
pIgA	polymeric IgA
RASi	renin-angiotensin system inhibition
RCTs	randomised controlled trials
S	segmental glomerulosclerosis
SGLT2i	sodium-glucose cotransporter 2 inhibitors
ST3Gal1	α2,3-sialyltransferase
ST6GalNAc2	α2,6-sialyltransferase
T	tubular atrophy
TfR1	transferrin receptor-1
TG2	transglutaminase 2
TLR	Toll-like receptors
TNF	tumor necrosis factor
Tregs	regulatory T cells
TRF-budesonide	targeted-release formulation of budesonide
UPCR	urinary protein-to-creatinine ratio

Introduction

French pathologist named Dr. Jean Berger, along with his colleague Dr. Nicole Hinglais in 1968 initially defined immunoglobulin A nephropathy (IgAN) as a kidney disorder marked by glomerular "intercapillary deposits of IgA-IgG" (Berger & Hinglais, 1968). The renal biopsy typically reveals predominant IgA glomerular deposits performing immunofluorescence, often accompanied by mesangial cell proliferation and matrix expansion. IgAN is the most prevalent primary glomerular disease in numerous countries and continues to be a significant contributor to chronic kidney disease (CKD) and end-stage kidney disease (ESKD) (Rajasekaran et al., 2021). Geographical variation in distribution and the galactose-deficient IgA1 (Gd-IgA1) inheritance trait highlight the potential influence of both environment and genetics on susceptibility to IgAN (Du et al., 2023).

IgAN clinically presents in diverse manifestations: episodes of macrohaematuria, microscopic changes in urinalysis, nephritic syndrome, nephrotic syndrome and rapidly progressive glomerulonephritis. The clinical course can vary, but on average 30–40 % of patients reach terminal kidney failure within 20–30 years of diagnosis, and the disease may recur in the kidney graft (Lai et al., 2016). As the prognosis of this disease varies according to its manifestation, the International IgAN Prediction Tool (IIgANPT) was investigated in 2019. It integrates validated clinicopathological prognostic factors with treatments received at the time of diagnosis to generate an individualised risk of disease progression, supporting patient counselling, and facilitating shared decision-making (Barbour et al., 2019). The IIgANPT has undergone external validation across different ethnic groups, producing varying results. Until 2021, it had only been validated in non-European cohorts: Chinese-Argentinian (Zhang et al., 2020) and Korean (Hwang et al., 2021).

According to existing evidence, IgA nephropathy is believed to result from a combination of various pathogenic factors rather than a singular cause. The increased presence of circulating polymeric Gd-IgA1 and the generation of O-glycan-specific antibodies contribute to the development of immune complexes containing IgA1. The subsequent deposition of these complexes in the mesangium triggers inflammation and leads to glomerular damage (Yeo et al., 2018). B cells play a central role in the pathogenesis by serving as the origin of Gd-IgA1 and producing autoantibodies against it (Scionti et al., 2022). However, significant gaps exist in our understanding of the activation and differentiation pathways of B cells that result in the secretion of pathogenic IgA antibodies in IgAN. Individual reports have delineated specific aspects of B cell activation, including a decrease in regulatory B cells (Wang et al., 2014), reduced proportion of pre-switched B cells and plasmablasts, an increase in long-lived plasma cells in the peripheral circulation (Sendic et al., 2021) and heightened expression of toll-like

receptor 7 (TLR) in circulating B cells (Zheng et al., 2020). However, the exploration of B cell activation pathways remains unexplored and awaits incorporation into the overall understanding of the etiopathogenesis of IgAN.

Increasing evidence establishes a significant connection between the gut mucosal system and IgAN. In line with this, genome-wide association studies have pinpointed numerous risk alleles for IgAN that also correlate with the immune response against intestinal pathogens, IgA synthesis within the gut, integrity of the intestinal epithelial barrier, and inflammatory bowel disease (Rehnberg et al., 2021). Interestingly, a cross-sectional study revealed variations in the gut microbiota profile among individuals with progressive IgAN (Kiryluk et al., 2014). While the sensitivity of the gut to diverse mucosal antigens has been documented in IgAN, there is currently no definitive evidence supporting the clinical efficacy of any specific dietary modification (Pei & Guo, 2025). However, there is a proven effect of the targeted-release formulation of budesonide, which releases the active drug at the distal ileum, specifically targeting the Peyer's patches where IgA, and particularly Gd-IgA1, is primarily produced (Barratt, Lafayette, Kristensen, et al., 2023).

Aim of the Thesis

The study was conducted to evaluate the risk of disease progression in patients with IgAN and to analyse B-cell differentiation and the gut microbiome as potential factors in the pathogenesis of the disease.

Objectives of the Thesis

1 To evaluate kidney survival in patients with histologically confirmed IgAN by calculating the risk of a 50 % decline in renal function or progression to ESKD over five years.
2 To compare these outcomes with risk predictions using IIgANPT.
3 To investigate alterations in B-cell differentiation in IgAN patients.
4 To compare the composition and functional pathways of the gut microbiome in IgAN patients and healthy individuals.

Hypotheses of the Thesis

1 IIgANPT accurately predicts the risk of disease progression in patients with IgAN and can be effectively applied in the Latvian population.
2 Alterations in B cell differentiation and gut microbiome composition contribute to the pathogenesis of IgA nephropathy.

Novelty of the Thesis

At present, the diagnosis of IgAN requires kidney biopsy and immunofluorescence analysis of the kidney tissue. In Latvia, the ability to diagnose this disease has been available since 2013. It is well recognised that the distribution and clinical presentation of IgAN vary across regions and ethnicities. Prior to this study, no published data existed on the course, clinical manifestations, or prognosis of glomerulonephritis specific to the Latvian population. Following the publication of the IIgANPT, which has since been validated in various countries, we evaluated its applicability in our Latvian patient cohort.

The mechanisms responsible for mesangial IgA1 deposition and subsequent renal injury remain incompletely understood. Searching for possible agents involved in the pathogenesis, our study group uncovered the previously unknown B cell activation pathway that is associated with pathogenic IgA secretion – differentiation of IgA-expressing plasmablasts via a CD21+ B cell intermediate, that lacks the classical memory B cell marker CD27, and is enhanced in patients with IgAN. $IgA^{+}CD27^{-}CD21^{+}$ B cell frequency correlates with serum lipopolysaccharide (LPS) levels, implicating mucosa in their activation. This pathway holds potential for further investigation to identify biomarkers and therapeutic targets in IgAN. These findings can be integrated into the multi-hit pathogenesis model of IgAN.

According to previous studies on gut microbiome changes in IgAN patients, no consistent significant variations in the abundance of specific bacteria have been reported. In our study, butyrate-producing bacteria (*Butyrococcus* and *Agathobacter rectalis*) were less abundant in IgAN patients than in healthy controls, along with a reduced presence of the sulfoquinovose degradation I pathway, in which these bacteria are involved. These findings suggest a potential avenue for further research – specifically, an interventional study to evaluate the effects of butyrate supplementation in IgAN.

When examining microbial community function, we observed increased biosynthesis of nucleosides (adenosine, guanosine, and inosine) in IgAN patients, suggesting a possible link to intestinal inflammation and barrier dysfunction. Gd-IgA1 accounted for a substantial portion of the observed variations in metabolic pathways. Notably, previous studies have not investigated metabolic pathway differences between IgAN progressors and non-progressors. In our study, pathways enriched in patients with progressive kidney function decline were associated with bacterial phospholipid synthesis, suggesting an adaptive process to enhance bacterial survival.

Personal contribution

The author personally organised patient recruitment according to the inclusion and exclusion criteria and informed all participants about the study; performed physical examinations and provided consultations for IgAN patients and healthy controls. The author

ensured proper collection, processing, and storage of biological material for further analysis, conducted statistical analyses, interpreted research findings, wrote scientific publications, and presented the results at national and international conferences.

1 Literature review

1.1 Epidemiology of immunoglobulin A nephropathy

Immunoglobulin A nephropathy (IgAN) is the most common primary glomerulonephritis worldwide, with significant differences in prevalence and clinical phenotypes in different populations (Zhang et al., 2022). Prevalence of the disease is lower in the United States (10–20 % of primary glomerulonephritis), higher in some European countries (20–30 %) and the highest in the developed Asian countries (40–50 %) (Woo et al., 2010). According to a recent epidemiological study across ten European countries, IgAN point prevalence is 2.53 per 10 000, ranging from 1.14 per 10 000 in Spain to 5.98 per 10 000 in Lithuania (Willey et al., 2023). The incidence of IgAN, based on kidney biopsy results over the past 10 years in Latvia, is the highest – 13.2 cases per million population per year, followed by rapidly progressive glomerulonephritis (8.4 cases) and membranoproliferative glomerulonephritis (4.3). For comparison, the incidence of IgAN is higher in countries with systematic population-wide urine screening programs, such as Japan (45 cases per million population per year) and Singapore (18 cases) (Schena & Nistor, 2018). Patients can exhibit symptoms at any age; however, there is a notable peak in incidence during the second and third decades of life. There is noted different male-to-female ratio among different ethnic populations: approximately a 2–3:1 in North American cohorts, while in Asia the sexes are equally affected (Yeo et al., 2019).

Epidemiologic variations can be attributed to several more factors: the lack of widespread urinalysis screening in Western countries; underestimation by general practitioners in Western nations of persistent microscopic haematuria and/or mild proteinuria in seemingly healthy individuals, resulting in delayed referrals to nephrologists; possibility in different nephrology centres to perform kidney biopsy in individuals with persistent urinary abnormalities (Schena & Nistor, 2018).

1.2 Pathogenesis of immunoglobulin A nephropathy

The majority of IgAN cases seem to occur randomly, but there are well-documented instances of familial IgAN in certain families. The initial successful use of genome-wide linkage analysis in disease affected families revealed a notable linkage peak on chromosome 6p22-23, consistent with an autosomal dominant inheritance pattern (Gharavi et al., 2000). Genetically determined factors play a role in the development of IgAN, even in cases that initially appear to be sporadic (Magistroni et al., 2015). Large genome-wide association study of 10 146 kidney-biopsy-diagnosed IgAN cases and 28 751 controls across 17 international cohorts defined 30 genome-wide significant risk loci that explain 11 % of disease risk. There is

a positive genetic correlation observed between IgAN and serum IgA levels. A higher polygenic score for IgAN is linked to an earlier onset of kidney failure (Kiryluk et al., 2023).

The prevailing understanding of IgAN pathogenesis is based on multiple “hits” model. These include an elevation in the levels of circulating poorly O-galactosylated IgA1, the production of specific IgG autoantibodies targeting this particular form of IgA1, the formation of IgA1-immune complexes, and their deposition in the glomerular mesangium, leading to renal damage (Scionti et al., 2022).

1.2.1 IgA1 O-Galactosylation

In humans and higher primates, there are two subclasses of IgA, namely IgA1 and IgA2, constituting approximately 84 % and 16 % of the total circulatory IgA, respectively (Woof & Russell, 2011). Production of circulatory IgA is mainly derived from B cells in bone marrow. The distribution of these subclasses shows significant variation across various external secretions. Typically, secretions from the upper respiratory and digestive tracts contain a higher proportion of IgA1 compared to IgA2. In contrast, secretions from the large intestine and female genital tract have a slightly higher abundance of IgA2 compared to IgA1 (Woof & Mestecky, 2005). IgA1 and IgA2 exhibit a difference wherein IgA1 possesses an elongated hinge region comprised of 18 amino acids.

Both subclasses can exist in two structural forms: monomeric IgA (mIgA) and polymeric IgA (pIgA). The pIgA variant includes a joining chain, a protein with a molecular weight of about 17 kDa. This joining chain forms disulfide bridges with cysteine residues located at the tail piece of the α heavy chain within the Fc region, thereby facilitating the linkage of two IgA monomers (Woof & Russell, 2011). The predominant composition of serum IgA is in the monomeric form. However, in IgAN there is an elevated presence of circulating IgA1 in a polymeric state, characterised by the absence of terminal galactose residues in its hinge region. This extended hinge region is situated between the first and second constant domains of the α1 heavy chain, which is variably glycosylated (Figure 1.1). Out of the nine potential glycosylation residues, six (highlighted in red) can undergo incremental elongation through the sequential addition of monosaccharides containing terminal galactose (Gal) to amino acids residues in O-linkage. The O-glycosylation process of IgA1 hinge regions commences with the addition of N-acetylgalactosamine (GalNAc) to serine or threonine by UDP-N-acetylgalactosaminyltransferase 2 (GalNAc-T2), forming Tn antigen (Iwasaki et al., 2003). Subsequently, the introduction of Gal is facilitated by core 1 β1,3-galactosyltransferase (C1GalT1). The stability of the C1GalT1 protein is reliant on its interaction with a specific molecular chaperone, Cosmc. Without Cosmc, the C1GalT1 protein undergoes rapid

degradation, rendering the attachment of Gal to GalNAc impossible (Ju & Cummings, 2005). Additionally, α2,3-sialyltransferase (ST3Gal1) and α2,6-sialyltransferase (ST6GalNAc2) transfer N-acetylneuraminic acid (NeuAc) to Gal and/or GalNAc (ST and STn antigens). If NeuAc is added to GalNAc before Gal attachment, this premature sialylation prevents subsequent addition of a Gal residue (Scionti et al., 2022; Suzuki et al., 2014).

Gd-IgA1 primarily are generated at mucosal surfaces in the respiratory and gut regions, specifically within the mucosa-associated lymphoid tissue (MALT). Key MALT locations linked to the pathogenesis IgAN include the tonsils and the gut, known as the gut-associated lymphoid tissue (GALT) (Knoppova et al., 2016).

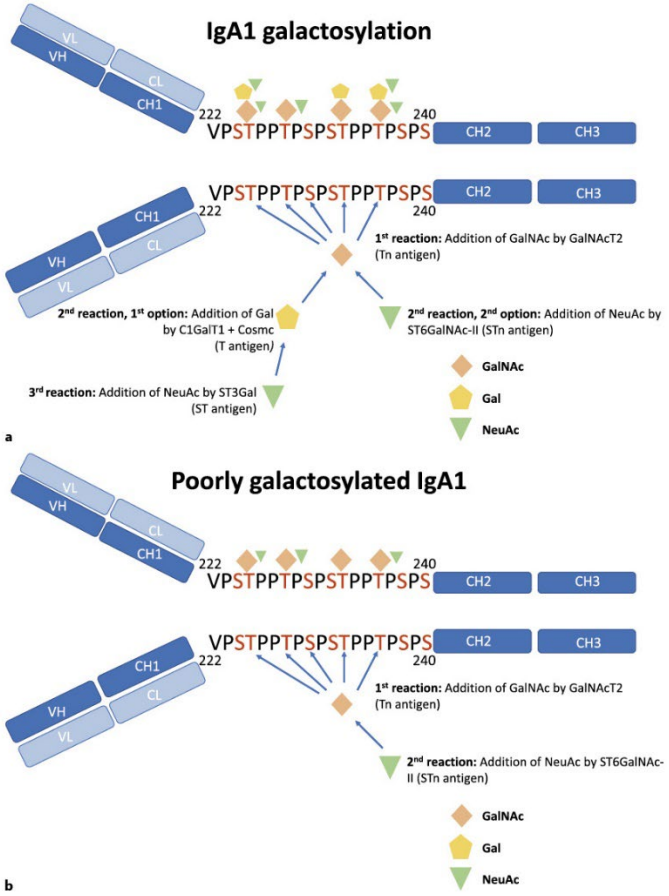

Figure 1.1 **IgA1 galactosylation** (Scionti et al., 2022)

1.2.2 Formation of autoantibodies

The hinge region of IgA1, lacking galactose, includes GalNac residues with or without terminal sialic acid. These IgA1 can be detected by lectins, such as *Helix aspersa* agglutinin, a lectin specific for terminal GalNAc. Levels of serum IgG specific for Gd-IgA1 are higher in IgAN patients comparing to placebo group and correlate with proteinuria (Suzuki et al., 2009). The majority (around 90 %) of individuals with IgAN exhibit heightened levels of these antibodies in their serum, that are also associated with renal histological grading. Although

Gd-IgA1 specific IgG are noticed in other origin CKD individuals, with a focus on those affected by immune-mediated glomerular diseases, reaching up to 25 % (Yanagawa et al., 2014).

Certain bacteria and viruses (*Streptococcus pneumoniae, Haemophilus influenzae, Actinomyces naeslundii* and others) display GalNac on their surfaces (Brockhausen et al., 2009), encountering these pathogens might serve as a stimulus for the generation of cross-reacting anti-glycan antibodies. Toll-like receptors (TLRs) are a class of proteins that have an ability to recognise numerous pathogens and initiate immune responses against them (Gao et al., 2017). Microbial antigens from external sources appear to trigger the activation of TLR9 in the mucosa. This activation, in turn, stimulates B cells in the tonsils, ultimately resulting in the production of nephritogenic Gd-IgA1 (Suzuki et al., 2011).

1.2.3 Circulating immune complexes

Increased concentrations of circulating immune complexes (CICs) that consist of inadequately galactosylated IgA1 are frequently observed in individuals diagnosed with IgAN. In laboratory settings, it has been demonstrated that both polymeric IgA1 and immune complexes formed by IgG-IgA1 stimulate human mesangial cell proliferation, whereas monomeric IgA1 does not exhibit the same effect (Novak et al., 2002). Cellular proliferation is induced by CICs of significant size (> 800 kDa), while smaller complexes can inhibit it (Novak et al., 2011). Not only IgG, but also IgA, IgM, and complement fractions are present in CICs (Nakamura et al., 2002). The presence of CICs containing polymeric IgA1 triggers the cleavage of the extracellular domain of FcaR (CD89), leading to the formation of an IgA1-CD89 complex. Animal experiments revealed that mice expressing both human IgA1 and CD89 exhibited the presence of IgA1–sCD89 complexes in both circulation and mesangial deposits, leading to kidney inflammation, haematuria, and proteinuria. In contrast, mice expressing IgA1 alone showed endocapillary IgA1 deposition without mesangial injury (Berthelot et al., 2012). The injection of recombinant soluble CD89 resulted in significant glomerular proliferation and proteinuria in mice that express human IgA1. Soluble CD89 was studied in humans as well. Levels of circulating IgA complexes (soluble CD89-IgA and IgG-IgA) and free soluble CD89 were markedly increased in children with IgAN. Biopsies revealed the presence of mesangial deposits containing soluble CD89 (Cambier et al., 2022).

1.2.4 Deposition of circulating immune complexes

Upon deposition, immune complexes containing IgA1 stimulate mesangial proliferation, release of angiotensin II and the local synthesis of cytokines, including interleukin-6, tumor necrosis factor (TNF) alpha, tumor growth factor beta, macrophage migration inhibitory factor (Chan et al., 2005; Lai et al., 2008; Lai et al., 2003). These molecules

play a role in fostering inflammatory responses by attracting leukocytes and contributing to both glomerular and tubulointerstitial fibrosis. Additionally, the complement system amplifies glomerular inflammation. Immunohistochemical observations of deposits such as complement component 3 (C3), properdin, C4d, mannose-binding lectin (MBL), and C5b-9 in the mesangium of IgAN biopsy samples, along with the notable absence of C1q, provide confirmation of the activation of the alternative and lectin pathways, as opposed to the classical pathway (Rizk et al., 2019).

Indications of lectin pathway activation are reinforced by the detection of glomerular staining for C4d in approximately 40 % of IgAN biopsies, when C1q is not present (Espinosa et al., 2014). In vitro, polymeric IgA has the ability to bind MBL in a Ca2+-dependent manner, likely facilitated by the N-linked glycans (Arnold et al., 2006). Additionally, there is reported mesangial deposition of MBL, L-ficolin and MBL-associated serine proteases in 25 % of IgAN biopsies (Roos et al., 2006).

In the majority of individuals with IgAN, C3 is deposited concomitantly with mesangial IgA1. Activation of the complement system via the alternative pathway results in the build-up of proteolytic fragments of C3, such as iC3b and C3d, induced by complement receptor 1, as well as the deficiency of factor I (FI) and factor H (FH) (Maillard et al., 2015). FH and FI serve as a plentiful regulator of complement activation, safeguarding host cells from self-attack by the complement system. FH facilitates the proteolytic degradation of C3b by serving as a binding platform for the FI, while concurrently stabilising the overall domain arrangement of C3b (Wu et al., 2009). Data from genome-wide association studies supports evidence of alternative pathway activation. The inheritance of a shared deletion in FH-related genes 1 and 3 (CFHR3,1-del) imparts additional protection against IgAN. Current data indicates an association between CFHR3,1-del and elevated circulating FH levels, as well as decreased levels of complement activation split products. Elevated FH levels also exhibit a positive correlation with circulating C3 and a negative correlation with mesangial C3 deposition (Zhu et al., 2015).

Activation of mesangial cells results in their proliferation, expansion of the matrix, and subsequent indirect injury and apoptosis of podocytes. This, in turn, exacerbates interstitial damage by enhancing the activation of tubular epithelial cells through increased TNF synthesis. Furthermore, aldosterone, released from mesangial cells subsequent to the deposition of IgA1 immune complexes, collaborates with angiotensin II to induce apoptosis in renal tubular epithelial cells. The angiotensin II derived from the mesangium sustains the tubulointerstitial injury (Lai, 2012).

1.2.5 Mucosal immune system

The gut microbiota, comprised of trillions of bacteria inhabiting the gut, plays a crucial role in the host's physiology and immunity (Sommer & Backhed, 2013). Mucosal secretory IgA is instrumental in the host-microbiota mutualism, as it actively excludes pathobionts and pathogens while limiting bacterial attachment to the epithelium (Mantis et al., 2011). Under homeostatic conditions, IgA is predominantly produced by plasma cells located in the lamina propria of the small intestine. Stimulation of the mucosal immune system can be prompted by environmental stimuli, including infectious pathogens, autoinflammatory diseases, and interactions with the existing mucosal microbiota (Haresh Selvaskandan, 2023). The modulation of secretory IgA is governed by the surrounding cytokines, generated by epithelial cells following TLR activation. Experimental findings indicate that the activation of mucosal immune cells through TLRs enhance the expression of B-cell activating factor (BAFF) and A Proliferation Inducing Ligand (APRIL), and facilitates the transformation of IgM+ B cells into IgA-producing plasma cells (Hand & Reboldi, 2021).

BAFF and APRIL can be produced by intestinal innate immune cells and epithelial cells and are necessary for B-cell maturation and survival. Multiple pieces of evidence support the involvement of APRIL in regulating IgA induction (He et al., 2007). Mice lacking APRIL and humans deficient in the APRIL receptor exhibited impaired IgA production (Castigli et al., 2004; Castigli et al., 2005). In genome-wide association studies of IgAN, TNFSF13 (the gene encoding APRIL) has been identified as a susceptibility locus. That correlated with elevated serum levels of IgA (Kiryluk et al., 2014). Mice with elevated BAFF expression exhibited increased levels of pIgA and displayed evidence of mesangial IgA deposition (McCarthy et al., 2011). In IgAN patients are observed elevated serum BAFF levels, that are linked to more severe renal histopathologic injury and lower eGFR (Xin et al., 2013). Genetic and epigenetic changes, including dysregulation of microRNAs in B-cells, have been demonstrated to influence the synthesis of Gd-IgA1. Therapies addressing these pathways are currently under investigation for their effectiveness in IgAN (Figure 1.2).

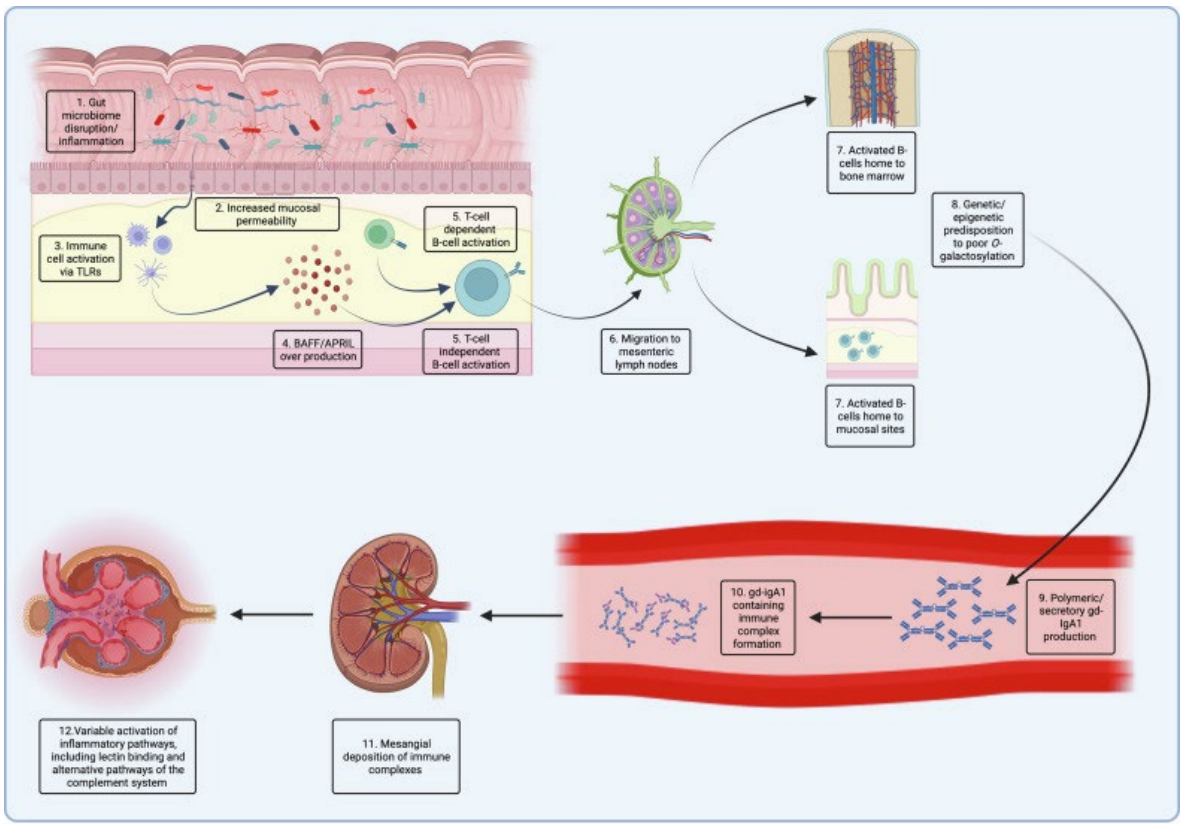

Figure 1.2 **An overview of the pathophysiology of IgAN** (Haresh Selvaskandan, 2023)

1.3 Clinical manifestations

The clinical manifestations of IgAN span a wide range, from asymptomatic microscopic haematuria to rapidly progressive glomerulonephritis and ESKD. The usual presentation mode varies based on age group and the practices employed in biopsy procedures.

Macroscopic haematuria can be the initial presentation of IgAN. The occurrence of visible blood in the urine typically happens during episodes of upper respiratory tract infections or, more rarely, after gastrointestinal tract infections. This situation is particularly distressing for patients. Haematuria can appear and persist 3–7 days after extensive exercise (Varma et al., 2014). This syndrome is observed in approximately 10–15 % of individuals, with a higher prevalence in patients under the age of 40 (D'Amico, 2004). Recurrent episodes of macroscopic haematuria are generally linked to a favourable short-term prognosis (Le et al., 2014).

Asymptomatic microscopic haematuria is seen in 70–100 % of patients, especially in children and young adults during the initial stages of their clinical progression (Coppo & Fervenza, 2017). Patients exhibiting microscopic haematuria more commonly presented active mesangial lesions with proliferative changes upon kidney biopsy. Nevertheless, subsequent development of **persistent proteinuria** is associated with the potential progression of the disease (Sevillano et al., 2017). Moderate proteinuria (1–3 g/day), haematuria with red cell casts, reduced kidney function, hypertension and oedema are signs of another IgAN manifestation – **nephritic syndrome** (Khanna, 2011).

Nephrotic syndrome is observed in 5–10 % of individuals with IgAN (Kim et al., 2012). Renal function is typically maintained, and overt nephrotic syndrome characterised by oedema, dyslipidaemia, and hypoalbuminemia is evident. Steroid responsiveness has been noted along with a positive outlook reminiscent of minimal change disease (MCD) lesions. In a particular group, 18 % out of 1368 individuals diagnosed with IgAN fulfilled the criteria of this form of the disease: the presence of diffuse IgA-dominant mesangial deposits, minimal changes in glomeruli as observed through light microscopy, and more than 50 % podocyte foot process effacement. Importantly, none of these patients progressed to ESKD, in contrast to 9.2 % of those with IgAN exhibiting other manifestations of the disease, over a median follow-up period of 2.6 years (Li et al., 2016). A comparable course of IgAN is observed in other studies, indicating a favourable clinical outcome likely attributable to minimal pathological changes (Qin et al., 2013). Serum Gd-IgA1 levels are lower in individuals with nephrotic syndrome compared to those with non-MCD-IgAN, but higher than in healthy participants. The deposition of Gd-IgA1 in the glomeruli of individuals with MCD-IgAN had a positive rate of only 13.7 %, contrasting with a significantly higher rate of 82.4 % in participants with non-MCD-IgAN (Guo et al., 2023).

Rapidly progressive IgAN, according to the Kidney Disease: Improving Global Outcomes (KDIGO) 2021 guidelines, is characterised by a ≥ 50 % decline in estimated glomerular filtration rate (eGFR) within a period of ≤ 3 months, after excluding other causes of rapidly progressive glomerulonephritis such as antineutrophil cytoplasmic antibodies (ANCA) -associated vasculitis, anti-glomerular basement membrane disease, and reversible factors like drug toxicity or common pre- and post-kidney disease causes. In such instances, a kidney biopsy is crucial and typically reveals increased cellularity in both mesangial and endocapillary regions, along with a significant percentage of glomeruli exhibiting crescents and focal necrotic areas (Kidney Disease: Improving Global Outcomes Glomerular Diseases Work, 2021). In one single centre study in China only 2.3 % (39/1677) of IgAN patients met mentioned KDIGO 2021 criteria, a quarter of them had ≥ 50 % crescents. Patients with rapidly progressive IgAN and ≥ 50 % crescents faced a greater risk of progressing to ESKD compared to those with other origin rapidly progressive glomerulonephritis and ≥ 50 % crescents (Yu et al., 2022).

1.4 Associated conditions

The incidence of secondary IgAN is not well established, particularly in comparison to primary IgAN. Other diseases may play a role in the development of IgAN due to changes in these pathogenesis components: exposure to antigen, synthesis of IgA, and the elimination of circulating IgA-containing immune complexes (Pouria & Barratt, 2008). Surprisingly, no

statistically significant differences were identified in plasma Gd-IgA1 or CIC levels between primary IgA nephropathy and secondary IgA nephropathy (Wang et al., 2020). Secondary IgAN can arise from various aetiologies, including liver, gastrointestinal, dermatological, oncological, autoimmune, and respiratory diseases, along with iatrogenic, infectious, and environmental factors (Saha et al., 2018).

Liver disease stands as the primary cause of secondary IgAN, with prevalence rates varying based on factors such as cohort size, the aetiology of liver disease, demographics, and criteria for kidney biopsy. In patients undergoing liver transplantation, IgAN has been detected in 9–25 % of cases upon kidney biopsy (Axelsen et al., 1995; Calmus et al., 2012). Liver serves as a primary site of catabolism of circulatory IgA. In patients with cirrhosis, hypergammaglobulinemia may arise due to elevated synthesis and/or reduced clearance of immunoglobulins. The liver's innate and adaptive immune systems actively engage in clearing pathogens, especially those originating from the gastrointestinal tract (Saha et al., 2018). Among liver diseases, those of alcoholic origin are notably widespread. But secondary IgAN was also described in Wilson`s disease, haemochromatosis, autoimmune hepatitis (Tota et al., 2023).

Gastrointestinal Diseases like celiac disease, inflammatory bowel disease, enterocolitis caused by *Clostridium difficile* has been linked to secondary IgAN.

Celiac disease is a chronic intolerance to gluten, marked by villous atrophy and indications of immunological activation in the lamina propria of the jejunum. In celiac disease, transglutaminase 2 (TG2) is an enzyme that oversees gliadin deamination and the retrotranscytosis of IgA, mediated by transferrin receptor-1 (TfR1) across the epithelial layer. Patients with celiac disease display elevated levels of circulating IgA and IgG antibodies directed against gliadin (a component of gluten), endomysium (a substrate of TG2), and TG2. The interaction between TG2 and TfR1 enhances the deposition of IgA1 in the mesangium, thereby promoting inflammation (Abbad et al., 2020).

Inflammatory bowel disease (IBD) is a condition marked by persistent inflammation of the gastrointestinal tract. Crohn's disease and ulcerative colitis are the two most common subtypes. IgAN is the most frequently identified finding on renal biopsy in individuals with IBD, followed by interstitial nephritis, arteriosclerosis, acute tubular injury, proliferative glomerulonephritis, and minimal-change disease (Ambruzs et al., 2014).

Other autoimmune diseases: Sjögren's syndrome, ankylosing spondylitis, Behcęt's disease, rheumatoid arthritis, psoriasis. In the majority of patients, the rheumatologic diagnosis either precede or is established concomitantly with the diagnosis of IgAN (Canetta, 2020). The pathomechanism connecting IgAN and one of the mentioned diseases remains unclear (Tota et al., 2023).

Regarding **viral infections**, human immunodeficiency virus, hepatitis B and C viruses, and Epstein-Barr virus (EBV) have been well-documented as triggers for secondary IgAN developpment. Additionally, there have been several case reports suggesting associations between hepatitis A and E viruses, and SARS-CoV-2 with IgAN (Tota et al., 2023). In a recent study, the role of the EBV in the pathogenesis of IgAN was investigated. Human B cells when infected with EBV *in vitro*, secreted Gd-IgA1. Upon polyclonal stimulation, these cells produced more Gd-IgA1 compared to cells from healthy controls. Surprisingly, in healthy African Americans, EBV was detected predominantly in surface IgM- and IgD-positive cells. This observation aligns with the fact that most African Blacks and African Americans acquire EBV within the first two years of birth, a period when the IgA system is naturally deficient, characterised by low serum IgA levels and a limited number of IgA-producing cells. Consequently, EBV tends to infect cells secreting immunoglobulins other than IgA during this early developmental stage. This study findings suggest that Epstein-Barr virus-infected IgA+ cells serve as the source of Gd-IgA1 and contribute to the expression of relevant homing receptors. Furthermore, the temporal sequence of racial-specific differences in EBV infection, in relation to the naturally delayed maturation of the IgA system, provides insights into the racial disparity observed in the prevalence of IgAN (Zachova et al., 2020).

1.5 The diagnosis of IgAN

The patient's history, clinical presentation, and laboratory results indicate a potential case of IgAN, but the definitive diagnosis is confirmed through renal biopsies, revealing diffuse IgA deposits in the mesangium by routine immunofluorescence microscopy (Roberts, 2014).

1.5.1 Pathology

Glomerular changes observed under light microscopy vary widely. These features include no or minimal abnormalities when examined under light microscopy, mesangial hypercellularity, focal endocapillary proliferative changes (involving $<$ 50 % of glomeruli), diffuse endocapillary proliferative changes (involving $\geqslant$50 % of glomeruli), necrotising and crescentic lesions, and, less commonly, patterns of injury resembling membranoproliferative glomerulonephritis. Red blood cell casts may be linked with acute tubular injury. In the chronic stages, there is a progression to focal or diffuse segmental and global glomerulosclerosis accompanied by tubular atrophy and interstitial fibrosis (Working Group of the International Ig, the Renal Pathology, Roberts, et al., 2009). The term 'minimal abnormality' is defined by the absence of hypercellularity, sclerosis, or hyalinosis, and its frequency ranges between 8–55 %, reflecting differences in biopsy practices. This condition is typically observed in renal biopsies from patients with isolated haematuria (Roberts, 2014).

Under electron microscopy, there is evidence of electron-dense material aligning with IgA immune complexes in the mesangial and paramesangial regions of the glomeruli. In some cases, additional deposits may be observed in the sub-endothelial and sub-epithelial areas of the glomerular basement membranes (Kimura et al., 1984).

Immunofluorescent microscopy may detect glomerular IgG in addition to IgA deposits. The prevalence of IgG positivity varies significantly among different cohorts, with reported frequencies ranging from 15 % to 85 % within published series (Roberts, 2014). C3 is found in over 90 % of biopsy samples, but typically C1q is absent (Medjeral-Thomas et al., 2021). Glomerular deposits of the complement degradation product C4d are observed in 20–55 % of IgA nephropathy biopsy samples (Barratt, Lafayette, Zhang, et al., 2023).

The pathological features of IgAN contain valuable prognostic information for healthcare providers. An international collaboration between nephrologists and nephropathologists introduced the Oxford Classification of IgAN, initially in 2009 with an update in 2016 (Table 1.1). This classification system provides a structured and reproducible scoring method for assessing the light-microscopy features observed in renal biopsy samples (Trimarchi et al., 2017; Working Group of the International Ig, the Renal Pathology, Roberts, et al., 2009).

Table 1.1

Updated Oxford classification of immunoglobulin A nephropathy

Histological Feature	Definition	Score
Mesangial hypercellularity	Percentage of glomeruli with > 3 mesangial cells per mesangial area	M0: ≤ 50 % M1: > 50 %
Endocapillary hypercellularity	Increased number of cells within glomerular capillary lumina causing narrowing of the lumina	E0: Absent E1: Present
Segmental glomerulosclerosis	Any amount of the glomerular tuft involved in sclerosis, but not involving the whole tuft, or the presence of an adhesion	S0: Absent S1: Present
Tubular atrophy / Interstitial fibrosis	Percentage of cortical area involved by tubular atrophy or interstitial fibrosis, whichever is greater	T0: 0–25 % of cortical area T1: 26–50 % of cortical area T2: > 50 % of cortical area
Cellular or fibrocellular crescent	Percentage of glomeruli with cellular or fibrocellular crescent	C0: Absent C1: < 25 % of glomeruli C2: ≥ 25 % of glomeruli

Focal or diffuse **mesangial hypercellularity** (M) is commonly observed in the majority of renal biopsies from patients with IgAN. This heightened mesangial cellularity often coincides with an accompanying increase in mesangial matrix, which may be disproportionate to the increase in cellularity.

Endocapillary hypercellularity (E), characterised by an elevated number of cells within glomerular capillaries leading to luminal narrowing, is present in approximately one-third of biopsy samples, usually in a focal pattern. This hypercellularity may indicate proliferation, inflammatory cell infiltration, or swelling of endothelial cells.

Segmental glomerulosclerosis (S), defined histologically as the occlusion of capillary lumina by extracellular matrix in a portion of a glomerulus, is a common observation in IgAN.

Tubular atrophy (T) stands out as the most dependable histological indicator of an unfavourable outcome. The tubulointerstitium may appear nearly normal in the early stages of the disease. Elevated levels of proteinuria are linked to the presence of protein resorption droplets in tubular epithelial cells and tubular injury. As disease progresses, tubular injury leads to a fibroproliferative peritubular response, infiltration of mononuclear inflammatory cells, and eventually the establishment of interstitial fibrosis and tubular atrophy.

Cellular crescents (C) are extracapillary proliferative lesions, characterised by the proliferation of cells in Bowman's space with more than two cell layers. These lesions are found in approximately one-third of IgA nephropathy biopsies. However, the percentage of glomeruli containing crescents is typically low (Roberts, 2014; Working Group of the International Ig, the Renal Pathology, Roberts, et al., 2009).

Validation studies conducted globally have revealed regional variations in histopathological findings as well. Active lesions like endocapillary hypercellularity (E) and crescents (C) are more commonly observed in Asian cohorts compared to those in Europe. In the Japanese cohort, E lesion was present in 42 % of cases, contrasting with 10–14 % in the European cohort. Similarly, C lesion was observed in 48 % of cases in the Chinese cohort and 63 % in the Japanese cohort, in contrast to only 17 % in the European cohort (Lee et al., 2023).

1.5.2 Diagnostic biomarkers

Biopsy, essential for confirming the diagnosis, is an invasive procedure requiring a hospital visit and carrying a risk of complications. Consequently, the exploration of non-invasive diagnostic markers is crucial for evaluating disease severity and progression.

Serum Gd-IgA1 levels stand out as promising candidates to potentially become diagnostic biomarkers for IgA nephropathy due to their pivotal role in the initial stages of the disease pathogenesis. They correlate with disease severity and can predict CKD progression (Kim et al., 2020; Zeng et al., 2023). However, it is not Gd-IgA1 but the normalised serum **IgG antiglycan autoantibody level** that indicates a higher risk of IgAN recurrence in transplant recipients (Berthoux et al., 2017).

Serum markers of complement activation have also been explored: diminished circulating levels of **C3** (likely reflecting more intense C3 deposition) have been associated with a decline in kidney function (Wu et al., 2021). However, combining Gd-IgA1 with C3 concentration may enhance its prognostic value. The plasma Gd-IgA1:C3 ratio has been identified as an independent marker of IgAN progression, particularly in Asian population (Chen et al., 2019). An elevated ratio is apparently an effective predictor of IgAN diagnosis, especially in patients with proteinuria ≤ 1 g/d (Gong et al., 2019).

MicroRNAs (miRNA) in urinary sediment have been explored as noninvasive biomarkers of IgAN. MiRNAs, ranging from 21 to 23 nucleotides in length, are short noncoding RNA molecules. They play a crucial role in regulating gene expression at the post-transcriptional level and are involved in diverse cellular functions, such as cellular proliferation, apoptosis, and differentiation (Szeto & Li, 2014). The urinary miR-106a has been found to be a potential liquid biomarker, that demonstrated 100 % sensitivity, but only 14.8 % specificity in detecting IgAN (Szeto et al., 2022). In another research study, 39 miRNAs were identified for the diagnosis of IgAN, and among them, urinary miR-204 exhibited the highest diagnostic accuracy. It showed 100 % sensitivity and 55 % specificity (Szeto et al., 2019). Furthermore, the expression of miR-204 correlated with established clinicopathological prognostic risk factors and exhibited distinct expression patterns in kidney tissue, showing increased interstitial expression in non-progressive IgAN (Pawluczyk et al., 2021).

1.5.3 Differential diagnosis

IgA vasculitis (IgAV), formerly known as Henoch-Schönlein purpura, is the most prevalent systemic vasculitis in children. The annual incidence of IgAV is 10–20 per 100 000 in children under 17 years of age, peaking at 4–6 years of age (Gardner-Medwin et al., 2002). Although adults of all ages may be affected, they account for only 10 % of all IgAV cases (Blanco et al., 1997). IgAV is characterised by the combination of cutaneous manifestations (palpable purpura), gastrointestinal symptoms (colicky pain, bloody stools), and joint involvement (arthralgia). More infrequently, there may be observed neurological, pulmonary, or urological involvement (Ozen et al., 2010). The long-term prognosis of the disease is contingent on the presence of renal impairment and its progression. From a histological standpoint, it is not possible to differentiate a glomerulonephritis as part of IgAV from IgAN (Pillebout, 2021).

Staphylococcus infection-associated glomerulonephritis is termed based on the apparent cause of infection, while it is referred to as immunoglobulin A-dominant deposition infection-related glomerulonephritis considering its histopathology. This form of

glomerulonephritis typically manifests as rapidly progressive glomerulonephritis or acute kidney injury, featuring diverse levels of proteinuria and microscopic haematuria in conjunction with an ongoing *S. epidermidis*, *S. aureus* or methicillin-resistant *S. aureus* infection. Renal pathology reveals several types of mesangial and/or endocapillary proliferative glomerulonephritis, varying degrees of crescent formation, and tubulointerstitial nephritis. Immunofluorescence examinations have detected IgA, IgG, and C3 staining in the mesangium and along the glomerular capillary walls (Takayasu et al., 2022).

1.6 Prognosis in IgAN

Some individuals with IgAN achieve sustained clinical remission characterised by normal renal function, normal urinalysis, and blood pressure. Despite this clinical improvement, subsequent renal biopsies typically show persistent glomerular IgA deposits (Hotta et al., 2002). However, in children, who experienced clinical remission and underwent a second biopsy, there was an observed improvement in glomerular changes under light microscopy, with the disappearance or reduction of IgA deposits in the mesangium (Yoshikawa et al., 1990).

Adverse outcomes, such as dialysis or death, have been associated with the simultaneous presence of three risk factors at the time of renal biopsy: proteinuria ≥ 1 g/day, sustained hypertension, and the severity of renal involvement determined by the revised Oxford Classification (Berthoux et al., 2011). The M, S, and T scores demonstrated independent predictive capabilities for the rate of GFR decline and the composite endpoint of either progression to ESKD or a 50 % decline in eGFR. This association was also observed with endocapillary hypercellularity in patients who had not undergone immunosuppression, indicating potential responsiveness to immunosuppressive therapy (Working Group of the International Ig, the Renal Pathology, Cattran, et al., 2009). In 2016, a fifth feature, glomerular crescents, was incorporated into the Oxford Classification following multiple studies suggesting its status as an independent predictor of renal outcomes (Trimarchi et al., 2017). The identification of crescents in 1–24 % of glomeruli indicates patients who are at risk of experiencing adverse renal outcomes if not subjected to immunosuppressive treatment (Haas et al., 2017).

The Original International IgAN Prediction Tool (IIgANPT) was published in 2019, based on a large international multiethnic cohort that included 3927 patients with biopsy-confirmed idiopathic IgAN. Using data from the derivation cohort, two models (one that included patient race/ethnicity, and one that did not) were developed to predict a primary composite outcome of a 50 % decline in eGFR or kidney failure (defined as eGFR < 15 ml/min/1.73 m^2 by CKD-EPI, dialysis or kidney transplantation) at the time of biopsy

(Barbour et al., 2019). The IIgANPT is awaiting validation as a tool for guiding treatment decisions. Nevertheless, the KDIGO Management of Glomerular Diseases 2021 guidelines recognise its value in assisting patient counselling and facilitating shared decision-making (Kidney Disease: Improving Global Outcomes Glomerular Diseases Work, 2021).

To perform the aforementioned calculation, the following patient clinical data are necessary: eGFR, systolic and diastolic arterial blood pressure, daily proteinuria, age, race, history of angiotensin-converting enzyme inhibitor (ACEI) or angiotensin II receptor blocker (ARB) use at the time of biopsy, MEST score of the renal biopsy according to the Oxford classification, use of immunosuppressive therapy at or before the time of biopsy, and the time period (usually between 12 and 60 months) over which we aim to determine the risk of progression of kidney injury. By inputting this clinical data, the calculator provides an estimate of the risk over a specified time period (e. g. 60 months) for eGFR to decline by 50 % or for the disease to progress to kidney failure (Barbour et al., 2019).

In 2021, the IIgANPT was updated for use in children (age < 18 years) with data collected from a multiethnic cohort recruited from Asia, Russia, Europe, and North America predicting the risk of a 30 % decline in eGFR or ESKD in children with IgAN (Barbour et al., 2021). Following additional external validation analyses, an updated Prediction Tool was published. This tool can be utilised for risk stratification one- or two-years post-biopsy, specifically for those patients who did not receive immunosuppressive treatment during this period (Barbour et al., 2022).

Initially considered a benign disease, it is now understood that IgAN leads to progressive renal disease in approximately 20–40 % of patients within 5 to 25 years after diagnosis, culminating in end-stage renal disease (Rychlik et al., 1999). In a single-centre study conducted in Japan, 50 % of the 1012 analysed patients progressed to ESKD within 30 years, despite receiving treatment (Moriyama et al., 2014).

1.7 Treatment of IgA nephropathy

Independently of the initial injury, renal disease progression is sustained by common mechanisms that, initiated by nephron loss, result in compensatory glomerular haemodynamic changes. Elevated intraglomerular capillary pressure and perfusion pressure cause mechanical damage to the three major cell types in the glomerulus – podocytes, endothelial cells, and mesangial cells – resulting in impaired selectivity of the glomerular capillary wall and excessive protein ultrafiltration (Hostetter, 1995). Certainly, glomerular capillary hypertension is frequently sustained by angiotensin-dependent mechanisms, achieved through heightened systemic blood pressure and constriction of the efferent arterioles. In addition to inducing

glomerular hypertension, angiotensin II has been proposed to directly contribute to progressive renal damage through various means, such as enhanced extracellular matrix deposition, immune activation, and the release of growth factors (Ma & Fogo, 2007).

1.7.1 Supportive care

The 2021 KDIGO guidelines recommend optimised supportive care as the initial management approach. This involves reducing proteinuria by optimising blood pressure and maximising single-agent renin-angiotensin system inhibition (RASi). There are no specific blood pressure targets specifically aimed at improving disease progression; however, is recommended a blood pressure target of < 120/80 mmHg for all glomerular diseases. Additionally, addressing lifestyle and cardiovascular risk factors, such as body weight, dietary salt restriction to < 2 g/day, smoking cessation, and lipid management, is emphasised. Apart from restricting dietary sodium, there is no evidence to support the effectiveness of any specific dietary intervention in influencing outcomes.

If the patient exhibits proteinuria exceeding 0.5 g/d, it is recommended to initiate therapy with either an **ACEI** or an **ARB**, regardless of whether hypertension is present (Kidney Disease: Improving Global Outcomes Glomerular Diseases Work, 2021). This recommendation is supported by randomised controlled clinical trials in IgAN, indicating a superior renal outcome in patients treated with enalapril compared to non-RASi antihypertensives, or benazepril compared to placebo (Coppo et al., 2007; Praga et al., 2003). The STOP-IgAN trial revealed no additional benefit with dual blockade and, in fact, observed higher levels of proteinuria throughout the 3-year trial phase (Lennartz et al., 2020).

The addition of **sodium-glucose cotransporter 2 inhibitors** (SGLT2i) to standard treatment has been suggested as a safe and effective approach for patients with IgAN. Renoprotective effects include influencing renal haemodynamics through the inhibition of sodium and glucose reabsorption in the proximal tubule. Natriuresis induces a reduction in preload and plasma volume, resulting in a modest decrease in blood pressure. Furthermore, the vasoconstriction of the afferent arteriole, mediated by tubuloglomerular negative feedback, leads to a decrease in intraglomerular pressure, glomerular hyperfiltration and proteinuria level (Heerspink et al., 2016). Drawing from the outcomes of the DAPA-CKD trial, it was observed that dapagliflozin mitigated albuminuria by 26 % and notably diminished the risk of major adverse kidney events by 71 % in patients with IgAN compared to the placebo group (Wheeler et al., 2021). In one recent study a significant reduction in proteinuria was observed, with a decrease of 22.9 % at three months and 27.1 % at six months after SGLT2i administration in IgAN patients receiving full dose of ACEI or ARB therapy (Dong et al., 2023).

In the EMPA-KIDNEY trial, 6609 patients with chronic kidney disease were enrolled, including 817 (12.4 %) with IgAN. Among IgAN patients receiving Empagliflozin 10 mg/day, the mean eGFR slope was –2.33, compared to –4.12 in the placebo group. This led to a relative reduction in the chronic rate of eGFR decline of 43 % (Group, 2024).

Sparsentan is an oral dual endothelin and angiotensin II receptor antagonist that was approved by the Food and Drug Administration (FDA) in 2023 for high-risk IgAN patients. Endothelin 1 (ET1) is a potent vasoconstricting agent produced by endothelial cells and others, in kidneys by glomerular epithelial, mesangial and medullar collecting duct cells (Dhaun et al., 2006). In the Phase 3 PROTECT trial, patients with IgAN exhibiting proteinuria of at least 1 g per day, despite maximal RASi for a minimum of 12 weeks, were randomly assigned to receive either sparsentan or irbesartan. The substantial reduction in proteinuria observed at 36 weeks was sustained throughout the study duration. After 2 years, the urinary protein-to-creatinine ratio (UPCR) was 40 % lower in the sparsentan group compared to the irbesartan group, with percentages of −42.8 % and −4.4 %, respectively (Rovin et al., 2023).

1.7.2 Immunosuppressive treatment

Glucocorticoid therapy despite decades of reports is still debated and controversial. Guidelines suggest that patients with eGFR > 30 ml/min/1.73 m^2 who persist at a high risk of progressive CKD, currently defined as proteinuria > 0.75–1 g/d in period over 90 days of optimised supportive care, should be evaluated for a 6-month course of glucocorticoid therapy. Additional indications for glucocorticoids include their use in cases of rapidly progressive IgAN, often in combination with cyclophosphamide. Glucocorticoids are also considered for IgAN cases that present with nephrotic syndrome and exhibit features resembling minimal change disease in both light and electron microscopy (Kidney Disease: Improving Global Outcomes Glomerular Diseases Work, 2021).

Multiple randomised controlled trials (RCTs) have assessed the impact of glucocorticoids in IgAN, yielding diverse outcomes. The two latest of them are:

- In the STOP-IgA trial after a 6-month of supportive care patients who had persistent proteinuria with urinary protein excretion of at least 0.75 g per day were randomly assigned to receive supportive care alone (supportive-care group) or supportive care plus immunosuppressive therapy (immunosuppression group) for 3 years. In the immunosuppression group, individuals with an eGFR > 60 ml/min were administered glucocorticoids, while those with an eGFR between 30–60 ml/min received prednisolone and cyclophosphamide for 3 months and azathioprine from 3 to 36 months. The introduction of immunosuppressive therapy to comprehensive

supportive care in high-risk IgAN patients did not lead to a significant improvement in outcomes. Over the 3-year study period, individuals receiving immunosuppressive therapy experienced more adverse effects, with no alteration in the rate of decline in the eGFR (Rauen et al., 2015). The rates of eGFR loss over 40 % and annual eGFR loss did not differ between groups over a follow-up of up to ten years (Rauen et al., 2020).

- The Therapeutic Evaluation of Steroids on IgA Nephropathy Global (TESTING) study was a multicentre, double-blind, randomised controlled clinical trial involving patients with IgAN, proteinuria exceeding 1 g/day despite 3 months of optimised RASi supportive care, and an eGFR between 20 and 120 ml/min per 1.73 m^2. Participants were randomly assigned to receive oral corticosteroids 0.6–0.8 mg/kg/day or a placebo for 6 months. The study was terminated prematurely following an interim analysis that revealed a higher risk of death with corticosteroid treatment, including two fatalities in the corticosteroid group compared to none in the placebo group. There appeared to be a benefit of corticosteroids, showing a significant reduction in the risk of a 40 % drop in eGFR or progression to ESKD. While the results suggested potential renal benefits, definitive conclusions regarding treatment benefits cannot be drawn due to the premature termination of the trial (Lv et al., 2017). Due to safety concerns, the TESTING study group modified the trial protocol for treatment with a reduced dose of methylprednisolone (0.4 mg/kg/day). Steroids demonstrated effectiveness in delaying major kidney events (ESKD) and improving kidney outcomes (eGFR and proteinuria decline) in IgAN, with benefits that seem to outweigh the slightly increased risk of steroid-related serious adverse events compared to the placebo (Lv et al., 2022).

The use of **mycophenolate mofetil** (MMF) is currently recommended only in Chinese population as a steroid-sparing agent (Kidney Disease: Improving Global Outcomes Glomerular Diseases Work, 2021). It is an antimetabolite that reduces the B- and T-cell proliferation and decreases antibody production. A Chinese trial comparing MMF with steroids showed greater reduction in proteinuria in the MMF group patients with protein excretion exceeding 2 g/day (Chen et al., 2002). Similar results demonstrating the benefits of the combination of MMF and steroids were recently published based on a retrospective analysis with a 6-year follow-up of IgAN patients at Mayo Clinic (USA) (Miao, 2024). After 12 months of treatment with MMF and RASi, there was a significantly reduced risk of disease progression compared with RASi alone in patients with progressive IgAN: 8.2 % and 27.1 %, respectively (Hou et al., 2023). However, placebo-controlled randomised studies conducted in Europe showed no beneficial effects of MMF (Maes et al., 2004).

The targeted-release formulation of **budesonide** (TRF-budesonide) is an oral variant of budesonide specifically engineered to release its contents at the terminal ileum. This design allows for localised delivery of the medication to Peyer's patches, the gut-associated lymphoid tissue, recognised as a key site for IgA production. After absorption, roughly 70 % of the active compounds in TRF-budesonide are released in the distal ileum and proximal colon. These compounds undergo initial metabolism in the liver through the cytochrome P450 isoenzyme CYP3A4/CYP3A5, resulting in the production of compounds with low glucocorticoid activity (16α-hydroxyprednisone and 6-β -hydroxybudesonide) (Smerud et al., 2011). FDA and European Medicine Agency (EMA) gave conditional approval for this medication for IgAN at risk of rapid disease progression with a UPCR ≥ 1.5 g/g (Liao et al., 2022). Three randomised controlled trials showed eGFR and proteinuria treatment benefit. The initial results of TRF-budesonide use over a 6-month period were published in 2011, revealing a median reduction in albuminuria by 40 % (Smerud et al., 2011). In a dose-dependent manner, the addition of TRF-budesonide at a dosage of 16 mg/day, combined with optimised RAS blockade, demonstrated greater efficacy (Fellstrom et al., 2017). After 9 months of therapy, TRF-budesonide significantly decreased UPCR compared to placebo (27 % lower in the budesonide group). Additionally, there was a benefit in eGFR preservation corresponding to a 3.87 ml/min/1.73 m^2 difference versus placebo (Barratt, Lafayette, Kristensen, et al., 2023).

As per KDIGO guidelines, there is no documented evidence of efficacy for **azathioprine**, **cyclophosphamide** (unless rapidly progressive IgAN), **calcineurin inhibitors**, or **rituximab** (Kidney Disease: Improving Global Outcomes Glomerular Diseases Work, 2021).

1.7.3 Medications under clinical development

Several new drugs are now under clinical development for treating IgAN. These include agents inhibiting the complement system, the GAS6-AXL signalling pathway, anti-APRIL, anti-B-lymphocyte stimulator monoclonal antibodies, tyrosine kinase and NLR Family Pyrin Domain Containing 3 inflammasome inhibitors (Locatelli et al., 2023). The results of the trials mentioned in Figure 1.3 could significantly alter the future of IgAN's treatment paradigm (Noor et al., 2023).

Drug	Mechanism	Sponsor	Study	Phase
Methylprednisolone	Glucocorticoid	George Institute	**TESTING-ON**	-
TRF- budesonide	Targeted glucocorticoid	Calliditas	**NefIgArd-OLE**	3
Sparsentan	endothelin A + angiotensin II type 1 receptor antagonist	Retrophin	**PROTECT**	3
Atrasentan	endothelin A receptor antagonist	Chinook	**ALIGN**	3
Narsoplimab (OMS721)	Anti MASP-2 mAb	Omeros	**Artemis-IgAN**	3
Iptacopan (LNP023)	Factor B inhibitor	Novartis	**APPLAUSE**	3
IONIS-FB-LRx	antisense inhibitor of factor B mRNA	Ionis		2
Cemdisiran	anti-C5 siRNA	Alnylam		2
Ravulizumab	Anti-C5 mAb	Alexion	**SANCTUARY**	2
Vemircopan (ALXN2050)	Oral Factor D inhibitor	Alexion		2
KP104	C5 / Factor H Bifunctional mAb	Kira		2
Sibeprenlimab (VIS649)	humanized mIgG2 anti-APRIL	Visterra/Otsuka	**Visionary**	3
Telitacicept	Fusion TACI-Ig anti-APRIL/BAFF	Remegen		3
Atacicept	Fusion TACI-Ig anti-APRIL/BAFF	VeraTh-peutics	**ORIGIN**	2
BION-1301	humanized mIgG4 anti-APRIL	Chinook		1-2
Felzartamab	Human Anti-CD38 Antibody	Morphosys	**IGNAZ**	2

Figure 1.3 **Clinical trials of novel medications**

1.7.4 Tonsillectomy

According to KDIGO guidelines, the performance of tonsillectomy as a treatment for IgAN in Caucasian patients is not recommended due to a lack of sufficient data in this population. The existing data fail to provide support for the efficacy of tonsillectomy in both Caucasian and Chinese patients. However, in Japan, tonsillectomy is routinely conducted as multiple cohort studies have reported enhanced kidney survival and partial or complete remission of haematuria and proteinuria following tonsillectomy alone or usually in combination with pulsed glucocorticoids (Kidney Disease: Improving Global Outcomes Glomerular Diseases Work, 2021).

2 Materials and methods

2.1 Patient characteristics

This study consists of three parts: the first, a retrospective cohort study, focuses on kidney survival analysis and its prediction; the second, a case-control study, addresses B cell differentiation pathways; and the third, a cross-sectional study with an embedded prospective cohort component, examines gut microbiome features in patients with IgA nephropathy. The study structure, including the number of patients involved in each part and the analyses performed, is illustrated in Annex 1. Adults with histologically proven IgAN selected from renal biopsy registry at Pauls Stradins Clinical University Hospital, Latvia, were enrolled in the study. This is the only centre for kidney diseases in Latvia that confirms the diagnosis of IgAN in adults; therefore, the IgAN patient cohort is representative of Latvian adults with IgAN. Pathohistological investigation of kidney samples was performed using light microscopy, immunofluorescence, and electron microscopy. Kidney biopsy results were analysed according to revised Oxford classification (MEST-C score): mesangial hypercellularity (M), endocapillary cellularity (E), segmental sclerosis (S), interstitial fibrosis/tubular atrophy (T) and crescents (C) (Trimarchi et al., 2017). The decision to perform the biopsy as well as a treatment management were determined by the treating physician based on the specific indications: impaired kidney function or changes in urinalysis.

Renal survival and validation of IIgANPT (Barbour et al., 2019) was studied in adults diagnosed with IgAN from 2013 to 1 November 2019. Kidney survival was defined as having a GFR > 15 ml/min/1.73 m^2 within 5 years after the biopsy. Predicted risk was defined as a risk of 50 % decline in eGFR or ESKD calculated by the IIgANPT. Observed risk was a risk of 50 % decline in eGFR or ESKD calculated by the Cox regression model considering the same parameters as in the IIgANPT.

To study gut microbiome characteristics and mechanisms of IgA+ B cell differentiation, IgAN patients and age- and sex-matched healthy volunteers were enrolled from January 2020 to December 2022. The healthy controls (HC) had age-appropriate kidney function, with no active urine sediment and no presence of proteinuria. Exclusion criteria of both group individuals were pregnancy, diabetes mellitus, severe organ dysfunction, acute cardiovascular disease, hepatic diseases, acute or chronic autoimmune or infectious diseases, immunodeficiency, malignancies, alcohol abuse. All participants provided written informed consent.

Medical records were reviewed up to March 2024 to calculate the decrease in eGFR during the follow-up period. Progressors were defined as patients with an eGFR decline

of more than 5 ml/min/1.73 m^2/year, whereas nonprogressors had an eGFR slope of 5 ml/min/1.73 m^2/year or less.

Ethics Committee permission. Retrospective and prospective studies were approved by Clinical Research Ethics Committee of Pauls Stradins Clinical University Hospital (Approval No 100118-10L and No 191219-6L) (Annexes 2 and 3).

2.2 Clinical laboratory tests

Serum creatinine, albumin and total cholesterol were measured on Atellica CH (Siemens Healthineers, Erlangen, Germany). eGFR was calculated using the CKD-EPI Creatinine 2009 (in data analysis before 2021) and 2021 Equation. Serum IgA was measured on Atellica NEPH 630 (Siemens Healthineers, Erlangen, Germany). Proteinuria was determined by spot protein-to-creatinine ratio (UPCR). Assessment of protein in urine was performed on Cobas Integra 400 Plus (Roche Diagnostics GmbH, Mannheim, Germany). Red blood cell count in urine was determined on Atellica 1500 automated urinalysis system (Siemens Healthineers, Erlangen, Germany). The complete blood counts were performed on ethylenediaminetetraacetic acid (EDTA)-treated peripheral blood samples using UniCel DxH cellular analysis system (Beckman Coulter, Miami, FL, USA). The Gd-IgA1 level in the serum was measured using ELISA kit (Gd-IgA1 Assay Kit-IBL 30111694, IBL International GmBH, Germany) following the manufacturer's instructions. The samples were diluted 200-fold with the provided EIA buffer, to obtain biomarker levels within the measurement range of the kit (1.56–100 ng/ ml). Serum LPS levels were detected by ELISA (MyBioSource MBS702450, San Diego, CA, USA).

2.3 Analysis of the differentiation of IgA+ plasmablasts

Peripheral blood mononuclear cell (PBMC) isolation and serum collection

Peripheral blood was obtained from study participants. Serum from tubes with coagulation activator was isolated by centrifugation and was either used immediately or stored at −80C. PBMCs were isolated from heparinised blood by density gradient centrifugation using Histopaque-1077 (Sigma-Aldrich, St. Louis, USA). After washing with complete RPMI-1640 (10 % foetal bovine serum and 1 % penicillin-streptomycin in RPMI-1640), PBMCs were resuspended in freezing media (90 % foetal bovine serum and 10 % dimethyl sulfoxide) and cryopreserved. All laboratory analyses were done in the Joint Laboratory at Pauls Stradins Clinical University Hospital (Riga, Latvia).

Immunophenotyping of peripheral blood B cells by flow cytometry

Using antibodies against CD24, CD27, CD38 and IgD we were able to enumerate transitional (CD24hiCD38hi), mature naive (CD24intCD38int), activated (CD24loCD38lo), and total memory (CD24hiCD38lo) B cells, including class-switched (IgDneg) and unswitched

(IgDpos) subsets, double negative (IgDnegCD27neg) B cells, pre-plasmablasts (CD24loCD38hi) and plasmablasts (CD27posCD38hi). We further interrogated IgA, CD21, T-bet, Ki-67 expression in B cell subsets. Briefly, for PBMC viability LIVE/DEAD Fixable Near-IR Dead Cell Stain Kit was used (Invitrogen, MA, USA). Nonspecific staining was prevented with Fc receptor blocking reagent (Miltenyi Biotec, Bergisch Gladbach, Germany). The cells were incubated with antibodies for 40 minutes at 4°C, afterwards unbound antibodies were removed by two washes with flow cytometry staining buffer (2 % fetal bovine serum and 2 mM EDTA in phosphate-buffered solution (PBS)) and fixed with PBS containing 2 % formaldehyde.

For intracellular and transcription factor staining, cells were fixed and permeabilised using the Foxp3/Transcription Factor Staining Buffer Set (00-5523-00, eBioscience) followed by incubation with antibodies diluted in permeabilisation buffer for 50 minutes at 4°C. Samples were acquired on the Navios EX flow cytometer (Beckman Coulter, Inc., Brea, CA, USA) and analysed with FlowJo software (BD Life Sciences). For the detection of GdIgA1 in B cells, we stained PBMCs with AlexaFluor 488-labelled (ReadyLabel Antibody Labeling Kit R10712, Invitrogen, Waltham, MA, USA) monoclonal antibody [Gd-IgA1 (KM55)-IBL 30117066, IBL Japan, Gunma, Japan] or isotype control (MAB0061, R&D Systems, Minneapolis, MN, USA) for 40 min at 37 °C. To evaluate Gd-IgA1 expression in plasmablasts, cells were permeabilised using the Foxp3/Transcription Factor Staining Buffer Set and stained intracellularly with anti-Gd-IgA1 antibody or isotype control. Both anti-Gd-IgA1 and isotype control were used at a final concentration of 67 µg/ml.

2.4 Gut microbiome analysis

Stool sample collection, microbial DNA isolation and shot-gun sequencing of DNA libraries

Stool samples were collected in two aliquots using sterile, buffer-free collection tubes. Each participant documented the precise date and time of sample collection. The samples were then aliquoted into cryogenic storage vials and stored at −80 °C until analysis. The extraction of microbial DNA was carried out using the MGISP-960 Automated Sample Preparation System (MGI Tech Co., Ltd., Wuhan, China) and the MagPure Stool DNA LQ Kit (Angen Biotech Co., Ltd., Guangzhou, China). DNA library preparation was performed using the MGIEasy Universal DNA Library Prep Set (MGI Tech Co., Ltd., Wuhan, China) following the manufacturer's instructions. The subsequent sequencing was conducted on the DNBSEQ-G400RS platform using the DNBSEQ-G400RS high-throughput sequencing set (PE 150) (MGI Tech Co., Ltd., Wuhan, China), which provides at least 20 million 150 bp paired-end sequencing reads per sample. The quantity and quality of the DNA were assessed

using a Qubit 2.0 fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) and the Agilent 2100 Bioanalyzer System (Agilent Technologies, Santa Clara, CA, USA), respectively.

2.5 Statistical analysis

Statistical analyses were conducted using GraphPad Prism 9 (La Jolla, CA, USA), IBM SPSS Statistics 22.0 and 26.0 0 (IBM Corp., USA), and R-Studio version 4.0.1 (R Core Team (2020): R: A language and environment for statistical computing; R Foundation for Statistical Computing, Vienna, Austria. Data distribution was assessed by the Shapiro-Wilk test and normal Q-Q plots. Normal distributed data were expressed as mean ± SD. Not normally distributed data were presented as median and interquartile range. For normally distributed and homogenous data, independent samples t-test was used; when data were not normally distributed, Mann-Whitney U test was used. Spearman's rank correlation test was used to interrogate statistical significance in correlations. Results were considered statistically significant at $p < 0.05$.

Kidney survival was analysed using the Kaplan-Meier method, and statistical comparisons were made using the log-rank test. Episode rates per patient-year were calculated in two patient groups in the five-year analysis: (1) those included in the Kaplan–Meier analysis and (2) Cox regression model.

To compare real patient data with the IIgANPT, the following analyses were performed:

1 Observed risk was calculated using the Cox regression model.
2 Intraclass correlation coefficient was used to assess the strength of agreement between the IIgANPT predictions and the Cox regression results.
3 Differences between predicted and observed risks were calculated and interpreted as follows:
   - a negative difference indicated that the IIgANPT assigned a lower risk than the observed risk,
   - a positive difference indicated that the IIgANPT assigned a higher risk than the observed risk.

For metagenomic shotgun sequencing data read quality evaluation and trimming was done using fastp v0.20.0 (Chen et al., 2018) with default parameters and by retaining only those reads that passed the 100 bp length requirements after the trimming. Host read contamination was controlled by mapping the reads against a Homo sapiens GRCh38 reference genome with Bowtie2 v2.3.5.1 (Langmead & Salzberg, 2012) and removing the mapped reads. Taxonomic classification was performed by using Kracken2 v2.0.8 (Wood et al., 2019) with a confidence parameter set at 0.1 in accordance with the Unified Human Gastrointestinal Genome (UHGG)

(Almeida et al., 2021) collection. Functional profiling was carried out using the HUMAnN 3.0 (Beghini et al., 2021) software.

Statistical analysis for the phylum, genus and species taxonomic levels was performed by using the phyloseq v1.48.0 (McMurdie & Holmes, 2013) microbiome analysis environment. First, we performed frequency and prevalence filtering to exclude taxa that had no counts in any of the samples and samples that had no counts in any of the detected taxa. Alpha diversity indices were calculated using the microbiome v1.26.0 (Lahti & Shetty, 2012–2019) package, which was subsequently used as an input for the Wilcoxon rank sum test in the ggpubr v0.6.0 (Kassambara, 2023) package with Benjamini-Hochberg p-value correction. Sample and group-level taxon proportion plots were produced from proportionally transformed counts using the MicrobiotaProcess v1.16.1 (S. Xu et al., 2023) package. Aitchison's distance was calculated and plotted as a principal component analysis (PCA) plot for taxa passing the 10 % sample prevalence threshold using the MicroViz v0.12.5 (Barnett et al., 2021) package to represent the beta diversity results. Significant covariate detection was performed via the canonical correspondence analysis (CCA) ordinance method and PERMANOVA test within the MicroViz package, with 99999 permutations. The resulting significant covariates were included in the MaAsLin2 v1.18.0 (Mallick et al., 2021) design formula for the negative binomial analysis method of differentially abundant taxa detection with trimmed mean normalisation applied beforehand, while the default values of the remaining parameters were used, except for increasing the taxon prevalence threshold from 10 % to 33 % in the case-control species level test, to reduce the number of sporadic results. The same analysis methodology was applied to investigate the functional alterations in our experimental group. The default false discovery rate (FDR) threshold in MaAsLin2 is < 0.25, with taxon-level associations below this value considered statistically significant in microbiome studies, requiring further validation (Stewart et al., 2018). Visualisations were created using ggplot2 v3.5.1 (Wickham, 2016) or the previously specified packages.

3 Results

3.1 Kidney survival and its associated clinical and histological risk factors

A total of 105 newly diagnosed IgAN patients were identified between January 2013 and November 2019. Of these, 90.5 % (n = 95) were included in the subsequent kidney survival analysis, while 9.5 % (n = 10) were excluded due to ESKD (eGFR < 15 ml/min/1.73 m²) at the time of biopsy. Demographic and clinical characteristics of the included patients are summarised in Table 3.1. All patients were of white ethnicity and middle-aged, with a mean age of 38.6 ± 11.2 years (range:18–72 years). The majority (40 %) belonged to the 30–39-year age group (Figure 3.1).

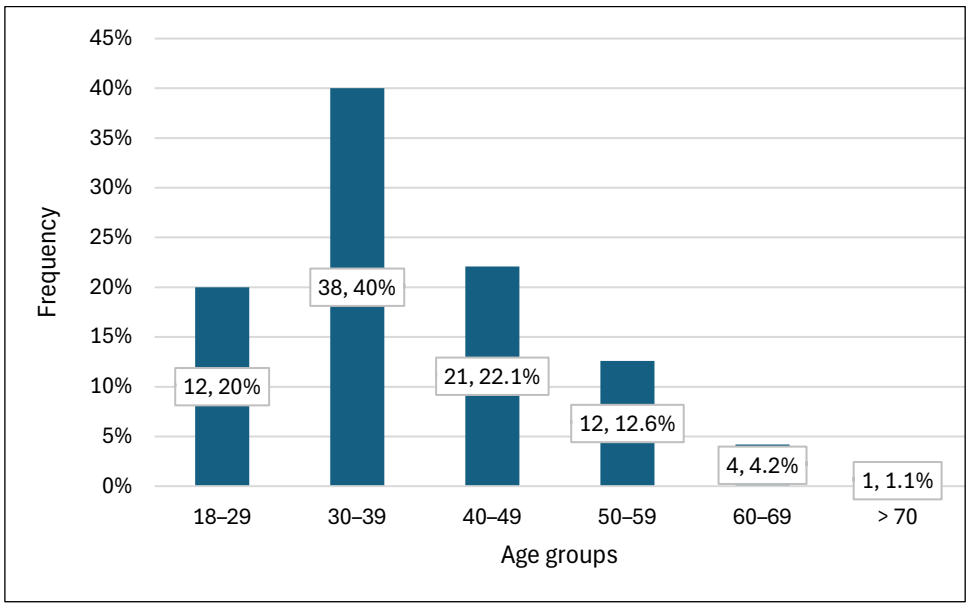

Figure 3.1 **Distribution of IgAN cases by age group**

Nephritic syndrome was the most common manifestation of IgAN, observed in 73.4 % of patients, followed by a combined presentation of nephrotic and nephritic syndrome, characterised by nephrotic-range proteinuria and haematuria (13.8 %), and asymptomatic urinary abnormalities (11.7 %). Nephrotic syndrome was identified in only one patient. Median proteinuria was 1.2 g/g, measured by the UPCR. eGFR ranged from 16 to 130 ml/min/1.73 m², with a median of 56 ml/min/1.73 m². Patients were categorised into different CKD stages based on their eGFR (Figure 3.2). A normal eGFR (> 90 ml/min/1.73 m²) with urinalysis abnormalities was observed in 32 patients (33.7 %). Moderate to severe kidney function loss (eGFR < 60 ml/min/1.73 m²) was present in 47 patients (49.5 %).

On physical examination, hypertension was detected in 56.4 % of patients (defined as SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg), and 39.6 % of them were using ACEI/ARB at the time of biopsy.

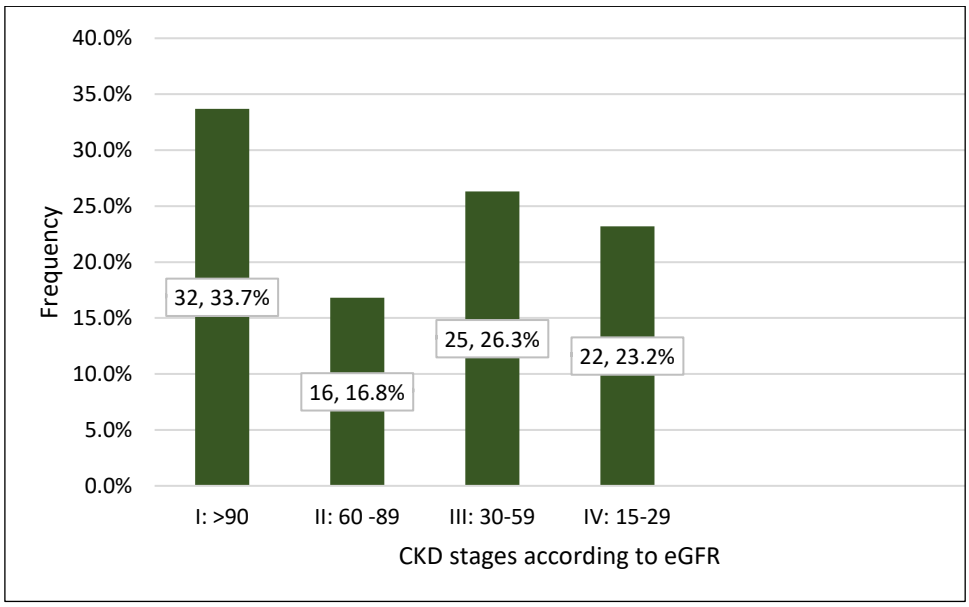

Figure 3.2 **Distribution of IgAN patients by stage of CKD**

In this study cohort, a significant proportion of IgAN patients exhibited mesangial hypercellularity (87.4 %) and segmental glomerulosclerosis (72.6 %). Various degrees of tubular atrophy were identified in 17 (17.9 %) patients.

Table 3.1

Demographic and clinical characteristics of patients included in the kidney survival analysis

Parameter	n (%)
Male	53 (55.8)
Age, years	37 (31–45)
SBP, mmHg	135 (120–150)
DBP, mmHg	80 (71.8–93.0)
GFR at biopsy, ml/min/1.73 m^2	56 (33–98)
Daily proteinuria, g/24 h	1.2 (0.5–2.33)
Oxford classification M1 E1 S1 T1 T2	 83 (87.4) 3 (3.2) 69 (72.6) 11 (11.6) 6 (6.3)
Use of ACEI or ARB at the time of biopsy	37 (38.9)
Immunosuppression at or prior the biopsy	9 (9.5)
Glomerular manifestations Nephritic syndrome Nephrotic syndrome Nephrotic and nephritic syndrome Isolated haematuria Isolated proteinuria	 69 (73.4) 1 (1.1) 13 (13.8) 7 (7.4) 4 (4.3)

Values are shown as frequencies and proportions (%) or median and interquartile range. SBP – systolic blood pressure. DBP – diastolic blood pressure. ACEI – angiotensin-converting enzyme inhibitors. ARB – angiotensin II receptor blockers.

The median follow-up duration was 18 months (ranging from 0 to 60 months). In this study, the overall median kidney survival during the follow-up period (60 months) was not reached; however, the third quartile (Q3) was 24 months. Among the 95 patients included in the analysis, 14 (14.7 %) progressed to ESKD, corresponding to an incidence rate of 0.11 episodes per patient-year. Gender, MEST-T score, and DBP were identified as significant risk factors for reduced kidney survival, while SBP showed a borderline statistically significant association (p = 0.059).

Women had a longer kidney survival time (exceeding 60 months) compared to men (58 months). A log-rank test was performed to assess differences in survival distribution between genders; $\chi^2(1) = 4.03$, p = 0.045 (Figure 3.3).

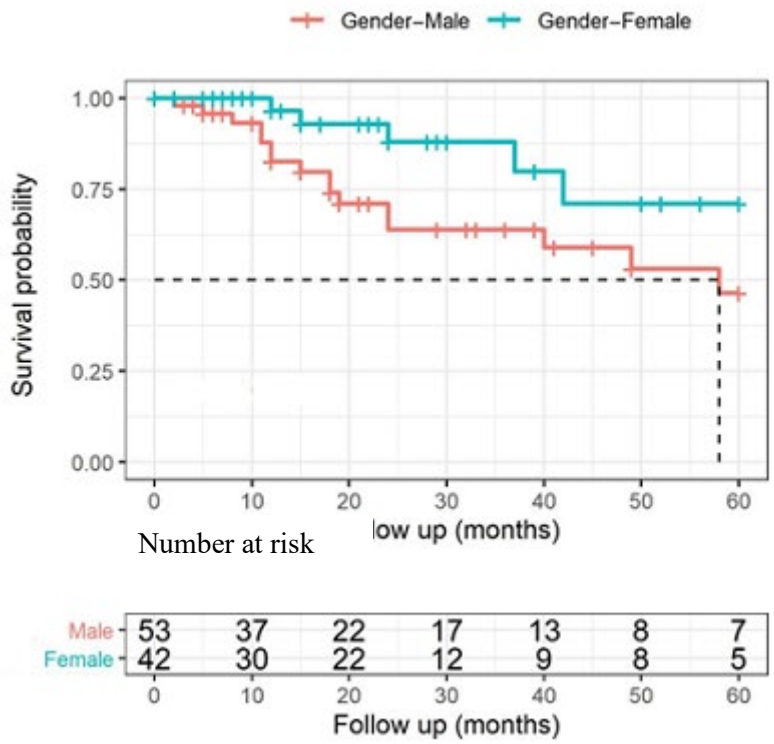

Figure 3.3 **Cumulative kidney-survival rates categorised by gender**

Men had worse kidney survival rates than women during the follow-up period. *p = 0.045

The analysis of kidney survival based on to the MEST score showed that patients with a MEST T0 score had a longer median kidney survival time throughout the follow-up period (60 months) compared to those with T1 (40 months) and T2 (18 months). A statistically significant difference in kidney survival distribution was observed between T0 and T1 scores ($\chi2(2) = 9.09$, p = 0.01; Figure 3.4). T1 also demonstrated a tendency toward better overall survival compared to T2 (χ^2 for trends = 8.80, p = 0.003).

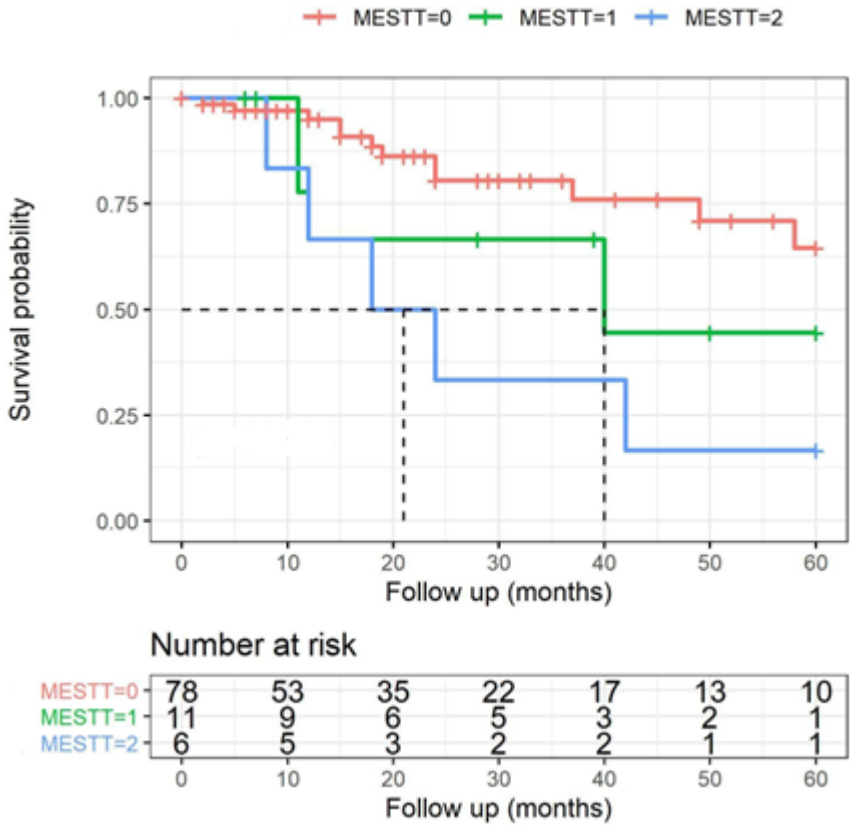

Figure 3.4 **Cumulative kidney survival rates categorised by tubular atrophy/ interstitial fibrosis**

Kidney survival according to the MEST T score revealed that patients with MEST T2 have a poorer prognosis compared to those with T1 and T0. *p = 0.01

Participants with a DBP less than 99 mmHg had a longer median kidney survival of 60 months compared to those with DBP levels of 100–109 mmHg (40 months) and those with DBP greater than 110 mmHg (24 months). A statistically significant difference in survival distribution was observed between the DBP categories ($\chi^2(4) = 11.46$, $p = 0.022$; Figure 3.5), indicating an association between higher blood pressure and worse kidney survival (χ^2 for trend = 9.21, $p = 0.002$).

In this study, survival distribution for age, proteinuria, use of ACEI/ARB at the time of biopsy, the use of immunosuppression at or prior to biopsy, and MEST M, E, and S scores were not statistically significantly affecting kidney survival.

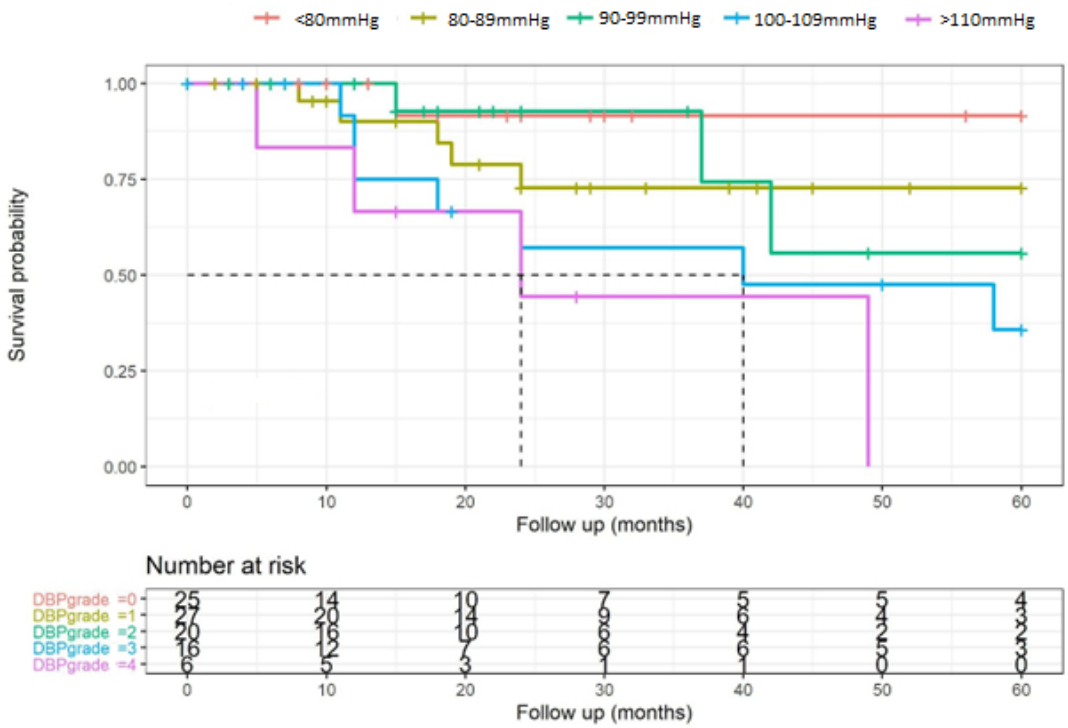

Figure 3.5 **Cumulative kidney survival rates categorised by diastolic blood pressure**

Kidney survival rate is better in patients with lower DBP. *p = 0.022
DBP grades 0: < 80 mmHg; grade 1: 80–89 mmHg; grade 2: 90–99 mmHg,
grade 3: 100–109 mmHg, grade 4: > 110 mmHg

3.2 Prediction of kidney function decline by Cox regression and the International IgAN Prediction Tool

A total of 68 patients were included in the Cox regression analysis, of whom 37 were men. The mean patient age was 39.1 ± 12.0 years (ranging from 18 to 72 years). We excluded 27 patients due to missing data (mostly absence of 24-hour proteinuria), required for prognosis calculation using IIgANPT (23 patients) and secondary IgAN (4 patients) related to IgA vasculitis or liver disease. The median follow-up duration for the five-year analysis was 21.5 months (ranging from 0 to 60 months). The event rate (50 % decline in GFR or progression to ESKD) was 0.15 episodes per patient-year over five years.

The analysis of hazard ratios revealed that DBP, MEST-E, and MEST-T were associated with the risk of a 50 % decline in GFR or progression to ESKD (Table 3.2). Each subsequent increase in DBP grade was associated with a 6 % rise in risk. Additionally, the risk increased fourfold when moving from E0 to E1 in the MEST-E score, and more than doubled for each unit increase in the MEST-T score. Conversely, age showed a protective effect, with the risk decreasing by 5 % for each additional year.

Table 3.2

Hazard ratio of various clinical parameters in IgAN patients

Factor	Hazard ratio (95 % confidence interval)	p-value
Age	0.95 (0.91–0.99)	0.023
SBP	0.99 (0.96–1.03)	0.683
DBP	1.06 (1.00–1.12)	0.04
MEST E	4.12 (0.83–20.47)	0.084
MEST T	2.52 (1.38–4.62)	0.003

Further analysis compared the observed and predicted five-year risks using the IIgANPT. The intraclass correlation coefficient (ICC) indicated a moderate level of reliability between predicted and observed risk, with an average-measure ICC of 0.70 (95 % confidence interval: 0.52 to 0.82; F(67.67) = 3.38, p = 0.001). Most differences between predicted and observed data fell within the range of 0 to 10 % (n = 33), with a median difference of 2.54 %, suggesting good reliability of the IIgANPT. While the IIgANPT underestimated risk in 23 patients compared to our estimates, it assigned similar or slightly higher risk in 45 patients. However, the maximum negative and positive differences were 77.35 % and 59.84 %, respectively. A closer examination of these outliers revealed no exceptional features in their disease course or histology (Figure 3.6).

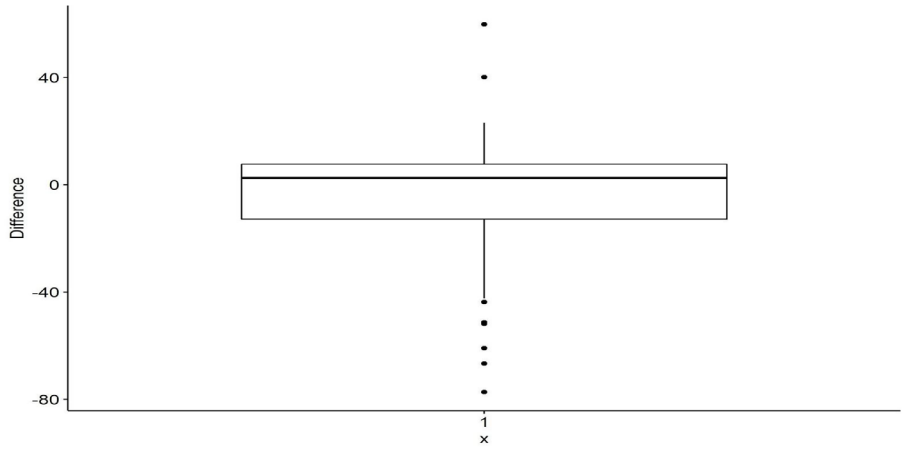

Figure 3.6 **The difference in estimated risks between Cox regression and IIgANPT**

An insignificant difference in estimated risk was observed between the two methods, with a median difference of 2.54 %, with an average-measure intraclass correlation coefficient of 0.70.

3.3 B cell activation pathways in IgAN patients

To uncover B cell activation pathways and how peripheral B cell composition may be impacted in patients with IgAN, we recruited 36 patients with IgAN and 19 HCs. The demographic, clinical and laboratory data of the cohort are summarised in Table 3.3. Study participants were sex- and age-matched. Both groups had normal and comparable leukocyte and lymphocyte counts. As expected, IgAN patients had higher serum creatinine levels and

lower eGFR than HC. IgAN patients represented all four chronic kidney disease stages (from 1 to 4) based on eGFR. The median proteinuria of patients with IgAN was 0.48 g/g (interquartile range 0.26–1.35), 11 patients had moderate proteinuria (1–3 g/g) and only one patient had nephrotic-range proteinuria (> 3 g/g). According to the Oxford classification of IgAN, a frequent histological finding was secondary glomerulosclerosis (69.4 %). Only in rare cases tubular atrophy or crescents in < 25 % of glomeruli were seen. There were no significant differences in body mass index (BMI) between IgAN patients and controls.

Table 3.3

Demographic, clinical and laboratory characteristics of the study cohort

Baseline characteristics	IgAN patients, n = 36	HC, n = 19	p-value
Age, yr	44.5 (22–65, IQR 37–50)	49 (23–66, IQR 38–53)	0.906
Male	23 (63.9)	13 (68.4)	0.775
BMI, kg/m^2	25.5 (17.6–44.1, IQR 23.4–29.4)	26.8 (17.82–40.63, IQR 23.4–31.1)	0.816
Systolic BP, mmHg	139 (120–174, IQR 130–152)	122 (98–145, IQR 120–129)	< 0.001
Diastolic BP, mmHg	85 (70–110, IQR 80–95)	80 (60–98, IQR 74–85)	0.024
Serum creatinine, µmol/l	126.5 (49–402, IQR 95.5–214)	83 (55–102, IQR 74–87)	< 0.001
eGFR, ml/min per 1.73m^2	56 (15–131, IQR 25.5–85.5)	100 (70–128, IQR 96–107)	< 0.001
CKD stage 1	6 (16.7)		
CKD stage 2	12 (33.3)		
CKD stage 3	7 (19.4)		
CKD stage 4	11 (30.6)		
Proteinuria, g/g	0.48 (0.07–6.18, IQR 0.26–1.35)		
Haematuria, RBC/µl	21.17 (0–1421, IQR 10–65)		
Serum albumin, g/l	46 (29–51, IQR 42.5–48)		
Serum total cholesterol, mmol/l	5.46 (3.79–8.11, IQR 4.94–5.9)		
Absolute leukocyte count, 10^9/l	7.15 (4.2–12.8, IQR 5.8–7.5)	5.3 (4.1–8.4, IQR 4.4–6.5)	0.07
Absolute lymphocyte count, 10^9/l	1.8 (0.9–3.1, IQR 1.5–2.2)	1.9 (1.4–3.0, IQR 1.8–2.4)	0.291
Oxford classification			
M1	29 (80.6)		
E1	1 (2.8)		
S1	25 (69.4)		
T1	2 (5.6)		
T2	2 (5.6)		
C1	2 (5.6)		

Values are presented as median, minimum–maximum, interquartile range (IQR), or as n (%).
BP: blood pressure; CKD: chronic kidney disease; RBC: red blood cells.

We first carried out B cell phenotyping based on CD24, CD27, CD38 and IgD surface expression. This allowed us to enumerate transitional (CD24^{hi}CD38^{hi}), mature naive

($CD24^{int}CD38^{int}$), activated ($CD24^{lo}CD38^{lo}$), and total memory ($CD24^{hi}CD38^{lo}$) B cells, including class-switched (IgD^{-}) and unswitched (IgD^{+}) subsets, double negative ($IgD^{-}CD27$) B cells, pre-plasmablasts ($CD24^{lo}CD38^{hi}$) and plasmablasts ($CD27^{pos}CD38^{hi}$). We found that IgAN patients had a significant increase in mature naive B cells with a reciprocal decrease in the frequency of total memory B cells (Figure 3.7 a). Frequencies of switched and unswitched memory B cells were comparable (Figure 3.7 b). A total of $IgD^{-}CD27^{-}$ B cells termed double negative (DN) or atypical memory B cells, that has been recently described as the precursors of autoantibody-producing plasmablasts, were comparable to plasmablasts in patients with IgAN and HC (Figure 3.7 c).

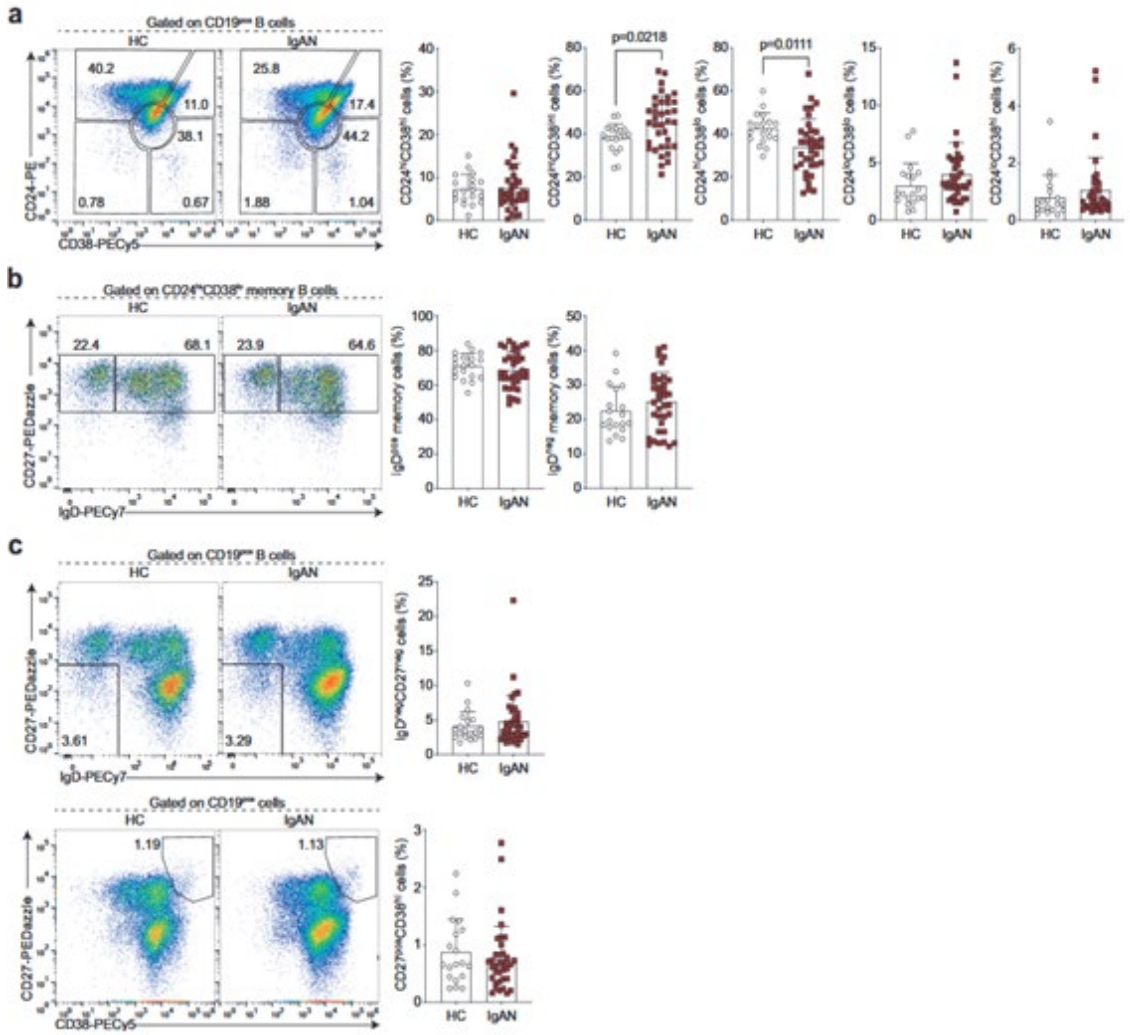

Figure 3.7 **IgAN-associated changes in the peripheral B cell landscape**

Representative flow cytometry plots and summary bar charts demonstrating (a) the frequencies of transitional (CD24hiCD38hi), mature (CD24intCD38int), memory (CD24hiCD38lo), activated B cells (CD24loCD38lo) and pre-plasmablasts (CD24loCD38hi), (b) the distribution of memory B cells into IgD^{+} unswitched and IgD^{-} class-switched subsets, (c) the frequencies of total double negative (DN; $IgD^{-}CD27^{-}$) B cells and plasmablasts ($CD27^{+}CD38hi$) in IgAN patients and healthy controls. Data are mean ± SD and each circle/square represents a study participant. For normally distributed CD24intCD38int, CD24hiCD38lo, IgD^{+} memory and IgD^{-} memory B cell populations independent samples t-test was used to compare IgAN patients and healthy controls. For non-normally distributed CD24hiCD38hi, CD24loCD38lo, CD24loCD38hi, $IgD^{-}CD27^{-}$ and $CD27^{+}CD38hi$ B cell subsets Mann-Whitney U test was used for the comparison of IgAN patients and HC.

The cellular origin and pathway that gives rise to IgA-producing B cells in IgAN is unknown. We wanted to determine whether systemic changes in the activation and differentiation of IgA-expressing B cells could be detected in IgAN patients. Among B cells, the frequency of IgA-expressing classical memory ($CD27^+$) B cells was similar between IgAN patients and healthy controls (HC) (Figure 3.8 a). However, we noted that in addition to CD27 expressing B cells, there was a smaller population of IgA class-switched B cells that lacked CD27 expression, which was particularly pronounced in IgAN patients. Indeed, there was a significant expansion of these IgA-expressing $CD27^-$ B cells in patients with IgAN (Figure 3.8 a). We also detected a significantly higher frequency of IgA class-switched antibody-secreting cells (ASC) in IgAN patients compared to controls (Figure 3.8 b). Supporting a lineage relationship between IgA-expressing plasmablasts and IgA-expressing $CD27^-$ B cells, we found a correlation between these two subsets (Figure 3.8 c).

We next wanted to address whether the IgA-expressing B cells were indeed expressing Gd-IgA1. We found that IgA^+CD27^- B cells co-expressed Gd-IgA1 (Figure 3.8 d). Reciprocally, in IgAN patients the majority of all Gd-$IgA1^+$ B cells were $CD27^-$ (Figure 3.8 e and f). IgA^+ plasmablasts also expressed high levels of Gd-IgA1 (Figue 3.8 g). Finally, implicating the IgA-expressing plasmablast as a potential functional contributor to pathogenesis, we observed a correlation between IgA^+ plasmablasts and circulating IgA levels (Figure 3.8 h). Nevertheless, despite the correlation between IgA and Gd-IgA1, we did not find a relationship between IgA^+ plasmablasts and serum Gd-IgA1 levels (Figure 3.8 i).

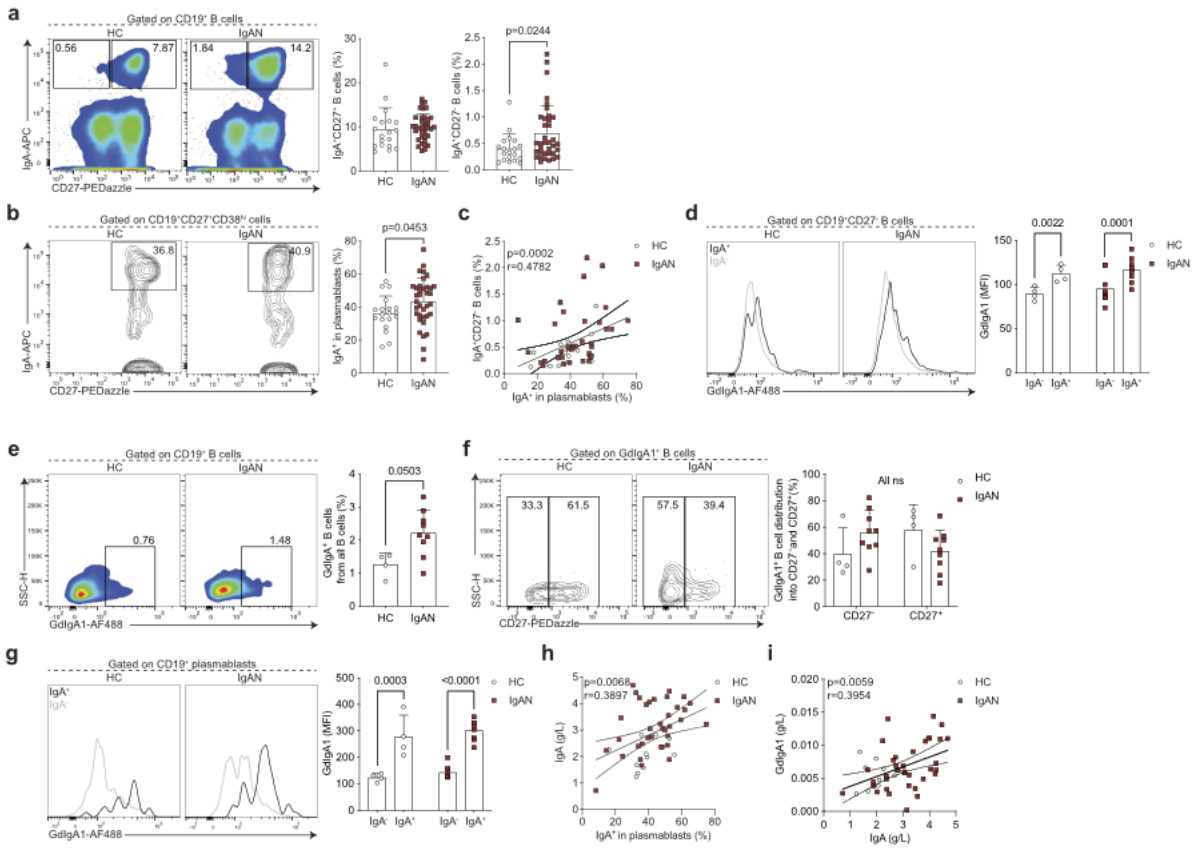

Figure 3.8 **Enhanced differentiation of IgA$^+$ CD27$^-$ B cells and IgA$^+$ plasmablasts in IgAN**

Representative flow cytometry plots and summary bar charts demonstrating the frequencies of (a) IgA$^+$ CD27$^+$ and IgA$^+$CD27$^-$ B cells, and (b) IgA$^+$ plasmablasts, (c) linear regression analysis of IgA$^+$CD27$^-$ B cells versus IgA$^+$ plasmablasts, and (d) representative flow cytometry histograms and summary bar charts demonstrating the median fluorescence intensity of Gd-IgA1 in IgA$^+$ and IgA$^-$ CD19$^+$ CD27$^-$ B cells. (e, f) Representative flow cytometry plots and summary bar charts demonstrating the frequencies of (e) GdIgA1$^+$ B cells and (f) the distribution of Gd-IgA1$^+$ B cells into CD27$^-$ and CD27$^+$ subsets. (g) Representative flow cytometry histograms and summary bar charts demonstrating the median fluorescence intensity of Gd-IgA1 in IgA$^+$ and IgA$^-$ plasmablasts. (h, i) Linear regression analysis of (h) serum IgA levels versus IgA$^+$ plasmablasts and (i) serum Gd-IgA1 versus serum IgA levels in IgAN patients and HC. Data are mean ± standard deviation and each circle/square represents a study participant. For non-normally distributed subsets Mann-Whitney U test was used for the comparison of IgAN patients and HC. Spearman's rank correlation tests were used to interrogate statistical significance in the correlation between IgA$^+$CD27$^-$ B cells and IgA$^+$ plasmablasts, serum IgA levels and IgA$^+$ plasmablasts, and serum Gd-IgA1 and serum IgA levels in IgAN patients and HC.

CD27$^-$ antigen-experienced B cells comprise two subsets, termed double negative (DN) 1 and 2. DN1 cells are defined by their expression of CD21 and CXCR5, while DN2 B cells lack CD21 and CXCR5 expression and instead express CD11c and are transcriptionally regulated by T-bet. Based on their transcriptional signatures these two subsets of B cells arise from different activation pathways (Jenks et al., 2018). While DN2 B cells arise through extrafollicular B cell activation, DN1 B cells represent precursors of classical memory B cells that have recently emerged from the germinal centre reaction (and have not yet upregulated CD27). Of note, germinal centres are the microanatomical structures that allow the evolution (by somatic hypermutation of antibody-encoding genes) and selection (affinity maturation) of B cells that enable the production of high-affinity antibodies. The understanding of which

pathway B cells are activated through can elucidate factors that regulate the response (e. g. T cell help) or properties of the compartment (e. g. longevity) (Elsner & Shlomchik, 2020). We found that the $IgA^{-}CD27^{+}$ B cells were phenotypically $CD21^{hi}$ and T-bet^{lo} corresponding to DN1 phenotype (Figure 3.9 a and b), suggesting they may indeed be the precursors of IgA^{+} $CD27^{+}$ classical memory B cells. $IgA^{+}CD27^{+}$ memory B cells were used as a control for high expression of CD21 and lack of T-bet. To further interrogate this developmental relationship, we reasoned that most of the classical memory compartment ($CD27^{+}$) in patients with IgAN and HC would be composed of foreign antigen-specific B cells generated throughout the lifetime. The majority of this pool should be resting cells, apart from those that report and participate in an ongoing immune response. We then carried out Ki-67 staining and found a significant positive correlation between proliferating IgA^{+}DN1 and Ki-67+ $IgA^{+}CD27^{+}$ memory B cells (Figure 3.9 c). Therefore, the shared phenotypic and proliferation characteristics of these subsets suggest a developmental relationship and support increased generation of IgA-expressing ASC through the germinal centre pathway.

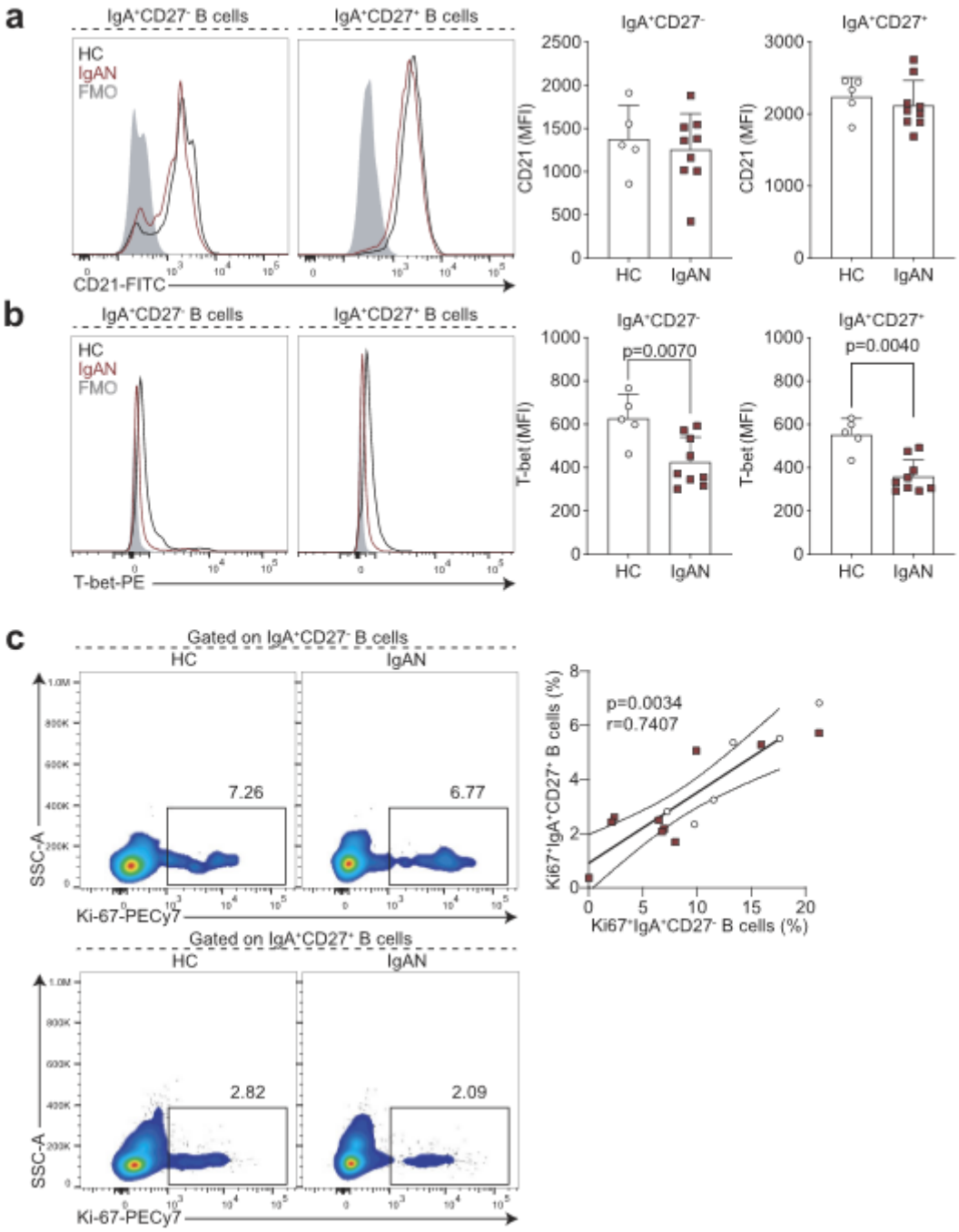

Figure 3.9 **IgA^{+}CD27^{-} B cells are phenotypically CD21^{+}T-bet^{-}**

Representative flow cytometry histograms and summary bar charts demonstrating the median fluorescence intensity of CD21 (a) and T-bet (b) in IgA^{+}CD27^{-} and IgA^{+}CD27^{+} B cells. (c) Representative flow cytometry plots and linear regression analysis of Ki-67^{+} IgA^{+}CD27^{+} B cells versus Ki-67^{+} IgA^{+}CD27^{-} B cells in HC and IgAN patients. Data are mean ± standard deviation and each circle/square represents a study participant.

Finally, we wanted to explore the mucosal–kidney axis in relation to B cell activation in IgAN and ask if previously reported perturbations at mucosal surfaces were linked to this DN1 B cell differentiation pathway. LPS is known not only to influence B cell class-switching to IgA (Cerutti, 2008), but is also used as a surrogate marker of dysbiosis and gut permeability (Mohr et al., 2022). LPS was significantly elevated in the serum of IgAN patients compared with HC (Figure 3.10 a). We further found that IgA-expressing CD27^{-} B cells correlated with

serum LPS levels (Figure 3.10 b). This suggests LPS may either directly drive their expansion/class-switching or that the observed correlation is because both increased LPS and IgA^+CD27^- B cells are a consequence of mucosal dysbiosis/reduced barrier function, which contributes to IgA-expressing plasmablast differentiation. Finally, we wanted to ask how the expansion of IgA^+ $CD27^-$ B cells related to clinical features of IgAN. We found that specifically in patients with reduced kidney function (eGFR < 90 ml/min) there was an inverse correlation between eGFR and the frequency of IgA^+CD27^- B cells (Figure 3.10 c).

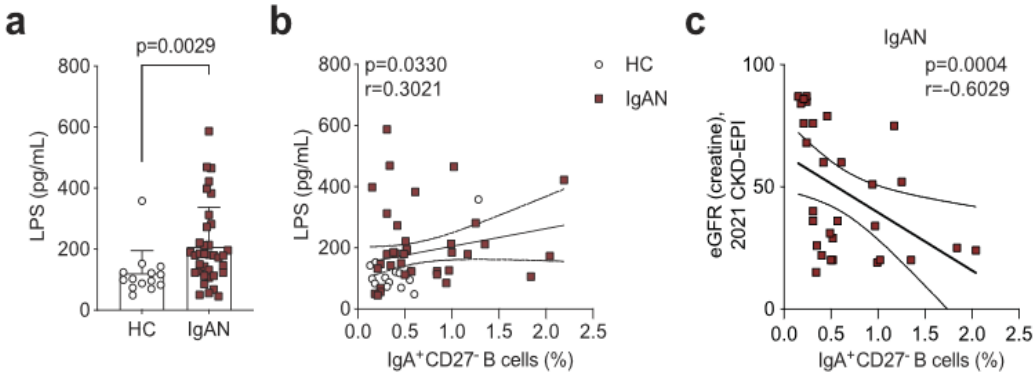

Figure 3.10 **Serum lipopolysaccharides correlation with IgA^+CD27^- B cell frequency**

(a) Serum LPS levels in IgAN patients and HC (b) linear regression analysis of serum LPS levels versus IgA^+CD27^- B cells in IgAN patients and HC (c) Linear regression analysis of eGFR versus IgA^+CD27^- B cells in IgAN patients. Data are mean ± standard deviation and each circle/square represents a study participant. For non-normally distributed serum LPS levels Mann-Whitney U test was used for the comparison of IgAN patients and HC. Spearman's rank correlation test was used to interrogate statistical significance in the correlation between serum LPS levels and IgA^+ $CD27^-$ B cells in IgAN patients and HC.

3.4 Characteristics of gut microbiome composition in IgAN patients

Forty-eight patients with IgAN and twenty-three healthy controls were enrolled in the study for gut microbiome analysis. The baseline and clinical characteristics are summarised in Table 3.4. The study participants were sex- and age-matched, with more than 60 % being men, and ages ranging from 22 to 65 years. Both groups had a similar median BMI of 26 kg/m². As expected, serum creatinine levels were higher in patients with IgAN than in healthy controls. The distribution of IgAN patients across CKD stages was approximately 20 %, with the majority in the CKD stage 1 group (33.3 %). According to the pathological Oxford classification, approximately 70 % of IgAN patients had mesangial hypercellularity (M1) and segmental glomerulosclerosis (S1) findings in their biopsy results. Patients had mild proteinuria, with a median UPCR of 0.349 g/g. Gd-IgA1 levels were greater in IgAN patients than in healthy individuals.

Table 3.4

Clinical, laboratory and pathological characteristics of IgAN patients and healthy controls

Baseline characteristics	IgAN patients, n = 48	HC, n = 23	p-value
Age, yr	41 (35–47.7)	46 (33–53)	0.36
Male	30 (62.5 %)	14 (60.9 %)	0.89
BMI, kg/m^2	26.1 (23.6–29.3)	26 (23–28)	0.93
Systolic BP, mmHg	130 (121–145)	127 (120–138)	0.2
Diastolic BP, mmHg	80 (72.2–90)	80 (70–80)	0.3
Serum creatinine, μmol/l	105 (84–195.5)	80 (66–88)	0.001
eGFR, ml/min per 1.73m^2	72.5 (32–97.7)	101 (96–108)	0.001
Gd-IgA1, ng/ ml	6568 (4916–9335.5)	4577.5 (3470.3–6292.8)	0.02
Lipopolysaccharides, pg/ ml	132.7 (107.2–189.5)	115 (89.9–230)	0.15
Indoxyl sulfate, ng/ ml	240.7 (195.5–373.6)	230 (185–291)	0.69
Mesangial hypercellularity (M1)	35 (72.9 %)	–	–
Endocapillary hypercellularity (E1)	2 (4.2 %)	–	–
Segmental glomerulosclerosis (S1)	33 (68.8 %)	–	–
Tubular atrophy/interstitial fibrosis 26–50 % (T1)	3 (6.3 %)	–	–
Tubular atrophy/interstitial fibrosis > 50 % (T2)	2 (4.2 %)	–	–
Cellular/fibrocellular crescents < 25 % (C1)	3 (6.3 %)	–	–

The results are expressed as the median (interquartile range) or n (%).

To evaluate the IgAN-related alterations in the gut microbiome profiles, we compared the shotgun sequencing-derived gut microbiome profiles of faecal samples from IgAN patients (n = 48) with those of samples collected from healthy controls (n = 23). We observed no statistically significant differences in alpha diversities estimated by several common indices (Table 3.5) between the interest groups.

Table 3.5

Comparison of various alpha diversity measures between IgAN patients and healthy controls

Method	IgAN cases (median, IQR)	Controls (median, IQR)	p	p.adj
Observed taxa	2042.50 (1900.75– 120.25)	2044.00 (1919.00–2181.00)	0.7	7.00E–01
Pielou's evenness	0.65 (0.62–0.68)	0.65 (0.64–0.69)	0.3	3.10E–01
Shannon index	4.92 (4.72–5.19)	5.01 (5.25–4.79)	0.4	3.60E–01
Inverse Simpson index	54.88 (78.21–39.50)	61.30 (82.88–50.55)	0.5	4.50E–01
Chao1	2042.50 (1900.75–2120.25)	2044.00 (1919.00–2181.00)	0.7	7.00E–01

In addition, no significant difference in the intersample variability in the microbial community composition among the analysed samples between the case and control groups was observed (Figure 3.11).

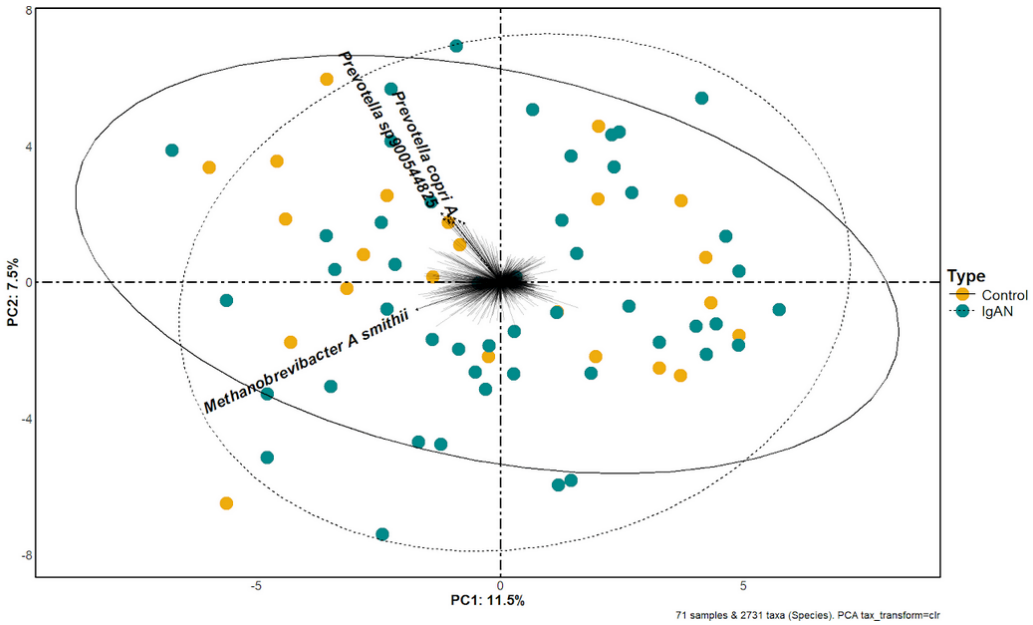

Figure 3.11. **Principal component analysis plot showing inter-sample variability in microbial community composition among IgA nephropathy patients and healthy controls**

Prevotella was the most prevalent genus in both IgAN patients (7.57 %) and controls (9.27 %), with *Faecalibacterium* (IgAN = 6.71 %, controls = 8.11 %) and *Blautia* (IgAN = 6.73 %, controls = 7.02 %) also among the most common genera. Notably, *Bacteroides* and *Bifidobacterium* were more abundant in IgAN patients than in controls (IgAN = 4.99 % and 2.84 %, controls = 4.56 % and 1.85 %). *Prevotella sp00900557255* (IgAN = 2.95 %, controls = 4.34 %), *Fusicatenibacter saccharivorans* (IgAN = 2.53 %, controls = 2.58 %), and *Phocaeicola dorei* (IgAN = 2.51 % and controls = 2.28 %) were the three most abundant species in both groups (Figure 3.12).

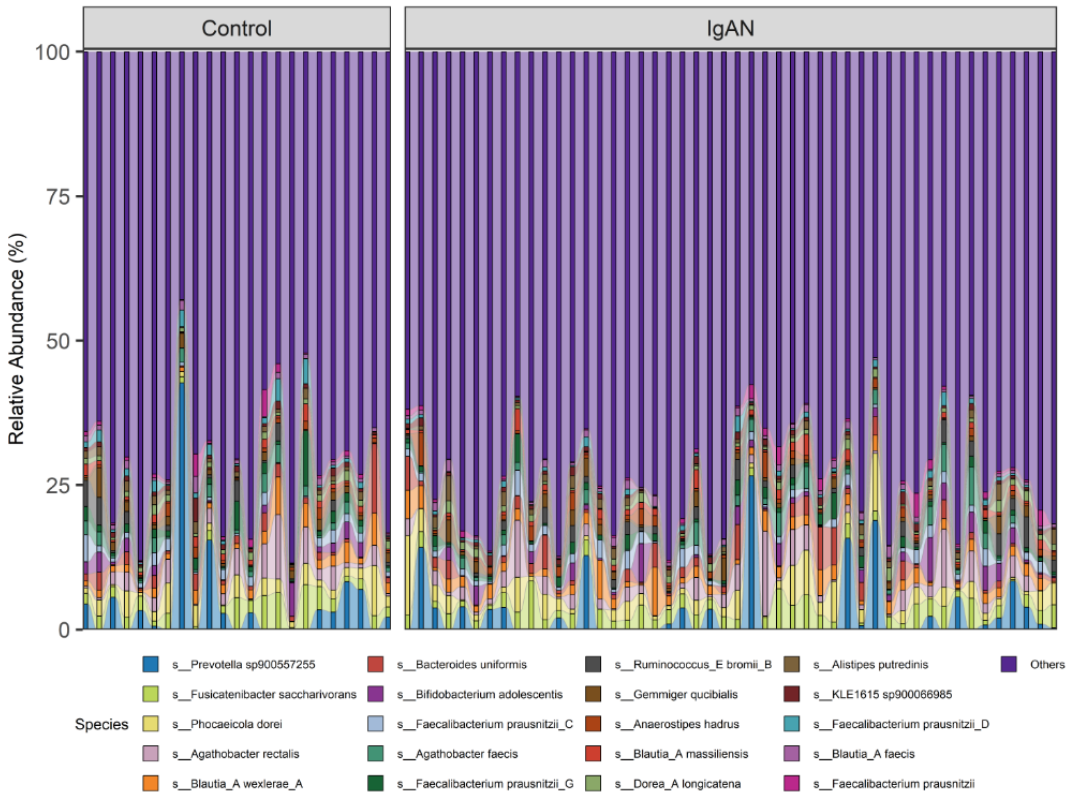

Figure 3.12. **Taxonomy bar plot depicting 20 of the most representative species of every individual stratified by study group**

Differential abundance analysis at the species level revealed 371 differentially abundant taxa (false discovery rate (FDR) < 0.25) between the IgAN patients and healthy controls, with *Absicoccus sp000434355* (coef. = 3.69, FDR = 3.53E-13), *Bacteroides ndongoniae* (coef. = 3.28, FDR = 4.59E-08), *Akkermansia muciniphila C* (coef. = 2.76, FDR = 1.66E-03) showing higher abundance, while *Eubacterium R sp000433975* (coef. = –3.28, FDR = 9.09E-07), and *CAG:462 sp900291465* (coef. = –3.23, FDR = 4.90E-09), *Olsenella E sp900540955* (coef. = –2.98, FDR = 1.75E-03) and *Butyricicoccus A sp002395695* (coef. = –2.52, FDR = 5.00E-08) had a lower abundance in IgAN patients (Figure 3.13 a and b).

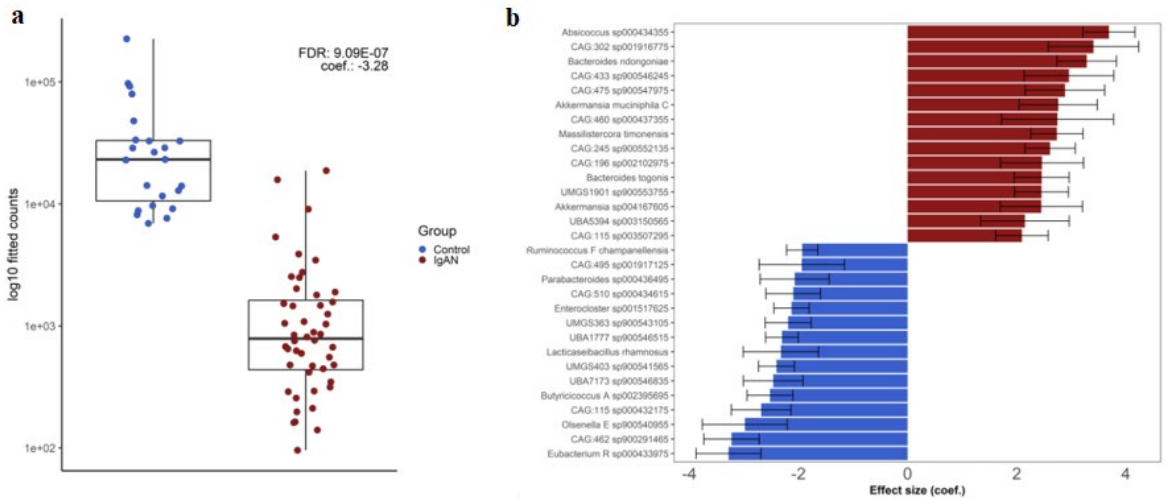

Figure 3.13 **Alterations in the gut microbiome in IgAN patients and healthy individuals**

(a) Relative abundance of Eubacterium R sp000433975 in control subjects (blue) and IgAN patients (red). Boxplots show the median, 25th, and 75th percentiles and outliers. (b) Bar plot representing the effect size (logFC) of the top 20 differentially abundant species between control subjects and IgAN patients. A positive coefficient (red) represents taxa with increased abundance in the IgAN patients, and a negative coefficient (blue) represents taxa in the control patients.

The functional consequences derived from gene mapping were supported by comparative MetaCyc metabolic pathway profiling between the gut metagenomic sample pools of control subjects and IgAN patients, revealing 34 significantly differentially abundant gut microbiome functional pathways. 4-Hydroxyphenylacetate degradation was the most enriched pathway in healthy controls compared with that in IgAN patients (coef. = –3.97, FDR = 1.00E-01), followed by the superpathway of lipopolysaccharide biosynthesis (coef. = –3.53, FDR = 2.24E-01) and the sulfoquinovose degradation I pathway (coef. = –3.51, FDR = 1.43E-01). However, multiple nucleotide- and nucleoside-biosynthesis-related pathways (including adenosine, guanosine, inosine, and pyrimidine biosynthesis) were more prominent in IgAN patients, as was glycolysis from glucose-6-phosphate (Figure 3.14).

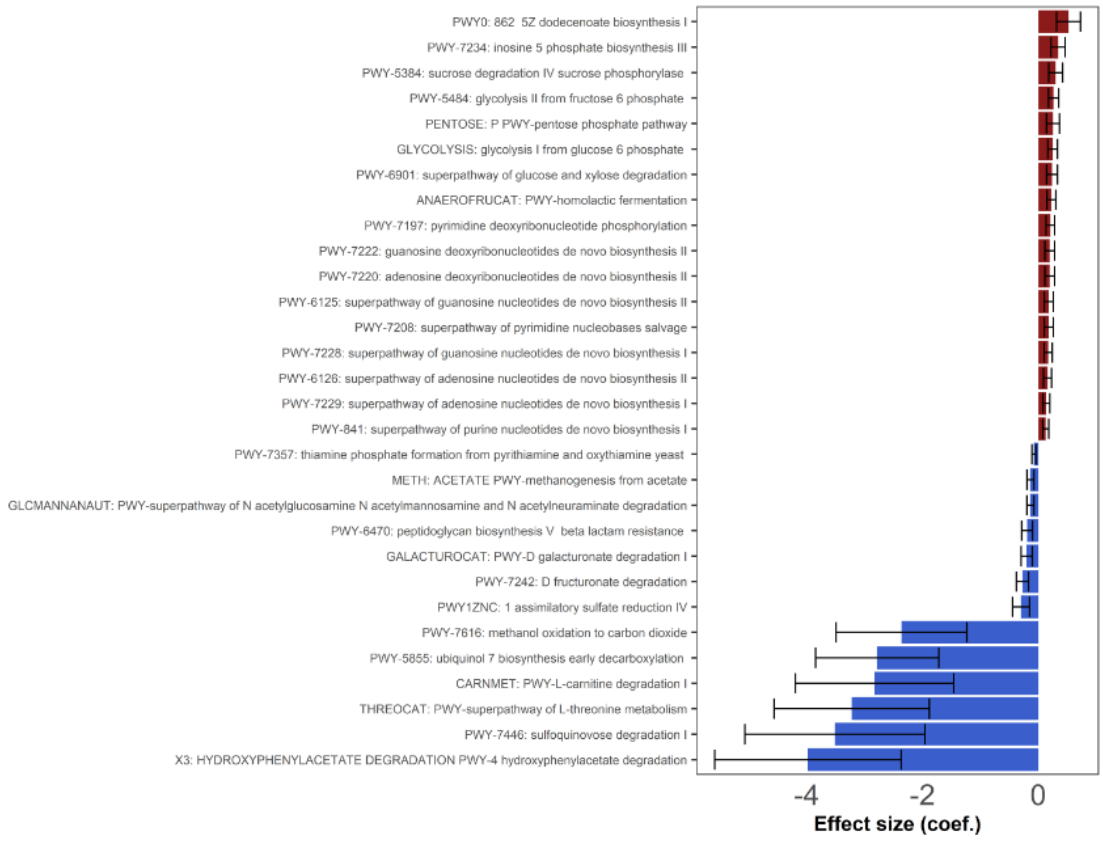

Figure 3.14. **Metabolic differential pathways in IgAN patients and healthy individuals**

The effect size is expressed as a coefficient with positive values for pathways enriched in IgAN patients, while negative values correspond to pathways enriched in control subjects.

Surprisingly, Gd-IgA1 accounted for a greater portion of the observed variations in metabolic pathways (PERMANOVA $R^2 = 0.061$, $p = 0.001$). This influence was greater than that attributed to the eGFR ($R^2 = 0.039$, $p = 0.015$) or BMI ($R^2 = 0.036$, $p = 0.02$) (Table 3.6).

Table 3.6

Contributing factors determining the observed variation in the gut microbiome-derived metabolic pathways among IgA nephropathy patients and healthy controls

Variable	Df	Sum Of Sqs	R2	F	Pr (> F)
Gd-IgA1, ng/ ml	1	0.04	0.06	4.52	1.60E-03
eGFR, ml/min/1.73 m^2	1	0.03	0.04	2.93	1.53E-02
BMI, kg/m^2	1	0.02	0.04	2.68	2.36E-02
Indoxyl sulphate, ng/ml	1	0.02	0.03	2.16	5.63E-02
Sex	1	0.02	0.02	1.76	1.01E-01
Type	1	0.01	0.02	1.58	1.33E-01
LPS, pg/ ml	1	0.01	0.01	0.70	6.21E-01
Residual	60	0.54	0.82	NA	NA
Total	67	0.67	1.00	NA	NA

Next, we aimed to assess the potential differences in the gut microbiome composition by focusing solely on the patient group. We retrospectively stratified the patients into two

groups based on their disease progression status: 23 were progressors with an eGFR decrease of more than 5 ml/min per year, and 23 were nonprogressors. Two patients lacked follow-up eGFR data and were excluded from further analysis. The mean follow-up time was 24 months (ranging from 2 to 43 months), with a mean eGFR reduction of 5.8 ml/min/year (ranging from a decline of 30 ml/min/year to an improvement in kidney function by 9 ml/min/year). There was no statistically significant difference in the alpha diversity metrics between the two groups (Table 3.7).

Table 3.7

Comparison of various alpha diversity measures between IgAN nonprogressors and progressors
(Wilcoxon test, Benjamini-Hochberg procedure)

Method	Nonprogressors (median, IQR)	Progressors (median, IQR)	p	p.adj
Observed taxa	2046.00 (1956.00 – 2097.00)	2043.00 (1862.00 – 2133.00)	0.74	7.40E-01
Pielou's evenness	0.65 (0.63 – 0.68)	0.65 (0.62 – 0.68)	0.36	3.60E-01
Shannon index	4.92 (4.82–5.24)	4.95 (4.69–5.19)	0.36	3.60E-01
Inverse Simpson index	61.85 (51.14–86.54)	53.86 (35.98–75.85)	0.2	2.00E-01
Chao1	2046.00 (1956.00–2097.00)	2043.00 (1862.00 – 2133.00)	0.74	7.40E-01

Nevertheless, distinct beta diversity clustering was observed between the two groups according to the PERMANOVA test ($R^2 = 0.03$; $p = 0.04$), with notably greater dispersion in progressors. While *Prevotella*, *Faecalibacterium*, and *Blautia* were the most prevalent genera in both groups, the order of abundance differed between them. In progressors, *Prevotella* was the most abundant genus (9.48 %), followed by *Blautia* (6.50 %) and *Faecalibacterium* (6.12 %), whereas *Faecalibacterium* (7.33 %) was the most common genus in nonprogressors. At the species level, *Prevotella sp00900557255* was the most abundant in progressors, with *Phocaeicola dorei* and *Fusicatenibacter saccharivorans* also prevalent. Among the nonprogressors, *Agathobacter rectalis* was the most abundant species (2.67 %), followed by *Fusicatenibacter saccharivorans* (2.18 %) and *Phocaeicola dorei* (2.02 %) (Figure 3.15).

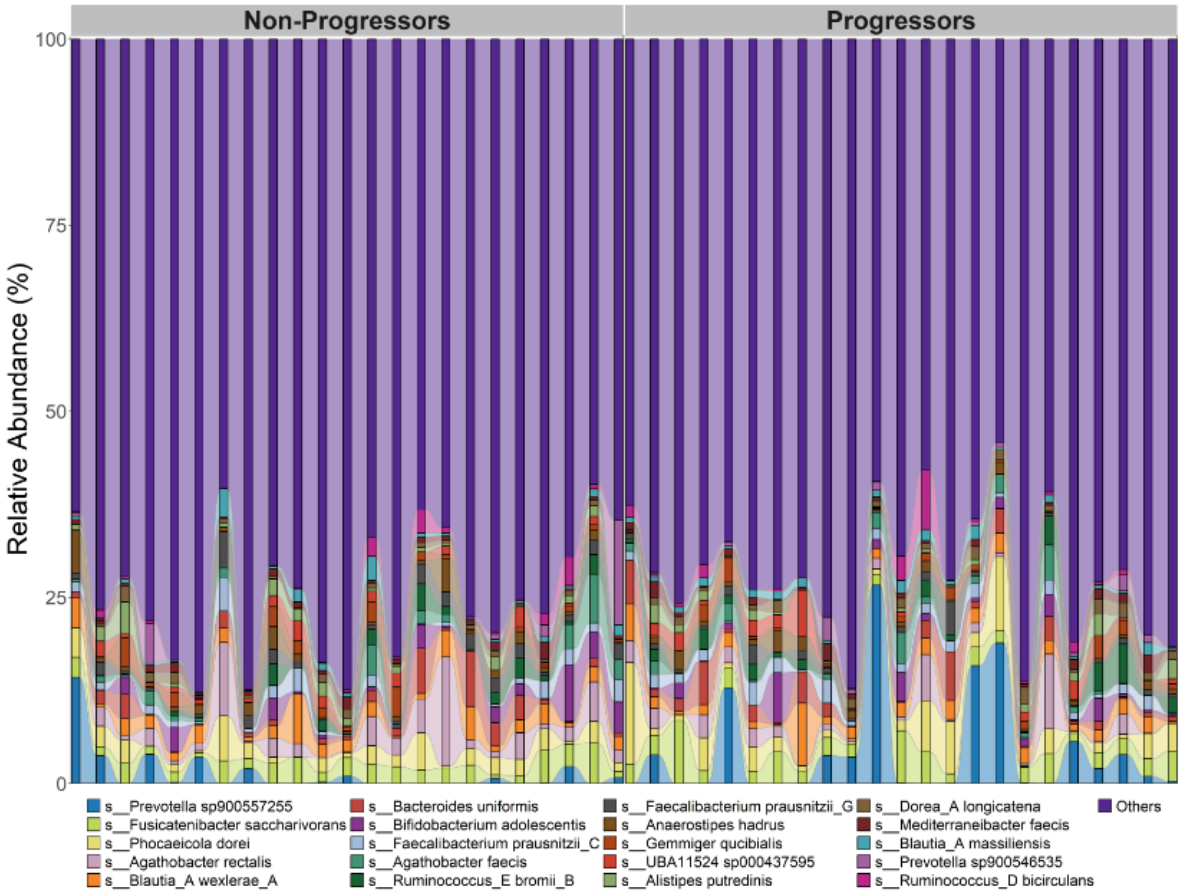

Figure 3.15 **Metabolic Taxonomy bar plot depicting the 20 most representative species for each individual, stratified by study group**

The differential abundance analysis at the species level comparing the gut microbiome compositions of progressors and nonprogressors revealed statistically significant differences in 455 taxa. Notably, *Staphylococcus hominis* (coef. = –8.39, FDR = 5.31E-02) and *UBA7173 sp900548705* (coef. = –6.50, FDR = 4.18E-03) were significantly more abundant in nonprogressors, whereas *Dialister hominis* (coef. = 6.38, FDR = 1.85E-08) was significantly more abundant in progressors (Figure 3.16). Among the 455 progression-related taxa, 69 (9 %) showed differential abundance also in the comparison of controls versus IgAN patients (e. g. *Akkermansia sp004167605, Dialister hominis*).

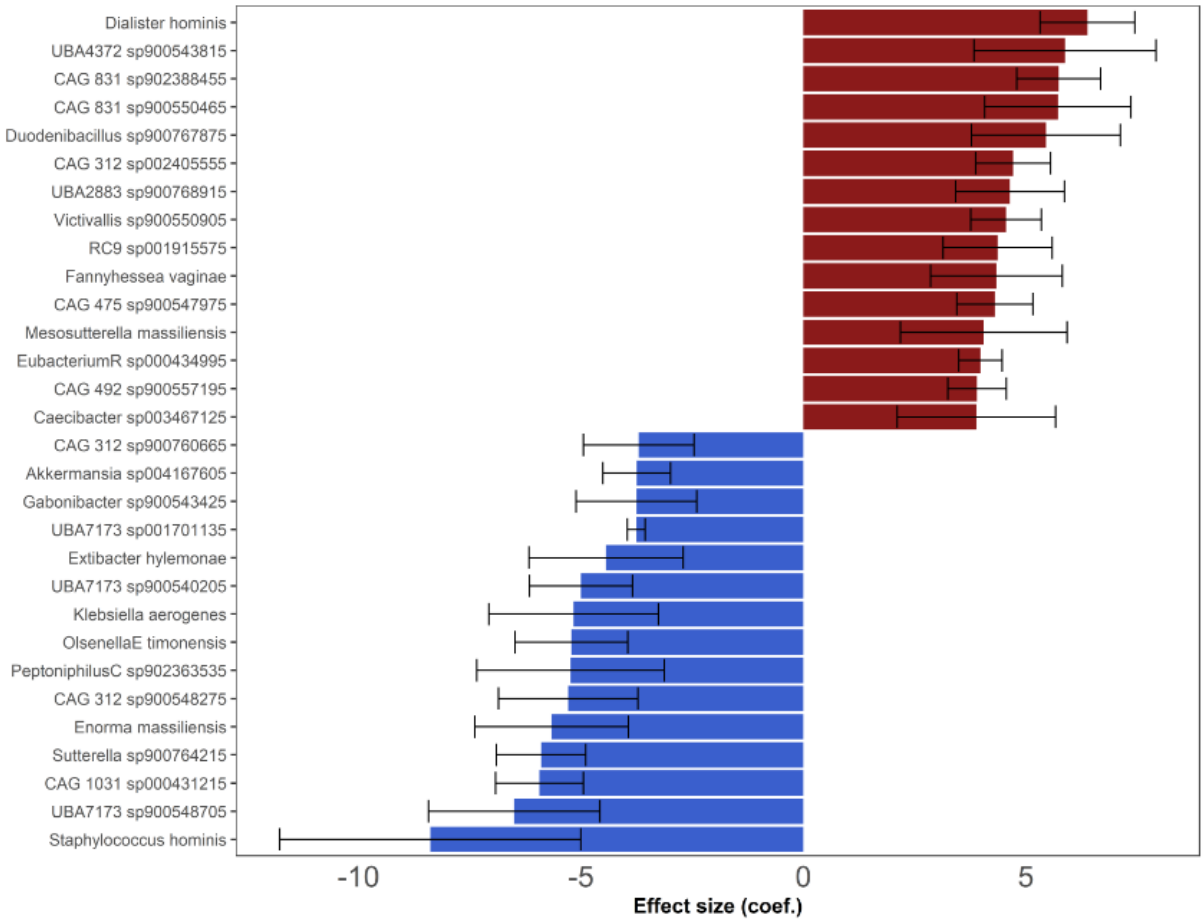

Figure 3.16. **Bar plot representing the effect size (logFC) of the top 20 differentially abundant species between IgAN progressors and nonprogressors**

A positive coefficient (red) represents taxa with increased abundance in the progressor group, and a negative coefficient (blue) represents taxa with increased abundance in the nonprogressors group.

Differential pathway analysis between nonprogressors and progressors revealed 21 metabolic pathways with the N-acetyl D glucosamine biosynthesis II pathway (coef. = −4.64, FDR = 1.27E-01), showing the strongest enrichment in nonprogressors. Progressors exhibited more active isopropanol biosynthesis (coef. = 1.08, FDR = 1.67E-1), followed by biotin biosynthesis II (coef. = 0.52, FDR = 2.28E-1) and phospholipid biosynthesis (Figure 3.17). Consistent with the case-control comparison, Gd-IgA1 accounted for a larger proportion of the observed variations in metabolic pathways (PERMANOVA $R^2 = 0.06$, $p = 0.018$) specifically among IgAN patients (Table 3.8). Furthermore, serum Gd-IgA1 levels in IgAN patients were significantly associated with the abundance of 88 metabolic pathways, including 57 positively associated pathways – such as phospholipid remodeling in yeast (coef. = 1.97; FDR = 1.17E-01) – and 31 negatively associated pathways, such as the superpathway of UDP-N-acetylglucosamine-derived O-antigen building blocks biosynthesis (coef. = −0.69; FDR = 4.33E-29) or creatinine degradation I (coef. = −0.67; FDR = 3.03E-54).

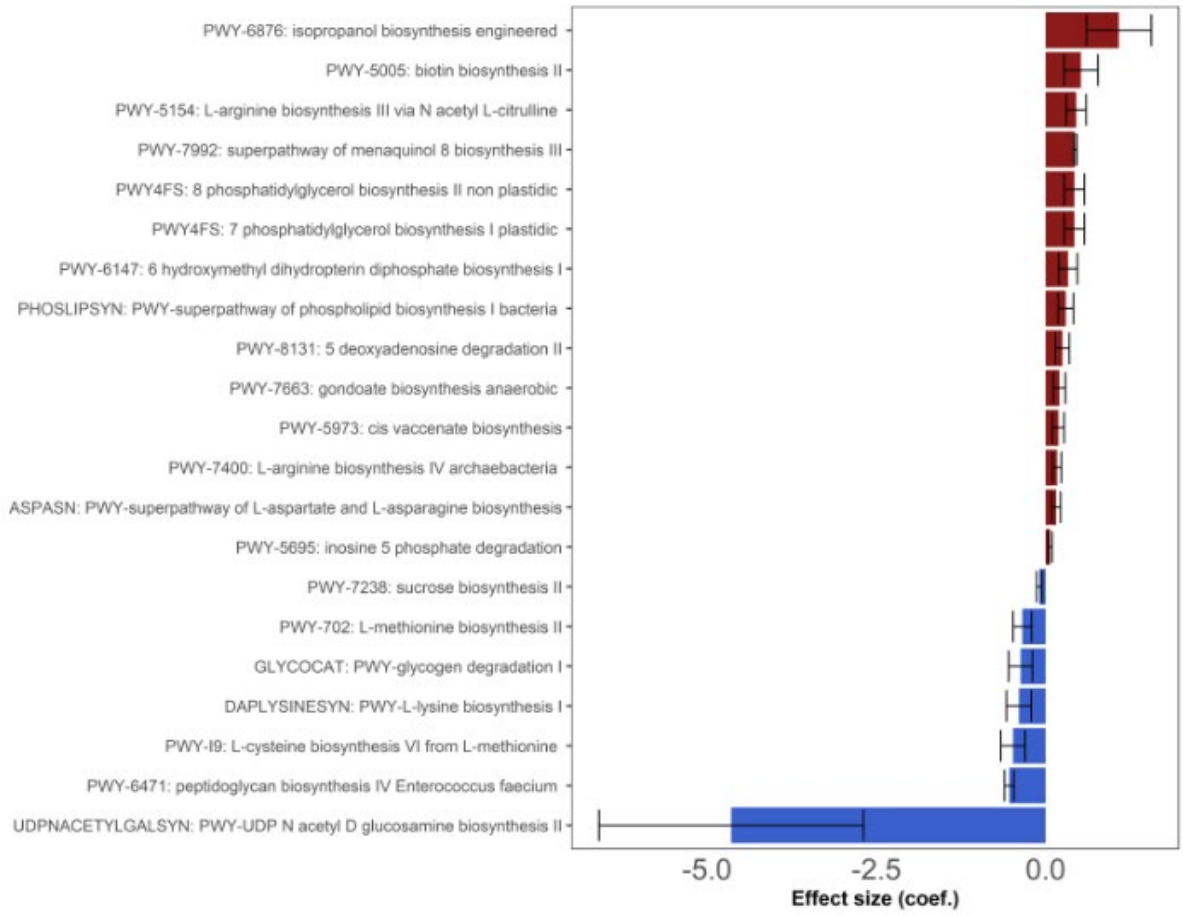

Figure 3.17 **Bar plot of MetaCyc metabolic differential pathways**

The effect size is expressed as a coefficient with positive values for pathways enriched in IgAN progressors and negative values for nonprogressors-related pathways

Table 3.8

Contributing factors determining the observed variation in the gut microbiome-derived metabolic pathways among IgA nephropathy patients

Variable	Df	Sum Of Sqs	R2	F	Pr (> F)
Gd-IgA1, ng/ml	1	0.03	0.06	2.80	1.81E-02
BMI, kg/m^2	1	0.02	0.04	1.83	8.74E-02
eGFR, ml/min/1.73 m^2	1	0.01	0.03	1.56	1.35E-01
Indoxyl sulphate, ng/ml	1	0.01	0.03	1.36	2.02E-01
Haematuria	1	0.01	0.02	1.10	3.22E-01
Sex	1	0.01	0.02	0.73	6.25E-01
Progression	1	0.01	0.02	0.73	6.30E-01
LPS, pg/ml	1	0.01	0.01	0.71	6.31E-01
Proteinuria range, g/g	1	0.01	0.01	0.58	7.69E-01
Prognosis	1	0.00	0.01	0.42	9.25E-01
Residual	35	0.33	0.73	NA	NA
Total	45	0.46	1.00	NA	NA

4 Discussion

The present work provides evidence on risk factors for kidney survival over a five-year period. The IIgANPT was applied to the study population, and the observed and predicted risks were similar, indicating that this tool can be effectively used in the Latvian population. Our results reveal that dysregulation of mucosal immunity drives increased naïve B cell activation in germinal centres, giving rise to $IgA^{+}CD27^{-}CD21^{+}$ B cells and subsequently to IgA-producing plasmablasts. The gut microbiome of patients with IgA nephropathy was analysed to identify possible mechanisms: these patients exhibited a reduction in butyrate-producing bacteria, and functional profiling indicated signs of immune activation and inflammation.

The diagnosis of IgAN in Latvia became possible after 2013, following the initiation of collaboration with the Vilnius Pathology Center in Vilnius, Lithuania. This partnership enabled the analysis of kidney biopsies using the immunofluorescence method, where the detection of positive IgA deposits in biopsy material confirms the diagnosis. IgAN can present in heterogeneous clinicopathological forms, ranging from asymptomatic urinalysis abnormalities to rapidly progressive glomerulonephritis. Across all discussed above IgAN patient cohorts, a male predominance is observed, with a median age of 40 years, and histological findings commonly showing mesangial hypercellularity and segmental glomerulosclerosis. Patients typically present with nephritic syndrome – characterised by mild to moderate proteinuria – or a nephritic-nephrotic syndrome, often accompanied by progressive glomerulosclerosis, which naturally affects disease prognosis. Approximately 20 % of patients diagnosed with IgAN at Pauls Stradins Clinical University Hospital initiated kidney replacement therapy on average 11 months after diagnosis.

The five-year kidney survival time was 57 % in our study group, while the Japanese study of 30-year analysis of kidney survival (n = 1012) showed that five-year kidney survival was more than 90 % (Moriyama et al., 2014). Another retrospective study on kidney survival in IgAN patients analysed long-term outcomes across four cohorts from different countries and three continents. The 10-year kidney survival rates reported were 95.7 % in Helsinki (Finland), 87.0 % in Sydney (Australia), 63.9 % in Glasgow (United Kingdom), and 61.6 % in Toronto (Canada). Additionally, five-year survival rates were 98 % in Helsinki, 95 % in Sydney, and 79 % in both Glasgow and Toronto, as demonstrated by survival curve analysis (Geddes et al., 2003). When comparing our findings to those of the Japanese and tricontinental studies, it appears that the disease course in Latvian patients is more aggressive, with a faster progression to ESKD. Differences in kidney biopsy indications across countries can contribute to this observation. In Japan, kidney biopsies are routinely performed in all patients with

suspected glomerulonephritis. In contrast, in Latvia, urinalysis is not routinely performed in the general population. As a result, many cases remain undiagnosed in time, and individuals often do not consult general practitioners until noticeable symptoms appear. Biopsies are typically conducted only when additional kidney manifestations are present, such as proteinuria, hypertension, or reduced GFR. Patients presenting with isolated haematuria are often not referred for biopsy. IgAN progression may vary by geographic region, proving genetic influence on the course of the disease. The genome-wide association studies have identified over 30 risk loci that may contribute to the pathogenesis of IgAN by affecting the dysregulation of mucosal innate immunity, immune cell proliferation, production of Gd-IgA1, formation of autoantibodies against Gd-IgA1, and activation of compliment system (L. L. Xu et al., 2023). A genetic risk score derived from replicated genome-wide association studies loci is highest among Asian populations, intermediate in Europeans, and lowest in Africans, reflecting the known global differences in IgAN prevalence. Notably, the genetic risk score also revealed an unexpectedly higher prevalence of IgAN-related kidney failure in Northern Europe (Kiryluk et al., 2013). Alongside genetic predisposition, there is also evidence of a subtly dysregulated mucosal immune response to antigens in IgAN, as reflected by alterations in gut permeability. Dietary habits, which differ between sexes and across ethnic groups, may also play a role in disease pathogenesis and further prognosis.

Until 2019, there was no validated tool to predict IgAN progression. The treatment decision was based on patient clinicopathologic features; however, as mentioned in the authors' publication, clinical trials suggested that 33 % of patients who did not meet clinical-treatment criteria but had high MEST scores did not receive treatment and experienced a decrease of kidney function, and more than 75 % of patients with low-risk nonprogressive disease were unnecessarily treated. The study population of "evaluating a new international risk-prediction tool in IgA nephropathy" was patients from multiethnic cohorts of adults with biopsy-proven IgAN, and it included patients from Oxford, North American, and VALIGA studies. In VALIGA study, patients from 53 centres in 13 countries were enrolled, and it included only one centre from the Baltic States (Estonia, Tartu) two centres from Scandinavian countries (Sweden), but many centres from Italy; this could indicate that the study population was nonhomogeneous (Barbour et al., 2019; Coppo et al., 2014). Considering that the course of IgAN may vary across different geographical regions, and that the Latvian population may differ from the general populations of Central and Southern Europe, it was important to clarify the disease course specifically within the Latvian population.

Our study focused on actual kidney survival analysis and validation of the IIgANPT in study population. To our knowledge, this was the first study in a European population to

compare predicted and observed risks of kidney disease progression since the IIgANPT became available online, followed later by analyses in Greek, Norwegian and French populations (Bon et al., 2023; Haaskjold et al., 2023; Papasotiriou et al., 2022).

In our study, the statistically significant factors affecting kidney survival were gender, MEST-T score, and diastolic blood pressure. Patients with T0 had a median kidney survival time longer than five years; those with T1 had a median survival of 40 months, while patients with T2 had the shortest survival – 18 months. The study and secondary analysis of the STOP-IgAN study also showed that T scores, with no effect modification by age, were associated with lower GFR and worse kidney outcomes (Coppo et al., 2020; Schimpf et al., 2018). Several studies indicated that hypertension prevalence in IgAN patients may vary between 19 % and 53 % at the time of kidney biopsy (Nagy et al., 2005). A large, multicentre study from China found an overall hypertension prevalence of 63.3 % among 1055 IgAN patients (Zheng et al., 2018). In our study group, 57.3 % (n = 59) of patients had hypertension defined as BP ≥ 140/90 mmHg, yet only 22 were using ACEI/ARB at the time of biopsy. This may be explained by late referral to a nephrologist in the absence of other clinical symptoms, or by caution in prescribing these medications to patients with low GFR. In this study, we found that the blood pressure affects kidney survival; patients with lower blood pressure had longer kidney survival. Zheng *et al*. supports a benefit of intensive control of BP < 130/80 mmHg for patients with proteinuria ≥ 1 g/d (Zheng et al., 2018). If the patient exhibits proteinuria exceeding 0.5 g/d, it is recommended to initiate therapy with either an ACEI or an ARB, regardless of whether hypertension is present (Kidney Disease: Improving Global Outcomes Glomerular Diseases Work, 2021).

Gender affected kidney survival, with women having longer kidney survival than men, and there were differences between predicted and observed data; for females, predicted and observed risks were more similar compared with those for males. Men tend to have poorer prognosis than women in IgAN mainly because male patients often present with worse clinical and pathological features at diagnosis: lower eGFR, greater proteinuria, higher prevalence of hypertension, hypertriglyceridemia, and hyperuricemia, more severe histopathological damage, including segmental sclerosis and tubular atrophy/interstitial fibrosis, higher proportion of global glomerulosclerosis (Deng et al., 2018).

In this study, we found that the predicted and observed risks of a 50 % decline in GFR or progression to ESKD were comparable. Furthermore, the five-year analysis showed a moderate concordance between predicted and actual outcomes, supporting the reliability of the IIgANPT for use in the Latvian population. Other external validation studies of the prediction tool conducted in Norwegian, Chinese, and Chinese Argentinian cohorts also

demonstrated good discrimination and model fit overall. However, in the Chinese Argentinian cohort, the full model (including race) overestimated the risk of progression over a 3-year period (Zhang et al., 2020). In an Indian cohort, the tool showed reasonable discriminatory ability but consistently underestimated risk across all groups (Bagchi et al., 2022). In an increasingly multicultural world, where defining ethnicity is complex, risk stratification based on genetic profiles rather than self-reported ethnicity may offer a more precise approach to predicting outcomes.

This shift toward genetic profiling aligns with growing insights into the molecular and immunological underpinnings of IgAN. Current concept of the pathogenesis of IgAN has been referred to as the "multi-hit" hypothesis (Scionti et al., 2022), and advances have been made particularly in uncovering the mechanisms operating within the kidney that contribute to organ damage. These include the dissection of how immune complexes containing IgA bind to mesangial cells, triggering proliferation and increased synthesis of extracellular matrix components as well as the role of the CD89 in regulating tissue damage (Launay et al., 2000). However, these mechanistic insights are unlikely to lead to major therapeutic breakthroughs, as they reflect processes that occur after B cell tolerance has already been broken.

Our findings reveal alterations in the B cell compartment among patients with IgAN, notably an expansion of naïve B cells and a reduction in total memory B cells. Similar B cell patterns are characteristic of systemic autoimmune diseases such as systemic sclerosis, rheumatoid arthritis (Bugatti et al., 2014; Sato et al., 2004). This observation is particularly significant because IgAN is considered an organ-specific autoimmune disease, and these data suggest that systemic immunity is dysregulated beyond previously characterised mucosal immune abnormalities. However, we did not observe an expansion of total double-negative (DN) B cells, which is typically associated with more severe systemic autoimmune and inflammatory phenotypes. Determining whether the systemic alterations in the overall B cell landscape observed in this study are a cause of IgAN or a consequence of autoimmunity-related inflammation or bystander effects remains an important question.

As the source of pathogenic Gd-IgA1 antibodies, B cells are a promising target in IgAN, but B cell activation and differentiation pathways that lead to pathogenic IgA production remain uncharacterised. Understanding how B cells are affected and become activated is important for the complete understanding of IgAN pathogenesis, prognosis, and treatment. Here we provide mechanistic insight into the early steps of IgAN pathogenesis by uncovering that IgA-expressing B cells that lack the classical memory marker CD27, but express CD21 are expanded in patients with IgAN and appear to be the cellular precursors of IgA-producing plasmablasts (Annex 5) (Popova et al., 2025). Our findings indicate that these ASC precursors

likely originate from active germinal centres, suggesting a continuous production of pathogenic cells. This points to an ongoing process rather than a predominant contribution from memory B cells or plasmablasts formed earlier. DN1 B cells have not been previously associated with disease states, unlike the extensively characterised DN2 subset. Therefore, our study may help uncover more about this B cell population. This observation of the increased recent germinal centre emigrant DN1 B cells also fits with the report of increased frequency of T follicular helper cells in IgAN patients (Zhang et al., 2014).

We found a correlation between the $CD27^-$ precursors of IgA-expressing plasmablasts and serum LPS levels, that implicates mucosal immunity in the differentiation of these cells. LPS could directly contribute to class-switching in activated B cells as previously observed (Xu et al., 2008). In support of direct activation, tonsillar B cells from IgAN patients have been shown to produce increased IgA in response to LPS stimulation (Liu et al., 2011). Therefore, LPS may act at multiple stages of B cell activation to not only induce class-switching and IgA production, but to also regulate IgA glycosylation. An alternative explanation for the observed correlation between IgA^+CD27^- B cells and LPS could be that both are elevated because of intestinal inflammation, which may promote the generation of IgA^+CD27^- B cells and increase intestinal permeability. Supporting the mucosa as a site of origin for these IgA expressing B cells, IgAN patients have an increased frequency of IgA^+ B cells that express the mucosal homing marker CC chemokine receptor 9 (CCR9) (Sallustio et al., 2021a). Tonsillectomy, which has shown some therapeutic benefit in IgAN (Hirano et al., 2019), may potentially eliminate the site where these B cells are generated or where they migrate to (Wu et al., 2013). This may also help explain why B cell depletion therapies have shown inconsistent efficacy in IgAN, despite the central role of B cells in its pathogenesis – as mucosal niches may retain a significant proportion of precursor cells that are not effectively targeted by such treatments (Lafayette et al., 2017). Recent findings of disease-modifying effects of enteral budesonide in IgAN further implicate mucosal sites in driving immunopathogenesis (Smerud et al., 2011).

In our cohort of IgAN patients, the distribution of men, as well as the age and BMI, was found to be like that reported in other studies focusing on IgAN. Clinically, patients exhibited lower protein excretion, yet their eGFR was worse when compared to the results of other studies evaluating B cells in IgAN (Sallustio et al., 2021b; Wang et al., 2014). Of note, a limitation of the study is that our cohort consisted entirely of white Eastern Europeans and thus the B cell activation pathway we have uncovered remains to be investigated in non-European cohorts.

Now that we have identified the aberrant B cell differentiation pathways in IgAN, important further questions can be addressed. Key questions remain about the site of induction and long-term residence of IgA-expressing plasmablast precursors – are these cells generated

and maintained in mucosal tissues, the bone marrow, or both, and is this pattern consistent among IgAN patients? Can specific targets be identified to selectively deplete or inhibit the differentiation of the pathogenic $IgA^{+}CD27^{-}$ B cells subset?! What antigens are IgA-expressing B cells in IgAN responding to – dietary or microbial components – and how diverse is the IgA response within an individual and across patients?

To explore whether microbial composition might influence the antigenic targets of IgA-expressing B cells in IgAN, we examined the gut microbiome profiles of affected individuals. It was a non-interventional study that did not establish causal relationships between findings. Our results aligned with previous gut microbiome studies in the Latvian population, which have identified *Prevotella* as the most common genus, showing a similar microbial community composition between the IgAN and HC groups. The high consumption of a plant-based, high-fibre diet is plausibly the primary reason for the high abundance of *Prevotella*. Interestingly, the data from our cohort in Latvia are like those of non-western populations (India, Peru, Tanzania, and Madagascar) (De Filippis et al., 2019). In contrast, the gut microbiome of Western populations, such as those in the United States and Spain, which consume a typical Westernised diet (rich in protein and fat and low in plant-based fibres), is enriched mainly in *Bacteroides* and *Ruminococcus*, with a very low abundance of *Prevotella* (Prasoodanan et al., 2021). According to a recent meta-analysis of gut microbiome characteristics in IgAN patients, which involved 11 cross-sectional studies and included 652 individuals, no consistent significant variations in the abundance of specific bacteria were noted across the different studies. For example, the *Bacteroides* genus had a greater relative abundance in patients with IgAN than in healthy individuals in three studies, a lower abundance in three studies, and no significant differences in four studies. Similarly, ambiguous data were found for Streptococcus, with higher abundances in patients with IgAN in two studies, lower abundances in one study, and no significant changes in eight studies (Han et al., 2022).

The differential abundance analysis in our study revealed that *Butyrococcus* and *Eubacterium rectale* (*Agathobacter rectalis*) were less abundant in IgAN patients than in healthy controls. Both bacteria produce butyrate from carbohydrates (Geirnaert et al., 2014). *E. rectale* preferentially colonises the mucus layer, thereby enhancing the bioavailability of butyrate for colon epithelial cells (El Aidy et al., 2013). Butyrate promotes intestinal barrier function by strengthening tight junctions and producing mucin and antimicrobial peptides and is essential for the differentiation of CD4-positive regulatory T cells (Tregs), which suppress immune responses (Furusawa et al., 2013; Riviere et al., 2016). According to Yang et al, the number of Tregs is decreased in IgAN patients (Yang et al., 2015). Moreover, *Agathobacter rectalis* was the most prevalent bacteria in nonprogressors, patients with a favourable prognosis.

These results highlight the potential importance of butyrate substitutions or dietary interventions. Butyrate-producing bacteria are predominantly slow-growing anaerobes, and their reduced abundance may be influenced by uremic toxins, gut mucosal inflammation, and the overgrowth of other bacterial species. In the intestinal microbiota of patients with IgAN, we observed an increased relative abundance of mucin-degrading bacteria, including *Akkermansia muciniphila*. This Gram-negative bacterium is an important modulator of the gut immune system, promoting the stimulation of cytokines such as IL-10 and enhancing secretory IgA production (Rodrigues et al., 2022). Another recent study found that IgA1 is deglycosylated by *Akkermansia muciniphila* both in vitro and within the intestinal lumen of mice, generating neo-epitopes that are recognised by autoreactive IgG in the sera of patients with IgAN. Following deglycosylation in the mouse gut, IgA1 underwent retrotranscytosis across the intestinal epithelium, entered the circulation, and was subsequently deposited in the glomeruli of the kidneys (Gleeson et al., 2024).

We found no significant difference in gut microbial diversity between IgAN patients and healthy individuals. These findings suggest that not the gut microbiome composition or microbial diversity plays a crucial role in IgAN pathogenesis but, hypothetically, changes in microbial community function.

Increased nucleoside (adenosine, guanosine, and inosine) biosynthesis pathways were found in IgAN patients. Extracellular adenosine is increased during intestinal inflammation. A key contributing factor to the development of intestinal inflammation is intestinal barrier dysfunction, which is associated with an altered microbiome composition, bacterial infiltration, impaired cell junctions, loss of the mucus layer and acidification. This contributes to the release of ATP from injured cells, which is converted to the alarm molecule adenosine (Stepanova & Aherne, 2024). Adenosine is a potent endogenous anti-inflammatory agent that regulates the function of inflammatory cells via interaction with specific receptors expressed on lymphocytes, inhibiting Th17 differentiation and stimulating Treg differentiation (Hasko & Cronstein, 2013). Inosine is formed from the breakdown of adenosine and has been shown to exert powerful anti-inflammatory effects related to the activation of adenosine receptors (Gomez & Sitkovsky, 2003).

Guanosine is a fundamental component of nucleotides and is essential for DNA and RNA synthesis (Balan et al., 2019). These processes are vital in rapidly dividing cells, such as immune cells (e. g. lymphocytes and plasma cells), which require substantial amounts of nucleotides to support their proliferation during immune response. Lymphocytes and plasma cells are highly metabolically active during activation and expansion, as they produce antibodies and mediate immune responses. The increased demand for guanosine and other

purine nucleotides in these cells aligns with their role in immune activation and inflammation, particularly in conditions such as IgAN, where aberrant immune responses are implicated. Another pathway that is involved in the proliferative and inflammatory process, glycolysis from glucose 6-phosphate, was also increased in IgAN patients compared with healthy controls. Glycolysis is typically used as an energy source and is known to be upregulated in dividing cells (Ghazi et al., 2019).

The 4-hydroxyphenylacetate degradation pathway was more prominent in healthy controls than in IgAN patients. 4-Hydroxyphenylacetate is a common product during the fermentation of aromatic amino acids, particularly in the L-tyrosine degradation pathway, which is carried out by several Escherichia coli strains and generates pyruvate and succinate as end products (Kim et al., 2025). Higher succinate production by the gut microbiota is promoted by a high-protein diet (Tan et al., 2022). Usually, chronic kidney disease patients are recommended to decrease protein intake in their diet, resulting in potentially increased protein consumption in healthy individuals.

Another pathway that was decreased in IgAN patients – the sulfoquinovose degradation I pathway. Sulfoquinovose is a plant-derived sulfonated monosaccharide that serves as the polar headgroup of the sulfolipids in photosynthetic membranes. The final product of this pathway is glycerone phosphate, which powers the cell via glycolysis from glucose 6-phosphate, and 2,3-dihydroxypropane-1-sulfonate in most individuals fermented mainly by *E. rectale* (Hanson et al., 2021). Afterwards, it is excreted and can be further degraded by other bacteria and serves as a source of sulphite (Burrichter et al., 2018). As mentioned previously, *E. rectale* in our study was less abundant in IgAN patients, which confirms the possibility of delayed sulfoquinovose degradation. To date, there are no other studies in IgAN patients showing this pathway alteration.

Previous studies have analysed the bacterial composition in progressors and nonprogressors. Nonprogressor patients were defined as having a 50 % decrease in daily proteinuria and stable kidney function (eGFR) after six months compared with baseline (Angelis de et al., 2014); however, metabolic pathways were not studied between the two groups. In our study, the progression rate was evaluated according to a significant GFR slope of 5 ml/min/1.73 m^2/year at a mean follow-up time of 24 months, which is usually irreversible. Pathways enriched in progressors were connected to phospholipid synthesis in bacteria, which are essential components of bacterial cell membranes. Gram-negative bacteria have a cell envelope composed of three distinct layers: the inner membrane, a periplasmic space containing the peptidoglycan cell wall, and the outer membrane, with its inner leaflet containing phospholipids and its outer leaflet predominantly composed of lipopolysaccharides (Morris &

Mitchell, 2023). Phospholipid synthesis may be adapted to increase bacterial survival, colonisation, or competition.

The main limitation of the microbiome study part is its small sample size, which restricts confounder control and the detection of less prevalent microbial taxa. Additionally, while we employed a data-driven approach – using PERMANOVA – to select covariates for differential abundance and pathway analyses, we acknowledge that this method does not account for potential unmeasured confounders. Factors not captured in our dataset, such as dietary patterns, medication use, or environmental exposures, could influence microbiome composition and function and thus may impact our findings. We fully acknowledge that metabolic pathways inferred from bacterial DNA through metagenomic analysis represent only the putative functional potential of the microbiome, rather than direct evidence of metabolite activity. Given the strong evidence of IgAN-related shifts in gut microbiome functions, future studies may benefit from integrating metabolomics or metatranscriptomics data to provide deeper insights into the functional consequences of these microbial alterations. Additionally, investigating the effects of diet and probiotic supplementation on IgAN progression and immune cell dynamics remains an important avenue for future research.

The renoprotective effects of butyrate have been observed in various types of kidney diseases: in cisplatin-induced kidney injury (Favero et al., 2024), diabetic kidney disease (Zhou et al., 2022), obesity-related glomerulopathy (Shi et al., 2025), septic kidney injury modelled by lipopolysaccharide administration. In the latter model, sodium butyrate exerted nephroprotective effects by modulating TLR 2/4 signalling to regulate β-defensin 2 expression, thereby reducing inflammation (Dou et al., 2022). Another proposed mechanism involves the inhibition of both pyroptosis and apoptosis (Tian et al., 2025). As lipopolysaccharides derived from gut bacteria contribute to IgAN pathogenesis by promoting IgA1 overproduction and hypogalactosylation through TLR4 activation (Zhu et al., 2024), and given the decreased abundance of butyrate-producing bacteria observed in IgAN patients, future studies should investigate the potential therapeutic benefits of butyrate supplementation in IgAN management.

We have reported associations between specific microbial taxa in IgAN patients compared to healthy individuals, as well as in relation to kidney function decline. However, such associations do not imply causation. It remains essential to determine whether these microbiome alterations are a causal factor in IgAN development or a consequence of disease process. A combination of advanced computational approaches, animal models, and clinical trials is essential to clarify the role of gut microbiome alterations in disease pathogenesis. These efforts should also account for the critical influence of diet on the gut–kidney axis.

Can the observed changes in the gut microbiome and B cells be the result of CKD? To minimise potential CKD-related effects, patients receiving kidney replacement therapy were excluded. With respect to B cells, CKD in general is associated with reduced memory B cells and impaired adaptive immunity. However, the specific pattern of increased naïve B cells and defective IgA memory differentiation leading to the formation of Gd-IgA1-secreting plasmablasts is more characteristic of IgAN. Some gut microbiome changes can indeed be associated with CKD; however, galactose-deficient IgA1 was the major factor accounting for a greater proportion of the observed variations in metabolic pathways, suggesting that these changes may be characteristic of patients with IgAN specifically. For future microbiome studies, detailed inclusion criteria should be applied to minimise possible confounding effects – for example, including only CKD patients with an eGFR > 60 ml/min.

Our IgAN research group plans to continue investigating the disease's pathogenesis by integrating analyses of B cell composition, with a particular focus on the interplay between the previously described $IgA^{+}CD27^{-}$ B cells and functional pathways of the gut microbiome. The next project will combine flow cytometry, single-cell transcriptional profiling, and confocal microscopy of mucosa-associated lymphoid tissue – specifically Peyer's patches – to identify the factors driving altered B cell activation in IgAN. We will employ cultures of sorted B cells and *in vitro* Peyer's patches models to explore what drives the differentiation of Gd-$IgA1^{+}$ B cells and antibody-secreting cells. Together, these approaches aim to fill a critical knowledge gap concerning the source and differentiation pathways of B cells involved in IgAN pathogenesis.

Critical analysis of the study

This study has several limitations that should be considered. First, the IgAN patient group was relatively small and heterogeneous, particularly with respect to CKD distribution. However, given the resources required for microbiome analyses, single-centre studies typically include a similar number of patients. During patient enrolment and sample collection, IgAN patients had no access to SGLT2 inhibitors, budesonide, or participation in clinical trials; therefore, the medications received by the included patients did not interfere with IIgANPT results and likely had no significant impact on B cell characteristics.

The COVID-19 pandemic also affected study progress. Patient and control group recruitment, as well as collaborative work within the research team, were slowed by epidemiological regulations and variable staff employment conditions. Many patients declined hospital visits due to fear of infection. Additionally, delays in the microbiome analyses occurred

due to differing perspectives between nephrologists and biostatistics specialists regarding analysis methodology.

Dietary influence on the gut microbiome represents another potential confounding factor. Study participants completed three-day food diaries, but these have not yet been analysed. This provides an opportunity for a future study combining dietary habits, microbiome profiles, and B cell characteristics to better understand disease-associated microbiome changes.

Despite these limitations, up-to-date methods were employed, including immunophenotyping of peripheral blood B cells by flow cytometry, shotgun sequencing of DNA libraries, and functional profiling using specialised software. The results are reproducible and have been published in open-access journals. Overall, this research represents a collaborative team effort, and the findings provide a strong foundation for further studies in IgAN pathogenesis and the gut-kidney-immune axis.

Conclusions

1 Patients with IgAN who have risk factors such as male gender, higher scores for tubular atrophy/interstitial fibrosis, and elevated diastolic blood pressure have an unfavourable prognosis for kidney survival over a five-year period, resulting in faster decline of renal function and progression to ESKD.

2 The observed and predicted risks using IIgANPT were similar, indicating that this tool can be effectively applied in the Latvian population.

3 Dysregulation of mucosal immunity drives the increased naïve B cell activation in germinal centres, giving rise to $IgA^{+}CD27^{-}CD21^{+}$ B cells and subsequently IgA-producing plasmablasts.

4 Compared to healthy controls, patients with IgAN exhibit a reduction in butyrate-producing bacteria, which are essential for maintaining intestinal barrier function. Functional profiling of the gut microbiome in these patients suggests immune activation and inflammation, as indicated by increased activity in various nucleoside biosynthesis pathways.

Proposals

For better IgAN diagnostics in Latvia, urinalysis should be performed as a screening method at least once in adults aged 18–30 years and in all patients with hypertension. Patients with any grade of proteinuria or haematuria should be evaluated for kidney biopsy and receive follow-up care from a general practitioner or nephrologist, depending on the clinical manifestations.

As IgAN is a heterogeneous disease with an unfavourable prognosis, risk assessment based on clinical factors and the IIgANPT results should be performed at diagnosis and every six months during follow-up. For patients at higher risk of progression, more aggressive lifestyle modifications, including dietary guidance, along with optimal blood pressure control and the use of maximally tolerated agents to inhibit angiotensin II activity, should be initiated.

Publications and reports on topics of doctoral thesis

Publications

1. **Popova, A**., Racenis, K., Briviba, M., Saksis, R., Saulite, M., Slisere, B., Berga-Svitina, E., Oleinika, K., Saulite, A. J., Seilis, J., Kroica, J., Cernevskis, H., Petersons, A., Klovins, J., Lejnieks, A., & Kuzema, V. (2025). Reduced butyrate-producing bacteria and altered metabolic pathways in the gut microbiome of immunoglobulin A nephropathy patients. *Sci Rep*, 15(1), 28011. doi: 10.1038/s41598-025-13629-5

2. **Popova, A**., Slisere, B., Racenis, K., Kuzema, V., Karklins, R., Saulite, M., Seilis, J., Saulite, A. J., Vasilvolfa, A., Vaivode, K., Pjanova, D., Kroica, J., Cernevskis, H., Lejnieks, A., Petersons, A., Oleinika, K. (2025). IgA class-switched CD27−CD21+ B cells in IgA nephropathy. *Nephrology Dialysis Transplantation*, Volume 40, Issue 3, March 2025, 505–515. doi: 10.1093/ndt/gfae173

3. Saulīte, A. J., **Popova, A**., Erts, R.. Rācenis, K., Kučāne, L., Pētersons, A., Laurinavičius, A., Čerņevskis, H., Kuzema, V. (2021). Renal Survival and Validation of Novel International Immunoglobulin A Nephropathy Prediction Tool in Latvian Population. Preliminary Data. *Proceedings of the Latvian Academy of Sciences. Section B. Natural. Exact. and Applied Sciences*. 75(5). 379–386. doi: 10.2478/prolas-2021-0055.

4. Kučāne, L., **Popova, A**., Kuzema, V., Lejnieks, A., Pētersons, A. (2020). Epidemiological, Clinical and Morphological Characteristics of Immunoglobulin A Nephropathy in Latvia. *Proceedings of the Latvian Academy of Sciences. Section B. Natural. Exact. and Applied Sciences*. 74(4): 227–231. doi: 10.2478/prolas-2020-0036.

Oral Presentations at international congresses and published abstracts in *Nephrology Dialysis Transplantation*

1. **Popova, A**. Halturina, M., Rācenis, K., Saulīte, M., Seilis, J., Saulite, A. J., Petersons, A., Kroiča, J., Cernevskis, H., Oļeiņika, K., Šlisere, B., Lejnieks, A., Kuzema, V. (2023). Montreal Cognitive Assessment (MOCA) and Dysmorphic Red Blood Cells in Patients with Immunoglobulin a Nephropathy. *Nephrology Dialysis Transplantation*. vol. 38. no. Suppl.1. 5133. I338–I339. doi: 10.1093/ndt/gfad063c_5133

2. **Popova, A**., Rācenis, K., Kuzema, V., Saulīte, A. J., Saulīte, M., Broks, R., Oļeiņika, K., Šlisere, B., Petersons, A., Laurinavicius, A., Cernevkis, H., Lejnieks, A., Kroiča, J. (2022). Leucine-Rich Alpha-2-Glycoprotein as a Potential Marker of Mesangial Cell Proliferation in Immunoglobulin a Nephropathy. *Nephrology Dialysis Transplantation*. 37(Supplement_3). i293–i293. doi: 10.1093/ndt/gfac070.019

3. **Popova, A**., Rācenis, K., Saulite, A. J., Vasiļvolfa, A., Pētersons, A., Čerņevskis, H., Kuzema, V. (2021). Bedside Urine Sediment Examination in Immunoglobulin A Nephropathy Patients Performed by Nephrologists. *Nephrology Dialysis Transplantation*. Volume 36. Issue Supplement_1. doi: 10.1093/ndt/gfab104.0077

Oral Presentations at other international congresses and conferences

1. **Popova, A**. Are There Specific Changes in Microbial Composition in Patients with Immunoglobulin A Nephropathy? Rīga Stradiņš University International Research Conference on Medical and Health Care Sciences "Knowledge for Use in Practice". Abstracts. 26–28 March 2025. 122.

2. **Popova, A**. Gut Microbiome Characteristics in IgA nephropathy patients. XVII Baltic Nephrology Conference. 4 October 2024, Tartu, Estonia.

3. **Popova, A**., Rācenis, K., Saulīte, M., Seilis, J., Šlisere, B., Oļeinika, K., Saulīte, J. A., Kuzema, V., Kroiča, J., Lejnieks, A., Pētersons, A., Čerņevskis, H. (2023). The Role of Microbiome in Pathogenesis of Immunoglobulin A Nephropathy. Rīga Stradiņš University International Research Conference on Medical and Health Care Sciences "Knowledge for Use in Practice". Abstracts. 27–31 March 2023.

4. Halturina, M., **Popova, A**., Rācenis, K., Saulīte, M., Pētersons, A., Saulīte, A. J., Seilis, J., Čerņevskis, H., Kroiča, J., Lejnieks, A., Oļeiņika, K., Šlisere, B., Kuzema, V. (2022). Montreal Cognitive Assessment (MoCA) for Cognitive Screening in Patients with IgA Nephropathy compared with a control group. XVI Baltic Nephrology Conference. Abstracts. 6–8 October 2022. 49, Latvia.
5. **Popova, A**., Rācenis, K., Šlisere, B., Oleinika, K., Vasiļvolfa, A., Saulīte, A. J., Kuzema, V., Kroiča, J., Lejnieks, A., Berga-Švītiņa, E., Pētersons, A., Čerņevskis, H. (2021). Dissecting the interplay between intestinal dysbiosis and B cell function in the pathogenesis of immunoglobulin A nephropathy. Rīga Stradiņš University International Research Conference on Medical and Health Care Sciences "Knowledge for Use in Practice". Abstracts. 22–26 March 2021. 434.
6. **Popova, A**. IgA nephropathy research in Latvia – national project. XV Baltic Nephrology Conference "UpToDate in Nephrology, Dialysis and Kidney Transplantation". 12 December 2020, Lithuania.

Theses at international congresses and conferences

1. Saulīte, M., Matusevica, L., **Popova, A**., Kuzema, V., Oļeiņika, K., Šlisere, B., Pētersons, A., Kroiča, J., Lejnieks, A., Rācenis, K. 2025. Risk Factors for IgA Nephropathy Progression: Insights beyond Galactose-Deficient IgA1 Levels. Rīga Stradiņš University International Research Conference on Medical and Health Care Sciences "Knowledge for Use in Practice". Abstracts. 26–28 March 2025. 123.
2. Saulīte, M., Matuseviča, L., **Popova, A**., Kuzema, V., Oļeiņika, K., Šlisere, B., Pētersons, A., Kroiča, J., Rācenis, K. 2024. The Ambiguous Role of Serum Gd-IgA1 in IgA Nephropathy: An Evaluation of Its Biomarker Potential. XVII Baltic Nephrology Conference. 4 October 2024, Tartu, Estonia.
3. Rācenis, K., Saulīte, A. J., **Popova, A**., Saulīte, M., Kroiča, J., Petersons, A., Cernevskis, H., Lejnieks, A., Oļeiņika, K., Šlisere, B., Seilis, J., Kuzema, V. (2022). Sars-COV-2 Vaccination DID Not Affect the Clinical Course of IgA Nephropathy in Latvian Adult Cohort. *Nephrology Dialysis Transplantation*. 37(Supplement_3). i145–i146. doi: 10.1093/ndt/gfac067.012
4. Halturina, M., **Popova, A**. (2022). Montreal Cognitive Assessment (MoCA) for Cognitive Screening in Patients with IgA Nephropathy. 17th Warsaw International Medical Congress. 06–08 May 2022. 100.
5. **Popova, A**., Saulīte, A. J., Rācenis, K., Vasiļvolfa, A., Kroiča, J., Pētersons, A., Lejnieks, A., Oleinika, K., Čerņevskis, H., Kuzema, V. (2021). Serum complement C3 and C4 levels: prognostic factors in immunoglobulin A nephropathy. Rīga Stradiņš University International Research Conference on Medical and Health Care Sciences "Knowledge for Use in Practice". Abstracts. 22–26 March 2021. 524.
6. Šlisere, B., **Popova, A**., Rācenis, K., Vasiļvolfa, A., Berga-Švītiņa, E., Imša, K., Saulīte, A. J., Kuzema, V., Laurinavicius, A., Lejnieks, A., Kroiča, J., Pētersons, A., Čerņevskis, H., Oleinika, K. (2021). Altered B-cell populations in IgA nephropathy. Rīga Stradiņš University International Research Conference on Medical and Health Care Sciences "Knowledge for Use in Practice". Abstracts. 22–26 March 2021. 565.
7. Bernšteine, A. J., **Popova, A**., Kučāne, L., Rācenis, K., Kuzema, V., Pētersons, A. (2020). Immunoglobulin A nephropathy prediction tool validation in Latvian population. XV Baltic Nephrology Conference UpToDate in Nephrology. Dialysis and Kidney Transplantation. Abstracts. 8. Lithuania.
8. Šlisere, B., **Popova, A**., Rācenis, K., Vasiļvolfa, A., Berga-Švītiņa, E., Kuzema, V., Lejnieks, A., Kroiča, J., Pētersons, A., Čerņevskis, H., Oļeiņika, K. (2020). Characterization of peripheral B-cells in IgA nephropathy. XV Baltic Congress in Laboratory Medicine. Riga. Latvia.
9. Saulīte, A. J., **Popova, A**., Kučāne, L., Kuzema, V., Pētersons, A. (2020). Kidney survival analysis in immunoglobulin A nephropathy patients. International Scientific Conference of the University of Latvia. 12 March 2020. 171.
10. Babičeva, P., **Popova, A**. (2021). Assessment of dietary habits in patients with immunoglobulin A nephropathy. Warsaw International Medical Congress. 29–30 May 2021. 68

References

1. Abbad, L., Monteiro, R. C., & Berthelot, L. (2020). Food antigens and Transglutaminase 2 in IgA nephropathy: Molecular links between gut and kidney. *Mol Immunol*, *121*, 1–6. https://doi.org/10.1016/j.molimm.2020.02.019
2. Almeida, A., Nayfach, S., Boland, M., Strozzi, F., Beracochea, M., Shi, Z. J., Pollard, K. S., Sakharova, E., Parks, D. H., Hugenholtz, P., Segata, N., Kyrpides, N. C., & Finn, R. D. (2021). A unified catalog of 204,938 reference genomes from the human gut microbiome. *Nat Biotechnol*, *39*(1), 105–114. https://doi.org/10.1038/s41587-020-0603-3
3. Ambruzs, J. M., Walker, P. D., & Larsen, C. P. (2014). The histopathologic spectrum of kidney biopsies in patients with inflammatory bowel disease. *Clin J Am Soc Nephrol*, *9*(2), 265–270. https://doi.org/10.2215/CJN.04660513
4. Arnold, J. N., Dwek, R. A., Rudd, P. M., & Sim, R. B. (2006). Mannan binding lectin and its interaction with immunoglobulins in health and in disease. *Immunol Lett*, *106*(2), 103–110. https://doi.org/10.1016/j.i mlet.2006.05.007
5. Axelsen, R. A., Crawford, D. H., Endre, Z. H., Lynch, S. V., Balderson, G. A., Strong, R. W., & Fleming, S. J. (1995). Renal glomerular lesions in unselected patients with cirrhosis undergoing orthotopic liver transplantation. *Pathology*, *27*(3), 237–246. https://doi.org/10.1080/00313029500169053
6. Bagchi, S., Upadhyay, A. D., Barwad, A., Singh, G., Subbiah, A., Yadav, R. K., Mahajan, S., Bhowmik, D., & Agarwal, S. K. (2022). The International IgA Nephropathy Network Prediction Tool Underestimates Disease Progression in Indian Patients. *Kidney Int Rep*, *7*(6), 1210–1218. https://doi.org/10.1016/j.ekir.2022.03.016
7. Balan, M., Chakraborty, S., & Pal, S. (2019). Signaling Molecules in Posttransplantation Cancer. *Clin Lab Med*, *39*(1), 171-183. https://doi.org/10.1016/j.cll.2018.10.006
8. Barbour, S. J., Coppo, R., Er, L., Russo, M. L., Liu, Z. H., Ding, J., Katafuchi, R., Yoshikawa, N., Xu, H., Kagami, S., Yuzawa, Y., Emma, F., Cambier, A., Peruzzi, L., Wyatt, R. J., Cattran, D. C., & International Ig, A. N. N. (2021). Updating the International IgA Nephropathy Prediction Tool for use in children. *Kidney Int*, *99*(6), 1439–1450. https://doi.org/10.1016/j.kint.2020.10.033
9. Barbour, S. J., Coppo, R., Zhang, H., Liu, Z. H., Suzuki, Y., Matsuzaki, K., Er, L., Reich, H. N., Barratt, J., Cattran, D. C., & International Ig, A. N. N. (2022). Application of the International IgA Nephropathy Prediction Tool one or two years post-biopsy. *Kidney Int*, *102*(1), 160–172. https://doi.org/10.1016/j.kint.2022.02.042
10. Barbour, S. J., Coppo, R., Zhang, H., Liu, Z. H., Suzuki, Y., Matsuzaki, K., Katafuchi, R., Er, L., Espino-Hernandez, G., Kim, S. J., Reich, H. N., Feehally, J., Cattran, D. C., & International Ig, A. N. N. (2019). Evaluating a New International Risk-Prediction Tool in IgA Nephropathy. *JAMA Intern Med*, *179*(7), 942–952. https://doi.org/10.1001/jamainternmed.2019.0600
11. Barnett, D. J. M., Arts, I. C. W., & Penders, J. (2021). microViz: an R package for microbiome data visualization and statistics. *Journal of Open Source Software*, *6*(63). https://doi.org/10.21105/joss.03201
12. Barratt, J., Lafayette, R., Kristensen, J., Stone, A., Cattran, D., Floege, J., Tesar, V., Trimarchi, H., Zhang, H., Eren, N., Paliege, A., Rovin, B. H., & NefIgArd Trial, I. (2023). Results from part A of the multi-center, double-blind, randomised, placebo-controlled NefIgArd trial, which evaluated targeted-release formulation of budesonide for the treatment of primary immunoglobulin A nephropathy. *Kidney Int*, *103*(2), 391–402. https://doi.org/10.1016/j.kint.2022.09.017
13. Barratt, J., Lafayette, R. A., Zhang, H., Tesar, V., Rovin, B. H., Tu mlin, J. A., Reich, H. N., & Floege, J. (2023). IgA nephropathy: the lectin pathway and implications for targeted therapy. *Kidney Int*, *104*(2), 254–264. https://doi.org/10.1016/j.kint.2023.04.029

14. Beghini, F., McIver, L. J., Blanco-Miguez, A., Dubois, L., Asnicar, F., Maharjan, S., Mailyan, A., Manghi, P., Scholz, M., Thomas, A. M., Valles-Colomer, M., Weingart, G., Zhang, Y., Zolfo, M., Huttenhower, C., Franzosa, E. A., & Segata, N. (2021). Integrating taxonomic, functional, and strain-level profiling of diverse microbial communities with bioBakery 3. *Elife*, *10*. https://doi.org/10.7554/eLife.65088

15. Berger, J., & Hinglais, N. (1968). [Intercapillary deposits of IgA-IgG]. *J Urol Nephrol (Paris)*, *74*(9), 694-695. https://www.ncbi.nlm.nih.gov/pubmed/4180586 (Les ddpots intercapillaires d'IgA-IgG.)

16. Berthelot, L., Papista, C., Maciel, T. T., Biarnes-Pelicot, M., Tissandie, E., Wang, P. H., Tamouza, H., Jamin, A., Bex-Coudrat, J., Gestin, A., Boumediene, A., Arcos-Fajardo, M., England, P., Pillebout, E., Walker, F., Daugas, E., Vrtosvnik, F., Flamant, M., Benhamou, M., . . . Monteiro, R. C. (2012). Transglutaminase is essential for IgA nephropathy development acting through IgA receptors. *J Exp Med*, *209*(4), 793–806. https://doi.org/10.1084/jem.20112005

17. Berthoux, F., Mohey, H., Laurent, B., Mariat, C., Afiani, A., & Thibaudin, L. (2011). Predicting the risk for dialysis or death in IgA nephropathy. *J Am Soc Nephrol*, *22*(4), 752–761. https://doi.org/10.1681/ASN.2010040355

18. Berthoux, F., Suzuki, H., Mohey, H., Maillard, N., Mariat, C., Novak, J., & Julian, B. A. (2017). Prognostic Value of Serum Biomarkers of Autoimmunity for Recurrence of IgA Nephropathy after Kidney Transplantation. *J Am Soc Nephrol*, *28*(6), 1943–1950. https://doi.org/10.1681/ASN.2016060670

19. Blanco, R., Martinez-Taboada, V. M., Rodriguez-Valverde, V., Garcia-Fuentes, M., & Gonzalez-Gay, M. A. (1997). Henoch-Schonlein purpura in adulthood and childhood: two different expressions of the same syndrome. *Arthritis Rheum*, *40*(5), 859–864. https://doi.org/10.1002/art.1780400513

20. Bon, G., Jullien, P., Masson, I., Sauron, C., Dinic, M., Claisse, G., Pelaez, A., Thibaudin, D., Mohey, H., Alamartine, E., Mariat, C., & Maillard, N. (2023). Validation of the international IgA nephropathy prediction tool in a French cohort beyond 10 years after diagnosis. *Nephrol Dial Transplant*, *38*(10), 2257–2265. https://doi.org/10.1093/ndt/gfad048

21. Brockhausen, I., Schachter, H., & Stanley, P. (2009). O-GalNAc Glycans. In A. Varki, R. D. Cummings, J. D. Esko, H. H. Freeze, P. Stanley, C. R. Bertozzi, G. W. Hart, & M. E. Etzler (Eds.), *Essentials of Glycobiology* (2nd ed.). https://www.ncbi.nlm.nih.gov/pubmed/20301232

22. Bugatti, S., Vitolo, B., Caporali, R., Montecucco, C., & Manzo, A. (2014). B cells in rheumatoid arthritis: from pathogenic players to disease biomarkers. *Biomed Res Int*, *2014*, 681678. https://doi.org/10.1155/2014/681678

23. Burrichter, A., Denger, K., Franchini, P., Huhn, T., Muller, N., Spiteller, D., & Schleheck, D. (2018). Anaerobic Degradation of the Plant Sugar Sulfoquinovose Concomitant With H(2)S Production: Escherichia coli K-12 and Desulfovibrio sp. Strain DF1 as Co-culture Model. *Front Microbiol*, *9*, 2792. https://doi.org/10.3389/fmicb.2018.02792

24. Calmus, Y., Conti, F., Cluzel, P., Hill, G., Antoine, C., Scatton, O., Soubrane, O., Glotz, D., Pillebout, E., & Nochy, D. (2012). Prospective assessment of renal histopathological lesions in patients with end-stage liver disease: effects on long-term renal function after liver transplantation. *J Hepatol*, *57*(3), 572–576. https://doi.org/10.1016/j.jhep.2012.04.028

25. Cambier, A., Gleeson, P. J., Abbad, L., Canesi, F., da Silva, J., Bex-Coudrat, J., Deschenes, G., Boyer, O., Rabant, M., Ulinski, T., Hogan, J., Peuchmaur, M., Berthelot, L., & Monteiro, R. C. (2022). Soluble CD89 is a critical factor for mesangial proliferation in childhood IgA nephropathy. *Kidney Int*, *101*(2), 274–287. https://doi.org/10.1016/j.kint.2021.09.023

26. Canetta, P. (2020). Toward a Clearer Picture of IgA Nephropathy in Spondyloarthritis. *Kidney Int Rep*, *5*(6), 766–768. https://doi.org/10.1–kir.2020.04.016

27. Castigli, E., Scott, S., Dedeoglu, F., Bryce, P., Jabara, H., Bhan, A. K., Mizoguchi, E., & Geha, R. S. (2004). Impaired IgA class switching in APRIL-deficient mice. *Proc Natl Acad Sci U S A*, *101*(11), 3903–3908. https://doi.org/10.1073/pnas.0307348101

28. Castigli, E., Wilson, S. A., Garibyan, L., Rachid, R., Bonilla, F., Schneider, L., & Geha, R. S. (2005). TACI is mutant in common variable immunodeficiency and IgA deficiency. *Nat Genet*, *37*(8), 829–834. https://doi.org/10.1038/ng1601

29. Cerutti, A. (2008). The regulation of IgA class switching. *Nat Rev Immunol*, *8*(6), 421–434. https://doi.org/10.1038/nri2322

30. Chan, L. Y., Leung, J. C., Tsang, A. W., Tang, S. C., & Lai, K. N. (2005). Activation of tubular epithelial cells by mesangial-derived TNF-alpha: glomerulotubular communication in IgA nephropathy. *Kidney Int*, *67*(2), 602–612. https://doi.org/10.1111/j.1523-1755.2005.67116.x

31. Chen, P., Yu, G., Zhang, X., Xie, X., Wang, J., Shi, S., Liu, L., Lv, J., & Zhang, H. (2019). Plasma Galactose-Deficient IgA1 and C3 and CKD Progression in IgA Nephropathy. *Clin J Am Soc Nephrol*, *14*(10), 1458–1465. https://doi.org/10.2215/CJN.13711118

32. Chen, S., Zhou, Y., Chen, Y., & Gu, J. (2018). fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics*, *34*(17), i884-i890. https://doi.org/10.1093/bioinformatics/bty560

33. Chen, X., Chen, P., Cai, G., Wu, J., Cui, Y., Zhang, Y., Liu, S., & Tang, L. (2002). [A randomised control trial of mycophenolate mofeil treatment in severe IgA nephropathy]. *Zhonghua Yi Xue Za Zhi*, *82*(12), 796–801. https://www.ncbi.nlm.nih.gov/pubmed/12126522

34. Coppo, R., D'Arrigo, G., Tripepi, G., Russo, M. L., Roberts, I. S. D., Bellur, S., Cattran, D., Cook, T. H., Feehally, J., Tesar, V., Maixnerova, D., Peruzzi, L., Amore, A., Lundberg, S., Di Palma, A. M., Gesualdo, L., Emma, F., Rollino, C., Praga, M., . . . Group, E.-E. I. W. (2020). Is there long-term value of pathology scoring in immunoglobulin A nephropathy? A validation study of the Oxford Classification for IgA Nephropathy (VALIGA) update. *Nephrol Dial Transplant*, *35*(6), 1002–1009. https://doi.org/10.1093/ndt/gfy302

35. Coppo, R., & Fervenza, F. C. (2017). Persistent Microscopic Hematuria as a Risk Factor for Progression of IgA Nephropathy: New Floodlight on a Nearly Forgotten Biomarker. *J Am Soc Nephrol*, *28*(10), 2831–2834. https://doi.org/10.1681/ASN.2017060639

36. Coppo, R., Peruzzi, L., Amore, A., Piccoli, A., Cochat, P., Stone, R., Kirschstein, M., & Linne, T. (2007). IgACE: a placebo-controlled, randomised trial of angiotensin-converting enzyme inhibitors in children and young people with IgA nephropathy and moderate proteinuria. *J Am Soc Nephrol*, *18*(6), 1880–1888. https://doi.org/10.1681/ASN.2006040347

37. Coppo, R., Troyanov, S., Bellur, S., Cattran, D., Cook, H. T., Feehally, J., Roberts, I. S., Morando, L., Camilla, R., Tesar, V., Lunberg, S., Gesualdo, L., Emma, F., Rollino, C., Amore, A., Praga, M., Feriozzi, S., Segoloni, G., Pani, A., . . . Group, V. s. o. t. E.-E. I. W. (2014). Validation of the Oxford classification of IgA nephropathy in cohorts with different presentations and treatments. *Kidney Int*, *86*(4), 828–836. https://doi.org/10.1038/ki.2014.63

38. D'Amico, G. (2004). Natural history of idiopathic IgA nephropathy and factors predictive of disease outcome. *Semin Nephrol*, *24*(3), 179–196. https://doi.org/10.1016/j.semnephrol.2004.01.001

39. De Angelis, M., Montemurno, E., Piccolo, M., Vannini, L., Lauriero, G., Maranzano, V., Gozzi, G., Serrazanetti, D., Dalfino, G., Gobbetti, M., & Gesualdo, L. (2014). Microbiota and metabolome associated with immunoglobulin A nephropathy (IgAN). *PLoS One*, *9*(6), e99006. https://doi.org/10.1371/journal.pone.0099006

40. De Filippis, F., Pasolli, E., Tett, A., Tarallo, S., Naccarati, A., De Angelis, M., Neviani, E., Cocolin, L., Gobbetti, M., Segata, N., & Ercolini, D. (2019). Distinct Genetic and Functional Traits of Human Intestinal Prevotella copri Strains Are Associated with Different Habitual Diets. *Cell Host Microbe*, *25*(3), 444–453 e443. https://doi.org/10.1016/j.chom.2019.01.004

41. Deng, W., Tan, X., Zhou, Q., Ai, Z., Liu, W., Chen, W., Yu, X., & Yang, Q. (2018). Gender-related differences in clinicopathological characteristics and renal outcomes of Chinese patients with IgA nephropathy. *BMC Nephrol*, *19*(1), 31. https://doi.org/10.1186/s12882-018-0829-1

42. Dhaun, N., Goddard, J., & Webb, D. J. (2006). The endothelin system and its antagonism in chronic kidney disease. *J Am Soc Nephrol*, *17*(4), 943–955. https://doi.org/10.1681/ASN.2005121256

43. Dong, Y., Shi, S., Liu, L., Zhou, X., Lv, J., & Zhang, H. (2023). Effect of SGLT2 inhibitors on the proteinuria reduction in patients with IgA nephropathy. *Front Med (Lausanne)*, *10*, 1242241. https://doi.org/10.3389/fmed.2023.1242241

44. Dou, X., Yan, D., Ma, Z., Gao, N., & Shan, A. (2022). Sodium butyrate alleviates LPS-induced kidney injury via inhibiting TLR2/4 to regulate rBD2 expression. *J Food Biochem*, *46*(7), e14126. https://doi.org/10.1111/jfbc.14126

45. Du, Y., Cheng, T., Liu, C., Zhu, T., Guo, C., Li, S., Rao, X., & Li, J. (2023). IgA Nephropathy: Current Understanding and Perspectives on Pathogenesis and Targeted Treatment. *Diagnostics (Basel)*, *13*(2). https://doi.org/10.3390/diagnostics13020303

46. El Aidy, S., Van den Abbeele, P., Van de Wiele, T., Louis, P., & Kleerebezem, M. (2013). Intestinal colonization: how key microbial players become established in this dynamic process: microbial metabolic activities and the interplay between the host and microbes. *Bioessays*, *35*(10), 913–923. https://doi.org/10.1002/bies.201300073

47. Elsner, R. A., & Shlomchik, M. J. (2020). Germinal Center and Extrafollicular B Cell Responses in Vaccination, Immunity, and Autoimmunity. *Immunity*, *53*(6), 1136–1150. https://doi.org/10.1016/j.immuni.2020.11.006

48. Espinosa, M., Ortega, R., Sanchez, M., Segarra, A., Salcedo, M. T., Gonzalez, F., Camacho, R., Valdivia, M. A., Cabrera, R., Lopez, K., Pinedo, F., Gutierrez, E., Valera, A., Leon, M., Cobo, M. A., Rodriguez, R., Ballarin, J., Arce, Y., Garcia, B., . . . Spanish Group for Study of Glomerular, D. (2014). Association of C4d deposition with clinical outcomes in IgA nephropathy. *Clin J Am Soc Nephrol*, *9*(5), 897–904. https://doi.org/10.2215/CJN.09710913

49. Favero, C., Pintor-Chocano, A., Sanz, A., Ortiz, A., & Sanchez-Nino, M. D. (2024). Butyrate promotes kidney resilience through a coordinated kidney protective response in tubular cells. *Biochem Pharmacol*, *224*, 116203. https://doi.org/10.1016/j.bcp.2024.116203

50. Fellstrom, B. C., Barratt, J., Cook, H., Coppo, R., Feehally, J., de Fijter, J. W., Floege, J., Hetzel, G., Jardine, A. G., Locatelli, F., Maes, B. D., Mercer, A., Ortiz, F., Praga, M., Sorensen, S. S., Tesar, V., Del Vecchio, L., & Investigators, N. T. (2017). Targeted-release budesonide versus placebo in patients with IgA nephropathy (NEFIGAN): a double-blind, randomised, placebo-controlled phase 2b trial. *Lancet*, *389*(10084), 2117–2127. https://doi.org/10.1016/S0140-6736(17)30550-0

51. Furusawa, Y., Obata, Y., Fukuda, S., Endo, T. A., Nakato, G., Takahashi, D., Nakanishi, Y., Uetake, C., Kato, K., Kato, T., Takahashi, M., Fukuda, N. N., Murakami, S., Miyauchi, E., Hino, S., Atarashi, K., Onawa, S., Fujimura, Y., Lockett, T., . . . Ohno, H. (2013). Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature*, *504*(7480), 446–450. https://doi.org/10.1038/nature12721

52. Gao, W., Xiong, Y., Li, Q., & Yang, H. (2017). Inhibition of Toll-Like Receptor Signaling as a Promising Therapy for Inflammatory Diseases: A Journey from Molecular to Nano Therapeutics. *Front Physiol*, *8*, 508. https://doi.org/10.3389/fphys.2017.00508

53. Gardner-Medwin, J. M., Dolezalova, P., Cummins, C., & Southwood, T. R. (2002). Incidence of Henoch-Schonlein purpura, Kawasaki disease, and rare vasculitides in children of different ethnic origins. *Lancet*, *360*(9341), 1197–1202. https://doi.org/10.1016/S0140-6736(02)11279-7

54. Geddes, C. C., Rauta, V., Gronhagen-Riska, C., Bartosik, L. P., Jardine, A. G., Ibels, L. S., Pei, Y., & Cattran, D. C. (2003). A tricontinental view of IgA nephropathy. *Nephrol Dial Transplant*, *18*(8), 1541–1548. https://doi.org/10.1093/ndt/gfg207

55. Geirnaert, A., Steyaert, A., Eeckhaut, V., Debruyne, B., Arends, J. B., Van Immerseel, F., Boon, N., & Van de Wiele, T. (2014). Butyricicoccus pullicaecorum, a butyrate producer with probiotic potential, is intrinsically tolerant to stomach and small intestine conditions. *Anaerobe*, *30*, 70–74. https://doi.org/10.1016/j.anaerobe.2014.08.010

56. Gharavi, A. G., Yan, Y., Scolari, F., Schena, F. P., Frasca, G. M., Ghiggeri, G. M., Cooper, K., Amoroso, A., Viola, B. F., Battini, G., Caridi, G., Canova, C., Farhi, A., Subramanian, V., Nelson-Williams, C., Woodford, S., Julian, B. A., Wyatt, R. J., & Lifton, R. P. (2000). IgA nephropathy, the most common cause of glomerulonephritis, is linked to 6q22-23. *Nat Genet*, *26*(3), 354–357. https://doi.org/10.1038/81677

57. Ghazi, S., Polesel, M., & Hall, A. M. (2019). Targeting glycolysis in proliferative kidney diseases. *Am J Physiol Renal Physiol*, *317*(6), F1531–F1535. https://doi.org/10.1152/ajprenal.00460.2019

58. Gleeson, P. J., Benech, N., Chemouny, J., Metallinou, E., Berthelot, L., da Silva, J., Bex-Coudrat, J., Boedec, E., Canesi, F., Bounaix, C., Morelle, W., Moya-Nilges, M., Kenny, J., O'Mahony, L., Saveanu, L., Arnulf, B., Sannier, A., Daugas, E., Vrtovsnik, F., . . . Monteiro, R. C. (2024). The gut microbiota posttranslationally modifies IgA1 in autoimmune glomerulonephritis. *Sci Transl Med*, *16*(740), eadl6149. https://doi.org/10.1126/scitranslmed.adl6149

59. Gomez, G., & Sitkovsky, M. V. (2003). Differential requirement for A2a and A3 adenosine receptors for the protective effect of inosine in vivo. *Blood*, *102*(13), 4472–4478. https://doi.org/10.1182/blood-2002-11-3624

60. Gong, W. Y., Liu, M., Luo, D., Liu, F. N., Yin, L. H., Li, Y. Q., Zhang, J., & Peng, H. (2019). High serum IgA/C3 ratio better predicts a diagnosis of IgA nephropathy among primary glomerular nephropathy patients with proteinuria < / = 1 g/d: an observational cross-sectional study. *BMC Nephrol*, *20*(1), 150. https://doi.org/10.1186/s12882-019-1331-0

61. Group, E.-K. C. (2024). Impact of primary kidney disease on the effects of empagliflozin in patients with chronic kidney disease: secondary analyses of the EMPA-KIDNEY trial. *Lancet Diabetes Endocrinol*, *12*(1), 51–60. https://doi.org/10.1016/S2213-8587(23)00322-4

62. Guo, W. Y., Sun, L. J., Dong, H. R., Wang, G. Q., Xu, X. Y., Cheng, W. R., Zhao, Z. R., Ye, N., Liu, Y., & Cheng, H. (2023). Characterization of patients with IgA nephropathy with and without associated minimal change disease. *Front Nephrol*, *3*, 1105933. https://doi.org/10.3389/fneph.2023.1105933

63. Haas, M., Verhave, J. C., Liu, Z. H., Alpers, C. E., Barratt, J., Becker, J. U., Cattran, D., Cook, H. T., Coppo, R., Feehally, J., Pani, A., Perkowska-Ptasinska, A., Roberts, I. S., Soares, M. F., Trimarchi, H., Wang, S., Yuzawa, Y., Zhang, H., Troyanov, S., & Katafuchi, R. (2017). A Multicenter Study of the Predictive Value of Crescents in IgA Nephropathy. *J Am Soc Nephrol*, *28*(2), 691–701. https://doi.org/10.1681/ASN.2016040433

64. Haaskjold, Y. L., Lura, N. G., Bjorneklett, R., Bostad, L., Bostad, L. S., & Knoop, T. (2023). Validation of two IgA nephropathy risk-prediction tools using a cohort with a long follow-up. *Nephrol Dial Transplant*, *38*(5), 1183–1191. https://doi.org/10.1093/ndt/gfac225

65. Han, S., Shang, L., Lu, Y., & Wang, Y. (2022). Gut Microbiome Characteristics in IgA Nephropathy: Qualitative and Quantitative Analysis from Observational Studies. *Front Cell Infect Microbiol*, *12*, 904401. https://doi.org/10.3389/fcimb.2022.904401

66. Hand, T. W., & Reboldi, A. (2021). Production and Function of Immunoglobulin A. *Annu Rev Immunol*, *39*, 695–718. https://doi.org/10.1146/annurev-immunol-102119-074236

67. Hanson, B. T., Dimitri Kits, K., Loffler, J., Burrichter, A. G., Fiedler, A., Denger, K., Frommeyer, B., Herbold, C. W., Rattei, T., Karcher, N., Segata, N., Schleheck, D., & Loy, A. (2021). Sulfoquinovose is a select nutrient of prominent bacteria and a source of hydrogen sulfide in the human gut. *ISME J*, *15*(9), 2779–2791. https://doi.org/10.1038/s41396-021-00968-0

68. Haresh Selvaskandan, J. B., Chee Kay Cheung. (2023). Novel Treatment Paradigms: Primary IgA Nephropathy. *Kidney International Reports*. https://doi.org/10.1016/j.ekir.2023.11.026

69. Hasko, G., & Cronstein, B. (2013). Regulation of inflammation by adenosine. *Front Immunol*, *4*, 85. https://doi.org/10.3389/fimmu.2013.00085

70. He, B., Xu, W., Santini, P. A., Polydorides, A. D., Chiu, A., Estrella, J., Shan, M., Chadburn, A., Villanacci, V., Plebani, A., Knowles, D. M., Rescigno, M., & Cerutti, A. (2007). Intestinal bacteria trigger T cell-independent immunoglobulin A(2) class switching by inducing epithelial-cell secretion of the cytokine APRIL. *Immunity*, *26*(6), 812–826. https://doi.org/10.1016/j.immuni.2007.04.014

71. Heerspink, H. J., Perkins, B. A., Fitchett, D. H., Husain, M., & Cherney, D. Z. (2016). Sodium Glucose Cotransporter 2 Inhibitors in the Treatment of Diabetes Mellitus: Cardiovascular and Kidney Effects, Potential Mechanisms, and Clinical Applications. *Circulation*, *134*(10), 752–772. https://doi.org/10.1161/CIRCULATIONAHA.116.021887

72. Hirano, K., Matsuzaki, K., Yasuda, T., Nishikawa, M., Yasuda, Y., Koike, K., Maruyama, S., Yokoo, T., Matsuo, S., Kawamura, T., & Suzuki, Y. (2019). Association Between Tonsillectomy and Outcomes in Patients With Immunoglobulin A Nephropathy. *JAMA Netw Open*, *2*(5), e194772. https://doi.org/10.1001/jamanetworkopen.2019.4772

73. Hostetter, T. H. (1995). Progression of renal disease and renal hypertrophy. *Annu Rev Physiol*, *57*, 263–278. https://doi.org/10.1146/annurev.ph.57.030195.001403

74. Hotta, O., Furuta, T., Chiba, S., Tomioka, S., & Taguma, Y. (2002). Regression of IgA nephropathy: a repeat biopsy study. *Am J Kidney Dis*, *39*(3), 493–502. https://doi.org/10.1053/ajkd.2002.31399

75. Hou, F. F., Xie, D., Wang, J., Xu, X., Yang, X., Ai, J., Nie, S., Liang, M., Wang, G., Jia, N., & Investigators, M. T. (2023). Effectiveness of Mycophenolate Mofetil Among Patients With Progressive IgA Nephropathy: A Randomised Clinical Trial. *JAMA Netw Open*, *6*(2), e2254054. https://doi.org/10.1001/jamanetworkopen.2022.54054

76. Hwang, D., Choi, K., Cho, N. J., Park, S., Yu, B. C., Gil, H. W., Lee, E. Y., Choi, S. J., Park, M. Y., Kim, J. K., Hwang, S. D., Kwon, S. H., Jeon, J. S., Noh, H., Han, D. C., & Kim, H. (2021). Validation of an international prediction model including the Oxford classification in Korean patients with IgA nephropathy. *Nephrology (Carlton)*, *26*(7), 594–602. https://doi.org/10.1111/nep.13865

77. Iwasaki, H., Zhang, Y., Tachibana, K., Gotoh, M., Kikuchi, N., Kwon, Y. D., Togayachi, A., Kudo, T., Kubota, T., & Narimatsu, H. (2003). Initiation of O-glycan synthesis in IgA1 hinge region is determined by a single enzyme, UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 2. *J Biol Chem*, *278*(8), 5613–5621. https://doi.org/10.1074/jbc.M211097200

78. Jenks, S. A., Cashman, K. S., Zumaquero, E., Marigorta, U. M., Patel, A. V., Wang, X., Tomar, D., Woodruff, M. C., Simon, Z., Bugrovsky, R., Blalock, E. L., Scharer, C. D., Tipton, C. M., Wei, C., Lim, S. S., Petri, M., Niewold, T. B., Anolik, J. H., Gibson, G., . . . Sanz, I. (2018). Distinct Effector B Cells Induced by Unregulated Toll-like Receptor 7 Contribute to Pathogenic Responses in Systemic Lupus Erythematosus. *Immunity*, *49*(4), 725–739 e726. https://doi.org/10.1016/j.immuni.2018.08.015

79. Ju, T., & Cummings, R. D. (2005). Protein glycosylation: chaperone mutation in Tn syndrome. *Nature*, *437*(7063), 1252. https://doi.org/10.1038/4371252a

80. Kassambara, A. (2023). *ggpubr: 'ggplot2' Based Publication Ready Plots*. In https://rpkgs.datanovia.com/ggpubr/

81. Khanna, R. (2011). Clinical presentation & management of glomerular diseases: hematuria, nephritic & nephrotic syndrome. *Mo Med*, *108*(1), 33–36. https://www.ncbi.nlm.nih.gov/pubmed/21462608

82. Kidney Disease: Improving Global Outcomes Glomerular Diseases Work, G. (2021). KDIGO 2021 Clinical Practice Guideline for the Management of Glomerular Diseases. *Kidney Int*, *100*(4S), S1–S276. https://doi.org/10.1016/j.kint.2021.05.021

83. Kim, J. K., Kim, J. H., Lee, S. C., Kang, E. W., Chang, T. I., Moon, S. J., Yoon, S. Y., Yoo, T. H., Kang, S. W., Choi, K. H., Han, D. S., Kie, J. H., Lim, B. J., Jeong, H. J., & Han, S. H. (2012). Clinical features and outcomes of IgA nephropathy with nephrotic syndrome. *Clin J Am Soc Nephrol*, *7*(3), 427–436. https://doi.org/10.2215/CJN.04820511

84. Kim, J. S., Hwang, H. S., Lee, S. H., Kim, Y. G., Moon, J. Y., Kong, J. Y., & Jeong, K. H. (2020). Clinical Relevance of Serum Galactose Deficient IgA1 in Patients with IgA Nephropathy. *J Clin Med*, *9*(11). https://doi.org/10.3390/jcm9113549

85. Kim, S., Chen, J., Cheng, T., Gindulyte, A., He, J., He, S., Li, Q., Shoemaker, B. A., Thiessen, P. A., Yu, B., Zaslavsky, L., Zhang, J., & Bolton, E. E. (2025). PubChem 2025 update. *Nucleic Acids Res*, *53*(D1), D1516–D1525. https://doi.org/10.1093/nar/gkae1059

86. Kimura, M., Sato, I., Shiratori, Y., Sugawara, N., Ozawa, K., & Yamabe, H. (1984). Electron microscopic studies on IgA nephropathy. *Acta Pathol Jpn*, *34*(2), 289–304. https://doi.org/10.1111/j.1440-1827.1984.tb07557.x

87. Kiryluk, K., Li, Y., Scolari, F., Sanna-Cherchi, S., Choi, M., Verbitsky, M., Fasel, D., Lata, S., Prakash, S., Shapiro, S., Fischman, C., Snyder, H. J., Appel, G., Izzi, C., Viola, B. F., Dallera, N., Del Vecchio, L., Barlassina, C., Salvi, E., . . . Gharavi, A. G. (2014). Discovery of new risk loci for IgA nephropathy implicates genes involved in immunity against intestinal pathogens. *Nat Genet*, *46*(11), 1187–1196. https://doi.org/10.1038/ng.3118

88. Kiryluk, K., Novak, J., & Gharavi, A. G. (2013). Pathogenesis of immunoglobulin A nephropathy: recent insight from genetic studies. *Annu Rev Med*, *64*, 339–356. https://doi.org/10.1146/annurev-med-041811-142014

89. Kiryluk, K., Sanchez-Rodriguez, E., Zhou, X. J., Zanoni, F., Liu, L., mladkova, N., Khan, A., Marasa, M., Zhang, J. Y., Balderes, O., Sanna-Cherchi, S., Bomback, A. S., Canetta, P. A., Appel, G. B., Radhakrishnan, J., Trimarchi, H., Sprangers, B., Cattran, D. C., Reich, H., . . . Gharavi, A. G. (2023). Genome-wide association analyses define pathogenic signaling pathways and prioritise drug targets for IgA nephropathy. *Nat Genet*, *55*(7), 1091–1105. https://doi.org/10.1038/s41588-023-01422-x

90. Knoppova, B., Reily, C., Maillard, N., Rizk, D. V., Moldoveanu, Z., Mestecky, J., Raska, M., Renfrow, M. B., Julian, B. A., & Novak, J. (2016). The Origin and Activities of IgA1-Containing Immune Complexes in IgA Nephropathy. *Front Immunol*, *7*, 117. https://doi.org/10.3389/fimmu.2016.00117

91. Lafayette, R. A., Canetta, P. A., Rovin, B. H., Appel, G. B., Novak, J., Nath, K. A., Sethi, S., Tu mlin, J. A., Mehta, K., Hogan, M., Erickson, S., Julian, B. A., Leung, N., Enders, F. T., Brown, R., Knoppova, B., Hall, S., & Fervenza, F. C. (2017). A Randomised, Controlled Trial of Rituximab in IgA Nephropathy with Proteinuria and Renal Dysfunction. *J Am Soc Nephrol*, *28*(4), 1306–1313. https://doi.org/10.1681/ASN.2016060640

92. Lahti, L., & Shetty, S. (2012-2019). *Microbiome R package*. In https://doi.org/10.18129/B9.bioc.microbiome

93. Lai, K. N. (2012). Pathogenesis of IgA nephropathy. *Nat Rev Nephrol*, *8*(5), 275-283. https://doi.org/10.1038/nrneph.2012.58

94. Lai, K. N., Leung, J. C., Chan, L. Y., Saleem, M. A., Mathieson, P. W., Lai, F. M., & Tang, S. C. (2008). Activation of podocytes by mesangial-derived TNF-alpha: glomerulo-podocytic communication in IgA nephropathy. *Am J Physiol Renal Physiol*, *294*(4), F945–955. https://doi.org/10.1152/ajprenal.00423.2007

95. Lai, K. N., Tang, S. C., Guh, J. Y., Chuang, T. D., Lam, M. F., Chan, L. Y., Tsang, A. W., & Leung, J. C. (2003). Polymeric IgA1 from patients with IgA nephropathy upregulates transforming growth factor-beta synthesis and signal transduction in human mesangial cells via the renin-angiotensin system. *J Am Soc Nephrol*, *14*(12), 3127–3137. https://doi.org/10.1097/01.asn.0000095639.56212.bf

96. Lai, K. N., Tang, S. C., Schena, F. P., Novak, J., Tomino, Y., Fogo, A. B., & Glassock, R. J. (2016). IgA nephropathy. *Nat Rev Dis Primers*, *2*, 16001. https://doi.org/10.1038/nrdp.2016.1

97. Langmead, B., & Salzberg, S. L. (2012). Fast gapped-read alignment with Bowtie 2. *Nat Methods*, *9*(4), 357–359. https://doi.org/10.1038/nmeth.1923

98. Launay, P., Grossetete, B., Arcos-Fajardo, M., Gaudin, E., Torres, S. P., Beaudoin, L., Patey-Mariaud de Serre, N., Lehuen, A., & Monteiro, R. C. (2000). Fcalpha receptor (CD89) mediates the development of immunoglobulin A (IgA) nephropathy (Berger's disease). Evidence for pathogenic soluble receptor-Iga complexes in patients and CD89 transgenic mice. *J Exp Med*, *191*(11), 1999–2009. https://doi.org/10.1084/jem.191.11.1999

99. Le, W., Liang, S., Chen, H., Wang, S., Zhang, W., Wang, X., Wang, J., Zeng, C. H., & Liu, Z. H. (2014). Long-term outcome of IgA nephropathy patients with recurrent macroscopic hematuria. *Am J Nephrol*, *40*(1), 43–50. https://doi.org/10.1159/000364954

100. Lee, M., Suzuki, H., Nihei, Y., Matsuzaki, K., & Suzuki, Y. (2023). Ethnicity and IgA nephropathy: worldwide differences in epidemiology, timing of diagnosis, clinical manifestations, management and prognosis. *Clin Kidney J*, *16*(Suppl 2), ii1–ii8. https://doi.org/10.1093/ckj/sfad199

101. Lennartz, D. P., Seikrit, C., Wied, S., Fitzner, C., Eitner, F., Hilgers, R. D., Rauen, T., & Floege, J. (2020). Single versus dual blockade of the renin-angiotensin system in patients with IgA nephropathy. *J Nephrol*, *33*(6), 1231–1239. https://doi.org/10.1007/s40620-020-00836-8

102. Li, X. W., Liang, S. S., Le, W. B., Cheng, S. Q., Zeng, C. H., Wang, J. Q., & Liu, Z. H. (2016). Long-term outcome of IgA nephropathy with minimal change disease: a comparison between patients with and without minimal change disease. *J Nephrol*, *29*(4), 567–573. https://doi.org/10.1007/s40620-015-0242-9

103. Liao, J., Zhou, Y., Xu, X., Huang, K., Chen, P., Wu, Y., Jin, B., Hu, Q., Chen, G., & Zhao, S. (2022). Current knowledge of targeted-release budesonide in immunoglobulin A nephropathy: A comprehensive review. *Front Immunol*, *13*, 926517. https://doi.org/10.3389/fimmu.2022.926517

104. Liu, H., Peng, Y., Liu, F., Xiao, W., Zhang, Y., & Li, W. (2011). Expression of IgA class switching gene in tonsillar mononuclear cells in patients with IgA nephropathy. *Inflamm Res*, *60*(9), 869–878. https://doi.org/10.1007/s00011-011-0347-0

105. Locatelli, F., Del Vecchio, L., & Ponticelli, C. (2023). Systemic and targeted steroids for the treatment of IgA nephropathy. *Clin Kidney J*, *16*(Suppl 2), ii40–ii46. https://doi.org/10.1093/ckj/sfad224

106. Lv, J., Wong, M. G., Hladunewich, M. A., Jha, V., Hooi, L. S., Monaghan, H., Zhao, M., Barbour, S., Jardine, M. J., Reich, H. N., Cattran, D., Glassock, R., Levin, A., Wheeler, D. C., Woodward, M., Billot, L., Stepien, S., Rogers, K., Chan, T. M., . . . Group, T. S. (2022). Effect of Oral Methylprednisolone on Decline in Kidney Function or Kidney Failure in Patients With IgA Nephropathy: The TESTING Randomised Clinical Trial. *JAMA*, *327*(19), 1888–1898. https://doi.org/10.1001/jama.2022.5368

107. Lv, J., Zhang, H., Wong, M. G., Jardine, M. J., Hladunewich, M., Jha, V., Monaghan, H., Zhao, M., Barbour, S., Reich, H., Cattran, D., Glassock, R., Levin, A., Wheeler, D., Woodward, M., Billot, L., Chan, T. M., Liu, Z. H., Johnson, D. W., . . . Group, T. S. (2017). Effect of Oral Methylprednisolone on Clinical Outcomes in Patients With IgA Nephropathy: The TESTING Randomised Clinical Trial. *JAMA*, *318*(5), 432–442. https://doi.org/10.1001/jama.2017.9362

108. Ma, L. J., & Fogo, A. B. (2007). Modulation of glomerulosclerosis. *Semin Immunopathol*, *29*(4), 385–395. https://doi.org/10.1007/s00281-007-0087-y

109. Maes, B. D., Oyen, R., Claes, K., Evenepoel, P., Kuypers, D., Vanwalleghem, J., Van Damme, B., & Vanrenterghem, Y. F. (2004). Mycophenolate mofetil in IgA nephropathy: results of a 3-year prospective placebo-controlled randomised study. *Kidney Int*, *65*(5), 1842–1849. https://doi.org/10.1111/j.1523-1755.2004.00588.x

110. Magistroni, R., D'Agati, V. D., Appel, G. B., & Kiryluk, K. (2015). New developments in the genetics, pathogenesis, and therapy of IgA nephropathy. *Kidney Int*, *88*(5), 974–989. https://doi.org/10.1038/ki.2015.252

111. Maillard, N., Wyatt, R. J., Julian, B. A., Kiryluk, K., Gharavi, A., Fremeaux-Bacchi, V., & Novak, J. (2015). Current Understanding of the Role of Complement in IgA Nephropathy. *J Am Soc Nephrol*, *26*(7), 1503–1512. https://doi.org/10.1681/ASN.2014101000

112. Mallick, H., Rahnavard, A., McIver, L., Ma, S., Zhang, Y., & Nguyen, L. (2021). Multivariable association discovery in population-scale meta-omics studies. *PLoS Comput Biol*, *17*(11). https://doi.org/10.1371/journal.pcbi.1009442

113. Mantis, N. J., Rol, N., & Corthesy, B. (2011). Secretory IgA's complex roles in immunity and mucosal homeostasis in the gut. *Mucosal Immunol*, *4*(6), 603–611. https://doi.org/10.1038/mi.2011.41

114. McCarthy, D. D., Kujawa, J., Wilson, C., Papandile, A., Poreci, U., Porfilio, E. A., Ward, L., Lawson, M. A., Macpherson, A. J., McCoy, K. D., Pei, Y., Novak, L., Lee, J. Y., Julian, B. A., Novak, J., Ranger, A., Gommerman, J. L., & Browning, J. L. (2011). Mice overexpressing BAFF develop a commensal flora-dependent, IgA-associated nephropathy. *J Clin Invest*, *121*(10), 3991–4002. https://doi.org/10.1172/JCI45563

115. McMurdie, P. J., & Holmes, S. (2013). phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One*, *8*(4), e61217. https://doi.org/10.1371/journal.pone.0061217

116. Medjeral-Thomas, N. R., Cook, H. T., & Pickering, M. C. (2021). Complement activation in IgA nephropathy. *Semin Immunopathol*, *43*(5), 679–690. https://doi.org/10.1007/s00281-021-00882-9

117. Miao, J., Duriseti, P., Radhakrishnan, Y., Vaughan, L., Fervenza, F. C., & Zand, L. . (2024). Mycophenolate Mofetil and Steroid for Treatment of Patients With IgA Nephropathy. *Kidney International Reports*, *9*, 182–185. https://doi.org/10.1016/j.ekir.2023.10.015

118. Mohr, A. E., Crawford, M., Jasbi, P., Fessler, S., & Sweazea, K. L. (2022). Lipopolysaccharide and the gut microbiota: considering structural variation. *FEBS Lett*, *596*(7), 849–875. https://doi.org/10.1002/1873-3468.14328

119. Moriyama, T., Tanaka, K., Iwasaki, C., Oshima, Y., Ochi, A., Kataoka, H., Itabashi, M., Takei, T., Uchida, K., & Nitta, K. (2014). Prognosis in IgA nephropathy: 30-year analysis of 1,012 patients at a single center in Japan. *PLoS One*, *9*(3), e91756. https://doi.org/10.1371/journal.pone.0091756

120. Morris, K. N., & Mitchell, A. M. (2023). Phosphatidylglycerol Is the Lipid Donor for Synthesis of Phospholipid-Linked Enterobacterial Common Antigen. *J Bacteriol*, *205*(1), e0040322. https://doi.org/10.1128/jb.00403-22

121. Nagy, J., Kovacs, T., & Wittmann, I. (2005). Renal protection in IgA nephropathy requires strict blood pressure control. *Nephrol Dial Transplant*, *20*(8), 1533–1539. https://doi.org/10.1093/ndt/gfh920

122. Nakamura, I., Iwase, H., Ohba, Y., Hiki, Y., Katsumata, T., & Kobayashi, Y. (2002). Quantitative analysis of IgA1 binding protein prepared from human serum by hypoglycosylated IgA1/Sepharose affinity chromatography. *J Chromatogr B Analyt Technol Biomed Life Sci*, *776*(1), 101–106. https://doi.org/10.1016/s1570-0232(02)00176-9

123. Noor, S. M., Abuazzam, F., Mathew, R., Zhang, Z., Abdipour, A., & Norouzi, S. (2023). IgA nephropathy: a review of existing and emerging therapies. *Front Nephrol*, *3*, 1175088. https://doi.org/10.3389/fneph.2023.1175088

124. Novak, J., Raskova Kafkova, L., Suzuki, H., Tomana, M., Matousovic, K., Brown, R., Hall, S., Sanders, J. T., Eison, T. M., Moldoveanu, Z., Novak, L., Novak, Z., Mayne, R., Julian, B. A., Mestecky, J., & Wyatt, R. J. (2011). IgA1 immune complexes from pediatric patients with IgA nephropathy activate cultured human mesangial cells. *Nephrol Dial Transplant*, *26*(11), 3451–3457. https://doi.org/10.1093/ndt/gfr448

125. Novak, J., Vu, H. L., Novak, L., Julian, B. A., Mestecky, J., & Tomana, M. (2002). Interactions of human mesangial cells with IgA and IgA-containing immune complexes. *Kidney Int*, *62*(2), 465–475. https://doi.org/10.1046/j.1523-1755.2002.00477.x

126. Ozen, S., Pistorio, A., Iusan, S. M., Bakkaloglu, A., Herlin, T., Brik, R., Buoncompagni, A., Lazar, C., Bilge, I., Uziel, Y., Rigante, D., Cantarini, L., Hilario, M. O., Silva, C. A., Alegria, M., Norambuena, X., Belot, A., Berkun, Y., Estrella, A. I., . . . Paediatric Rheumatology International Trials, O. (2010). EULAR/PRINTO/PRES criteria for Henoch-Schonlein purpura, childhood polyarteritis nodosa, childhood Wegener granulomatosis and childhood Takayasu arteritis: Ankara 2008. Part II: Final classification criteria. *Ann Rheum Dis*, *69*(5), 798–806. https://doi.org/10.1136/ard.2009.116657

127. Papasotiriou, M., Stangou, M., Chlorogiannis, D., Marinaki, S., Xydakis, D., Sampani, E., Lioulios, G., Kapsia, E., Zerbala, S., Koukoulaki, M., Moustakas, G., Fokas, S., Dounousi, E., Duni, A., Papadaki, A., Damianakis, N., Bacharaki, D., Stylianou, K., Gakiopoulou, H., . . . Goumenos, D. S. (2022). Validation of the International IgA Nephropathy Prediction Tool in the Greek Registry of IgA Nephropathy. *Front Med (Lausanne)*, *9*, 778464. https://doi.org/10.3389/fmed.2022.778464

128. Pawluczyk, I., Nicholson, M., Barbour, S., Er, L., Selvaskandan, H., Bhachu, J. S., & Barratt, J. (2021). A Pilot Study to Predict Risk of IgA Nephropathy Progression Based on miR-204 Expression. *Kidney Int Rep*, *6*(8), 2179–2188. https://doi.org/10.1016/j.ekir.2021.05.018

129. Pei, T., & Guo, D. (2025). The relationship between gluten-free diet and IgA nephropathy: A review. *Medicine (Baltimore)*, *104*(24), e41964. https://doi.org/10.1097/MD.0000000000041964

130. Pillebout, E. (2021). IgA Vasculitis and IgA Nephropathy: Same Disease? *J Clin Med*, *10*(11). https://doi.org/10.3390/jcm10112310

131. Popova, A., Slisere, B., Racenis, K., Kuzema, V., Karklins, R., Saulite, M., Seilis, J., Saulite, A. J., Vasilvolfa, A., Vaivode, K., Pjanova, D., Kroica, J., Cernevskis, H., Lejnieks, A., Petersons, A., & Oleinika, K. (2025). IgA class-switched CD27-CD21+ B cells in IgA nephropathy. *Nephrol Dial Transplant*, *40*(3), 505–515. https://doi.org/10.1093/ndt/gfae173

132. Pouria, S., & Barratt, J. (2008). Secondary IgA nephropathy. *Semin Nephrol*, *28*(1), 27–37. https://doi.org/10.1016/j.semnephrol.2007.10.004

133. Praga, M., Gutierrez, E., Gonzalez, E., Morales, E., & Hernandez, E. (2003). Treatment of IgA nephropathy with ACE inhibitors: a randomised and controlled trial. *J Am Soc Nephrol*, *14*(6), 1578–1583. https://doi.org/10.1097/01.asn.0000068460.37369.dc

134. Prasoodanan, P. K. V., Sharma, A. K., Mahajan, S., Dhakan, D. B., Maji, A., Scaria, J., & Sharma, V. K. (2021). Western and non-western gut microbiomes reveal new roles of Prevotella in carbohydrate metabolism and mouth–gut axis. *NPJ Biofilms Microbiomes*, *7*(1), 77. https://doi.org/10.1038/s41522-021-00248-x

135. Qin, J., Yang, Q., Tang, X., Chen, W., Li, Z., Mao, H., Jiang, Z., Huang, F., & Yu, X. (2013). Clinicopathologic features and treatment response in nephrotic IgA nephropathy with minimal change disease. *Clin Nephrol*, *79*(1), 37–44. https://doi.org/10.5414/CN107682

136. Rajasekaran, A., Julian, B. A., & Rizk, D. V. (2021). IgA Nephropathy: An Interesting Autoimmune Kidney Disease. *Am J Med Sci*, *361*(2), 176–194. https://doi.org/10.1016/j.amjms.2020.10.003

137. Rauen, T., Eitner, F., Fitzner, C., Sommerer, C., Zeier, M., Otte, B., Panzer, U., Peters, H., Benck, U., Mertens, P. R., Kuhlmann, U., Witzke, O., Gross, O., Vielhauer, V., Mann, J. F., Hilgers, R. D., Floege, J., & Investigators, S. T.-I. (2015). Intensive Supportive Care plus Immunosuppression in IgA Nephropathy. *N Engl J Med*, *373*(23), 2225–2236. https://doi.org/10.1056/NEJMoa1415463

138. Rauen, T., Wied, S., Fitzner, C., Eitner, F., Sommerer, C., Zeier, M., Otte, B., Panzer, U., Budde, K., Benck, U., Mertens, P. R., Kuhlmann, U., Witzke, O., Gross, O., Vielhauer, V., Mann, J. F. E., Hilgers, R. D., Floege, J., & Investigators, S. T.-I. (2020). After ten years of follow-up, no difference between supportive care plus immunosuppression and supportive care alone in IgA nephropathy. *Kidney Int*, *98*(4), 1044–1052. https://doi.org/10.1016/j.kint.2020.04.046

139. Rehnberg, J., Symreng, A., Ludvigsson, J. F., & Emilsson, L. (2021). Inflammatory Bowel Disease Is More Common in Patients with IgA Nephropathy and Predicts Progression of ESKD: A Swedish Population-Based Cohort Study. *J Am Soc Nephrol*, *32*(2), 411–423. https://doi.org/10.1681/ASN.2020060848

140. Riviere, A., Selak, M., Lantin, D., Leroy, F., & De Vuyst, L. (2016). Bifidobacteria and Butyrate-Producing Colon Bacteria: Importance and Strategies for Their Stimulation in the Human Gut. *Front Microbiol*, *7*, 979. https://doi.org/10.3389/fmicb.2016.00979

141. Rizk, D. V., Maillard, N., Julian, B. A., Knoppova, B., Green, T. J., Novak, J., & Wyatt, R. J. (2019). The Emerging Role of Complement Proteins as a Target for Therapy of IgA Nephropathy. *Front Immunol*, *10*, 504. https://doi.org/10.3389/fimmu.2019.00504

142. Roberts, I. S. (2014). Pathology of IgA nephropathy. *Nat Rev Nephrol*, *10*(8), 445–454. https://doi.org/10.1038/nrneph.2014.92

143. Rodrigues, V. F., Elias-Oliveira, J., Pereira, I. S., Pereira, J. A., Barbosa, S. C., Machado, M. S. G., & Carlos, D. (2022). Akkermansia muciniphila and Gut Immune System: A Good Friendship That Attenuates Inflammatory Bowel Disease, Obesity, and Diabetes. *Front Immunol*, *13*, 934695. https://doi.org/10.3389/fimmu.2022.934695

144. Roos, A., Rastaldi, M. P., Calvaresi, N., Oortwijn, B. D., Schlagwein, N., van Gijlswijk-Janssen, D. J., Stahl, G. L., Matsushita, M., Fujita, T., van Kooten, C., & Daha, M. R. (2006). Glomerular activation of the lectin pathway of complement in IgA nephropathy is associated with more severe renal disease. *J Am Soc Nephrol*, *17*(6), 1724–1734. https://doi.org/10.1681/ASN.2005090923

145. Rovin, B. H., Barratt, J., Heerspink, H. J. L., Alpers, C. E., Bieler, S., Chae, D. W., Diva, U. A., Floege, J., Gesualdo, L., Inrig, J. K., Kohan, D. E., Komers, R., Kooienga, L. A., Lafayette, R., Maes, B., Malecki, R., Mercer, A., Noronha, I. L., Oh, S. W., . . . Investigators, P. (2023). Efficacy and safety of sparsentan versus irbesartan in patients with IgA nephropathy (PROTECT): 2-year results from a randomised, active-controlled, phase 3 trial. *Lancet*, *402*(10417), 2077–2090. https://doi.org/10.1016/S0140-6736(23)02302-4

146. Rychlik, I., Andrassy, K., Waldherr, R., Zuna, I., Tesar, V., Jancova, E., Stejskalova, A., & Ritz, E. (1999). Clinical features and natural history of IgA nephropathy. *Ann Med Interne (Paris)*, *150*(2), 117–126. https://www.ncbi.nlm.nih.gov/pubmed/10392260

147. Saha, M. K., Julian, B. A., Novak, J., & Rizk, D. V. (2018). Secondary IgA nephropathy. *Kidney Int*, *94*(4), 674–681. https://doi.org/10.1016/j.kint.2018.02.030

148. Sallustio, F., Curci, C., Chaoul, N., Fonto, G., Lauriero, G., Picerno, A., Divella, C., Di Leo, V., De Angelis, M., Ben Mkaddem, S., Macchia, L., Gallone, A., Monteiro, R. C., Pesce, F., & Gesualdo, L. (2021a). High levels of gut-homing immunoglobulin A+ B lymphocytes support the pathogenic role of intestinal mucosal hyperresponsiveness in immunoglobulin A nephropathy patients. *Nephrol Dial Transplant*, *36*(9), 1765. https://doi.org/10.1093/ndt/gfaa344

149. Sallustio, F., Curci, C., Chaoul, N., Fonto, G., Lauriero, G., Picerno, A., Divella, C., Di Leo, V., De Angelis, M., Ben Mkaddem, S., Macchia, L., Gallone, A., Monteiro, R. C., Pesce, F., & Gesualdo, L. (2021b). High levels of gut-homing immunoglobulin A+ B lymphocytes support the pathogenic role of intestinal mucosal hyperresponsiveness in immunoglobulin A nephropathy patients. *Nephrol Dial Transplant*, *36*(3), 452–464. https://doi.org/10.1093/ndt/gfaa264

150. Sato, S., Fujimoto, M., Hasegawa, M., & Takehara, K. (2004). Altered blood B lymphocyte homeostasis in systemic sclerosis: expanded naive B cells and diminished but activated memory B cells. *Arthritis Rheum*, *50*(6), 1918–1927. https://doi.org/10.1002/art.20274

151. Schena, F. P., & Nistor, I. (2018). Epidemiology of IgA Nephropathy: A Global Perspective. *Semin Nephrol*, *38*(5), 435–442. https://doi.org/10.1016/j.semnephrol.2018.05.013

152. Schimpf, J. I., Klein, T., Fitzner, C., Eitner, F., Porubsky, S., Hilgers, R. D., Floege, J., Groene, H. J., & Rauen, T. (2018). Renal outcomes of STOP-IgAN trial patients in relation to baseline histology (MEST-C scores). *BMC Nephrol*, *19*(1), 328. https://doi.org/10.1186/s12882-018-1128-6

153. Scionti, K., Molyneux, K., Selvaskandan, H., Barratt, J., & Cheung, C. K. (2022). New Insights into the Pathogenesis and Treatment Strategies in IgA Nephropathy. *Glomerular Dis*, *2*(1), 15–29. https://doi.org/10.1159/000519973

154. Sendic, S., Mansouri, L., Lundberg, S., Nopp, A., Jacobson, S. H., & Lundahl, J. (2021). B cell and monocyte phenotyping: A quick asset to investigate the immune status in patients with IgA nephropathy. *PLoS One*, *16*(3), e0248056. https://doi.org/10.1371/journal.pone.0248056

155. Sevillano, A. M., Gutierrez, E., Yuste, C., Cavero, T., Merida, E., Rodriguez, P., Garcia, A., Morales, E., Fernandez, C., Martinez, M. A., Moreno, J. A., & Praga, M. (2017). Remission of Hematuria Improves Renal Survival in IgA Nephropathy. *J Am Soc Nephrol*, *28*(10), 3089–3099. https://doi.org/10.1681/ASN.2017010108

156. Shi, Y., Xing, L., Zheng, R., Luo, X., Yue, F., Xiang, X., Qiu, A., Xie, J., Russell, R., & Zhang, D. (2025). Butyrate attenuates high-fat diet-induced glomerulopathy through GPR43-Sirt3 pathway. *Br J Nutr*, *133*(1), 1–10. https://doi.org/10.1017/S0007114524002964

157. Smerud, H. K., Barany, P., Lindstrom, K., Fernstrom, A., Sandell, A., Pahlsson, P., & Fellstrom, B. (2011). New treatment for IgA nephropathy: enteric budesonide targeted to the ileocecal region ameliorates proteinuria. *Nephrol Dial Transplant*, *26*(10), 3237–3242. https://doi.org/10.1093/ndt/gfr052

158. Sommer, F., & Backhed, F. (2013). The gut microbiota--masters of host development and physiology. *Nat Rev Microbiol*, *11*(4), 227–238. https://doi.org/10.1038/nrmicro2974

159. Stepanova, M., & Aherne, C. M. (2024). Adenosine in Intestinal Epithelial Barrier Function. *Cells*, *13*(5). https://doi.org/10.3390/cells13050381

160. Stewart, C. J., Ajami, N. J., O'Brien, J. L., Hutchinson, D. S., Smith, D. P., Wong, M. C., Ross, M. C., Lloyd, R. E., Doddapaneni, H., Metcalf, G. A., Muzny, D., Gibbs, R. A., Vatanen, T., Huttenhower, C., Xavier, R. J., Rewers, M., Hagopian, W., Toppari, J., Ziegler, A. G., . . . Petrosino, J. F. (2018). Temporal development of the gut microbiome in early childhood from the TEDDY study. *Nature*, *562*(7728), 583-588. https://doi.org/10.1038/s41586-018-0617-x

161. Suzuki, H., Fan, R., Zhang, Z., Brown, R., Hall, S., Julian, B. A., Chatham, W. W., Suzuki, Y., Wyatt, R. J., Moldoveanu, Z., Lee, J. Y., Robinson, J., Tomana, M., Tomino, Y., Mestecky, J., & Novak, J. (2009). Aberrantly glycosylated IgA1 in IgA nephropathy patients is recognised by IgG antibodies with restricted heterogeneity. *J Clin Invest*, *119*(6), 1668–1677. https://doi.org/10.1172/JCI38468

162. Suzuki, H., Raska, M., Yamada, K., Moldoveanu, Z., Julian, B. A., Wyatt, R. J., Tomino, Y., Gharavi, A. G., & Novak, J. (2014). Cytokines alter IgA1 O-glycosylation by dysregulating C1GalT1 and ST6GalNAc-II enzymes. *J Biol Chem*, *289*(8), 5330–5339. https://doi.org/10.1074/jbc.M113.512277

163. Suzuki, Y., Suzuki, H., Nakata, J., Sato, D., Kajiyama, T., Watanabe, T., & Tomino, Y. (2011). Pathological role of tonsillar B cells in IgA nephropathy. *Clin Dev Immunol*, *2011*, 639074. https://doi.org/10.1155/2011/639074

164. Szeto, C. C., & Li, P. K. (2014). MicroRNAs in IgA nephropathy. *Nat Rev Nephrol*, *10*(5), 249–256. https://doi.org/10.1038/nrneph.2014.50

165. Szeto, C. C., Ng, J. K., Fung, W. W., Chan, G. C., Luk, C. C., Lai, K. B., Wang, G., Chow, K. M., & Mac-Moune Lai, F. (2022). Urinary mi-106a for the diagnosis of IgA nephropathy: Liquid biopsy for kidney disease. *Clin Chim Acta*, *530*, 81–86. https://doi.org/10.1016/j.cca.2022.03.006

166. Szeto, C. C., Wang, G., Ng, J. K., Kwan, B. C., Mac-Moune Lai, F., Chow, K. M., Luk, C. C., Lai, K. B., & Li, P. K. (2019). Urinary miRNA profile for the diagnosis of IgA nephropathy. *BMC Nephrol*, *20*(1), 77. https://doi.org/10.1186/s12882-019-1267-4

167. Takayasu, M., Hirayama, K., Shimohata, H., Kobayashi, M., & Koyama, A. (2022). Staphylococcus aureus Infection-Related Glomerulonephritis with Dominant IgA Deposition. *Int J Mol Sci*, *23*(13). https://doi.org/10.3390/ijms23137482

168. Tan, J., Ni, D., Taitz, J., Pinget, G. V., Read, M., Senior, A., Wali, J. A., Elnour, R., Shanahan, E., Wu, H., Chadban, S. J., Nanan, R., King, N. J. C., Grau, G. E., Simpson, S. J., & Macia, L. (2022). Dietary protein increases T-cell-independent sIgA production through changes in gut microbiota-derived extracellular vesicles. *Nat Commun*, *13*(1), 4336. https://doi.org/10.1038/s41467-022-31761-y

169. Tian, X., Yuan, L., & Zeng, Y. (2025). Butyrate attenuates SA-AKI by inhibiting pyroptosis via the STING-GSDMD axis. *Biochem Biophys Res Commun*, *743*, 151143. https://doi.org/10.1016/j.bbrc.2024.151143

170. Tota, M., Baron, V., Musial, K., Derrough, B., Konieczny, A., Krajewska, M., Turkmen, K., & Kusztal, M. (2023). Secondary IgA Nephropathy and IgA-Associated Nephropathy: A Systematic Review of Case Reports. *J Clin Med*, *12*(7). https://doi.org/10.3390/jcm12072726

171. Trimarchi, H., Barratt, J., Cattran, D. C., Cook, H. T., Coppo, R., Haas, M., Liu, Z. H., Roberts, I. S., Yuzawa, Y., Zhang, H., Feehally, J., Ig, A. N. C. W. G. o. t. I. I. N. N., the Renal Pathology, S., & Conference, P. (2017). Oxford Classification of IgA nephropathy 2016: an update from the IgA Nephropathy Classification Working Group. *Kidney Int*, *91*(5), 1014–1021. https://doi.org/10.1016/j.kint.2017.02.003

172. Varma, P. P., Sengupta, P., & Nair, R. K. (2014). Post exertional hematuria. *Ren Fail*, *36*(5), 701–703. https://doi.org/10.3109/0886022X.2014.890011

173. Wang, M., Lv, J., Zhang, X., Chen, P., Zhao, M., & Zhang, H. (2020). Secondary IgA Nephropathy Shares the Same Immune Features With Primary IgA Nephropathy. *Kidney Int Rep*, *5*(2), 165–172. https://doi.org/10.1016/j.ekir.2019.10.012

174. Wang, Y. Y., Zhang, L., Zhao, P. W., Ma, L., Li, C., Zou, H. B., & Jiang, Y. F. (2014). Functional implications of regulatory B cells in human IgA nephropathy. *Scand J Immunol*, *79*(1), 51–60. https://doi.org/10.1111/sji.12128

175. Wheeler, D. C., Toto, R. D., Stefansson, B. V., Jongs, N., Chertow, G. M., Greene, T., Hou, F. F., McMurray, J. J. V., Pecoits-Filho, R., Correa-Rotter, R., Rossing, P., Sjostrom, C. D., Umanath, K., Langkilde, A. M., Heerspink, H. J. L., Committees, D.-C. T., & Investigators. (2021). A pre-specified analysis of the DAPA-CKD trial demonstrates the effects of dapagliflozin on major adverse kidney events in patients with IgA nephropathy. *Kidney Int*, *100*(1), 215–224. https://doi.org/10.1016/j.kint.2021.03.033

176. Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. In Springer-Verlag New York. https://ggplot2.tidyverse.org

177. Willey, C. J., Coppo, R., Schaefer, F., Miserska-Wasiak, M., Mathur, M., & Schultz, M. J. (2023). The incidence and prevalence of IgA nephropathy in Europe. *Nephrol Dial Transplant*. https://doi.org/10.1093/ndt/gfad082

178. Woo, K. T., Chan, C. M., Mooi, C. Y., H., L. C., Tan, H. K., Foo, M., Lee, G. S., Anantharaman, V., Lim, C. H., Tan, C. C., Lee, E. J., Chiang, G. S., Tan, P. H., Boon, T. H., Fook-Chong, S., & Wong, K. S. (2010). The changing pattern of primary glomerulonephritis in Singapore and other countries over the past 3 decades. *Clin Nephrol*, *74*(5), 372–383. https://doi.org/10.5414/cnp74372

179. Wood, D. E., Lu, J., & Langmead, B. (2019). Improved metagenomic analysis with Kraken 2. *Genome Biol*, *20*(1), 257. https://doi.org/10.1186/s13059-019-1891-0

180. Woof, J. M., & Mestecky, J. (2005). Mucosal immunoglobulins. *Immunol Rev*, *206*, 64-82. https://doi.org/10.1111/j.0105-2896.2005.00290.x

181. Woof, J. M., & Russell, M. W. (2011). Structure and function relationships in IgA. *Mucosal Immunol*, *4*(6), 590–597. https://doi.org/10.1038/mi.2011.39

182. Working Group of the International Ig, A. N. N., the Renal Pathology, S., Cattran, D. C., Coppo, R., Cook, H. T., Feehally, J., Roberts, I. S., Troyanov, S., Alpers, C. E., Amore, A., Barratt, J., Berthoux, F., Bonsib, S., Bruijn, J. A., D'Agati, V., D'Amico, G., Emancipator, S., Emma, F., Ferrario, F., . . . Zhang, H. (2009). The Oxford classification of IgA nephropathy: rationale, clinicopathological correlations, and classification. *Kidney Int*, *76*(5), 534–545. https://doi.org/10.1038/ki.2009.243

183. Working Group of the International Ig, A. N. N., the Renal Pathology, S., Roberts, I. S., Cook, H. T., Troyanov, S., Alpers, C. E., Amore, A., Barratt, J., Berthoux, F., Bonsib, S., Bruijn, J. A., Cattran, D. C., Coppo, R., D'Agati, V., D'Amico, G., Emancipator, S., Emma, F., Feehally, J., Ferrario, F., . . . Zhang, H. (2009). The Oxford classification of IgA nephropathy: pathology definitions, correlations, and reproducibility. *Kidney Int*, *76*(5), 546–556. https://doi.org/10.1038/ki.2009.168

184. Wu, D., Li, X., Yao, X., Zhang, N., Lei, L., Zhang, H., Tang, M., Ni, J., Ling, C., Chen, Z., Chen, X., & Liu, X. (2021). Mesangial C3 deposition and serum C3 levels predict renal outcome in IgA nephropathy. *Clin Exp Nephrol*, *25*(6), 641–651. https://doi.org/10.1007/s10157-021-02034-7

185. Wu, G., Peng, Y. M., Liu, F. Y., Xu, D., & Liu, C. (2013). The role of memory B cell in tonsil and peripheral blood in the clinical progression of IgA nephropathy. *Hum Immunol*, *74*(6), 708–712. https://doi.org/10.1016/j.humimm.2012.10.028

186. Wu, J., Wu, Y. Q., Ricklin, D., Janssen, B. J., Lambris, J. D., & Gros, P. (2009). Structure of complement fragment C3b-factor H and implications for host protection by complement regulators. *Nat Immunol*, *10*(7), 728–733. https://doi.org/10.1038/ni.1755

187. Xin, G., Shi, W., Xu, L. X., Su, Y., Yan, L. J., & Li, K. S. (2013). Serum BAFF is elevated in patients with IgA nephropathy and associated with clinical and histopathological features. *J Nephrol*, *26*(4), 683–690. https://doi.org/10.5301/jn.5000218

188. Xu, H., Liew, L. N., Kuo, I. C., Huang, C. H., Goh, D. L., & Chua, K. Y. (2008). The modulatory effects of lipopolysaccharide-stimulated B cells on differential T-cell polarization. *Immunology*, *125*(2), 218–228. https://doi.org/10.1111/j.1365-2567.2008.02832.x

189. Xu, L. L., Zhou, X. J., & Zhang, H. (2023). An Update on the Genetics of IgA Nephropathy. *J Clin Med*, *13*(1). https://doi.org/10.3390/jcm13010123

190. Xu, S., Zhan, L., Tang, W., Wang, Q., Dai, Z., Zhou, L., Feng, T., Chen, M., Wu, T., Hu, E., & Yu, G. (2023). MicrobiotaProcess: A comprehensive R package for deep mining microbiome. *Innovation (Camb)*, *4*(2), 100388. https://doi.org/10.1016/j.xinn.2023.100388

191. Yanagawa, H., Suzuki, H., Suzuki, Y., Kiryluk, K., Gharavi, A. G., Matsuoka, K., Makita, Y., Julian, B. A., Novak, J., & Tomino, Y. (2014). A panel of serum biomarkers differentiates IgA nephropathy from other renal diseases. *PLoS One*, *9*(5), e98081. https://doi.org/10.1371/journal.pone.0098081

192. Yang, S., Chen, B., Shi, J., Chen, F., Zhang, J., & Sun, Z. (2015). Analysis of regulatory T cell subsets in the peripheral blood of immunoglobulin A nephropathy (IgAN) patients. *Genet Mol Res*, *14*(4), 14088–14092. https://doi.org/10.4238/2015.October.29.28

193. Yeo, S. C., Cheung, C. K., & Barratt, J. (2018). New insights into the pathogenesis of IgA nephropathy. *Pediatr Nephrol*, *33*(5), 763–777. https://doi.org/10.1007/s00467-017-3699-z

194. Yeo, S. C., Goh, S. M., & Barratt, J. (2019). Is immunoglobulin A nephropathy different in different ethnic populations? *Nephrology (Carlton)*, *24*(9), 885–895. https://doi.org/10.1111/nep.13592

195. Yoshikawa, N., Iijima, K., Matsuyama, S., Suzuki, J., Kameda, A., Okada, S., & Nakamura, H. (1990). Repeat renal biopsy in children with IgA nephropathy. *Clin Nephrol*, *33*(4), 160–167. https://www.ncbi.nlm.nih.gov/pubmed/2190720

196. Yu, B., Shi, S., Lv, J., Liu, L., Zhou, X., Zhu, L., Chen, P., Yang, H., Wang, Z., Wang, S., Barratt, J., & Zhang, H. (2022). Rapidly progressive IgA nephropathy: clinicopathological characteristics and outcomes assessed according to the revised definition of the KDIGO 2021 Guideline. *Nephrol Dial Transplant*, *37*(12), 2429–2437. https://doi.org/10.1093/ndt/gfac004

197. Zachova, K., Kosztyu, P., Zadrazil, J., Matousovic, K., Vondrak, K., Hubacek, P., Julian, B. A., Moldoveanu, Z., Novak, Z., Kostovcikova, K., Raska, M., & Mestecky, J. (2020). Role of Epstein-Barr Virus in Pathogenesis and Racial Distribution of IgA Nephropathy. *Front Immunol*, *11*, 267. https://doi.org/10.3389/fimmu.2020.00267

198. Zeng, Q., Wang, W. R., Li, Y. H., Liang, Y., Wang, X. H., Yan, L., & Yu, R. H. (2023). Diagnostic and prognostic value of galactose-deficient IgA1 in patients with IgA nephropathy: an updated systematic review with meta-analysis. *Front Immunol*, *14*, 1209394. https://doi.org/10.3389/fimmu.2023.1209394

199. Zhang, L., Wang, Y., Shi, X., Zou, H., & Jiang, Y. (2014). A higher frequency of CD4(+)CXCR5(+) T follicular helper cells in patients with newly diagnosed IgA nephropathy. *Immunol Lett*, *158*(1–2), 101–108. https://doi.org/10.1016/j.i mlet.2013.12.004

200. Zhang, Y., Guo, L., Wang, Z., Wang, J., Er, L., Barbour, S. J., Trimarchi, H., Lv, J., & Zhang, H. (2020). External Validation of International Risk-Prediction Models of IgA Nephropathy in an Asian-Caucasian Cohort. *Kidney Int Rep*, *5*(10), 1753–1763. https://doi.org/10.1016/j.ekir.2020.07.036

201. Zhang, Z., Zhang, Y., & Zhang, H. (2022). IgA Nephropathy: A Chinese Perspective. *Glomerular Dis*, *2*(1), 30–41. https://doi.org/10.1159/000520039

202. Zheng, N., Xie, K., Ye, H., Dong, Y., Wang, B., Luo, N., Fan, J., Tan, J., Chen, W., & Yu, X. (2020). TLR7 in B cells promotes renal inflammation and Gd-IgA1 synthesis in IgA nephropathy. *JCI Insight*, *5*(14). https://doi.org/10.1172/jci.insight.136965

203. Zheng, Y., Wang, Y., Liu, S., Wu, J., Duan, S., Zhu, H., Wu, D., Cai, G., & Chen, X. (2018). Potential Blood Pressure Goals in IgA Nephropathy: Prevalence, Awareness, and Treatment Rates in Chronic Kidney Disease Among Patients with Hypertension in China (PATRIOTIC) Study. *Kidney Blood Press Res*, *43*(6), 1786–1795. https://doi.org/10.1159/000495636

204. Zhou, T., Xu, H., Cheng, X., He, Y., Ren, Q., Li, D., Xie, Y., Gao, C., Zhang, Y., Sun, X., Xu, Y., & Huang, W. (2022). Sodium Butyrate Attenuates Diabetic Kidney Disease Partially via Histone Butyrylation Modification. *Mediators Inflamm*, *2022*, 7643322. https://doi.org/10.1155/2022/7643322

205. Zhu, L., Zhai, Y. L., Wang, F. M., Hou, P., Lv, J. C., Xu, D. M., Shi, S. F., Liu, L. J., Yu, F., Zhao, M. H., Novak, J., Gharavi, A. G., & Zhang, H. (2015). Variants in Complement Factor H and Complement Factor H-Related Protein Genes, CFHR3 and CFHR1, Affect Complement Activation in IgA Nephropathy. *J Am Soc Nephrol*, *26*(5), 1195–1204. https://doi.org/10.1681/ASN.2014010096

206. Zhu, Y., He, H., Sun, W., Wu, J., Xiao, Y., Peng, Y., Hu, P., Jin, M., Liu, P., Zhang, D., Xie, T., Huang, L., He, W., Wei, M., Wang, L., Xu, X., & Tang, Y. (2024). IgA nephropathy: gut microbiome regulates the production of hypoglycosilated IgA1 via the TLR4 signaling pathway. *Nephrol Dial Transplant*, *39*(10), 1624–1641. https://doi.org/10.1093/ndt/gfae052

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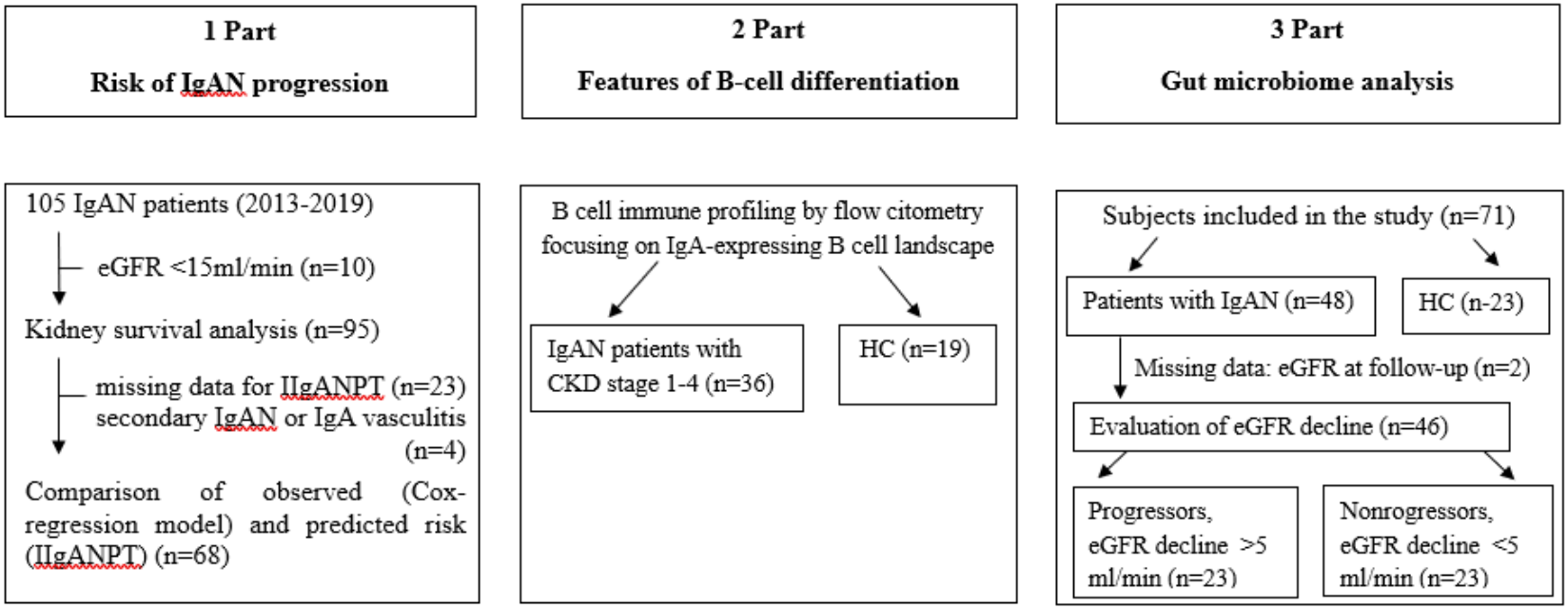
I would like to extend my sincere gratitude to *MD, PhD* Professor **Aivars Pētersons** – head of the Nephrology Center for providing the opportunity to engage in scientific exploration and work in the field of Nephrology.

Annexes



Saulīte A. J., Popova A., Erts R., Rācenis K., Kučāne L., Pētersons A., Laurinavičius A., Cernevskis H., Kuzema V. (2021). Renal Survival and Validation of Novel International Immunoglobulin A Nephropathy Prediction Tool in Latvian Population. Preliminary Data. Proceedings of the Latvian Academy of Sciences. Section B. Natural. Exact. and Applied Sciences. 75(5). 379–386. doi: 10.2478/prolas-2021-0055.

Popova A., Slisere B., Racenis K., Kuzema V., Karklins R., Saulite M., Seilis J., Saulite A.J., Vasilvolfa A., Vaivode K., Pjanova D., Kroica J., Cernevskis H., Leinieks A., Petersons A., Oleinika K. (2025). IgA class-switched CD27−CD21+ B cells in IgA nephropathy. *Nephrology Dialysis Transplantation*, Volume 40, Issue 3, March 2025, 505–515. https://doi.org/10.1093/ndt/gfae173

Popova A., Racenis, K., Briviba, M., Saksis, R., Saulite, M., Slisere, B., Berga-Svitina, E., Oleinika, K., Saulite, A. J., Seilis, J., Kroica, J., Cernevskis, H., Petersons, A., Klovins, J., Leinieks, A., & Kuzema, V. (2025). Reduced butyrate-producing bacteria and altered metabolic pathways in the gut microbiome of immunoglobulin A nephropathy patients. Sci Rep, 15(1), 28011. https://doi.org/10.1038/s41598-025-13629-5

IgAN – IgA nephropathy; **e-GFR** – estimated glomerular filtration rate; **IIgANPT** - International IgAN Prediction Tool; **CKD** – chronic kidney disease; **HC** – healthy controls.

Research Permission approved by Ethics Committee of Pauls Stradins Clinical University Hospital

Paula Stradiņa klīniskās universitātes slimnīcas Attīstības biedrības
KLĪNISKĀS IZPĒTES ĒTIKAS KOMITEJA

ATTĪSTĪBAS FONDS

Darbojas saskaņā ar SHK LKP un vietējām normatīvajām prasībām
ATZINUMS Nr.100118-10L

1. **Akadēmisks pētījums "IgA nefropātijas klīnikas, patoloģijas, terapijas un iznākumu analīze pacientiem, kuriem veikta nieres biopsija PSKUS 5 gadu laika periodā"**
2. **Pētījuma protokola numurs:** nav
3. **Pētījuma autors un pētījuma norises vietas:**
   Linda Kučāne - VSIA "Paula Stradiņa klīniskā universitātes slimnīca", Pilsoņu ielā 13, Rīgā, LV-1002, Latvija
   Darba vadītājs: Prof. Aivars Pētersons
4. **Izskatītie un apstiprinātie dokumenti:**
   4.1. Pētījuma protokols;
   4.2. VSIA "Paula Stradiņa klīniskā universitātes slimnīca" atļauja pētījuma veikšanai;
   4.3. Pētnieka CV.
5. **Ētikas komitejas atzinums – pozitīvs**
6. **Ētikas komitejas locekļi, kuri piedalījās balsošanā:**

Pēteris Stradiņš – kardioķirurgs	Juris Pokrotnieks – gastroenterologs
Ilze Aizsilniece – ģimenes ārsts	Inga Vīgante – filologs
Biruta Kupča – psihiatrs	Pēteris Ersts – jurists
Santa Purviņa – farmakologs	Daina Biseniece – ķīmiķe

7. **Ētikas komitejas sēdes datums: 2018. gada 10.janvāris**

Ētikas komitejas priekšsēdētājs **Pēteris Stradiņš**

PAULA STRADIŅA KLĪNISKĀS UNIVERSITĀTES SLIMNĪCAS
KLĪNISKĀS IZPĒTES ĒTIKAS KOMITEJA
ATTĪSTĪBAS BIEDRĪBA

Paula Stradiņa klīniskās universitātes slimnīcas Attīstības biedrība
Pilsoņu 13, Rīga, LV- 1002, Tel. +371 26380055 fakss +371 67069946; E – pasts: etikas-komiteja@stradini.lv

Paula Stradiņa klīniskās universitātes slimnīcas
Attīstības biedrības
KLĪNISKĀS IZPĒTES ĒTIKAS KOMITEJA

Darbojas saskaņā ar SHK LKP un vietējām normatīvajām prasībām
ATZINUMS Nr. 191219-6L

1.Pētījuma nosaukums: Zarnu disbakteriozes un B šūnu mijedarbības nozīme imūnglobulīna A nefropātijas patoģenēzē.

2.Pētījuma protokola numurs: n/a

3.Pētījuma komanda un norises vietas adreses:

3.1. **Pētījuma vadītājs: Asoc.prof. Harijs Čerņevskis** – Paula Stradiņa klīniskā universitātes slimnīca, Nefroloģijas centrs, Pilsoņu iela 13, Rīga, LV-1002, Latvija

3.2. **Pētnieks: Dr. Viktorija Kuzema** – Paula Stradiņa klīniskā universitātes slimnīca, Nefroloģijas centrs, Pilsoņu iela 13, Rīga, LV-1002, Latvija

3.3. **Pētījuma norises vieta:** Paula Stradiņa Klīniskās universitātes slimnīcas Nefroloģijas centrs, Slimnīcas Apvienotā laboratorija, Rīgas Stradiņa universitātes Onkoloģijas institūts, Bioloģijas un mikrobioloģijas katedra.

4.Izskatītie un apstiprinātie dokumenti:

4.1. Pētījuma protokols;
4.2. Pacienta informētās piekrišanas veidlapa latviešu un krievu valodā;
4.3. Pētnieka CV.

5.Ētikas komitejas atzinums – pozitīvs

6.Ētikas komitejas locekļi, kuri piedalījās balsošanā:

Ilze Aizsilniece – ģimenes ārsts
Pēteris Ersts – jurists
Dace Selecka– jurists
Daina Biseniece – ķīmiķe
Inga Vīgante – filologs
Santa Purviņa – farmakologs
Irina Vinnika – biologs
Juris Pokrotnieks – gastroenterologs
Sergejs Zadorožnijs – traumatologs - ortopēds

7.Ētikas komitejas datums: 2019. gada 19.decembris.

Ētikas komitejas priekšsēdētājs **Pēteris Stradiņš**

Paula Stradiņa klīniskās universitātes slimnīcas Attīstības biedrība
Pilsoņu 13, Rīga, LV- 1002, Tel. +371 26380055 fakss +371 67069946; E – pasts: etikas-komiteja@stradini.lv

Informed Consent Form

PACIENTA INFORMĒTĀ PIEKRIŠANA
par dalību Rīgas Stradiņa universitātes veiktajā pētījumā
"Zarnu disbakteriozes un B šūnu mijedarbības nozīme imūnglobulīna A nefropātijas patoģenēzē"

Pētījuma īstenotājs - Rīgas Stradiņa universitātes Bioloģijas un mikrobioloģijas katedra, Dzirciema iela 16, Rīga.

Pētījuma mērķis – noteikt B šūnu tipus un to funkcijas, kā arī zarnu mikrobioma (dažādu baktēriju populāciju) izmaiņas imūnglobulīna A nefropātijas pacientiem, salīdzinot ar veseliem cilvēkiem.

Personas datu apstrādes mērķis – noteikt imūnglobulīna A nefropātijas pacientu specifiskas imūnas sistēmas šūnu un mikrobioma izmaiņas, analizēt pētījuma laikā iegūtos datus par B šūnu funkciju un disbakteriozi saistībā ar patoloģiskām izmaiņām nieru biopsiju materiālā un slimības klīnisku gaitu.

Datu Pārzinis pētījuma ietvaros:
Rīgas Stradiņa universitāte
Adrese: Dzirciema iela 16, Rīga
Personas datu speciālista kontaktinformācija: personu.dati@rsu.lv
Personas datu apstrādes tiesiskais pamats: Vispārīgās datu aizsardzības regulas 9.panta 2.punkta a) apakšpunkts.
Ar sīkāku informāciju iespējams iepazīties RSU Privātuma politikā www.rsu.lv/privatuma-politika

Cienītā kundze!
Godātais kungs!

Mēs uzaicinām Jūs piedalīties pētījumā "Zarnu disbakteriozes un B šūnu mijedarbības nozīme imūnglobulīna A nefropātijas patoģenēzē", ko veic Rīgas Stradiņa universitāte (RSU) sadarbībā ar Paula Stradiņa Klīniskās Universitātes slimnīcas (PSKUS) ārstu - nefrologu komandu. Vēlamies Jūs iepazīstināt ar pētījuma mērķi, norisi un saturu. Pirms šī dokumenta parakstīšanas rūpīgi izlasiet visu informāciju! Pirms dokumenta parakstīšanas Jums ir tiesības uzdot jautājumus par pētījumu un saņemt uz tiem atbildes.

Imūnglobulīna A nefropātija (IgAN) ir autoimūna slimība un biežākais no glomerulonefrītiem, kas veicina nieru mazspējas attīstību. Slimība izpaužas ar dažādu klīnisku ainu, no izmaiņām urīna analīzē līdz ātri progresējošai gaitai. Termināla nieru mazspēja attīstās līdz 39% pacientu 20 gadu laikā. Ir aktuāli izprast IgAN iespējamus slimības attīstības mehānismus. Ir iespējams, ka disbakteriozei (nelabvēlīgo baktēriju pārsvaram zarnu traktā) ir loma slimības attīstībā. Nelabvēlīgas baktērijas ne tikai izstumj labvēlīgas baktērijas, neļaujot tām pildīt savas funkcijas – sašķelt uzturvielas, bet arī var novājināt imunitāti, rada zarnu caurlaidību. Imūnās sistēmas (B) šūnām ir centrālā nozīme IgAN attīstībā caur antivielu ražošanu un citiem veidiem. Mūsu pētījums būs pirmais, kas aprakstīs B šūnu sastāvu asiņu un nieru paraugos Eiropas populācijā.

Pētījuma norise:
Pētījums norisināsies no 2020.gada 1.janvāra līdz 2022.gada 31.decembrim Paula Stradiņa Klīniskajā Universitātes slimnīcā. Pacienti ar pierādītu IgAN diagnozi pēc nieru biopsijas ir uzrunāti piedalīties pētījumā, atnākot pie nefrologa uz ambulatoru konsultāciju. Iepriekšēja slimības vēsture tiks analizēta ar Jūsu atļauju no ambulatorām un stacionāra medicīniskām

kartēm, lai savāktu datus par kopēju veselības stāvokli un laboratoriskām izmaiņām biopsijas veikšanas brīdī.
Piekrītot piedalīties pētījumā:

- Pirmajā vizītē Jums tiks izskaidrota pētījuma gaita, analīžu nodošanas kārtība un sagatavošanās, tiks iedoti trauki analīžu materiāla nodošanai.
- Ar Jums vienojas par nākamās otrās vizītes datumu, kad Jūs atnāksiet tukšā dūšā uz PSKUS un atnesīsiet no mājām divus fēču analīžu paraugus, viens no tiem tiks izmantots mikrobioma analīzei, otrs būs glabāts biobankā (-80°C), urīna analīzi, lai precizētu nieru slimības izpausmi, kā arī 3 dienu laikā aizpildītu pārtikas uzņemšanas anketu.
- Uz vietas PSKUS otrās vizītes laikā Jums tiks uzdoti jautājumi par slimības attīstību, blakusslimībām, esošu pašsajūtu, lietotiem medikamentiem, nieru aizstājterapijas metodi (ja tāda ir), kaitīgiem ieradumiem, tiks veikta ārsta apskate. Jums paņems asins analīzes bioķīmiskai izmeklēšanai, B šūnu izdalīšanai, disbakteriozes noteikšanai, būs jānodod slimnīcā vēl viena urīna analīze šūnu mikroskopēšanai, disbakteriozes vielu noteikšanai arī urīnā.
- Lai varētu novērtēt dinamikā slimības gaitu, Jūs aicinās uz 3.vizīti PSKUS pēc 5-7 mēnešiem, kad būs veikta tāda pati izmeklēšana kā otrās vizītes laikā.

Ieguvumi:
Pētījuma dalībniekiem paziņos par mikrobioma sastāvu un nieru funkciju pēc analīžu veikšanas, ar pētnieku izrunās neskaidrus jautājumus par slimību un saņemtu ārstēšanu, kā arī tiks regulāri medicīniski novēroti.
Pētījuma ieguvums sabiedrībai – izpētīt mikrobioma un imūnas sistēmas šūnu sastāva izmaiņas, raksturīgas šai slimībai. Ja pētījuma laikā atklās vienādas mikrobioma vai B šūnu izmaiņu tendences, tad nākotnē, lai paildzinātu periodu līdz terminālai nieru mazspējai, apsverama jaunu medikamentu lietošana.

Risks un neērtības (diskomforts)
Pētījuma dalībniekiem ir zināmas neērtības. Ir jārezervē 40 minūtes laika katrai no slimnīcas vizītēm (kopā 3 vizītes). Piedaloties pētījumā vajag mājas savākt materiālu fēču analīzēm un dienas laikā to nogādāt līdz pētniekam, kā arī nodot materiālu (urīnu un asinis) uz vietas slimnīcā. Iespējamas sāpes dūriena vietā pie analīžu paņemšanas no vēnas un īslaicīgi pēc tās. Paaugstinātu risku dalībai pētījumā nav.

Konfidencialitāte:
Personas datu apstrāde notiks atbilstoši "Fizisko personu datu apstrādes likuma" prasībām.

Pētījuma dati tiks apkopoti vienotā anketā, kur nefigurēs vārds, uzvārds, dzimšanas dati, bet pacientam būs piešķirts kārtas numurs. Piekrišanas veidlapas tiks glabātas 75 gadus RSU mikrobioloģijas katedras telpā speciāli izveidotā arhīvā, kuram varēs piekļūt tikai projekta pētnieki. Bioloģiskie paraugi būs indificējami pētījuma laikā (3 gadus), pēc pētījuma beigām viens fēču un asins paraugi tiks pseidonimizēti. No 27-30 ml asins materiāla, daļa tiks izmantota B šūnu izdalīšanai, mazāk materiāla būs sasaldēti un glabāsies PSKUS RSU Onkoloģijas institūta laboratorijā piekrišanas formu uzglabāšanas laikā. Turpmāk atlikušais materiāls varētu būt izmantots citos Rīgas Stradiņa universitātes pētījumos, kas saņēmuši normatīvajos aktos noteiktās atļaujas (piemēram, IgAN genoma analīze). No diviem fēču paraugiem viens tiks izmantots mikrobioma analīzei, otrs glabāsies RSU Bioloģijas un mikrobioloģijas katedras biobankā. Urīna paraugi būs analizēti PSKUS Apvienotā laboratorijā, RSU Bioloģijas un mikrobioloģijas katedras laboratorijā, pēc rezultāta iegūšanas paraugi tiks utilizēti kā bioloģiskais materiāls.

Bioloģiskā materiāla paraugi tiks uzglabāti pseidonimizētā veidā laboratorijā piekrišanas formu uzglabāšanas laikā.
Pētījuma rezultāti būs pieejami pētījuma galvenajiem pētniekiem, par rezultātiem informāciju varēs saņemt tikai pats pacients atnākot pie ārsta uz konsultāciju. Telefoniski atbildes nebūs sniegtas. Ģimenes ārstam vai citam slimnīcas ārstam nebūs pieejas analīžu rezultātiem.

Tiesības atteikties vai pārtraukt piedalīšanos pētījumā. Piedalīšanās šajā pētījumā ir brīvprātīga. Jums ir tiesības atteikties piedalīties pētījumā vai pārtraukt dalību pētījumā jebkurā laikā. Jūsu atteikšanās piedalīties pētījumā vai dalības pārtraukšana neradīs nekādu nevēlamu ietekmi uz Jums sniegtās veselības aprūpes kvalitāti.

Ja Jūs pārdomāsiet un vēlēsieties atsaukt savu dalību pētījumā, lūdzu, sazinieties ar RSU pētnieku ..... pa telefonu (+371)..... vai e-pastu – .....@stradini.lv.

Jautājumi vai bažas. Jums vajadzētu jautāt ārstējošajam ārstam par visām neskaidrībām saistībā ar Jūsu dalību šajā pētījumā, kamēr atrodaties Ārstniecības iestādē. Jūs arī turpmāk varat uzdot jautājumus, ja Jūs neizprotat, kas tiek darīts, vai vēlaties iegūt papildu informāciju. Ja vēlaties saņemt konsultāciju, vai papildu informāciju par pētījumu, lūdzu sazināties ar RSU pētnieku.....
.....(aizpilda pētnieks dokumenta parakstīšanas dienā).

Šis dokuments ir sastādīts divos eksemplāros, no kuriem viens atrodas pie pētījuma veicēja, bet otrs – pie pētāmās personas.

Lūdzu, parakstiet šo piekrišanas veidlapu tikai tad, ja piekrītat dalībai šajā pētījumā un esat saņēmis izsmeļošas un apmierinošas atbildes uz visiem saviem jautājumiem!

Lūdzu, katrā rāmītī atzīmējiet „X", norādot, ka piekrītat dotajiem apgalvojumiem.

1.	Esmu saņēmis un iepazinies ar rakstisku informāciju par veiktā pētījuma mērķi, saturu un iespējamiem riskiem. Uz visiem maniem jautājumiem esmu saņēmis izsmeļošas atbildes.	☐
2.	Esmu saņēmis un iepazinies ar informāciju par manu personas datu apstrādes tiesisko pamatu, mērķi, apjomu, glabāšanas ilgumu un iznīcināšanu, kā arī esošo un iespējamiem personas datu saņēmējiem.	☐
3.	Esmu informēts, ka jebkura mani identificējoša informācija būs konfidenciāla, un manu personas datu apstrāde tiks veikta atbilstoši labas klīniskās prakses principiem un saskaņā ar spēkā esošo normatīvo aktu prasībām.	☐
4.	Piekrītu Pētījuma rezultātā iegūto personas datu apstrādei un uzglabāšanai, kas nepieciešama Pētījuma mērķa sasniegšanai, personas datu nodošanai Rīgas Stradiņa universitātei.	☐
5.	Esmu informēts, ka man ir tiesības jebkurā brīdī labot, papildināt vai pārtraukt manu personas datu apstrādi vai jautāt par savu personas datu iznīcināšanu, kā arī man ir tiesības iegūt visu informāciju, kas ir savākta par maniem personas datiem.	☐

6.	Apliecinu, ka brīvprātīgi piekrītu dalībai Pētījumā, un apzinos, ka jebkurā brīdī, nesniedzot paskaidrojumu, varu atcelt šo piekrišanu un tas neietekmēs manu turpmāko ārstēšanos. Kā arī, esmu informēts, ka šādā gadījumā jebkura mani identificējoša informācija tiks iznīcināta vai anonimizēta.	▫
7.	Piekrītu, ka mans bioloģiskais materiāls, kas savākts pētījuma realizēšanas gaitā, pēc pētījuma noslēgšanās pseidoanonimizētā veidā turpmāk tiek glabāts RSU Bioloģijas un mikrobioloģijas katedras un PSKUS RSU Onkoloģijas institūta laboratorijas biobankā un var tikt izmantots citos Rīgas Stradiņa universitātes pētījumos, kas saņēmuši normatīvajos aktos noteiktās atļaujas.	▫

Pētamās personas paraksts _____ Pētnieka paraksts _____

_____ _____

(vārds, uzvārds) (vārds, uzvārds)

Datums _____

Pathway of B cell activation in IgAN