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REVIEW

Omics studies for comprehensive understanding of immunoglobulin A nephropathy: state-of-the-art and future directions

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ABSTRACT

Immunoglobulin A nephropathy (IgAN) is the most common worldwide primary glomerulonephritis with a strong autoimmune component. The disease shows variability in both clinical phenotypes and endpoints and can be potentially subdivided into more homogeneous subtypes through the identification of specific molecular biomarkers. This review focuses on the role of omics in driving the identification of potential molecular subtypes of the disease through the integration of multilevel data from genomics, transcriptomics, epigenomics, proteomics and metabolomics. First, the identification of molecular biomarkers, including mapping of the full spectrum of common and rare IgAN risk alleles, could permit a more precise stratification of IgAN patients. Second, the analysis of transcriptomic patterns and their modulation by epigenetic factors like microRNAs has the potential to increase our understanding in the pathogenic mechanisms of the disease. Third, the specificity of urinary proteomic and metabolomic signatures and the understanding of their functional relevance may contribute to the development of new non-invasive biomarkers for a better molecular characterization of the renal damage and its follow-up. All these approaches can give information for targeted therapeutic decisions and will support novel clinical decision making. In conclusion, we offer a framework of omic studies and outline barriers and potential solutions that should be used for improving the diagnosis and treatment of the disease. The ongoing decade is exploiting novel high-throughput molecular technologies and computational analyses for improving the diagnosis (precision nephrology) and treatment (personalized therapy) of the IgAN subtypes.

Keywords: genomics, IgA nephropathy, metabolomics, proteomics, transcriptomics

INTRODUCTION

Immunoglobulin A nephropathy (IgAN) is the most common primary glomerulonephritis worldwide [1, 2], developing mainly in the second and third decade of life, and 40% of IgAN cases reach end-stage kidney disease after 20 years from the biopsy-proven diagnosis, implying a great socio-economic burden [3, 4]. In this review we describe the results of omics studies carried out in IgAN patients. We move from genomic studies focusing on common and rare variants linked to the disease, to transcriptomics carried out on circulating immune cells and kidney biopsy specimens and then to epigenomics, proteomics and metabolomics. Finally, we suggest an integrative approach for developing biological networks and identify potential diagnostic biomarkers for precision nephrology and personalized therapy.

GENOMICS

Two main genome-wide genetic approaches have been used in the study of this complex disease: genome-wide association studies (GWASs) and next-generation sequencing (NGS).

Common variants

The GWASs conducted on IgAN have identified several common variants that clearly show a strong participation of the human leucocyte antigen (HLA) system, genes involved in innate immunity and other loci summarized in Supplementary data, Table S1. Seven single-nucleotide polymorphisms (SNPs) identified through GWASs were used for constructing a genetic risk score, which explained 4.7% of overall IgAN risk [5]. This score was integrated with other eight recently associated SNPs, but it cumulatively explains only 5.37% of disease risk (http://www.columbiamedicine.org/divisions/gharavi/calc_genetic.php) [6]. In summary, IgAN risk loci identified through GWASs are common to other inflammatory and immune-mediated diseases, explain a proportion of the disease risk worldwide, contribute to the geographic variation in disease prevalence and confirm the polygenic and multiple-susceptibility-gene nature of IgAN. Future studies are needed to evaluate whether these

GWAS signals are effectively generated by causative common variants or due to rare variants in linkage disequilibrium with the common ones. Direct sequencing of the associated regions may lead to a finer mapping of these disease-related loci, as seen for example in chronic obstructive pulmonary disease [7].

Very recently two quantitative GWASs identified two loci, in core 1, β-1-3-galactosyltransferase-1 (C1GALT1) and C1GALT1-specific chaperone 1 (C1GALT1C1-cosmc) [8, 9], providing novel insights into the genetic regulation of *O*-glycosylation and providing evidence that also common genetic variations can influence *O*-glycosylation.

Copy number variants (CNVs) in IgAN have been reported in complement factor H–related proteins (CFHR1 and CFHR3) [10, 11] and have been associated with decreased IgAN risk (Supplementary data, Table S2). CNVs were also found enriched in the linked 17q12-22 locus [12, 13] and in the *Toll-like receptor 9* (*TLR9*) gene, where its potential role in disease progression was confirmed [13]. A lower copy number of three variants within the defensin highly variable locus correlated with renal dysfunction, increased serum IgA1 and Gd-IgA1 [14, 15].

Rare variants

Rare variants, usually found in the frequency range between 0.1% and 1%, are more likely to have a stronger effect in complex diseases compared with common variants. Three whole exome sequencing (WES) studies have been performed on IgAN (Supplementary data, Table S3). The first study evidenced seven common co-segregating deleterious variants within four genes (*CARD8*, *DEFA4*, *MYCT1* and *ZNF543*) [16]. The second WES study performed on two affected individuals and an unaffected familial control from a large Sicilian family with multiple affected individuals identified a novel missense variant in the *sprouty RTK signalling antagonist 2* (*SPRY2*) gene that segregated in all IgAN-affected individuals [17]. Functional analysis of the variant in B lymphoblastoid cell lines from affected members linked the *SPRY2* mutation with inhibition of the MAPK/ERK pathway. Recently a combined linkage analysis and an exome sequencing methodology have been successfully applied for pinpointing specific causative variants involved in familial goitre [18]. A similar procedure has been carried out by Cox *et al.* [19] and their results support a polygenic and a multiple-susceptibility-gene model for familial IgAN. Evident connections with previous gene expression studies were found, but further studies are needed to understand whether these variants can effectively disrupt gene function and validate their causality in contributing to the IgAN phenotype. Furthermore, these studies must be replicated in other independent cohorts of IgAN patients to confirm the validity of the results.

To date several efforts have been made to identify susceptibility variants, but NGS data for the identification of rare variants within IgAN GWAS areas have never been done. This strategy has been recently applied in the study of age-related macular degeneration in which rare causative coding variants were pinpointed within known associated genetic loci and could represent an innovative and promising approach for IgAN [20]. Association testing deployed in parallel or in combination with familial linkage could represent another innovative strategy for the identification and characterization of a full range of disease-susceptibility variants as seen in hearing impairment and cardiomyopathy [21–24].

TRANSCRIPTOMICS

Transcriptome is considered as the gene signature leading to a phenotype that is possibly influenced by genetic determinants. Specific gene expression patterns have been found in IgAN patients' blood cells (Supplementary data, Table S4). An aberrant modulation of genes belonging to the WNT-β-catenin and PI3K/AKT pathways was found in peripheral blood leucocytes (PBLs) of IgAN patients [25]. Monocytes were principally involved in the altered WNT signalling pathway and an expansion of the non-classical $CD16^{+}$ monocyte subset characterized by an enhanced apoptotic potential was demonstrated [26]. Moreover, transcriptomics was used to analyse gross haematuria episodes in concomitance to mucosal infections, an important time point for IgAN [27, 28]. Differently regulated genes during the gross haematuria episode were principally involved in interferon signalling and antigen presentation [28]. This network showed an up-regulation of genes involved in the immune-proteasome pathway and based on a series of additional experimental approaches, C-X3-C motif chemokine receptor 1 (CX3CR1) and its ligand fractalkine were found to promote macroscopic haematuria in IgAN patients. An important limitation of all these experimental approaches is that gene expression data have not been integrated with genome-wide genotype data. This integration could be useful for the identification of expression quantitative trait loci (eQTL), giving a direct genetic explanation of aberrant gene expression data useful for translational medicine [29].

Most transcriptomic findings have been obtained from complex starting material, i.e. whole blood, which is made up of different cell lineages. Future studies should propend towards the use of cell sorting technologies for analysing the transcriptomic profile of specific cell populations or single-cell analysis, which may give novel insights into health and disease status [30]. These novel technological approaches may help to obtain cell-lineage-specific expression data from IgA1-secreting cells, giving important insights into the pathogenic mechanisms involved in the disease.

The renal damage in IgAN is caused by mesangial deposition of polymeric IgA1 and/or IgA1–IgG immune complexes at the glomerular level leading to oversynthesis of extracellular matrix (ECM). Since alterations in mRNA levels could precede the histological damage, transcriptomics in kidney tissue might identify gene expression profiles involved in the development and progression of renal damage.

Investigators focused their attention on the gene expression of isolated glomeruli or tubulointerstitial tissue from IgAN patients' kidney biopsies. They observed an increased expression of some proteoglycans directly involved in renal damage and suggested their potential role as prognostic biomarkers and highlighted an 11 transcript proteinuria signature in the tubulointerstitial compartment [31, 32]. Differently expressed genes in microdissected glomeruli with endocapillary proliferation were

involved in innate immune response, classical complement pathway activation and matrix turnover. Interestingly, the *in silico* study demonstrated that the aberrantly expressed genes characterizing endocapillary proliferation were responsive to combined corticosteroid–resveratol therapy [33]. A subsequent study demonstrated that altered expressed genes were responsive to some organic substances such as doxorubicin and thapsigargin [34] (see Supplementary data, Table S5).

Data from these studies indicate that glomerular and tubulointerstitial gene expressions are independent and some genes involved in specific pathways are responsive to drugs. Gene expression data from isolated glomeruli or tubules are not correlated to the extent of renal damage, but it is a sterile description of up- and down-regulated genes. Future studies should be performed on the entire tissue as a whole since kidney disease involves all renal compartments globally and simultaneously. In particular, gene expression studies will need to be performed on well-characterized kidney biopsy specimens that have been accurately scored with the Mesangial hypercellularity (M), Endocapillary hypercellularity (E), Segmental glomerulosclerosis (S), Tubular atrophy/interstitial fibrosis (T) and Crescents (MEST-C) [35]. In this way, specific gene expression changes that characterize active renal lesions (E and C) that are more responsive to immunosuppressive therapy compared with chronic lesions (S and T) will be identified. Furthermore, differences in gene expression linked to the degree of histologic renal damage have yet to be evaluated. This information is important because glomerular lesions are always associated with tubulointerstitial damage during the progression of renal damage.

The emerging transcriptomic methodology not deployed in the study of IgAN is RNAseq. This methodology has brought a significant qualitative and quantitative improvement to transcriptome analysis, offering an unprecedented level of resolution able to detect genes expressed at low levels, splice isoforms and novel exons/genes [36]. In addition, allele-specific expression, RNA editing and fusion transcripts represent some of the information that do not emerge from hybridization-based platforms and may be crucial in complex diseases [37].

EPIGENOMICS

To date, there are principally two studies on DNA methylation in IgAN (Supplementary data, Table S6). The first study showed that the gene expression of *Cosmc*, whose activity is closely related to aberrantly glycosylated IgA1, could be regulated by DNA methylation in lymphocytes of children with IgAN [38]. Limitations of this study, in addition to the small sample size, are the lack of strong validation, functional assays and DNA methylation analysis using a mixed cell population of peripheral blood mononuclear cells (PBMCs). The second study showed that the two hypomethylated DNA regions contained the promoters for *Dual Specificity Phosphatase 3* (*DUSP3*) and *Tripartite Motif Containing 27* (*TRIM27*) and the most extensively hypermethylated region on chromosome 5 contained the *Vault RNA 2-1* (*VTRNA2-1*) gene promoter, known as the precursor of miR-886. These aberrantly methylated DNA regions were found to induce a T helper cell imbalance towards the Th1 subtype. Even if the sample size is not very large, this study can be considered with confidence due to the many validation experiments and functional assays.

MicroRNAs (miRNAs) are another epigenetic component that can modulate gene expression in tissue and biological fluids. Three different genome-wide miRNA gene expression studies on IgAN patients' blood cells have been performed (see Supplementary data, Table S7). The first study led to the identification of two aberrantly expressed miRNAs (let-7b and miR-148b) that regulate the gene expression of two key enzymes involved in the sequential *O*-glycosylation process of the IgA1 molecule (*GALNT2* and *C1GALT1*) [39]. These results were validated biologically with transient transfection experiments and demonstrated that the abnormal miRNA-based regulatory mechanism influences the *O*-glycosylation process determining the aberrant glycosylation of IgA1, main characteristic of IgAN [39, 40]. These findings could be exploited for therapeutic use because the inhibition of these up-regulated miRNAs could normalize the IgA1 *O*-glycosylation process.

Recently the miRNA expression profiling of B cells from IgAN patients demonstrated an up-regulation of miR-374b that is able to determine B-cell proliferation and aberrant IgA1 glycosylation by targeting *C1GALT1-Cosmc* and phosphatase and tensin homolog (PTEN); [41] the latter has already been found aberrantly modulated in IgAN [25]. Anti-miR blockers could be exploited for new therapeutic approaches and are being tested in *ex vivo* clinical studies on myocardial infarction and neoplasms and in phase III clinical trials for hepatitis C virus infection [41–44].

While the majority of miRNAs are found within the cell, a handful of circulating miRNAs, released from blood cells, have also been detected in various body fluids. In a multicentre international retrospective study, let-7b and miR-148b levels were measured in serum samples of Caucasian and Asian IgAN patients and their combined value was found to be a significant predictor of the disease status, showing a good sensitivity and specificity and discriminating IgAN patients from other primary glomerulonephritides [45]. This combined biomarker seems to be a novel, specific and non-invasive indicator to test the probability of being affected by IgAN, mainly in patients' relatives with persistent urinary abnormalities.

In recent years, several groups have studied the role of miRNAs in kidney tissue. A cluster of miRNAs has been observed in the human kidney tissue with a different distribution in renal cortex and medulla of rat kidney. To date, few studies have been carried out on IgAN renal biopsies (see Supplementary data, Table S8). Abnormal miRNA expression patterns in renal biopsies correlated with glomerular sclerosis and interstitial fibrosis. Various miRNAs have been found to be involved in the progression of renal damage or in the profibrotic processes in IgAN through E-cadherin and the epithelial-to-mesenchymal transition process. Recently miRNAs have also been found to have a role in the activation of mesangial cells by secretory IgA (SIgA) from IgAN patients [43]. In conclusion, regarding the use of miRNAs as biomarkers in the blood and kidney, many studies have been conducted in retrospective cohorts but prospective studies are required to confirm their clinical utility and diagnostic value in asymptomatic

individuals. In addition, several miRNAs have been identified as targets for the treatment but the use of miRNA modulators in pre-clinical settings is still missing.

PROTEOMICS

Urine is a biological fluid containing many cytokines that can be considered predictors of poor prognosis for the progression of renal damage [46, 47]. Today, proteomics-based techniques are promising approaches for uncovering new and more sensitive and relevant biomarkers that may be involved in the earlier phases of IgAN. Several studies have identified specific polypeptide patterns in patients' urine, as reported in Supplementary data, Table S9. Other proteins are able to differentiate IgAN from other diseases or distinguish specific proteomic profiles on the basis of disease severity or identify specific alterations during the progression of renal damage. Other urinary proteomics patterns distinguish progressor from non-progressor IgAN patients. Some urinary proteins are also predictive of inadequate response to angiotensin-converting enzyme inhibitors.

Data from the published articles do not evidence a common urinary pattern of polypeptides in the urine of IgAN patients, probably due to different cohorts of patients included in the studies. However, some common proteins such as fragments of albumin, alpha-1 antitrypsin, uromodulin and alpha-1 microglobulin have been reported by many investigators.

Limitations of the published studies are different cohorts of IgAN patients included in the studies, different grades of renal damage, different techniques used for detecting urinary polypeptides and the absence of validation in independent IgAN cohorts. Future studies should propend in the identification of a proteome-based classifier containing specific peptides able to discriminate IgAN patients with specific clinical patterns (i.e. microscopic haematuria or patients with recurrent episodes of macroscopic haematuria) from healthy individuals and from other types of glomerulonephritis. The same classifier could be used as a disease outcome indicator or could be helpful in the evaluation of a therapeutic response. A urinary proteome–based classifier, CKD273, has successfully been identified in diabetic nephropathy and used in a prospective randomized clinical trial [48]. This proteomic panel of 273 urinary peptides performed significantly better than albuminuria in predicting the early stages of CKD [49]; a similar approach is needed in IgAN.

A recent development in proteomic analysis is the new high-throughput imaging mass spectrometry (IMS) technique for the identification, quantization and distribution of proteins, lipids and chemical metabolites at a picomolar level in complex multicellular tissue [50]. This procedure could be applied for the evaluation of biopsy sections and help differentiate IgAN subphenotypes. Furthermore, IMS has the power to discover specific three-dimensional peptide profiles that are constitutively expressed in human cells and tissue types and in the future the IMS integration with other omics such as genomics and transcriptomics will generate specific gene-transcipt protein networks in health and disease [51, 52].

METABOLOMICS

Metabolomics studies the individual metabolic profiles and their changes over time due to physiological and pathological conditions (Supplementary data, Table S10). ^{1}H nuclear magnetic resonance (NMR) spectroscopy and pattern recognition analysis have been used by Del Coco *et al.* [53] to detect minor alterations in the metabolomic profile of urine from IgAN patients. Data from NMR urinalysis evidenced altered values of specific metabolites (increased values of creatinine, trimethylamine-N-oxide, betaine and acetate and decreased levels of hippurate, lactate and citrate). These results were confirmed in another study using the metabolomic urinary profiles of four primary glomerulonephrites where a specific urinary signature for IgAN patients was found [54]. Recently, Kalantari *et al.* [55] identified several urinary metabolite biomarkers correlating with proteinuria and the most significant pathway associated with disease severity was the phenylalanine metabolic pathway.

These limited number of studies demonstrate a specific metabolite signature in IgAN correlating with the severity of the renal damage. The presence of these metabolites in the urine reflects an altered metabolic pathway determined by an altered cell activity, highlighting new targets for specific therapy. However, to confirm these results, data should be validated in larger independent and well-phenotyped cohorts. Moreover, the integrated analysis of genomics and urinary metabolomics could be considered as a promising approach for uncovering novel gene–disease connections via metabolic traits highlighting novel hypotheses about molecular mechanisms involved in the a etiology of disease [56, 57].

CONCLUDING REMARKS

A summary of the results obtained from the omic studies, including points of weaknesses and strengths, are described in Table 1.

Large-scale GWASs have been successfully applied in IgAN with extensively replicated results. To date, GWASs have identified highly relevant associated loci containing major histocompatibility complex (MHC) regions, the complement system and genes involved in mucosal IgA production, innate immunity and inflammatory response [11]. Fine-mapping studies are needed to uncover the causal genetic variants underlying the signals. Furthermore, identified rare/common variants underlying the IgAN phenotype must be validated with classical biological experimental approaches.

The clinical approaches using flow cytometry, Western blot, enzyme-linked immunosorbent assay, immunohistochemistry, transfection experiments and others have been applied in transcriptomics/epigenomics/proteomics studies, but results require extensive replication. Moreover, metabolomics results must be replicated and applied to larger cohorts of well-phenotyped IgAN patients.

Results from different omics seem to be fragmented, but overlaps are evident when we focus on the four-hit pathogenic model (Table 2). High circulating levels of Gd-IgA1 (*hit 1*) are determined by a dysregulation in antigen handling and aberrancies in IgA1 production at mucosal sites (Figure 1A) [58]. All omics performed on PBLs show the involvement of altered

Table 1. Overview of omics studies in IgA nephropathy

Omics	Input material	Tissue origin	Number of studies	Results	Weaknesses	Strengths	Involved genes/pathways
Genomics	DNA	WB	5	51 CVs	• No validation with classical biological experimental approaches	• Large sample size 13 replicated CVs in Asian and European populations	HLA-DRB1, HLA-DQA, HLA-DQB, CFHC, FHR3, CFHR1, CFHR4, CFHR2, CFHR5, **TAP1, TAP2**, **PSMB8, PSMB9**, VAV3.HORMAD2, MTMR3, LIF, OSM, TNFSF13, MPDU1, EIF4A1.CD68, TP53, SOX15, **ITGAM-ITGAX**, HLA-DP, HLA-A, HLA-DQA/B, DEFA, ACCS, KLF10/ODF1, CARD9, HLA-DQ, ST6GAL1, HLA-DPB1, HLA-DPA2
	DNA	WB	4	CNVs	• No validation with classical biological experimental approaches	• CFH and defensin loci have been replicated • CNV real-time PCR assay validation for GALNT13, COL11A2, TLR9 and validation with classical biological experimental approaches and replication in Greek population	CFHR3, CFHR1, *HLA-DQB1, GALNT13*, *COL11A2*, ***TLR9***, *DEFA3*
	DNA	WB	3	31 RVs	• RVs causality validation is missing	• RVs within previously validated aberrant networks	MAPK/ERK pathway, **PI3K/AKT pathway**
Transcriptomics	RNA	PBL	3	470 dysregulated genes	Results have not been replicated Small sample size	• Validated with classical biological experimental approaches	WNT-β-catenin and **PI3K/Akt pathway (INVS, PTEN)**, **enhanced proliferation of PBMCs** **Innate immunity activation** of **PSMB8, PSMB9 and TAPBP**
	RNA	Blood $CD14^{+}$ cells	1	710 differently expressed genes	Results have not been replicated Small sample size	• Validated with classical biological experimental approaches	TNF, CD83, NDUFS3 and TNFRSF1A
	RNA	Kidney	4	140 differently expressed genes	Results have not been replicated Small sample size	• Some validated with classical biological experimental approaches	**Innate immune response**, **classical complement pathway** activation and matrix turnover. Role of pattern recognition receptors for the identification of bacteria and viruses, leucocyte extravasation signalling, **TREM signalling-ITGAX**, **NF-κB** activation by viruses, **Toll-like receptor signalling**, cell cycle: G2/M DNA damage checkpoint regulation, IL-8 signalling, production of nitric oxide and reactive oxygen species in macrophages

Continued

Table 1. Continued

Omics	Input material	Tissue origin	Number of studies	Results	Weaknesses	Strengths	Involved genes/pathways
Epigenomics	DNA	Blood $CD4^+$ cells	1	Aberrantly methylated genes: DUSP3, TRIM27, VTRNA2-1	• Results have not been replicated Small sample size	• Validated with classical biological experimental approaches	T helper cell imbalance towards the Th1 subtype
	DNA	PBMCs	1	Aberrantly methylated gene: COSMC	Results have not been replicated Small sample size Results obtained from an heterogeneous cell population	• Validated with classical biological experimental approaches	**COSMC** methylation involved in aberrant glycosylation of IgA1
	Total RNA including miRNA	PBMCs/SERUM	2	37 dysregulated miRNAs	Small sample size	Validated with classical biological experimental approaches Replicated CVs in Asian and European populations	miRNAs involved in aberrant glycosylation of IgA1 let-7d directly regulates **PTEN** and miR-148b regulates both **INVS** and **PTEN**
	Total RNA including miRNA	Circulating cells	1	1 up-regulated miRNA	Small sample size	• Validated with classical biological experimental approaches	**B-cell proliferation** by targeting **PTEN**miRNAs involved in aberrant glycosylation of IgA1 targeting **COSMC** in B cells of IgAN
	Total RNA including miRNA	Kidney	6	14	Small sample size No validation with classical biological experimental approaches		Abnormal miRNA expression patterns correlated with glomerular sclerosis and interstitial fibrosis and with progression of renal damage involving **complement pathway (ITGAM–ITGAX)**
Proteomics	Proteins	Urine	14	245	Small sample size	• Three studies have been performed validations in independent cohorts	Altered albumin, alpha-1 antitrypsin, uromodulin, alpha-1 microglobulin, kininogen
Metabolomics	Metabolites	Urine	3		Small sample size No validation with classical biological experimental approaches		Increased values of creatinine, TMAO, betaine and acetate, and decreased levels of hippurate, lactate and citrate

Bold font signifies that genes or pathways are observed in more than one study. CVs, common variants; RVs, rare variants; WB, whole blood.

Table 2. Omics studies in the context of the four-hit pathogenic model

Hit	Phase process	Molecular processes involved	Omics contributions	Related omics pathological processes
1	High circulating levels of galactose-deficient IgA1 (Gd-IgA1)	Dysregulation of IgA1 production at mucosal surfaces alterations in post-translational modification of *O*-glycans within IgA1-producing cells	Common genetic variants	*HORMAD2*, *LIF*, *OSM*, *DEFA* and, *TNFSF13* genes appear to modulate mucosal immunity and production of IgA1. *VAV3* essential for adaptive immune function and NF-κB activation in B cells, a process that stimulates IgA production. ITGAM and ITGAX are involved in intestinal inflammation and IgA production. *CARD9* activates NF-κB, which is responsible for both innate and adaptive immunity. TAP1, TAP2, PSMB8 and PSMB9 are involved in the antigen presentation pathway. MHC class II alleles loci (*HLA-DQA1*, *HLA-DQB1*, *HLA-DRB1* and *HLA-DP*) may participate in the regulation of intestinal inflammation and IgA production.
			Rare genetic variants	Multiple rare genetic variants co-segregating with familial IgA nephropathy all act within a single immune-related network where WNT-β-catenin, PI3K/Akt pathway and interferon signalling involve both innate and adaptive immunity.
			Gene expression studies	Hyperactivation of WNT-β-catenin and PI3K/Akt pathways (down regulation of *INVS* and *PTEN*) leads to a defect in antigen handling and to abnormal systemic responses to mucosally encountered antigens. Defect in antigen handling in PBMCs has been demonstrated during the macroscopic haematuria with a specific up-regulation of the immunoproteasome pathway (PSMB8, PSMB9, PSMB10 and TAPBP).
			miRNA and methilation studies	miRNA let-7b and miR-148b modulate two enzymes involved in the sequential *O*-glycosylation process of the IgA1 molecule. Abnormal miRNA-based regulatory mechanism influences the *O*-glycosylation process determining the aberrant glycosylation of IgA1. Furthermore, let-7d directly regulates PTEN and miR-148b regulates both INVS and PTEN. Up-regulation of miR-374b promotes B-cell proliferation and aberrant IgA1 glycosylation by targeting ***Cosmc*** and ***PTEN***. The gene expression of ***Cosmc***, whose activity is closely related to Gd-IgA1, is regulated by DNA methylation in lymphocytes. Aberrantly methylated regions in $CD4^+$ T cells of IgAN patients led to the altered expression of genes involved in ***TCR*** signal transduction and in the reduced TCR signal strength explaining the T-helper cell imbalance towards the Th1 subtype.
2	Production of anti-Gd-IgA1 autoantibodies of IgG and/or IgA isotype	High Gd-IgA1 elicits an autoimmune response, resulting in generation of anti-glycan antibodies that recognize *N*-acetylgalactosamine epitopes on Gd-IgA1	Common genetic variants	Anti-glycan response may be triggered by the exposure to infectious or dietary antigens that have been aberrantly processed by genetically predisposing MHC class II allele loci: *HLA-DQA1*, *HLA-DQB1*, *HLA-DRB1* and *HLA-DP* having a role in autoimmunity.
			Gene expression studies	Defect in antigen handling in PBMCs with an up-regulation of interferon signalling and immunoproteasome pathway (PSMB8, PSMB9, PSMB10 and TAPBP) has been demonstrated in IgAN patients. These pathways seem to be up-regulated in autoimmune diseases.
3	Formation of circulating IgA1–IgG and IgA1–IgA1 immune complexes and deposition	Immune complexes activate alternative complement pathway	Common genetic variants	ITGAM is essential for interactions between CD89 and secretory IgA.
			Gene expression studies	Hyperactivation of PI3K pathway in monocytes activates FcαRI and circulating immune complexes aggregate this receptor. This interaction induces shedding of the extracellular domain to form circulating IgA1–FcαRI complexes that could have a pathogenic role in IgAN. Gene expression studies on kidney tissue have evidenced ITGAM.

Continued

Table 2. Continued

Hit	Phase process	Molecular processes involved	Omics contributions	Related omics pathological processes
4	Local activation of inflammatory pathways and the complement system	Local inflammation, mesangial cell proliferation, secretion pro-inflammatory cytokinesleading to the interstitial infiltration of inflammatory cells and promoting glomerular and tubulointerstitial fibrosis	Common genetic variants	Glomerular inflammation is enhanced by the complement system—lectin or the alternative pathway (CFHR1 and CFHR3). ITGAM and ITGAX encode integrins that combine to form leucocyte-specific complement receptors CR3 and CR4. These may be involved in glomerular inflammation.
			Gene expression studies	Various gene expression studies conducted on kidney tissue show the involvement of relevant signalling pathways: activation complement system (*ITGAX–ITGAM*, *C1QA* and *C1QB*); Toll-like receptor signalling; NF-κB pathway; IL-8 signalling; production of nitric oxide and reactive oxygen species in macrophages. Kidney production of cytokines (mainly IL-6) promotes mesangial cell proliferation.
			miRNA and methylation studies	TLR9 CNV contributes to the deterioration of the renal function.
			Proteomics	Low levels of UMOD peptides cause progression of renal damage.

innate and adaptive immunity. The altered response to an antigenic challenge clearly emerges during the gross haematuria episode with the up-regulation of interferon-regulated genes involved in the degradation and processing of antigens (*TAP1*, *TAP2*, *PSMB8* and *PSMB9*) [28]. These genes are found in highly replicated GWAS loci [11] and have been validated with traditional experimental approaches by different authors [27, 59]. Another pathway that seems to be hyperactivated in this context is the WNT-β-catenin–PI3K/Akt pathway mainly determined by the down-regulation of the negative regulator *PTEN* and enhanced translocation of the effector molecule nuclear factor (NF)-κB [27, 59]. GWASs, rare genetic variants and epigenetic studies support this hyperactivation with NF-κB translocation occurring through *VAV3* [60], *CARD9* [61] and *miR-374b* [41], the latter modulating a B-cell proliferation and aberrant IgA1 glycosylation. Interestingly, miRNAs let-7b and miR-148b have also been demonstrated to regulate these pathways; let-7b directly regulates *PTEN* and miR-148b regulated both inversin (INVS) and PTEN [39]. The same let-7b, miR-148b and miR-374b participate directly in the aberrant glycosylation process targeting, respectively, GALNT2, C1GALT1 and C1GALT1-COSMC, important enzymes in the IgA1 glycosylation process.

Gd-IgA1 exposes *N*-acetylgalactosamine within the IgA1 hinge region and is an epitope for the anti-glycan response [62] that promotes the formation of circulating autoantibodies (*hit 2*, Figure 1B). This autoimmune phenotype could be regulated by MHC class II loci identified by GWASs (HLA-DQA1, HLA-DQB1, HLA-DRB1 and HLA-DP) and triggered by the exposure to infectious or dietary antigens that have been aberrantly processed. Furthermore, the immunoproteasome (PSMB8, PSMB9, PSMB10 and TAPBP) could be involved in this process, as it is highly up-regulated in a number of diseases, including autoimmune diseases [63].

The formation of circulating immune complexes and deposition (*hit 3*) may be elicited by hyperactivation of the PI3K/AKT pathway Figure 1C. This pathway activates FcαRI (CD89) in monocytes [64, 65]. Then, circulating immune complexes aggregate this receptor, inducing shedding of the extracellular domain to form circulating IgA1–FcαRI complexes. Other receptors that are known to be involved in the deposition process (such as transglutaminase-2, CD89, transferrin receptor and pIgR) have not been confirmed by omic studies.

Immunocomplex deposition induces mesangial cell proliferation, secretion of ECM chemokines and cytokines with activation of the alternative complement pathway (*hit 4*, Figure 1D). These pathways all seem to be evident in GWAS loci and gene expression studies on kidney tissue. In particular, activation of ITGAX–ITGAM, members of the complement system, encodes for integrins αM and αX that combine with the integrin β2 chain to form leucocyte-specific complement receptors 3 and 4; these receptors may augment glomerular inflammation. Toll-like receptor signalling is also common to different omics and may be involved in progression of the disease.

FUTURE DIRECTIONS

The use of molecular profiling technologies has identified different molecular signatures in IgAN associated with different phases of the disease and the progression of histologic lesions. Integration of data using an extensive computational support and statistical modelling is the key for connecting omic datasets with clinical data [66] and for decoding the pathophysiological disease processes; it is necessary for an accurate and precise diagnosis and a more targeted therapy (Figure 2). Future omics studies should be applied on well-phenotyped IgAN patients with extensive clinical data and MEST-C classification of renal damage. Common and rare gene variants located in the GWAS loci, PBMC gene expression data, molecular signature of renal lesions, clinical data (serum levels of aberrantly glycosylated IgA1, miRNAs, estimated glomerular filtration rate and proteinuria, morphologic pictures), urinary polypeptides and metabolites should all be integrated for the identification of

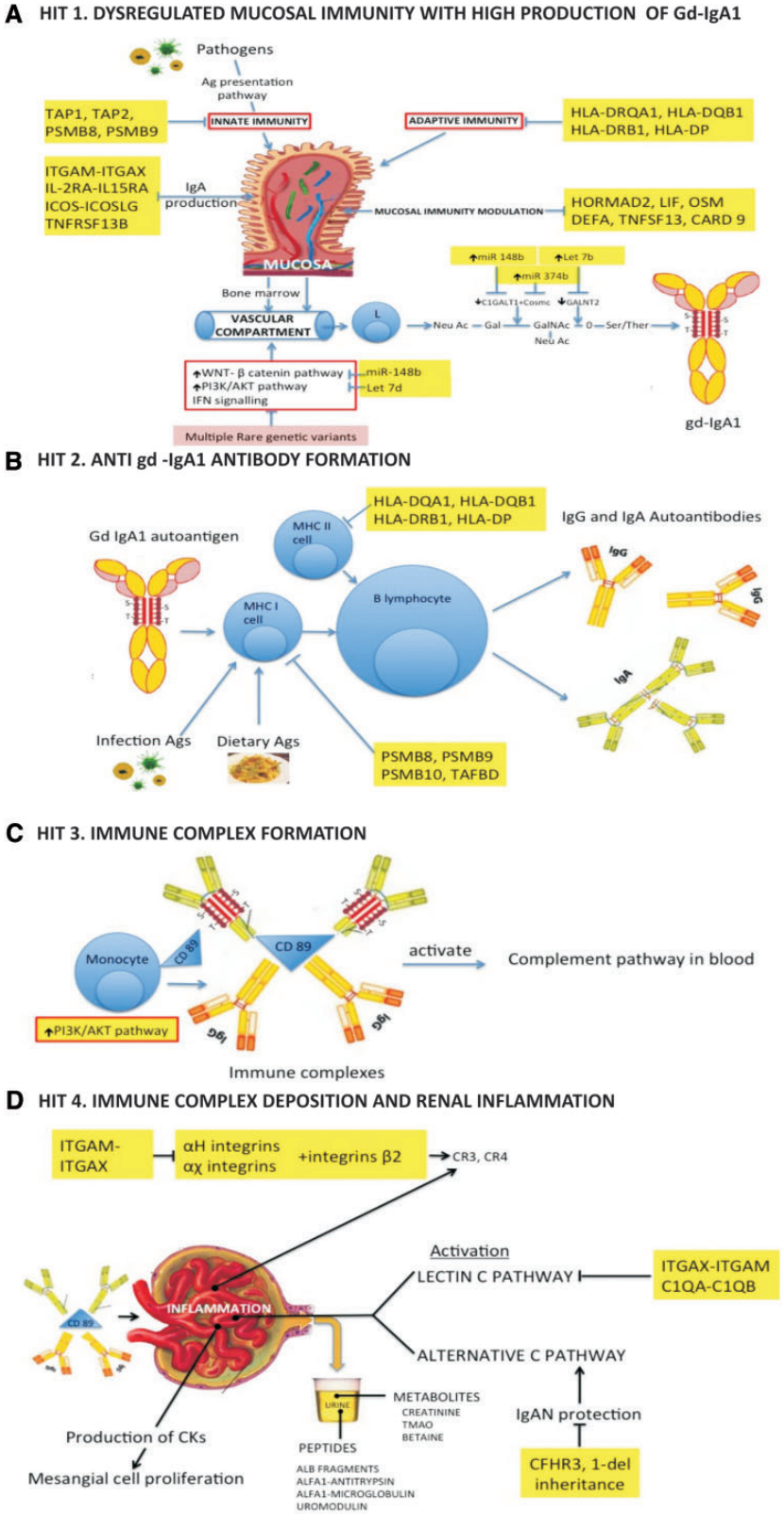

FIGURE 1: Figurative integration of omics data in the four hit pathogenic model. The yellow color highlights genes and pathways identified by omics studies.

diagnostic biomarkers in IgAN and risk prediction scores. The integrative analyses may be facilitated by the use of online resources like Nephroseq (https://www.nephroseq.org/resource/login.html) and Kidney and Urinary Pathway Knowledge Base (KUPKB, http://www.kupkb.org) and other tools based on '*in silico*' nanodissection (http://nano.princeton.edu). To date, high-throughput omics analyses are in progress; different issues have to be taken into consideration to discuss a

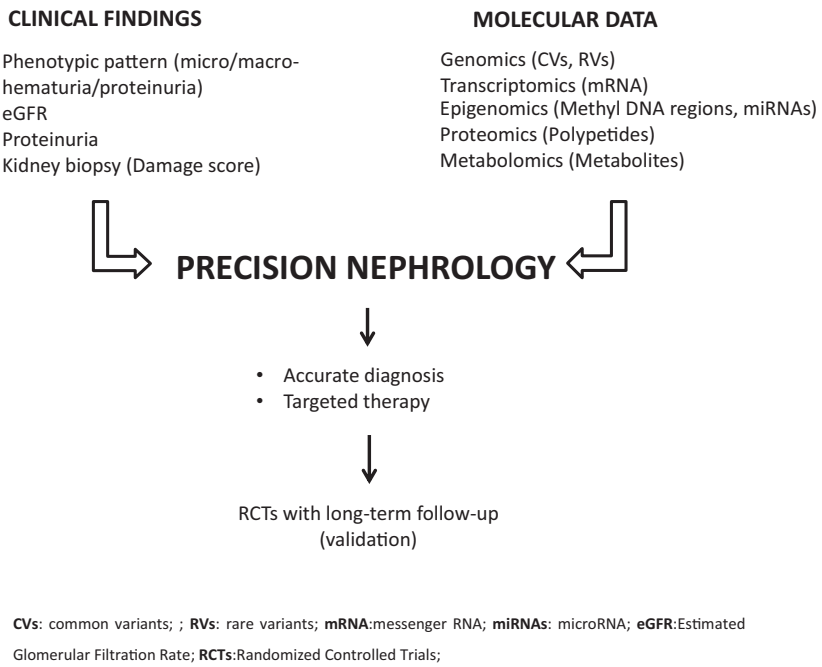

FIGURE 2: Integrative analysis of clinical findings and omics data for a better molecular understanding of IgAN leading towards precision nephrology and improvement in disease management (personalized therapy). RCTs, randomized controlled trials; eGFR, estimated glomerular filtration rate.

concrete methodology for personalized health care starting from omics data [67]. From a technical point of view, sample acquisition and data analysis should also be standardized and results need to be validated on different platforms in large cohorts of patients. Furthermore, the study of biological component systems via the computational and mathematical modelling of complex biological systems [68] is the missing link between omics and precision medicine in IgAN. This methodology is crucial to provide insights into new pathways and networks between omics to drive innovation through biology-based computational analysis that can detect aberrant networks at the onset of the disease and enable patient stratification on the basis of their individual genetic and molecular profiles [69]. The shift from the traditional trial-and-error approach to precision medicine is possible if systems biology and multidisciplinary approaches are undertaken as has occurred in other diseases [70].

In conclusion, the integration of results from multilevel, high-dimensional datasets is the next step for a better understanding of molecular networks and provides a unique resource for identifying new drug targets. Ultimately this methodology will lead to personalized therapeutic approaches in different subsets of IgAN patients, thus promoting precision nephrology.

SUPPLEMENTARY DATA

Supplementary data are available at ndt online.

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CONFLICT OF INTEREST STATEMENT

None declared.

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