Development of an IgA nephropathy molecular model based on an integrative analysis of -omics data

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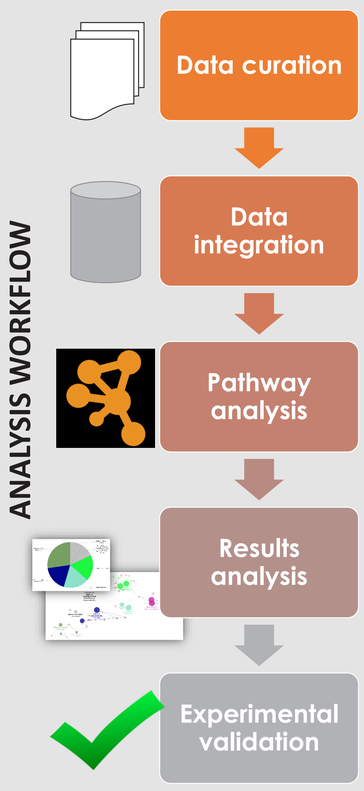
OBJECTIVES

IgA nephropathy (IgAN) is the most common type of primary glomerular disease worldwide. We applied a combinatorial approach for the analysis of 'omics data collected from published scientific literature and 'omics databases, aiming at the discovery of novel putative biomarkers of the disease.

METHODS



Mining of 'omics databases related to kidney pathologies and literature in search for high-throughput 'omics studies on IgAN (human case control-studies, including healthy controls and independent validation of findings).

Extraction of differentially expressed molecules ($p<0.05$) from each study and construction of one integrated dataset. Datasets preparation involved mapping identifiers to common format, assigning regulation and merging datasets.

Segregation of data into pathways in Cytoscape (ClueGO plug-in) through screening of the Reactome pathway database.

Multi-step assessment of predicted proteins - transcriptomics data association analysis, investigation of protein-protein interactions and expression in the kidney tissue, functional evaluation of proteins in the context of IgAN and selection of novel disease-relevant findings.

Pending validation of predicted targets in urine samples (IgAN and healthy) via multiple reaction monitoring (MRM).

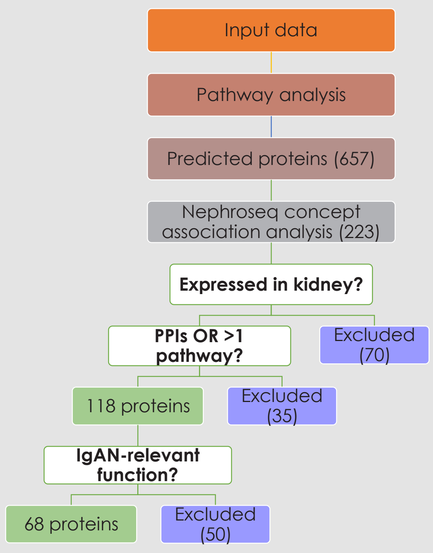
RESULTS

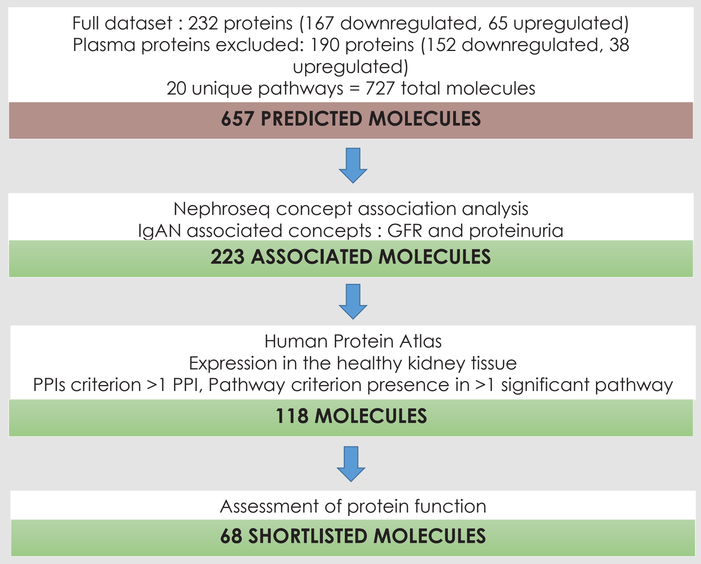
A set of 232 proteins was identified as differentially expressed in IgAN versus healthy controls and used for pathway enrichment analysis. Most significant pathways included *Platelet activation, signaling and aggregation*, *Formation of Fibrin Clot* and *Collagen formation*. **657 predicted molecules** building these pathways were subjected to multi-step assessment. This resulted in shortlisting of **68 proteins**, which might be novel molecules potentially implicated in IgAN pathology.

Table 1. Significant pathways yielded from the analysis of urine proteomics dataset.

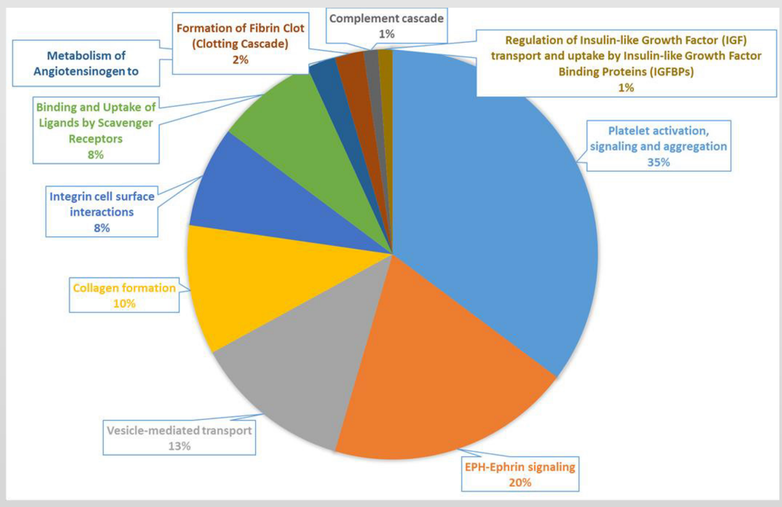
Pathway	#proteins	coverage %	p-value
Platelet activation, signaling and aggregation	23	10.45	2.55E-09
Formation of Fibrin Clot (Clotting Cascade)	6	15.38	6.81E-03
Binding and Uptake of Ligands by Scavenger Receptors	6	15.00	7.88E-03
Membrane Trafficking	13	6.47	1.06E-02
Metabolism of Angiotensinogen to Angiotensins	4	25.00	1.47E-02
Collagen formation	7	8.14	2.75E-02
Integrin cell surface interactions	6	9.09	3.91E-02
EPH-Ephrin signaling	7	7.53	4.42E-02
Regulation of Insulin-like Growth Factor (IGF) transport and uptake by Insulin-like Growth Factor Binding Proteins (IGFBPs)	4	19.05	4.49E-02
Complement cascade	5	13.51	4.90E-02

Steps followed in the assessment of molecules leading to the selection of validation candidates.





Distribution of 68 shortlisted proteins among pathways yielded from the pathway enrichment analysis.



Among the shortlisted molecules, many have been already identified in the literature as altered in IgA nephropathy, such as:

- alpha-actinin-1 (ACTN1),
- alpha-actinin-4 (ACTN4),
- growth arrest-specific protein 6 (GAS6) or
- matrix metalloproteinase-2 (MMP2)

confirming the validity of presented approach.
Validation of novel predicted findings via multiple reaction monitoring (MRM) is pending.

CONCLUSIONS

Integration of heterogeneous experimental data provides new insights, through comprehensive reconstruction of biological processes and mechanisms. Adopting this approach, we performed pathway analysis of urine proteomics dataset followed by an assessment of predicted molecules with regards to IgAN pathological hallmarks. The shortlisted molecules have a high probability of being successfully identified as differentially expressed in IgAN and their utility as potential disease biomarkers or drug targets should be further investigated. MRM validation of novel predicted targets in urine is ongoing.

ACKNOWLEDGEMENTS
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