1) A GENOME-WIDE ASSOCIATION STUDY (GWAS) FOR IGAN SEVERITY AND PROGRESSION

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Rationale

We recently completed a genome-wide association study (GWAS) of IgAN. We identified three independent loci in the major histocompatibility complex (MHC) on Chr. 6p21, a common deletion of CFHR1 and CFHR3 at Chr. 1q32 and a locus at Chr. 22q12 that each surpassed genome-wide significance (p-values for association between $1.6 \times 10^{-26}$ and $4.8 \times 10^{-9}$ and minor allele odds ratios of 0.63-0.80). These five loci explain 4-7% of the disease variance and up to a 10-fold variation in interindividual risk. Moreover, the IgAN risk allele frequencies closely parallel the variation in disease prevalence across continents, suggesting that genetic risk factors may in part explain variation in disease prevalence among Asian, European and African populations.

While these five loci explain the risk of development of IgAN and predict geographic patterns of disease, they are not associated with the severity of IgAN at presentation and progression of nephropathy. In the current proposal, we hypothesize that additional genetic variants influence the severity of disease at presentation and influence the risk of progression to end-stage renal failure. We propose to perform a GWAS in the VALIGA cohort to identify variants linked to Oxford pathology scores ad baseline

Protocol

We will examine association of genetic variants with clinico-pathological data in the VALIGA. We will perform a GWAS for the following phenotypes
- Oxford scores,
- Renal function at the time of biopsy
- Proteinuria at biopsy
- Renal function and proteinuria at latest follow-up
Logistics
We only require a one-time collection of whole blood that will be collected in special tubes that will enable isolation of serum, plasma, DNA. Clinical data will be obtained from the data coordinating center. We will provide all participating enters with tubes and supplies for collecting and shipping the samples. The shipment can be billed to our Fedex account.

References:


2) INTERFERON REGULATED IMMUNOPROTEASOMES IN CLINICAL AND PATHOLOGY FEATURES OF PATIENTS WITH IGAN. GENETIC AND PHENOTYPIC STUDY

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Rationale
In a recent GWAS we localized five IgAN susceptibility loci on Chr.6p21 (HLA-DQB1/DRB1, PSMB9/TAP1, and DPA1/DPB2 loci), Chr.1q32 (CFHR3/R1 locus), and Chr.22q12 (HORMAD2 locus) (Gharavi A. et al., Nat Genet. 2011 and PLoS Genet. 2012). One of these loci contains the PSMB8 and PSMB9 genes, which encode immunoproteasome units that were found to be upregulated in peripheral blood mononuclear cells from individuals with IgA nephropathy (Coppo, R. et al. Kidney Int. 2009: 75, 536–541). Immunoproteasomes (IPs) containing the interferon-inducible subunits β1i (LMP2), β2i (MECL-1), and β5i (LMP7) alter proteasomal cleavage preference, optimise the generation of peptide ligands of MHC class I molecules, alter cytokine profile, influence T-helper cell differentiation, and play a role in T-cell survival. Interferons elicited by pathogens are potent modulators of Toll-like receptors (TLR). We found an increased expression of TLR4 in peripheral blood mononuclear cells from patients with IgAN (Coppo, R. et al. Clin Exp Immunol. 2009)/

In the present proposal, we hypothesize that other IgAN risk loci are associated with additional sequential hits in the immune system that influence the development and progression of IgAN (Suzuki et al, JASN 2011). For example, we hypothesize that a switch to an immunoproteasome in peripheral blood mononuclear cells of patients with IgA nephropathy results in increased efficiency of antigen processing and presentation, leading to increased production of immune complexes.

Protocol
We would like to combine genetic, immunobiology and clinico-pathological data starting from VALIGA cohort to test these hypotheses. We will measure the following parameters and test association with Oxford scores:

1- PSMB8 and PSMB9 genes transcript levels from PBMCs
2- Serum levels of serum IgA-1 and Galctose deficient IgA1
3- Serum Levels of anti-IgA1 autoantibodies
4- Terminal complement activation
5- Genetic polymorphisms at five loci associated with risk of IgAN by GWAS

**Logistics**

We only require a one-time collection of whole blood that will be collected in specials tubes that will enable isolation of serum, plasma, DNA and RNA. We will provide all participating centers with tubes and supplies for collecting and shipping the samples. The shipment can be billed to our Fedex account.
3) The role of the complement system in determining outcome in IgA-nephropathy

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Background

Complement analyses such as C3, C4 or CH50 usually reveal no abnormalities in patients with IgA-nephropathy (IgAN). However, we and others have shown that using very sensitive techniques, systemic complement activation can be demonstrated in such patients (Zwirner 1997; Smailhodzic 2011). The prognostic relevance of such systemic complement activation, however, is unknown. In addition, it has now been demonstrated that IgA in IgAN can activate the MBL-pathway in addition to the alternative pathway of complement (Roos 2006). MBL exhibits a functionally relevant polymorphism, which, for example, determines the risk for infections following liver transplantation (de Rooij 2010). Again, the role of this polymorphism in IgAN is unknown.

As part of the STOP-IgAN study (Eitner 2008), we have established an extensive clinical database, a prospective biobank and have repetitively obtained serum samples. These samples so far have never been thawed and thus allow in-depth analyses of the complement system. The study currently comprises almost 450 patients with prospective 2-3 year follow-up.

We propose to measure C3, its catabolic fragment C3d and MBL in these samples. Additionally, since it has been shown recently that IgAN is associated with a novel N-terminal mutation of H (Schmitt 2011), we will assess H and its variant as well.

References

Research Plan

We plan to first generate data in the above STOP-IgAN population. In the meantime we have already measured the C3d/C3 values of all the sera. Based on these findings we can conclude that there is complement activation in the IGAN patients.

The following is to establish the pathways that are involved meaning:

1. The measurement of the activity of the alternative, classical and lectin pathways, including the levels of MBL and Ficolins using sensitive ELISA methods that have been established earlier. In particular given hints for a genetic role of factor H, we will assess H and H variants.

2. Measurement of C3a and C5a (to be done via the Gharavi-group in New York).

3. Depending on the outcome of C3a and C5a we will decide whether to measure the levels of soluble C5b-9.

If we can identify novel markers, we then plan to validate our findings in independent populations such as the VALIGA cohort.
Rationale

Immunoglobulin A nephropathy (IgAN) is one of the most common forms of primary glomerular disease. Recent insight suggests that the composition of the gut microbiota can globally influence the immune system and disease development in nonmucosal organs (Chervonsky AV et al Nat Immunol 2010). The human gut microbiota is estimated to consist of at least $10^{14}$ bacteria and archaea, composed of approximately 1,100 prevalent species, with approximately 160 such species per individual. In its entirety, the microflora is estimated to contain 150-fold more genes than our own host genomes.

Mucosal IgA production occurs in the Peyer's patches (PPs), mesenteric lymph nodes (MLNs), and isolated lymphoid follicles (ILF). These are the major inductive sites for IgA production and the balance of various factors in the mucosal microenvironment plays an important role in regulating the synthesis of IgA. In IgAN polymeric IgA-containing immune complexes are deposited in the kidney glomeruli, triggering renal injury. In the case of IgAN, the source of the IgA has been variously speculated to involve mucosal tissues, tonsils, and bone marrow. In IgAN, the IgA itself is not generally viewed as being autoreactive per se, but rather it has a strong propensity to form macromolecular complexes that accumulate as immuno-deposits in the glomerular mesangium. Despite considerable investigation into the biochemical abnormalities of IgA1 from patients with IgAN, the origin and localization of sites secreting the aberrant IgA1, and the downstream effector mechanisms triggered by mesangial IgA1 deposition, the aetiology of IgAN remains poorly understood. More recently, signaling induced by the TNF family members B cell activation factor (BAFF) has been implicated in the pathogenesis of IgAN. In the presence of excess BAFF, as in BAFF-Tg mice, some B cell subsets expand abnormally and B cell tolerance to self-antigen is perturbed and these mice develop an IgA-driven nephritis, and the development of this condition, which is commensal microbiota dependent, involves a breakdown in the normal barrier between the mucosal and peripheral compartments. The evidence supporting a role for the enteric flora in the gut-kidney axis has been increasing as reported by recent studies (Douglas D. et al., JCI 2011).

Purpose of the study
Along structural IgA abnormalities, hyperproduction of IgA is thought to play a role in the pathogenesis of primary IgA nephropathy. The generation of IgA in the mucosal compartments is a dominant immunological process that is crucial for homeostasis between the gut commensal flora and the local immunological environment.

We will test the hypothesis that IgA production in patients with IgA nephropathy is deregulated, in part, by mean of a quali-quantitative unbalancing in salivary and fecal microbiota. Moreover, we will study the hypothesis that changes in microbiota may influence the progression of IgAN and may be different in subclasses of IgAN patients (progressor vs not progressor vs patients with segmental necrotizing lesions).

Aim of our study will be to determine if changes in salivary and fecal microbiota could promote, at least in part, the Galactose-deficient IgA1 commonly observed in the primary IgA nephropathy patients, and contributing to kidney failure progression.

This new “integrated” approach may lead to better understand the “link” between human mucosal immunity and microbiota in the pathogenesis of IgAN.

Protocols and logistics

We would like to characterize the microbiota in IgAN patients starting from VALIGA cohort to test this hypothesis. We only require a one-time samples of saliva (shipped and collected in RNALater Solution), feces (shipped and collected in RNALater Solution) and whole blood for RNA, DNA, plasma and serum.

Received samples will be used for:
- Combining functional and structural genomic and metabonomic approaches based on culture-independent (e.g., pyrosequencing, DGGE) together with GC-MS/SPME analyses, in order to determine the metabolic changes triggered by salivary and fecal microbiota in the primary IgA nephropathy patients;
- Sequencing of circulating 16S rRNA, in order to characterize either Gram+ and Gram- Bacteria composition;
- Measuring circulating levels of BAFF and April;
- Serum Gd-IgA1 levels.

We will provide all participating centers with tubes and supplies for collecting and shipping the samples. The shipment will be billed to our courier account.
5) Soluble transferrin receptor in urine, a new biomarker for IgA nephropathy and Henoch-Schönlein purpura nephritis.

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IgA nephropathy (IgAN) and Henoch-Schönlein purpura nephritis (HSPN) might represent different ends of a continuous spectrum of glomerular disease. In both conditions, upregulated soluble transferrin receptor (sTfR) might be excreted in urine, which could be a potential biomarker to monitor disease activity and therapeutic response. In this cross-sectional study, 147 Caucasian patients consulting the Nephrology Department at the Ghent University Hospital and 50 controls were included. The value of urinary sTfR as biomarker in IgAN and HSPN was evaluated. sTfR was assayed in concentrated urine using a newly developed latex-enhanced immunonephelometric assay. The assay required a preconcentration step and was characterized by a within-run and between-run coefficient of variation of respectively 3.0 and 3.1%. The method provided linear results between 0.5 and 114 µg/L with an entirely satisfactory precision and analytical recovery (95-105%) over a wide pH range (4.76-7.6). Median urinary sTfR concentration was significantly higher in primary glomerulonephritis than in healthy subjects ($P < 0.0001$). Absolute median levels of urinary sTfR were markedly higher in patients with active IgAN or HSPN [10 µg/L, 95% confidence interval (CI), 9-19 µg/L] in comparison with those with other morphological types of GN [2 µg/L, 95% CI, 0-3 µg/L] ($P < 0.0001$). A statistical significant difference in urinary sTfR concentration was observed comparing patients with active IgAN or HSPN with patients who had achieved partial or complete remission [0 µg/L, 95% CI, 0-2 µg/L] ($P < 0.0001$). Multiple regression analysis with urinary sTfR as dependent variable revealed that proteinuria was the main predictor of urinary sTfR concentration ($r^2 = 0.46$, $P < 0.001$). Determination of sTfR in urine is a new and sensitive method, which can be used as a biomarker for IgAN and HSPN.

The data obtained in a limited population of our own outpatients seem promising (although the histological definition could be refined). We are interested in extending and/or validating these data in another set of samples of histologically classified IgA patients of whom also outcomes and data on progression are known. At the same time, all other suggestions in regard of this study are of course welcome as well. Thanks in advance for the consideration.