Have the "Newomics" Approaches Provided New Insights into the Pathogenesis and Management Lupus Nephritis

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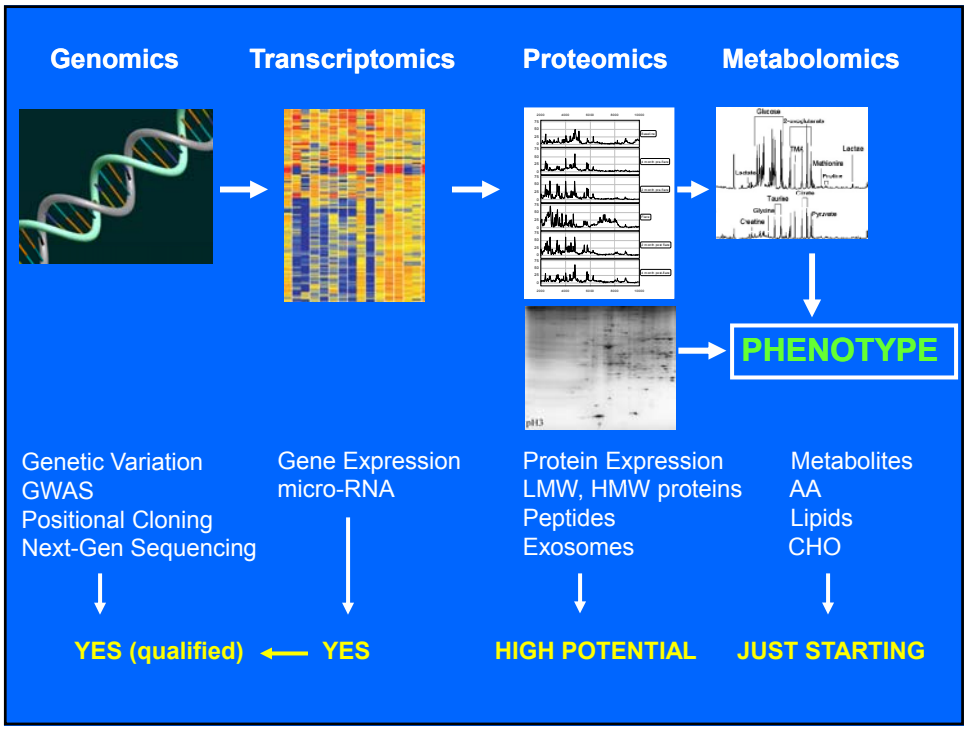
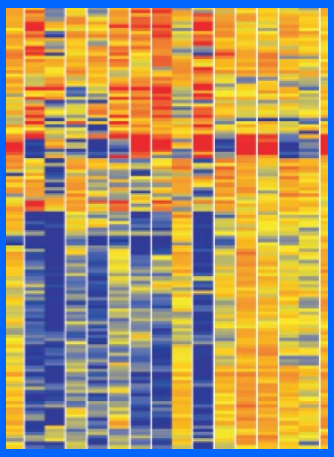


Transcriptomics

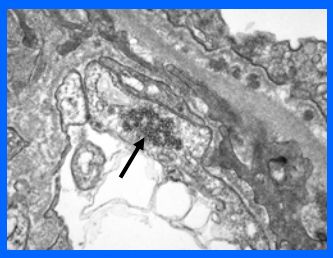


Type I IFN in SLE

Historical Considerations

•Therapeutic administration of IFNα was associated with the appearance of autoantibodies, and in some patients clinical SLE

•Tubuloreticular inclusions found in renal endothelial cells



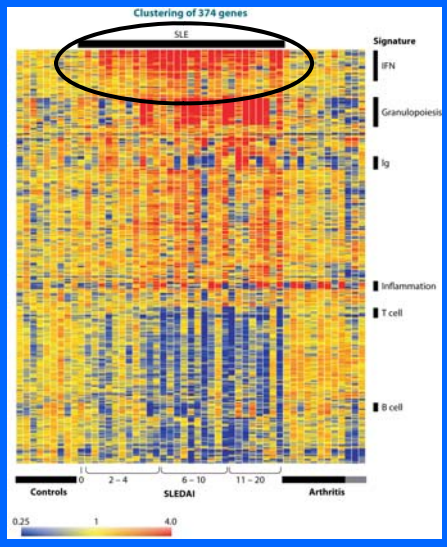
An endothelial tubuloreticular inclusion ("interferon footprint"). Often seen in kidney biopsies of lupus nephritis

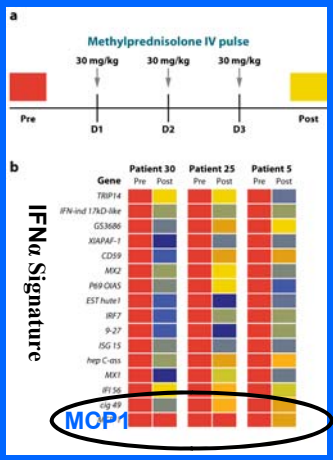
•SLE serum inhibits viral-induced cell death

•SLE serum induces monocyte→DC; this is blocked by anti-IFNα

•But…IFNα difficult to measure directly in serum

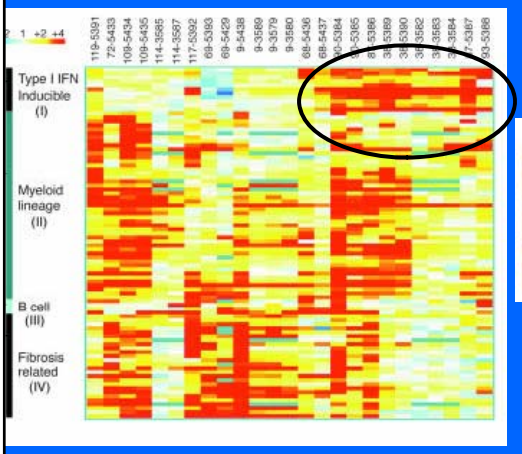
Transcriptomics of PBMCs from children with SLE and ~50% of unselected adults with SLE showed an IFNα signature. Not clear if IFNα negative SLE in adults is a distinct subset or if the IFNα pathway was inactive at sampling





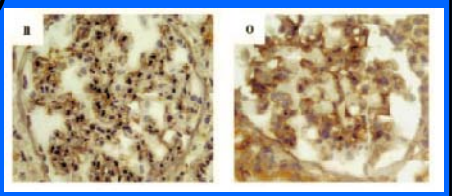
Pascual *et al;* Ann Rev Immunol, 2010

The IFNα Signature and Cells That Produce IFNα are Found in LN Kidneys



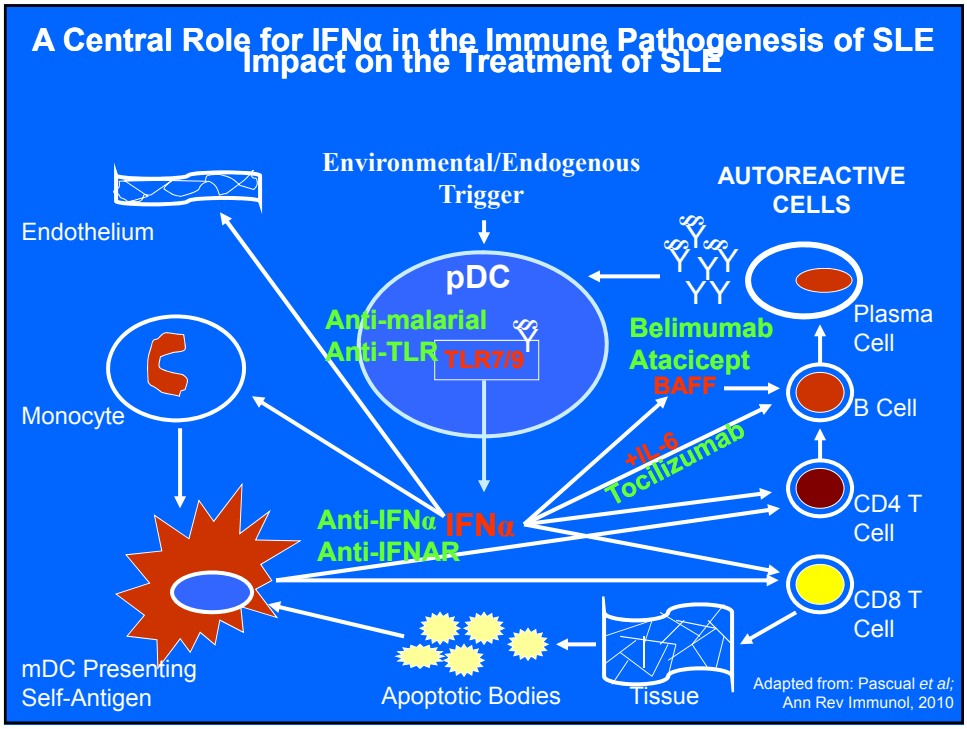
Gene expression in laser-captured SLE glomeruli

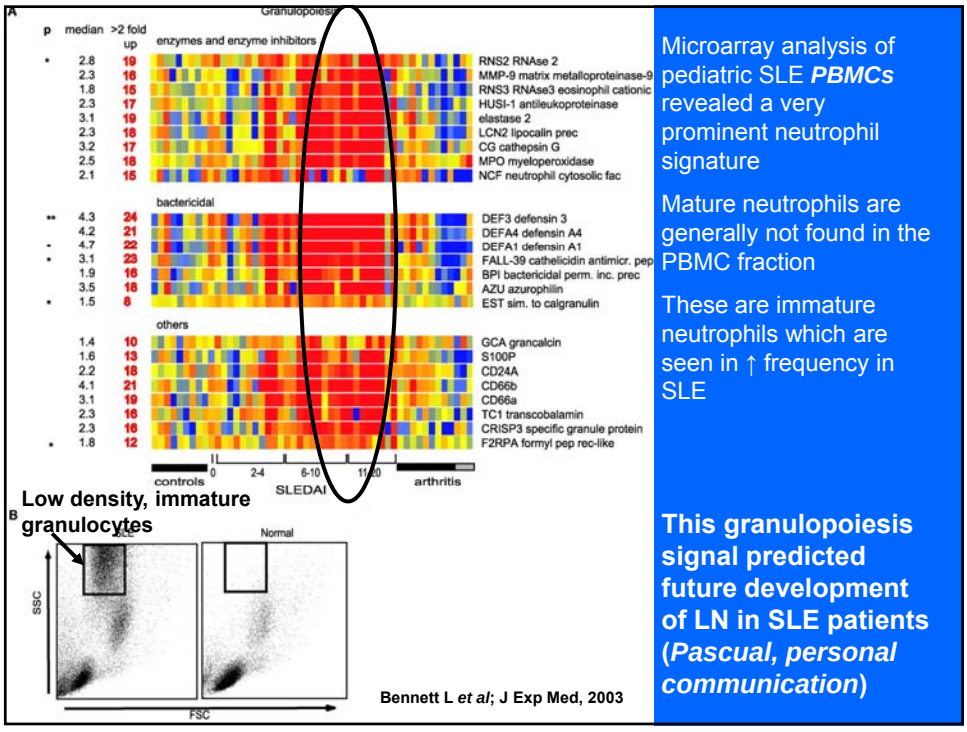
Peterson *et al*; JCI, 2004



pDC in Class IV and V SLE glomeruli

Tucci *et al*; A&R, 2008





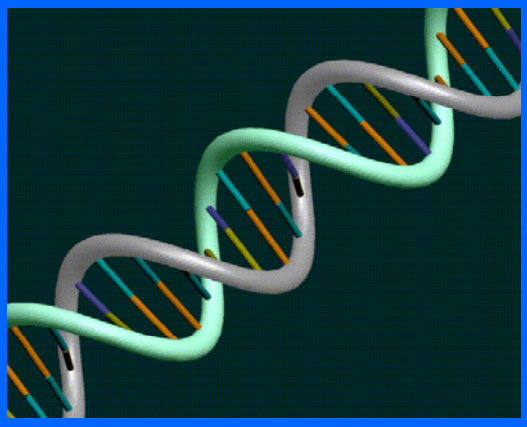
Microarray analysis of pediatric SLE ***PBMCs*** revealed a very prominent neutrophil signature

Mature neutrophils are generally not found in the PBMC fraction

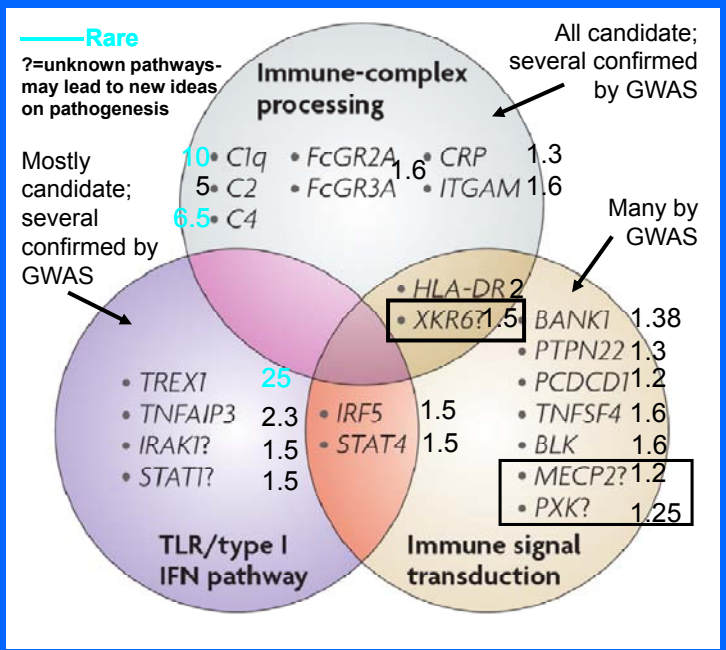
These are immature neutrophils which are seen in ↑ frequency in SLE

This granulopoiesis signal predicted future development of LN in SLE patients (*Pascual, personal communication*)

Genomics



Odds Ratios of SLE Candidate Genes

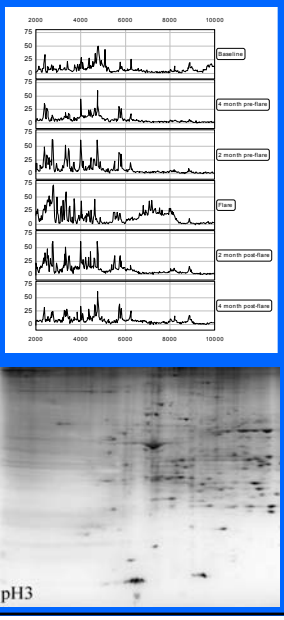


Harley et al; *Nat. Rev. Gen.*, 2009

Genomic Conclusions

- Genetic risk for SLE is derived from variations in a large number of genes with very modest effect sizes
- Many of the genes for SLE were discovered by a candidate approach based on predictions/knowledge of the biology-IFN story is a good example
- Thus far these analyses have generally confirmed our thoughts of the pathways involved in SLE pathogenesis
- Many studies were done on Caucasian populations and need to be expanded to other races/ethnicities

Proteomics



Urine Proteomics and Kidney Histology in LN

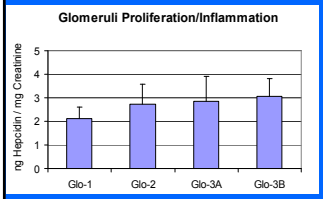
- Urine obtained at the time of diagnostic kidney biopsy for LN (n=47)
- Urine depleted of proteins >30 KDa
- Urine analyzed by SELDI-TOF MS
- Urine samples were grouped by semi-quantitatively graded pathologic findings

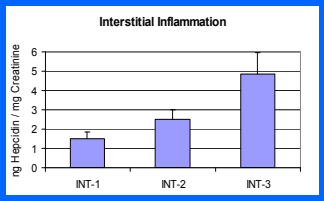
Glomerular Proliferation-Inflammation	Interstitial Inflammation	Interstitial Fibrosis/Atrophy
1=None	1=None	1=None
2=Endocapillary Prolif	2=Mild	2=Mild
3=Necrosis-Crescents	3=Mod-Severe	3=Mod-Severe

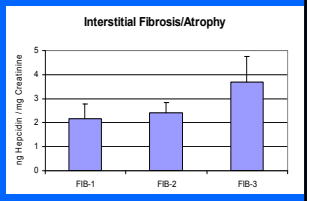
Results: 169 proteins identified, 2-20 kDa, signal:noise >15. Peptides present in ≥50% of samples were considered for statistical analysis. Differential protein expression was tested by ANOVA followed by t-test.

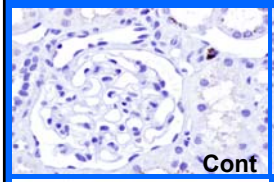
M/Z	Protein ID	
2376	ND	Glomerular Inflammation (Glom-1; Glom-2; Glom-3)
2938	Albumin Fragment	
4010	α-1 Antitrypsin precursor	
2391	A1AT	Interstitial Inflammation (Int-1; Int-2; Int-3)
2543	ND	
2754	Albumin	
4010	A1AT	
2790	Hepcidin	
2376	ND	Interstitial Fibrosis/Atrophy (Fib-1; Fib-2; Fib-3)
3388	ND	

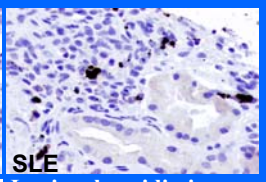
Hepcidin-An Iron Regulatory Peptide



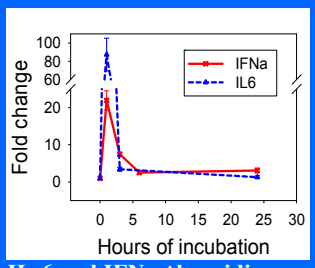








In vivo, hepcidin is expressed in interstitial leukocytes in LN

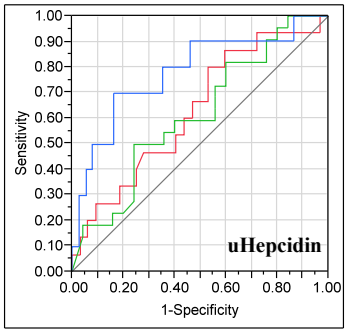


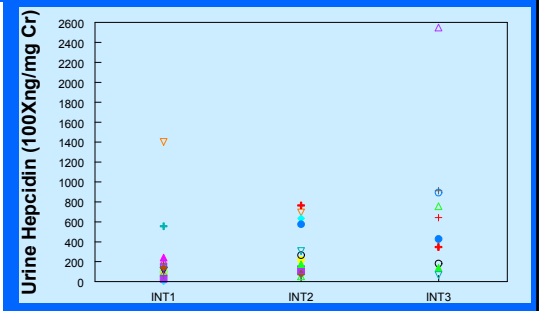
IL-6 and IFNα ↑hepcidin mRNA in human monocytes

Zhang X *et al*; Kid Int, 2008; Zhang X *et* al; BBA, 2010

Could uHepcidin Predict the Level of Interstitial Inflammation Without a Biopsy?

~~No-not well and not by itself~~



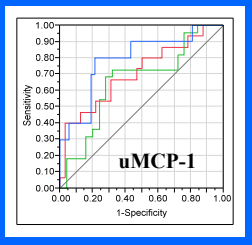


Legend	INT	AUC
Red	INT-1	0.630
Green	INT-2	0.615
Blue	INT-3	0.781

62% misclassified

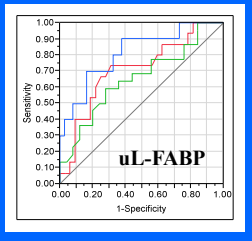
Other Individual Urine Proteins Suffered the Same Fate as uHepcidin as a Biomarker of Interstitial Inflammation

MCP-1 is a candidate biomarker, also seen as part of the INFα signature; LFABP is a candidate biomarker



Legend	INT	AUC
Red	INT-1	0.708
Green	INT-2	0.658
Blue	INT-3	0.792

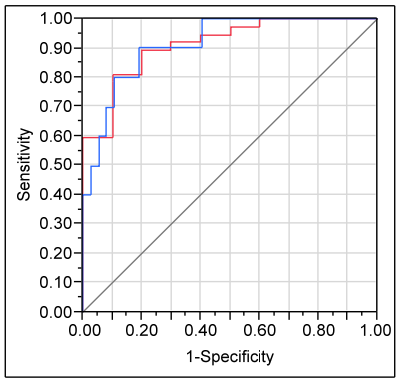
51% misclassified



Legend	INT	AUC
Red	INT-1	0.709
Green	INT-2	0.654
Blue	INT-3	0.813

47% misclassified

Combining 3 Urine Proteins Builds a Better Biomarker



Legend	INT	AUC
	1 (none+mild)	0.913
	3 (mod+severe)	0.913

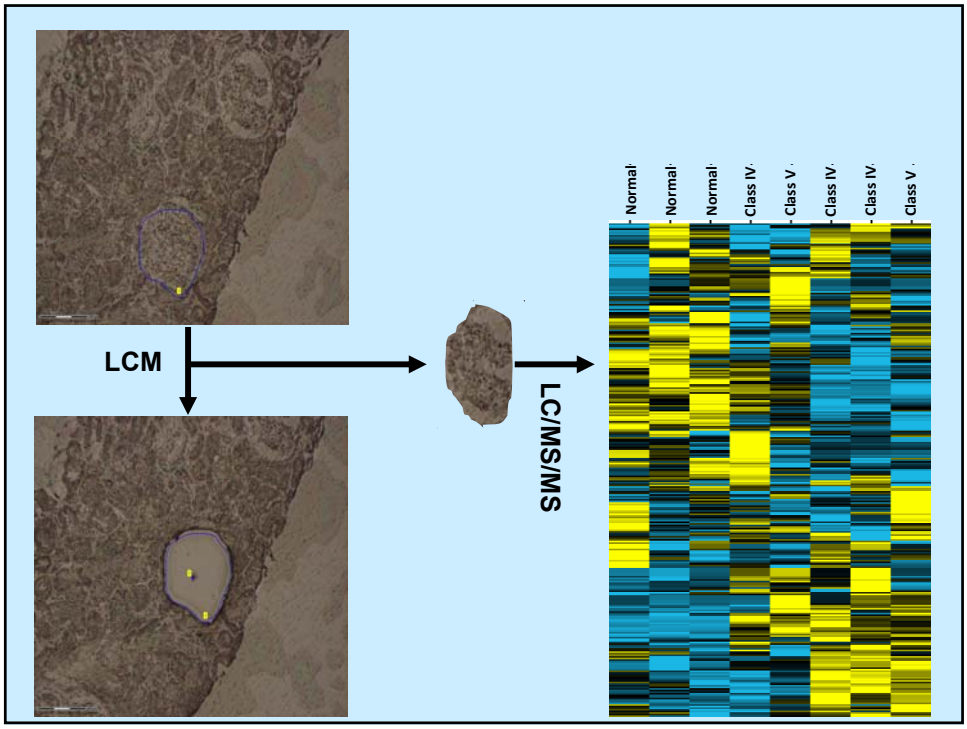
ROC curve based on a linear determinant model incorporating uHepcidin + uMCP-1 + uLFABP

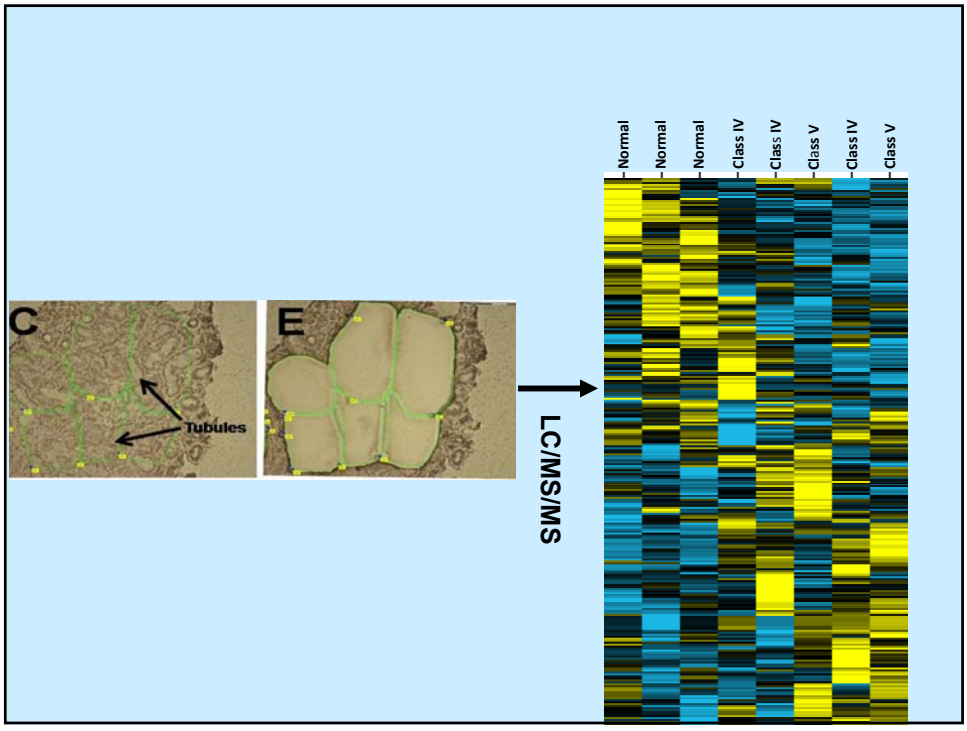
Using a cutoff of 18-

Specificity: 80%

Sensitivity: 90%

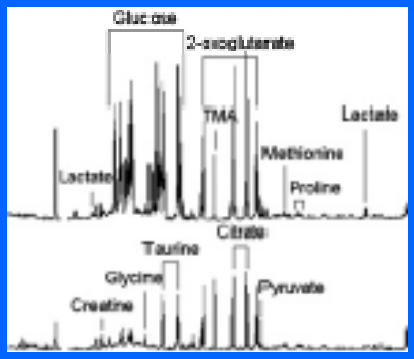
Misclassified: 16%


LCM
LC/MS/MS
Normal
Normal
Normal
Class IV
Class V
Class IV
Class IV
Class V


C
Tubules
E
LC/MS/MS
Normal
Normal
Normal
Class IV
Class IV
Class V
Class IV
Class V

Protein ID	Cont (n=3)	Class IV (n=3)	Class V (n=2)
Glomeruli-Average Spectral Counts			
C1q	0	23	4
C3	21	201	172
C5	0	27	46
Fibronectin	52	122	57
TGFβ-induced	1	3	12
IFN-induced	0	6	nd
Synaptopodin	84	29	40
Podocin	24	3	3
Tubulo-Interstitium-Average Spectral Counts			
MHC Class I	4	15	8
Tenascin0	21	13	
IFN-induced	0	34	5
Glut-S-Trans	93	23	1
Histone deacyl	8	1	0

Metabolomics



Metabolic Profiling

CONCEPT

A technology capable of detecting metabolic changes due to environmental or (patho-) physiological stimuli

APPROACH VARIATIONS

-Global Profiling-unfocused approach that examines spectral data for new biomarkers
-Focused Profiling-Pre-selection of chemical classes (e.g., amino acids, steroids, lipids) for profiling

STRENGTH

Biomarkers from metabolic profiling studies can be translated from pre-clinical (animal) investigations to the clinical setting as many endogenous metabolites (sugars, lipids, aa, steroids) are species-independent, whereas gene transcript and proteins often show inter-species variations

Berger R *et al*; Tox App Pharm, 2010

Metabolic Profiling

METHODS

I. Electrospray-MS
Advantage: no derivatization required
Disadvantage: identification of components can be very challenging

II. Gas Chromatography-MS/MS
Advantage: Large data banks allow immediate identification of many components
Disadvantage: requires that sample be derivatized

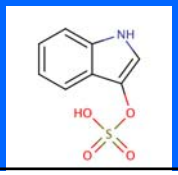
III. NMR
Advantage: directly quantitative, a traditional, long-standing method
Disadvantage: detects relatively few compounds

UTILITY

Metabolomics has been successfully applied to profiling for drug-induced nephrotoxicity

Metabolic Profiling in Diabetes

- Unbiased urine metabolomic screen in type 2 DM
- At baseline all had normal albuminuria; after 10 years cases progressed to macroalbuminuria and controls remained normoalbuminuric
- Differentially expressed metabolites were sought with LC-MS followed by identification with co-chromatography and MS
- 66 putative markers found; indoxyl sulfate was identified
- Its relative concentration was higher in cases than controls (3.1 *vs.* 1.8; p=5.6x10^{-4})
- Indoxyl sulfate is a protein-bound toxin excreted in the proximal tubule that increases glomerulosclerosis and interstitial fibrosis by up-regulating TGFβ1 expression



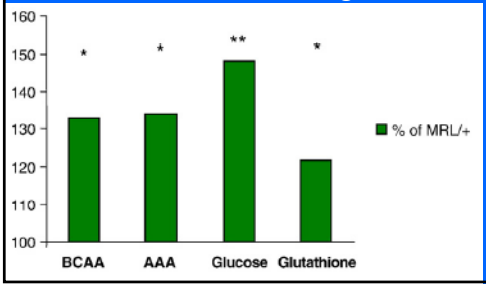
Nelson RG *et al*; F-PO1987

Metabolic Profiling in MRL/*lpr* LN

•NMR spectroscopy was done on extracts of MRL/lpr or control kidneys

•Several metabolites, particularly branched-chain and aromatic amino acids and glucose were increased in LN kidneys; this was due to increased glucose transport into the kidneys; blocking complement activation ↓ glucose transport in LN

•**Hypothesis**: C' activation → ↑ renal glucose content → ↑TGFβ, SMAD-3 → ↑ECM and glomerulosclerosis in SLE



These data, plus the diabetic nephropathy data provide proof-of-concept that metabolomics may yield relevant information in SLE

Quigg R *et al*; BBA, 2007