DYSREGULATION OF PPAR SIGNALING, FOCAL ADHESION PATHWAY AND DECREASED ILK ARE EARLY EVENTS IN COL4A3 -/- MICE MANIFESTING ALPORT SYNDROME

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OBJECTIVES

Alport syndrome (AS) is the second most common hereditary kidney disease caused by mutations in collagen IV genes. Patients present with microscopic hematuria that progressively leads to proteinuria and End Stage Renal Disease. Currently, no specific treatment exists for AS. **We aimed to identify alterations in molecular signaling, resulting from the Glomerular Basement Membrane (GBM) compositional change in AS, and in particular prior to the onset of proteinuria, that could also be amenable to therapeutic intervention.**

METHODS

We undertook comparative proteomic profiling of kidney tissues harvested from COL4A3+/+ (n=4) wild type and COL4A3-/- (n=4) mice (129/SvJ genetic background) sacrificed at 4 weeks using LC-MS/MS proteomic techniques. Following protein identification and quantitation, pathway analysis was performed using the WEB-based Gene Set Analysis Toolkit. Immunohistochemistry and immunofluorescence staining for Collagen IV and electron microscopy were used to confirm the results of histological and ultrastractural changes compatible with AS phenotype. Hematuria was defined as >5RBCs/HPGx20 magnification. Immunohistochemistry, Immunofluorescence and western-blot were used to confirm Integrin-Linked Kinase (ILK) downregulation in AS kidneys.

RESULTS

Among the significantly dysregulated pathways we focused on two pathways fulfilling our initial hypothesis:

(1) The Peroxisome-proliferator-activated receptors signaling pathway (PPAR) as this was also shown to be downregulated from other researches[1] and is amenable to therapeutic intervention (i.e. PPAR agonists). Associated pathways were also dysregulated (Table 1).

(2) The Focal Adhesion Pathway dysregulation as it reflects the response to the abnormal GBM composition in AS. ILK, an important signal transduction kinase at adhesion sites, showed decreased staining on immunofluorescence and immunohistochemistry in glomeruli from Col4a3-/- compared to Col4a3+/+ mice (Figures 1&2). Western blot also confirmed ILK reduction in AS mice (Figure 3).

TABLE 1: Significantly enriched KEGG pathways

Pathway name	# genes	Statistics*
Metabolic pathways	81	C=1175;O=81;E=14.65;R=5.53;rawP=3.77e37;adjP=4.00e-35
Oxidative phosphorylation	20	C=143;O=20;E=1.78;R=11.22;rawP=1.57e15;adjP=4.16e-14
Peroxisome	12	C=80;O=12;E=1.00;R=12.03;rawP=3.30e10;adjP=5.83e-09
PPAR signaling pathway	9	C=80;O=9;E=1.00;R=9.02;rawP=7.00e07;adjP=5.30e-06
Renin-angiotensin system	5	C=19;O=5;E=0.24;R=21.11;rawP=2.95e06;adjP=1.95e-05
Regulation of actin cytoskeleton	12	C=215;O=12;E=2.68;R=4.48;rawP=1.82e05;adjP=0.0001
Focal adhesion	10	C=199;O=10;E=2.48;R=4.03;rawP=0.0002;adjP=0.0008

*C: the number of reference genes in the category, O: the number of genes in the gene set and also in the category, E: the expected number in the category, R: ratio of enrichment, rawP: p value from hypergeometric test, adjP: p value adjusted by the multiple test adjustment

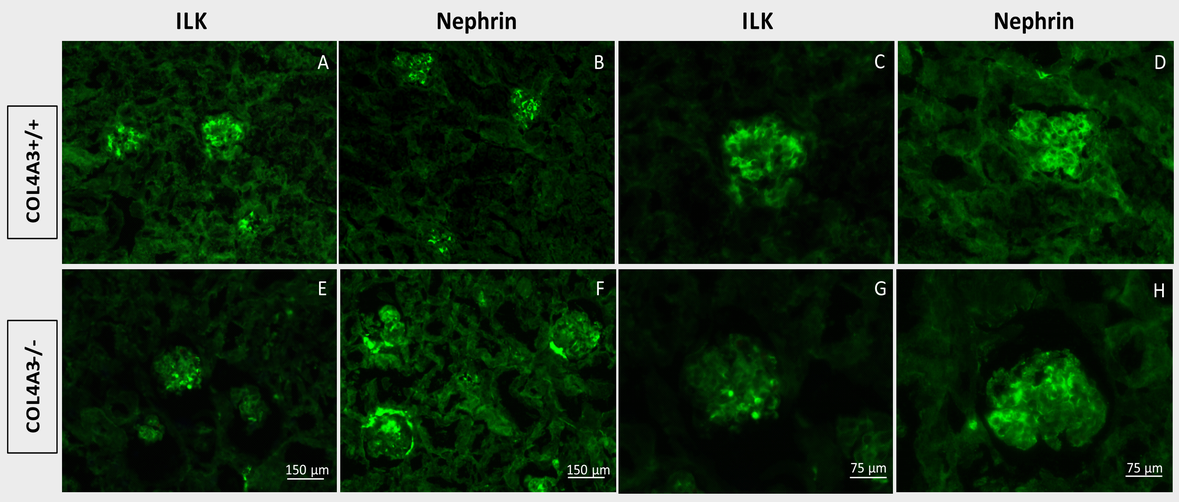

FIGURE 1: ILK expression levels in wildtype and Alport mice.
Immunofluorescence of frozen kidney tissues from COL4A3+/+ wild type and COL4A3-/- mice using antibodies against ILK protein (A-E; 20x and C-G; 40x). Nephrin was used as positive control (B-F; 20x and D-H; 40x). No differences in the expression levels of Nephrin were observed, while ILK expression levels were found to be decreased in the glomeruli of COL4A3-/- when compared to COL4A3+/+ wild type mice.

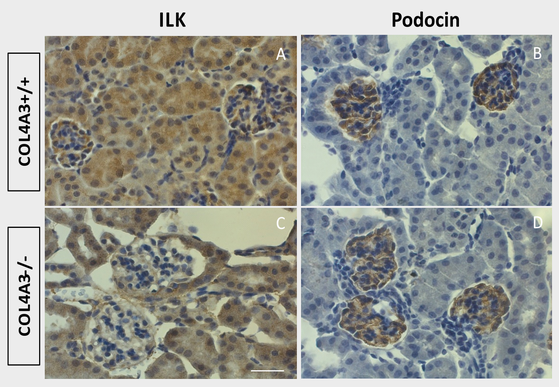

FIGURE 2: ILK expression levels in wildtype and Alport mice.
Immunohistochemistry of formalin-fixed paraffin-embedded kidneys tissues from COL4A3+/+ wild type and COL4A3-/- mice using antibodies against ILK protein (A-C). Podocin was used as positive control (B-D). No differences in the expression levels of Podocin were observed, while ILK expression levels were found to be decreased in the glomeruli of COL4A3-/- when compared to COL4A3+/+ wild type mice. Scale bar=75 µm.

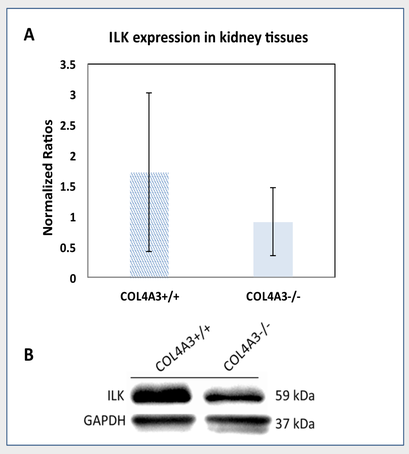

FIGURE 3: ILK expression levels in wildtype and Alport mice.
Immunoblot analysis of frozen kidney tissues from COL4A3+/+ wild type and COL4A3-/- mice using antibodies against ILK protein. Values were normalized using GAPDH housekeeping gene (A). Representatives bands of ILK and GAPDH expression (B). ILK expression levels were found to be decreased in COL4A3-/- when compared to COL4A3+/+ wild type mice.

CONCLUSIONS

These preliminary results identified two pathways that are significantly altered early in disease progression in Col4a3 -/- mice, particularly before proteinuria ensues. ILK downregulation was also an early event in these mice. Further studies are needed to determine whether targeting these pathways could delay disease progression in AS.

REFERENCES

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