The Tetraspanin CD151's Role in the Kidney and Mapping of Genetic Modifiers of Glomerular Disease

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Declaration

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Abbreviations

Abbreviations	In Full
ACE	angiotensin converting enzyme
ACR	albumin to creatinine ratio
ANOVA	Analysis of Variance
BMP	bone morphogenic protein
bp	base pairs
C57Bl/6	C57 black 6 (mouse strain)
Chr	Chromosome
Cm	centimorgans
Ct	cycle threshold
DAPI	4',6-diamidino-2-phenylindole
DNA	deoxyribonucleic acid
DOCA	deoxycorticosterone acetate
eGFR	estimated glomerular filtration rate
ERK	extracellular signal related kinase
F1	First filial generation
FAK	focal adhesion kinase
FDR	false discovery rate
FFPE	formalin-fixed paraffin-embedded
FVB/N	Friend-leukaemia-Virus-B-strain-sensitive (mouse strain)
GBM	glomerular basement membrane
GDNF	Glial derived neurotrophic factor
GSEA	Gene Set Enrichment Analysis
HBSS	Hank’s Buffered Salt Solution
HIV	Human Immunodeficiency Virus
HCl	Hydrochloric acid
ILK	integrin linked kinase
IPA	Ingenuity Pathways Analysis
KEGG	Kyoto Encyclopedia of Genes and Genomes
K-M	Kaplan-Meier
LOD	Logarithm of odds
LSD	least significant difference
MAPK	mitogen activated protein kinase
Mb	megabases
miRNA	microRNA
MMPs	matrix metalloproteinases
MQM	multiple-QTL mapping
mRNA	messenger RNA
N2	second backcrossed generation

Abbreviations	In Full
NFAT-c	nuclear factor of activated T-cells
PAS	periodic acid-Schiff
PBS	phosphate buffered saline
PBT	phosphate buffered saline with Tween 20
PCR	Polymerase-chain reaction
QTL	quantitative trait loci
RMSD	root mean squares deviation
RNA	Ribonucleic acid
ROP	Renal lesions in sclerosis-prone (mouse strain)
rpm	revolutions per minute
SAM	Significance Analysis for Microarrays
SDS	Sodium dodecyl sulfate
snoRNA	small nucleolar RNA
SNP	single nucleotide polymorphisms
TCR	T-cell receptor
VEGF	vascular endothelial growth factor

Abstract

Glomerular diseases represent a major burden for both patients and the community. They are responsible for a significant proportion of chronic kidney disease, which can ultimately progress to end stage renal disease, requiring dialysis or transplantation. Glomerular diseases are associated with leakage of proteins across the glomerular filtration barrier into the primary urine (proteinuria or albuminuria – as albumin is the major protein involved). The glomerular filtration barrier is composed of three interconnected layers: podocyte foot processes, the glomerular basement membrane (GBM) and a fenestrated endothelium.

The tetraspanin protein CD151 is a crucial component of the glomerular filtration barrier, where it is known to complex with integrins to strengthen podocyte foot process anchorage to the GBM. In addition, previous findings in our laboratory have shown that in FVB/N *Cd151*$^{-/-}$ mice the disruption and abnormal development of the GBM precedes podocyte foot process abnormalities, suggesting that CD151 also plays a role in the maturation and remodelling of the GBM. In other settings CD151 has been shown to regulate the proteolytic activity of matrix metalloproteinases (MMPs), important players in the homeostasis of basement membranes, and thus CD151 has many potential roles in glomerular homeostasis and disease.

Similar to human mutation, *Cd151* knockout in the FVB/N mouse strain leads to severe early-onset glomerular disease associated with GBM abnormalities, whereas knockout in the C57Bl/6 mouse strain does not lead to glomerular disease with kidneys presenting healthy. This strong influence of genetic background suggests the action of modifier genes, which may have important roles in human kidney disease where the course of glomerular diseases can vary significantly between patients. It is therefore important to identify the genes

modulating progression as they could be used as biomarkers to predict the course of these heterogeneous diseases in patients or as potential therapeutic targets.

In order to understand the molecular mechanisms contributing to progression and onset of glomerular disease in FVB/N *Cd151*$^{-/-}$ mice, whole genome mRNA expression profiles of glomeruli from *Cd151*$^{+/+}$ and *Cd151*$^{-/-}$ mice were investigated on both the C57Bl/6 and FVB/N backgrounds. Analysis was conducted at 3 weeks of age, at this stage in FVB/N *Cd151*$^{-/-}$ mice changes to the GBM are evident but secondary changes such as glomerulosclerosis are not yet significant or widespread, and therefore allows the identification of genes relevant to early stage disease.

The FVB/N *Cd151*$^{-/-}$ mouse glomeruli showed 24 highly significant transcript changes compared to FVB/N *Cd151*$^{+/+}$, including changes in transcription factors, inflammatory factors and extracellular matrix regulators. Many of these changes did not occur in the corresponding comparison in the C57Bl/6 strain (*Cd151*$^{-/-}$ versus *Cd151*$^{+/+}$) and therefore are likely specific to glomerular disease development. Following on from identified changes in transcript expression of MMPs, it was found that the proteolytic activities of pro-MMP-9, MMP-9 and MMP-2 were reduced in FVB/N *Cd151*$^{-/-}$ glomeruli compared to FVB/N *Cd151*$^{+/+}$, C57Bl/6 *Cd151*$^{+/+}$ and C57Bl/6 *Cd151*$^{-/-}$ mice. Furthermore the protein expression of MMP-10 was upregulated specifically in FVB/N *Cd151*$^{-/-}$ glomeruli. Therefore the GBM defects observed in FVB/N *Cd151*$^{-/-}$ mice may be due to reduced turnover of basement membrane proteins by MMPs in FVB/N *Cd151*$^{-/-}$ mice.

Eleven pathways were enriched specifically in FVB/N *Cd151*$^{-/-}$ mice, including robust changes in two cellular signalling gene networks: the T-cell receptor signalling network and the axon guidance network. Firstly, this suggests that the development of glomerular disease in this mouse model may have immune involvement. Secondly this finding supports recent

parallels that have been drawn between the signalling molecules involved in elongation and adhesion signalling of podocyte processes and axonal dendrites. Overall the loss of CD151 significantly affects the expression of molecules likely to influence inflammatory signalling in the glomerulus, and cytoskeletal organisation within podocyte foot processes.

Mindin, an inflammatory mediator, was significantly and specifically induced in the GBM of FVB/N *Cd151*$^{-/-}$ mice, as detected by immunofluorescence and was also observed in the urine of these mice by immunoblotting. The functions of mindin have not been investigated in the kidney; however, as it is known to be pro-inflammatory, the potential for mindin to be pathologically contributing to disease progression was investigated. Inflammatory infiltrates including lymphocytes, neutrophils, macrophages and eosinophils, were observed as early as 3 weeks of age in FVB/N *Cd151*$^{-/-}$ but not in FVB/N *Cd151*$^{+/+}$, C57Bl/6 *Cd151*$^{+/+}$ and C57Bl/6 *Cd151*$^{-/-}$ mice. This infiltration was progressive and more pronounced in 12 week old FVB/N *Cd151*$^{-/-}$ mice, which had developed extensive inflammatory lesions. It can be speculated therefore that mindin is involved in recruiting inflammatory cells into kidneys of FVB/N *Cd151*$^{-/-}$ mice early in disease, which may then contribute to disease progression.

As FVB/N *Cd151*$^{-/-}$ mice show severe early onset glomerular disease compared to C57Bl/6 *Cd151*$^{-/-}$ mice, which show a healthy kidney phenotype, this model lends itself to the identification of quantitative trait loci (QTL) influencing glomerular disease development. Therefore, a backcross of the resistant line (C57Bl/6) for two generations onto the permissive line (FVB/N) was carried out. F1 *Cd151*$^{-/-}$ mice (FVB/N × C57Bl/6) show complete absence of glomerular disease, suggesting that the protective alleles from the C57Bl/6 background are dominant. N2 (F1 x FVB/N) *Cd151*$^{-/-}$ mice were found to have a highly variable kidney phenotype, with 68% developing albuminuria.

Analysis of age at onset of albuminuria in N2 mice showed that there were three statistically distinct groups: no onset (followed up to 12 months of age), early onset (<2 months of age) and late onset (3-4 months of age). Linkage analysis identified 2 regions that account for >50% of the variability in inheritance of the trait. Specifically inheritance of an FVB/N homozygous genotype at a chromosome 14 QTL (45.34cM - 46.34cM) was strongly associated with early onset albuminuria. This region includes only 1 gene, protocadherin 9, which is known to be expressed in human foetal kidney tissue, suggesting a role in kidney development. Taken together, the data suggests that protocadherin 9 represents a modifier gene influencing glomerular phenotype in the absence of CD151. A second linked region on chromosome 1 (40.859cM-44.046cM) also strongly influenced the development of glomerular disease, with susceptibility associating with heterozygosity. Within this region 22 genes, including GBM proteins collagen IV chains α3 and α4 are located. The genetic modifiers responsible for influencing glomerular phenotype in the absence of CD151 in the two QTL regions remain undefined; however, protocadherin 9 and the collagen IV chains α3 and α4 represent likely candidate modifier genes, and require further investigation.

To further assess the relationship between genetic background and disease severity and progression, a multiple-trait analysis was performed using albuminuria, GBM defects, creatinine clearance and serum urea as quantitative traits. Using this approach a further 13 QTLs were identified which relate to *Cd151*$^{-/-}$ glomerular disease progression and severity. Several of the mapped QTLs demonstrated concordance with previously identified QTLs for rodent models of glomerular disease, and concordance with a human locus influencing diabetic nephropathy.

In conclusion this is the first reported comprehensive analysis of gene expression changes as well as QTLs in the *Cd151*$^{-/-}$ model of glomerular disease. The study has identified a number

of genes and proteins that likely contribute to disease onset or progression which now require further investigation to determine their function in human kidney diseases.

Published acknowledgements and conference abstracts arising from this work

Acknowledged for providing data

Renal disease as a potential compounding factor in carcinogenesis experiments with Cd151-null mice

LK Ashman, Oncogene, March 2013

Oral Presentation at the 3rd Inflammatory and Immunological Biomarkers Conference in San Diego, Mar 2014

Induction of mindin is associated with inflammation in the kidney of *Cd151*$^{-/-}$ mice

Crystal Passfield, Judith Weidenhofer, Danielle Bond, Kyra Minahan, Simon Keely, Leonie K. Ashman, and Séverine Roselli

Oral presentation at the Early Career Research Group Conference in Newcastle, Nov 2012

Glomerular disease development in CD151 knockout mice is dependent on the inheritance of modifier genes

Crystal Naudin, Leonie Ashman, Séverine Roselli, Judith Weidenhofer

Oral presentation at the 5th European Conference on Tetraspanins in Nijmegen Sept 2012

Glomerular disease development in mice lacking the tetraspanin CD151 is dependent on the inheritance of modifier genes

Crystal Naudin, Leonie Ashman, Séverine Roselli, Judith Weidenhofer

Oral Presentation at Australia and New Zealand Society of Nephrology (ANZSN) 47th Annual Scientific Meeting in Adelaide, Sept 2011

Induction of mindin expression is associated with GBM damage in *Cd151*$^{-/-}$ mice

Crystal Naudin, Judith Weidenhofer, Rodney Scott, Leonie Ashman, Séverine Roselli

Poster presentation at Australian Society for Medical Research (ASMR) NSW scientific meeting in Sydney, Jun 2012

Glomerular disease development in mice lacking the tetraspanin CD151 is dependent on the inheritance of modifier genes

Crystal Naudin, Leonie Ashman, Judith Weidenhofer, Séverine Roselli

Poster presentation at Australian Society for Medical Research (ASMR) NSW scientific meeting, in Sydney, Jun 2011

Damage of the GBM is associated with induction of mindin expression in *Cd151*$^{-/-}$ mice

Crystal Naudin, Judith Weidenhofer, Rodney Scott, Leonie Ashman, Séverine Roselli