CHINA SCHOLARSHIP COUNCIL

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Title of the research project :

Role of the cannabinoid receptor 1 (CB1) in non metabolic chronic kidney disease

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Research Unit

Name : Common and rare renal diseases

UMR_S1155

Doctorate School

Thesis supervisor's doctorate school (candidate's futur doctoral school) ED 394 Sorbonne Université, Ecole Doctorale Physiologie, Physiopathologie et Thérapeutique

PhD student currently supervised by the thesis supervisor (number, year of the first inscripBon) :
Myriam DAO, 3rd year (thesis defense scheduled december 12 2021)

Joint supervisor : N/A

Joint supervisor's doctorate school : Or, if non SU :

PhD student currently supervised by the joint supervisor (number, year of the first inscripBon) :

SORBONNE UNIVERSITÉ

1. Description of the research project: context, positioning and objective(s)

Renal diseases remain a burden for Public Health and chronic kidney disease (CKD) affects several millions of individuals worldwide. Independently of the cause (of immune, toxic or metabolic origin), CKD corresponds to the replacement of renal functional tissue by extra-cellular matrix proteins, i.e fibrosis, which ultimately impairs renal function. To date, despite considerable efforts and research, few therapeutic options remain available for clinicians, except for the classical optimal blockade of the renin-angiotensin system (RAS)[1] that barely slows the course of CKD. The cannabinoid system and its receptors cannabinoid 1 (CB1) and 2 (CB2), have recently emerged as potential targets in renal disease [2,3], CB1 being pro-fibrotic and CB2 anti-fibrotic in an opposite way. Initially discovered in the central nervous system, CB1 and CB2 receptors are best known for their psychoactive effects and are the receptors for both endogenous and exogenous cannabinoids [4]. CB1 and CB2 are also involved in the regulation of appetite and metabolism and are promising targets in diabetes and obesity-induced metabolic syndrome [5-6]. CB1 inhibition promotes a reduction in albuminuria, renal fibrosis and preserves renal function in obesity-induced nephropathy in mice [7] and in diabetes [8,9] through the improvement of metabolic parameters but also through a direct action of CB1 in podocytes [9] and/or proximal tubules [8] in diabetes and in proximal tubules in obesity-induced nephropathy [7]. In non-metabolic renal disease, we recently found that the Cnr1 gene, encoding for CB1, was among the 10 most up-regulated genes in an experimental model of renal fibrosis (Unilateral Ureteral Obstruction or UUO) [10] and that CB1 inhibition (genetic or pharmacological) profoundly reduces renal fibrosis [10], mainly through a direct action on renal interstitial myofibroblasts. However, there is still to date no proof that CB1 blockade can efficiently impairs fibrogenesis, protect kidney function and significantly slow the progression of non-metabolic CKD. In addition the cellular and molecular pathways involved in CKD, given that we found that most resident renal cells express CB1 (podocytes, tubules and myofibroblasts) during renal injury [7].

Our hypothesis is that CB1 receptor is inducing renal fibrosis regardless of the initial injury model, and that its inhibition will protect kidneys against the progression of CKD. Our ultimate goal will be to propose CB1 blockade as one of the most promising targets to treat renal fibrosis and CKD. Thus, our objectives are:

1) to demonstrate that CB1 inhibition reduces renal fibrosis and CKD in a model of non-metabolic renal fibrosis: the severe ischemia-reperfusion injury model which is a model of initial vascular and tubular injury with chronic development of renal fibrosis and renal failure (IR-CKD) in mice.

2) to check whether CB1 expression has the potential of being a marker of renal fibrosis in humans in metabolic and non-metabolic nephropathies.

1) Demonstrate that CB1 inhibition reduces renal fibrosis and improves renal function in a model of non-metabolic renal fibrosis in mice: the severe ischemia-reperfusion injury model which is a model of initial vascular and tubular injury with chronic development of renal fibrosis and renal failure (IR-CKD).

Preliminary results and scientific context: the PhD's supervisor has recently published that CB1 pharmacological blockade and genetic disruption reduces renal fibrosis by 30 to 50% in the UUO model [10]. However, although useful as "proof of concept" that CB1 is a key player in non-metabolic renal fibrosis, the UUO model does not allow renal function monitoring. Although CB1 expression is induced in tubules during UUO, it is the CB1 expressed in myofibroblasts that plays a key role in the UUO fibrotic process. However, we recently found that renal tubular cells seem to play a major role in the development of interstitial fibrosis in Chronic allograft nephropathy in humans [11]. Thus, to study this complex interaction between injured tubuli, myofibroblast activation and long-term development of tubulointerstitial fibrosis, we will use **the model of severe Ischemia Reperfusion injury with delayed unilateral nephrectomy allowing the development of renal fibrosis and CKD (IR-CKD).** Renal CB1 expression does not significantly increase early on after but does in the late phase after IR (4 weeks, n=7, $p<0,05$, data not shown) when renal fibrosis is detectable. Similarly, whereas renal function and lesions are not modified by CB1 genetic disruption 24h after IR (n=7 WT and *Cnr1*$^{-/-}$, p=ns, data not shown), *Cnr1*$^{-/-}$ mice displayed preserved renal function and less fibrosis 6 weeks after IR (***Figure 1, Dao et al, in preparation, current PhD student***).

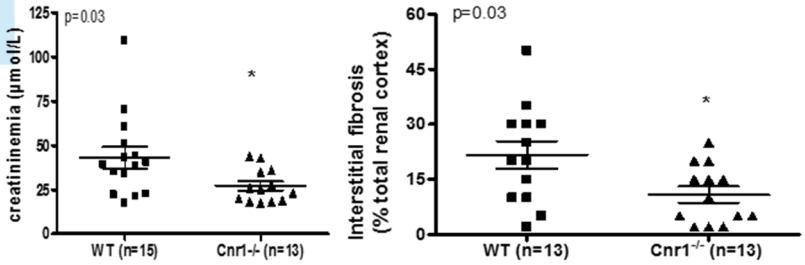


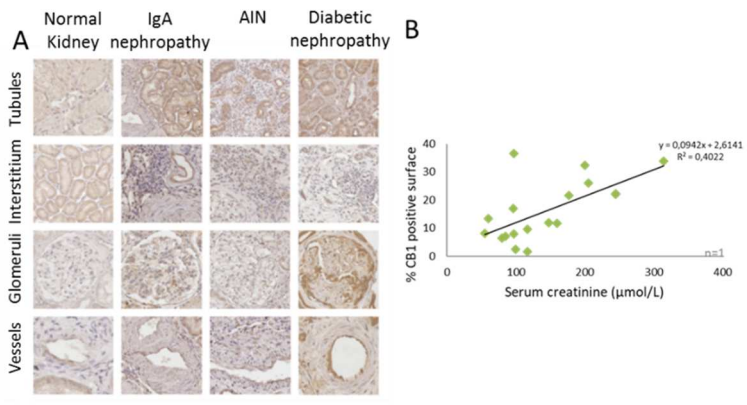
Figure 1: Mice lacking CB1 receptor are protected against long-term decline of renal function in the IR-CKD model. *Serum creatinine is significantly lower in Cnr1$^{-/-}$ mice 6 weeks after 30 min IR (p=0,03) n=15 WT versus n=13 Cnr1$^{-/-}$ mice) and renal fibrosis is significantly decreased (p=0,03) n=15 WT versus n=13 Cnr1$^{-/-}$ mice, interstitial fibrosis quantified seli-quantitatively using the Masson's trichrome staining and expressed as a % of interstitial fibrotic area compared to the total cortical surface, double independent quantification).*

Furthermore, renal function is preserved in the same model after specific CB1 peripheral blockade, ($p<0,05$, n=5 mice treated with vehicle compared with n=5 mice treated with JD5037, ChemExpress).

Methods and contingency plan: we will perform the IR-CKD model both during genetic disruption of the CB1 receptor (*Cnr1$^{-/-}$*) and during specific CB1 peripheral blockade (JD5037, ChemExpress). We will next use tissue specific CB1 knockout animals to study the relative contribution of tubules and myofibroblasts in those models. We will use CB1-floxed mice (provided by Dr J Egan from the NIA/NIH) intercrossed with Pax8-LC1-Cre mice locally bred. This inducible model allows time-specific deletion of CB1 in all tubular compartments. We will also generate P0-cre mice (locally bred, previously published [12] that will be intercrossed with our CB1-floxed mice to allow a specific myofibroblast deletion of CB1. These intercrosses will decipher what cell type plays a prominent role in non-metabolic CB1 dependent fibrosis. In addition, we will also explore the specific molecular signaling pathways involved in CB1 dependent collagen synthesis by **renal myofibroblasts *in vitro***. Cell proliferation, migration and energy balance will be studied using live microscopy imaging as recently used in the PhD's lab in both primary renal myofibroblasts from WT and *Cnr1$^{-/-}$* mice [13]. Additionally, we will study the downstream pathways involved in CB1 dependent-collagen secretion upon hypoxia or/and TGFβ stimulation such as PKC and PKA pathways since preliminary *in vitro* data indicate that the CB1/Arrestin pathway is involved in collagen synthesis by myofibroblasts. We will also explore other putative signaling using unbiased approaches to discover potential new and specific targets. As such, we will perform a RNASeq study in *Cnr1$^{-/-}$* and WT murine primary myofibroblasts after TGFβ stimulation. This approach will allow the selection of putative transcriptional factors downstream of CB1 and involved in TGFβ-induced collagen synthesis. We will also confirm the most promising new potential targets in human primary myofibroblasts using kidneys declared unsuited for human transplantation from our center in Tenon (approved protocol by the Agence Nationale de la Biomédecine PFS14-019, H. François as the PI) as there is no commercial source.

2) Demonstrate that CB1 expression is an early marker of metabolic and non-metabolic CKD

Figure 2: Cannabinoid receptor-1 (CB1) expression is increased in multiple human nephropathies.
(a) Immunostaining for the CB1



receptor in control kidneys, IgA nephropathy, acute interstitial nephritis (AIN), or diabetic nephropathy. (b) CB1 expression (immunohistochemistry) correlates with renal function assessed by serum creatinine.

Preliminary results and scientific context: we have previously shown that CB1 expression increases in IgA and in diabetic nephropathies and that its expression correlates with renal function in humans (Fig2, [10]) We have also recently published that CB1 expression increases very early-on in kidney grafts, especially in ischemic tubules and that CB1

expression correlates with interstitial fibrosis 3 months after kidney transplantation [11]. Our aim is to confirm that CB1 can be a marker of CKD. In addition, we will specify at what stage and in which nephropathies CB1 expression is the strongest i.e which patients would benefit the most of CB1 inhibitors to treat CKD.

Methods and contingency plan:

To this end, using biopsies from the nephrology transplant division of Tenon hospital. We will first perform a preliminary study of CB1 expression in 30 biopsies from diabetes (10 with CKD Stage IIIa, 10 with CKD stage IIIb, and 10 with CKD stage IV), 30 biopsies from IgA nephropathies (10 with CKD Stage IIIa, 10 with CKD stage IIIb, and 10 with CKD stage IV) and 10 normal kidneys. We will next confirm our finding in larger cohorts.Apart from clinical parameter, we will also quantify renal fibrosis using Sirius Red and our previously described morphometry software[14] and will correlate CB1 expression with renal fibrosis in various stages of CKD.

The team 4 of UMR INSERM S1155 to which the PhD supervisor's belongs has international notoriety in renal disease and has been evaluated at the top 5% of French INSERM labs in 2018 and include many experts in the field of acute, chronic and genetic kidney disease as well as specialists in tubule physiology. Hélène François is a MD-PhD nephrologist with both a clinical and scientific background. She works part time in the nephrology unit of Tenon hospital and Sorbonne university as well as in the INSERM UMR_S 1155 unit and has supervised more than 10 master degrees and lab students and 2 PhD students who published as first author original research and review articles in the last 10 years.

The PhD project will thus combine: (1) endocannabinoids in non-metabolic kidney diseases (2) well established techniques and technologies in the PhD's lab that are required to fulfil the objectives. We expect the PhD student to have a background in renal physiology and to be highly motivated by both in vivo and in vitro bench work, especially cellular biology and histology. The PhD will have access to animal facilities and gold-standard technical platforms including but not limited to a renal hemodynamic surgery unit, intra vital microscopy, live microscopy and anatomo-pathology platform. Trained personnel in charge of these platforms will participate to the PhD's training and help fulfil the PhD's scientific objectives, as well as all the team 4's members.

Overall, this translational project will allow the PhD student to decipher an important health care issue, namely CKD treatment. He/She will acquire very valuable skills when going back to China to help deciphering potential new treatment in CKD.

2. References related to the project

(the actual and former PhD students appear in **bold characters,** the PhD's supervisor publications as first/last/corresponding author are highlighted in yellow)

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