Genetic risk score and its utility in diabetic nephropathy management

Paul Hugues Guillaume Simon

Division of Experimental Medicine, McGill University, Montreal

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English Abstract:

Introduction: Diabetic nephropathy is the leading cause of chronic kidney disease and end stage renal disease in most industrialized countries and increasingly so in countries with transition economies. While major successes in the treatment of diabetic nephropathy have been obtained, a proportion of type 2 diabetic patients continues to progress along the path of renal disease despite receiving evidence-based recommended medications for the management of their diabetic nephropathy. This phenomenon is characteristic of renal non-response and is associated with unmet renal needs. We hypothesize that patients presenting evidence of renal non-response can be genetically distinguished from patients presenting evidence of renal response and that these genetic traits may differ between European Caucasian patients of different ethnic backgrounds. ***Design and method:*** the ADVANCE study is a 2x2 factorial randomized trial assessing the effect of blood pressure and glycemic control in 11,140 patients with type 2 diabetes (T2D) over a period of five years. 4,098 Caucasian participants from ADVANCE were genotyped in addition to having their urine: albumin creatine ratio (UACR) and estimated glomerular filtration rate (eGFR) measured at regular intervals during the study. We began by reviewing the best practices in the study of gene-environment interactions to propose an appropriate process to identify such interactions. The geo-ethnic origin of Caucasian genotyped patients of ADVANCE was then determined by principal component analysis on a set of 139,186 independent single nucleotide polymorphisms. Phenotypic comparisons were conducted between patients of different geo-ethnic backgrounds living in different geographic regions to assess the impact of genes and environment on renal and diabetic traits. We designed a classification algorithm based on longitudinal measures of UACR and eGFR to identify patients presenting evidence of renal non-response or renal response for UACR and eGFR separately. Genome wide associations studies were conducted between these patient groups to identify genetic determinants associated with UACR and eGFR non-response. Genetic risk scores were created using genetic determinants of renal non-response and their capacity to stratify patients according to their risk of developing unmet renal needs was evaluated. Following these analyses, the presence of geo-ethnic based genetic heterogeneity within the genetic determinants of renal non-response was assessed. ***Results:*** The first principal

component separated ADVANCE Caucasian participants in groups of Slavic and Celtic ethnic origins. Age of onset of T2D and albuminuria appear to have an important genetic component as the values of these traits were also different between Slavic and Celtic individuals living in the same countries. Our classification algorithm identified distinct groups of patients with and without renal non-response. GWAS analyses revealed distinct genetic determinants associated to UACR and eGFR non-response. Genetic risk scores based on renal non-response genetic determinants could stratify patients according to their risk of developing unmet renal needs, identify patients who benefit the most from blood pressure and glycemic control treatments at a renal level, as well as stratify patients according to their risk of experiencing DN onset for those without DN at study entry. Distinct genetic architectures between Slavic and Celtic patient groups of the Caucasian ADVANCE population were identified. These distinct architectures were also observed for the genetic determinants renal non-response. ***Conclusion:*** Data from our studies revealed the importance of gene-environment interactions as well as the best practice to identify these, and the presence of distinct genetic architectures between Caucasian ethnic groups. In addition, our data suggests that a genetic basis for renal non-response exists and can be harnessed through genetic tools to stratify patients according to their risk of renal non-response. Our results indicate that this genetic tool could distinguish patients likely to experience DN onset from those remaining free of DN, and could identify patients who will benefit the most from blood pressure and glycemic control beyond the capacity of common clinical risk factors.

French Abstract:

Introduction : La néphropathie diabétique est la cause principale d'insuffisance rénale chronique dans la plupart des pays occidentaux et de manière croissante dans les pays en voie de développement. Malgré le fait que plusieurs succès furent rapportés dans le traitement de la néphropathie diabétique, une proportion de patients diabétique de type 2, continue de progresser sur le chemin de l'insuffisance rénale et cela malgré leur traitement avec des médicaments recommandés. Ce phénomène est caractéristique de la non-réponse rénale et est associé aux besoins rénaux non satisfaits. Nous faisons l'hypothèse que les patients présentant des évidences de non-réponse rénale peuvent être génétiquement distingués des patients présentant des évidences de réponse rénale et que ces traits génétiques peuvent être différent chez les patients Caucasien avec des origines géo-ethniques différentes. ***Design et méthodologie :*** l'étude ADVANCE est un essai clinique factoriel 2x2 randomisé évaluant l'effet d'un control de la pression sanguine et de la glycémie chez 11,140 patients diabétique de type 2 sur une période de cinq ans. 4,098 patients caucasiens d'ADVANCE furent génotypés en plus d'avoir leur ratio d'albumine et de créatinine urinaire (UACR), et leur taux de filtration glomérulaire estimé (eGFR) mesurés à des intervalles de temps réguliers pendant l'étude. Nous avons commencé par faire une revue des meilleures méthodes pour l'étude des interactions gène-environnement afin de proposer un processus pour identifier ces interactions. L'origine géo-ethnique des patients Caucasien génotypés d'ADVANCE fut ensuite déterminée par analyse de component principale sur un panel de 139,187 polymorphismes d'un seul nucléotide. Des comparaisons phénotypiques furent complétées entre les patients d'origine géo-ethnique différente et habitant dans des régions différentes afin d'évaluer l'impact des gènes et de l'environnement sur des traits rénaux et diabétiques. Nous avons créé un algorithme de classification basé sur des valeurs longitudinales d'UACR et d'eGFR afin d'identifier les patients présentant des évidences de non-réponse rénale ou de réponse rénale pour UACR et eGFR séparément. Des études d'association pangénomiques furent complétées entre ces groupes de patients afin d'identifier des déterminants génétiques associés à la non-réponse UACR et eGFR. Des scores de risque génétique furent créés à partir de ces déterminants génétiques et leurs capacités à stratifier les patients par rapport à leur risque de développer des besoins rénaux

non satisfaits furent évaluées. Suite à ces analyses, la présence d'une hétérogénéité au sein des déterminants génétique de la non-réponse rénale fut évalué. ***Résultats :*** Le premier component principal sépara les patients Caucasien d'ADVANCE en groupe d'origine Slavic et Celtic. L'âge de début de diabète et l'albuminurie semble avoir un component génétique important car les valeurs de ces traits étaient différentes chez les patients Slavic et Celtic habitant dans le même pays. Notre algorithme de classification a identifié deux groupes distincts de patients avec et sans non-réponse rénale. Les études d'association pangénomiques ont révélées l'existence de déterminants génétiques distincts pour la non-réponse UACR et eGFR. Des scores de risque génétique créés à partir de ces déterminants génétiques, furent capable de stratifier des patients par rapport à leur risque de développer des besoins rénaux non satisfaits, d'identifier les patients qui bénéficient le plus à un niveau rénal des traitements de control de la pression sanguine et de la glycémie, ainsi que d'identifier au sein des patients sans néphropathie diabétique les patients les plus à risque de développer cette complication du diabète. Des architectures génétiques distinctes furent identifiées entre les patients slavic et celtique au sein des patients caucasiens d'AVANCE. Ces distinctions furent également observées au niveau des déterminants génétique de la non-réponse rénale. ***Conclusion :*** Les données de nos études révèlent l'importance des interactions gène-environnement ainsi que les meilleures méthodes pour les identifier, et révèle l'existence d'une architecture génétique différente entre des groupes Caucasiens. De plus, nos données suggèrent qu'une base génétique à la non-réponse rénale existe et que celle-ci peut être utilisée au sein d'un outil génétique afin de stratifier les patients par rapport à leur risque de non-réponse rénale. Nos résultats indiquent que cet outil génétique peut distinguer les patients susceptibles de développer la néphropathie diabétique de ceux susceptible de demeurer sans néphropathie diabétique, et qu'il peut identifier les patients qui bénéficient le plus à un niveau rénal de leurs traitements contre l'hypertension et l'hyperglycémie, et ceci au-delà des capacités des indicateurs cliniques conventionnels.

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First, I wish to thank the patients of the ADVANCE study, the Czech-post MONICA study, and of the numerous studies included in the CKDGen consortium for participating in these studies, and their component focusing on genetic discovery. I also wish to thank the funding agencies for financing my project and studies throughout the years. Specifically, the support of grant OPTI-THERA, a private-public initiative of Government of Quebec in "Optimization of Therapeutic Approaches in Personalized Primary Care" and Les Laboratoires Servier France and Servier Canada.

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Preface:

The current thesis presents original work concerning diabetic nephropathy in the context of type 2 diabetes. To our knowledge, this study is the first which approaches the topic of diabetic nephropathy with an angle focused on defining and identifying renal non-response in type 2 diabetic patients with the goal of identifying genetic determinants associated to unmet renal-needs. Our research project on the different genetic architectures in Celtic and Slavic Caucasian ethnic groups is the first to report the impact of genetic architecture heterogeneity on clinical traits such as diabetes age of onset, and diabetic nephropathy. Lastly, we are the first to report the use of genetic determinants associated with renal non-response and unmet renal needs in the context of a precision/personalized medicine project assessing the validity of such genetic information for primary prevention purposes.

Authors contribution:

ADVANCE project

Dr. John Chalmers, Dr. Mark Woodward, Dr. Michel Marre, Dr. Stephen Harrap, and Dr. Pavel Hamet contributed to launching the ADVANCE study. Dr Pavel Hamet and Dr. Johanne Tremblay formed the Genomic Sub-study with these colleagues.

Dr. Carol Long and the laboratory and biostatics teem genotyped 4,098 patients from ADVANCE used in our study.

Czech post-Monica

Dr. Renata Cífková contributed to launching the Czech post-MONICA study.

Dr. Mounsif Haloui gentoyped 504 patients from the Czech post-MONICA study

Dr. Alena Krajčoviechová described the importance of uricemia in the development of microalbuminuria during her PhD study at Charles University in collaboration with the genomics team of Dr. Hamet's lab.

Paul Simon conducted all the experiments and analyses for the ADVANCE study of renal non-response as well as the validation of genetic determinants of this study in the Czech post-MONICA study. He wrote *Publication 1,* the *Manuscript* present in this thesis, and contributed as a co-author to the redaction of *Publication 2*.

Abbreviation list:

List of figures:

Introduction

A. Type 2 Diabetes and its renal complication: diabetic nephropathy

Nephropathy, as defined by the Merriam-Webster Medical Dictionary [1], refers to an abnormal state of the kidney; especially: one associated with or secondary to some other pathological process. Prolonged nephropathy will lead to chronic kidney disease (CKD), which ultimately and in the event of unsuccessful management, will progress to end-stage renal disease (ESRD) [2]. Several factors such as long term use of non-opioid analgesics [3], high fat and sodium diets [4], excess IgA deposits [5], ionated intravenous radiocontrast [6], long term lithium use [7], induced xanthine oxidase deficiency [8], polycystic kidney disease [9], and chemotherapy induced toxicity [10] can cause nephropathy. The most prevalent form of nephropathy; however, is caused by diabetes, and is referred to as diabetic nephropathy (DN) [11]. It is now well recognized that DN is the most common cause of CKD and ESRD in industrialized countries; with highest prevalence reported in Asian countries [12, 13], and increasingly so in countries completing their economic transition [14]. The first section of this thesis will focus on providing the reader with information required to have a clear understanding of DN. To that end, we will first look at some key epidemiological metrics on diabetes, type 2 diabetes (T2D) and DN. Next, we will review the basic anatomy and characteristics of the kidney, to then discuss DN pathogenesis, and DN management. We will conclude this section by answering the question: why should we care about DN?

A.a. Current state of the T2D pandemic, and DN epidemic

Being downstream consequences of DN, the prevalence of CKD and ERSD are closely linked to the prevalence of DN, which is itself directly affected by the prevalence of diabetes. T2D being the most prevalent form of diabetes [15], the majority of DN cases are therefore caused by this type of diabetes. To better understand the trends of the global prevalence of DN, one must look at the current state of the T2D pandemic. A pandemic is defined as a disease outbreak that

occurs over a wide geographic area and affects an exceptionally high proportion of the population [16]. By observing current trends of T2D, one will note that this non-communicable disorder fulfills all requirements of a pandemic. The number of individuals affected by diabetes rose from 108 to 422 million between 1980 and 2014, leading to an increase of global diabetes prevalence from 4.7% to 8.5% [15, 17]. If current trends continue, it is expected that 642 million people will be affected by diabetes by 2040 [15]. The largest increase in diabetes prevalence is expected to occur in countries completing their economic transition, undergoing a nutrition transition, experiencing an expansion of their middle class, and/or where urbanization trends continue to increase sedentary lifestyles [18]. In India, estimates place diabetes prevalence at 8.7% of the adult population (69.2 million individuals) [15], while in Brazil, a study based on complex diagnosis, estimated diabetes prevalence at 11,9% (23.6 million individuals) [19]. While these numbers concern all forms of diabetes, it is important to recall that T2D stands as the most prevalent form of diabetes and therefore accounts for the majority of these cases [15]. Estimating the exact prevalence of T2D is complicated, due to the large number of undiagnosed affected individuals, and consequently the lack of data on true incidence [17]. Thanks to the UK Quality and Outcomes Framework (QOF) - a general medical practise initiative collecting indicators of the level of care received by patients - a recent study in the UK, reported that 3,3 million individuals aged 17 or older are affected by diabetes [20]. This data enabled the authors to report an accurate 6.2% prevalence figure for diabetes in that age group. Of these patients, 90.4% have T2D, while 8.5% have Type 1 Diabetes (T1D), and 1.1% have other forms of diabetes. While the proportion of T2D cases observed amongst UK individuals cannot be generalized to other countries, it can however, once put in context with diabetes prevalence in these countries, provide an appreciation of the considerable place that T2D plays in the broader and global diabetic pandemic.

T2D is a condition rarely seen in isolation but rather along with one or several other components of the metabolic syndrome (MS); a constellation of interconnected physiological, biochemical, clinical, and metabolic factors [21]. A central component of MS is obesity, an established risk factor for T2D [22]. To truly understand the extent of the T2D pandemic, one

must also look at recent reports on obesity, for several mechanisms link obesity to insulin resistance and T2D [23]. Not surprisingly, obesity prevalence is also on the rise and has more than doubled since the 1980s affecting 1,9 billion adults and 42 million children under the age of five in 2014 [24]. As a result, and due to the mechanisms that link obesity and T2D [23], the global T2D pandemic can be explained in part, by the progression of the global obesity pandemic.

Achieving a thorough understanding of both pandemics; however, would not be complete without highlighting an important shift in paradigm in the pathogenesis of T2D and obesity. This shift is driven by a change in the age at which patients are diagnosed as overweight or having T2D. When considering both diseases, it is vital to note the increasing prevalence in young individuals; a trend which was unseen in past decades. The prevalence of overweight infants and young children increased globally by close to a third between 1990 and 2013 (from 32 million to 42 million affected children age five or under) [25]. Similarly, and since the mid-1990s, an increasing number of paediatric T2D cases have been reported globally and have been associated to the increasing degree of obesity in affected children and adolescents [26, 27]. The rise of early T2D onset presents an additional challenge for patients and physicians. Due to an early and lasting exposure to the deleterious effects of this metabolic disease, individuals with an early onset of the condition are more likely to develop adverse macrovascular and microvascular complications over time [28-30].

As the global prevalence of T2D increases in both adults and children, so will the number of patients affected by DN; thus, increasing the proportion of individuals at risk of CKD and ESRD. The etiology of DN being complex, it is important to understand the mechanisms involved in its pathogenesis, its clinical characteristics as well as its clinical management. To achieve such an understanding, one must first acquire a basic comprehension of the human kidney.

A.b. The Kidney

Like other nephropathies, DN is characterized by an abnormal state of the kidneys. Unlike other nephropathies, however, DN is caused by the metabolic and vascular consequences of diabetes. To appropriately discuss the topic of DN, one must first have a clear understanding of the anatomical structure and physiological functions of the kidney. While the functional unit of the kidney and its main anatomical features will be discussed below, we advise readers interested in acquiring an in depth understanding of kidney anatomy and renal physiology to consult *Box 1* in the work of Christian Kurts et al. [31], as well as the *Anatomy and physiology of the Kidney* from Wallace, M.A. [32].

A.b.i. Kidney anatomy and physiology

The kidneys are a pair of organs located in the back of the abdomen. From a histological perspective, and proceeding inwards from its connective tissue capsule, the kidney is divided in a cortex, a medulla organized in pyramidal shapes, the minor and major calix, and the renal pelvis. Their main functions are to excrete waste, reabsorb vital nutriments, maintain acid-base homeostasis, guarantee osmolality and blood pressure regulation, as well as hormone secretion [32]. The functional unit of the kidney, located in the cortex, is the nephron (Figure 1). Each kidney possesses on average a million [33].

As outlined in Figure 1, the nephron can be divided in two main functional areas: the renal corpuscle and the tubules. The renal corpuscle is the first part of the nephron (Figure 1, structure indicated in blue). This structure is composed of small blood vessels encased by Bowman's capsule, a structure composed of two epithelial cell layers separated by a space. At the vascular pole of Bowman's capsule, the renal corpuscle receives its blood supply from the afferent arteriole. Within the Bowman capsule, the afferent arteriole branches out into a capillary network: the glomerulus (also called tuft of capillaries) [32]. These capillaries then drain in the efferent arteriole, which exits the renal corpuscle at the vascular pole. The renal corpuscle is responsible for blood filtration. This function results from the interaction of the glomerulus with the inner/visceral layer of Bowman's capsule: specialized epithelial cells called

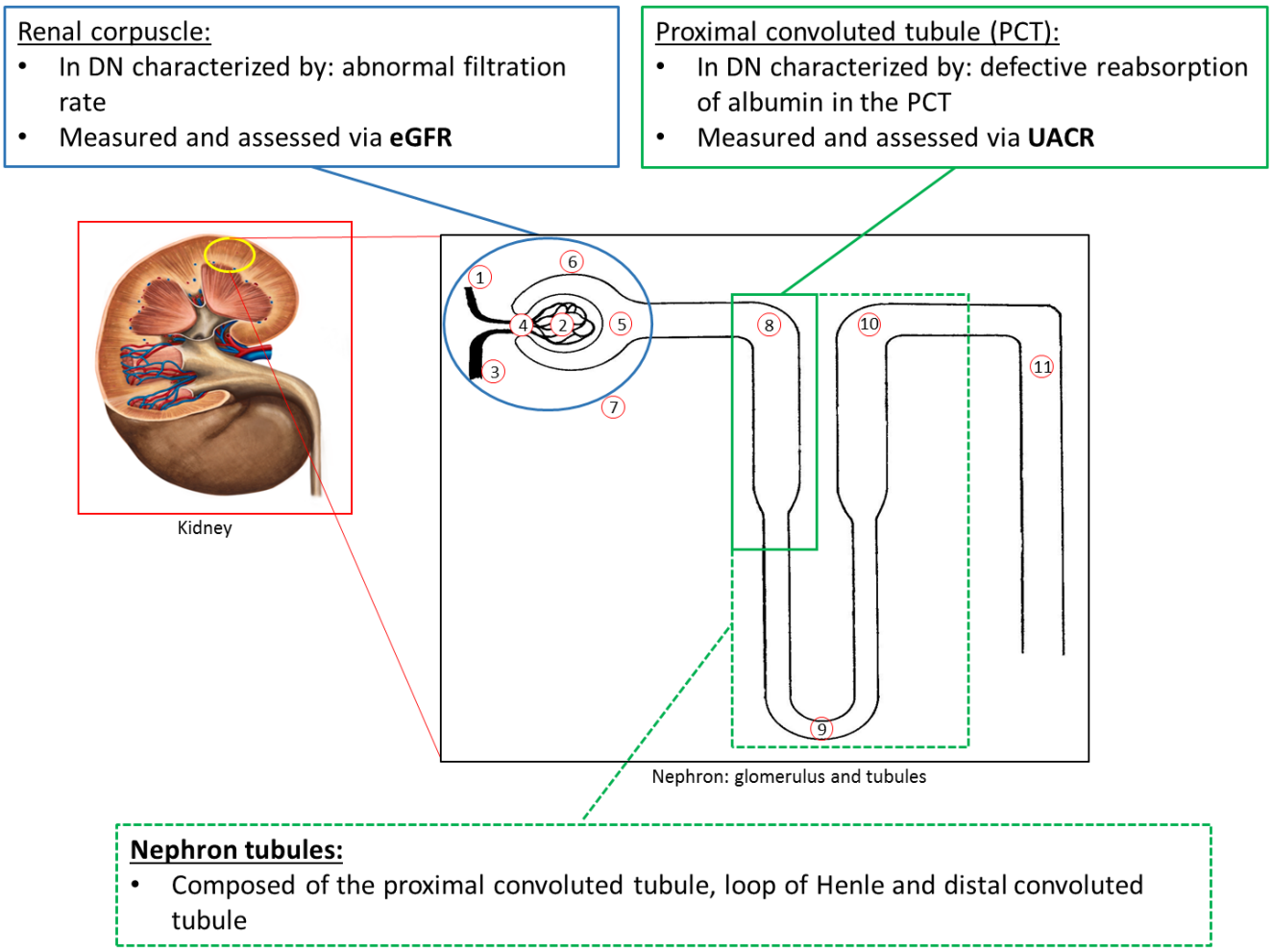

Figure 1: Diagram of the kidney and annotated schematic of the nephron. Taken and adapted from: © 2015 Health, Cancer, Liver, and Surgery. All Rights Reserved. Illustration of kidney and schematic representation of the nephron. (1) Afferent arteriole, (2) glomerulus/tuft of capillaries, (3) efferent arteriole, (4) vascular pole of Bowman's capsule, (5) urinary pole of Bowman's capsule, (6) Bowman's capsule composed of an outer parietal layer, Bowman's space, and an inner visceral layer/pedicel (all three sub-parts not shown), (7) renal corpuscle, (8) proximal convoluted tubule, (9) loop of Henle, (10) distal convoluted tubule, (11) collecting tubule

podocytes, which encapsulate the capillaries of the glomerulus. The processes of the podocytes, called pedicels, wrap around the basement membrane of the glomerular capillaries leaving thin filtration slits in between each pedicel. Blood is filtered through these filtration slits (also referred to as filtration diaphragm) allowing the passage of fluids into Bowman's space and the retention of macromolecules in the capillaries.
The resulting filtrate exits Bowman's space at the urinary pole and enters the tubular part of the nephron via the proximal convoluted tubule (Figure 1, structures indicated in green). The tubules are then responsible for reabsorption of salt, water, albumin and all filtered organic solutes [32]. The filtrate then leaves the nephron at the level of the distal convoluted tubule to enter the collecting tubule, which actively participates in electrolyte and fluid balance via hormonally regulated excretion or reabsorption of ions and water.

The activity of the nephron is regulated by a structure located near the vascular pole: the juxtaglomerular apparatus [32, 34]. This apparatus is composed of the macula densa cells, the juxtaglomerular cells, and the extraglomerular mesangial cells. Extraglomerular mesangial cells are to be distinguished from intraglomerular mesangial cells (also referred to as glomerular mesangial cells) [32]. The former are located between the afferent and efferent arteriole, while the latter are located in the glomerulus in between the capillaries. The juxtaglomerular apparatus plays a central role in the regulation of blood pressure due to its implications in the renin-angiotensin-aldosterone system (RAAS) [34]. This role is mediated by the capacity of the juxtaglomerular cells to sense changes (more precisely a decrease) in renal perfusion and ion concentrations within the afferent arteriole. These changes will lead the juxtaglomerular cells to release the enzyme renin, which triggers the RAAS. In short, and at a systemic level, renin is responsible for the conversion of liver produced angiotensinogen (AGT) to angiotensin I (Ang I), which is then converted by lung produced angiotensin-converting enzyme (ACE) to angiotensin II (Ang II), which acts on the sympathetic nervous system, the renal tubules, the adrenal cortex, the vasculature, and the pituitary gland to increase blood pressure and ion concentration [34]. One of the several actions of Ang II is to stimulate the release of hypothalamus secreted anti-diuretic hormone (ADH), which acts on the collecting tubules of the kidney to reabsorb water

[32]. The return to normal blood pressure is sensed by the juxtaglomerular cells, which then decrease their release of renin. In addition to this system, in which the juxtaglomerular cells play a key role, the macula densa cells are responsible for the tubuloglomerular feedback [35]. The location of macula densa cells allows them to be in close contact with cells of the thick ascending limb of the Loop of Henle (a segment of the nephron tubules), as well as with the afferent and efferent arterioles. Through their capacity to sense increased NaCl concentration and fluid flow in the thick ascending limb, the macula densa cells can then regulate glomerular filtration rate (GFR) via purinergic signaling [36]. This feedback system allows each nephron to obtain information about flow rate and ion concentration in the tubules and to modulate GFR when changes in the filtrate are perceived.

The maintenance of acid-base balance, the reabsorption of filtered organic solutes, and the excretion/reabsorption of water throughout the tubular system of the kidney are based on complex mechanisms. These functions are managed by numerous and diverse transporters, which vary according to the tubular segment under consideration. While tubular defects will be described in the following sections pertaining to DN pathogenesis, the specificities of the mechanisms involved in ion and fluid exchange will not be presented in this thesis. We recommend that readers interested in a more in depth understanding of this topic consult the work of Izzedine, H et al., as well as Gattineni, J. et al. [37, 38].

A.b.ii. Clinical measures to assess kidney health

Before reviewing the factors involved in the pathogenesis of DN, it is appropriate to present a brief overview and explanation of the two clinical measures used to evaluate kidney status in health and in disease. Due to its filtration and absorption/excretion functions being carried out in two distinct areas, the nephron offers the opportunity to evaluate kidney health with two distinct indicators. First, one can evaluate the rate at which the glomerulus filters blood and generates the filtrate. Second, one can assess the extent to which tubules absorb and excrete materials from, and to the filtrate by measuring urine concentrations of specific proteins. These two distinct methods enable researchers to respectively assess the health of the glomerulus

and its neighbouring structures, as well as the health of the tubular system [39]. The former is measured by the estimated glomerular filtration rate (eGFR), while the latter is measured by proteinuria.

eGFR – The measurement of eGFR is the most widely accepted standard for the assessment of renal function both in health and in disease [40]. As its name indicates, eGFR is an estimation of the GFR. This estimation is obtained by measuring the rate of endogenous creatinine clearance (a breakdown product of creatinine phosphate in muscle) by the kidney, and estimating the rate of GFR by means of equations [40]. Several equations have been developed (notably the Cockcroft-Gault, the Modification of Diet in Renal Disease (MDRD) Study, and the Chronic Kidney Disease Epidemiology Collaboration (CDK-EPI) equations [41]) to estimate GFR via creatinine measurements. Over the years, these equations have gained in accuracy by adjusting raw creatinine measurement with key variables which influence it, such as age, sex, and ethnicity [41]. While the CKD-EPI and MDRD equations are commonly used to calculate eGFR, it must be noted that inaccuracies remain [42]. These can result from measurement errors in GFR (which depend on the filtration marker used), variations in measurement of endogenous filtration markers, and modeling errors [42]. In addition, the secretion of creatinine by the tubules, can affect the precision of creatinine measurements and thus decrease the accuracy of GFR estimations [43]. While direct measurement of GFR is possible by constantly infusing a marker (such as ^{125}I-labeled iothalamate) and measuring the rate of clearance of that compound by the kidneys, these are not routinely used due to the lengthy and cumbersome procedure they impose on patients [40]. When estimated by the equations mentioned above, eGFR is expressed in mL/min per 1.73m^2. To stratify patients with CKD and facilitate the assessment of disease progression, the National Kidney Foundation developed a grading system as part of its 2002 Kidney Disease Outcomes Quality Initiative (KDOQI) [44]:

- Stage 1: normal eGFR ≥ 90 mL/min per 1.73 m^2
- Stage 2: eGFR between 60 to 89 mL/min per 1.73 m^2

- Stage 3: eGFR between 30 to 59 mL/min per 1.73 m^2
- Stage 4: eGFR between 15 to 29 mL/min per 1.73 m^2
- Stage 5: eGFR of < 15 mL/min per 1.73 m^2 or end-stage renal disease

Proteinuria – Kidney function and health can also be evaluated by the measurement of total urinary protein and is referred to as proteinuria measurement. A specific type of proteinuria measurement focuses on albumin and reports the urine-albumin-to-creatinine ratio (UACR) rather than the protein-to-creatinine ratio (PCR). The use of UACR is typically preferred over that of PCR in patients with T2D due to several studies reporting a greater sensitivity and specificity for UACR [45, 46]. Measurements of proteinuria or UACR are conducted with multi-reagent urinary dipstick testing and are most commonly reported in mg/g when measured by spot collection, mg/24h when measured by 24H collection or µg/min when measured by timed collection. The presence of urinary albumin is detected by a colorimetric reaction with the dipstick-impregnated reagent. Similarly, to measures of eGFR which enable the creation of stages for kidney function decline, UACR measurements enable the creation of classes for albuminuria progression:

- Normoalbuminuria: UACR < 30mg/g
- Microalbuminuria: UACR between 30 and 300 mg/g
- Macroalbuminuria: UACR > 300mg/g

In 2012, the Kidney Disease Improving Global Outcomes (KDIGO) clinical practice guidelines [47], updated the 2002 KDOQI by indicating the inclusion of UACR in the staging of CKD to more accurately measure CKD progression.

A.c. Diabetic nephropathy pathogenesis

As explained above, the kidney's functions are carried out by the glomerular and tubular parts of its nephrons, which are both modulated by the juxtaglomerular apparatus and the tubuloglomerular feedback. The deleterious effects of diabetes, which can lead to DN, CKD, and

ESRD, affect both the tubules and the glomerulus [48]. To understand why both parts of the nephron are affected during DN, one must consider the various elements involved in the pathogenesis of this complication of diabetes. The damages afflicted to the kidneys in DN are caused by hemodynamic and metabolic factors [49, 50]. These factors respectively result from downstream effects of two clinical risk factors of T2D, which are also commonly observed in patients with MS: hypertension and hyperglycaemia [21]. These directly affect kidney structures and modulate kidney activity via their effect on the RAAS and their disruption of the renal cellular, and extracellular environments [51, 52]. The resulting characteristic abnormalities of DN include glomerular hyperfiltration, hyperperfusion, glomerulosclerosis, thickening of glomeruli basement membrane, mesangial cell expansion, podocyte loss, alterations in the tubulo-instersitum, interstitial fibrosis, expansion of tubular basement membranes, tubular atrophy, and arteriosclerosis [49]. To understand the complex nature of DN pathogenesis, we will first examine the actors implicated in both the hemodynamic and metabolic factors which cause this complication of T2D, to then explore their areas of interactions. We will conclude this section by presenting the two current paradigms thought to cause DN pathogenesis.

Hemodynamic factors: two types of hypertension: As seen previously, the filtration function of the glomerulus is based - mechanistically - on an interaction between vasculature elements (namely the glomerular tuft of capillaries originating from the afferent arteriole) and the specialized cells of the visceral layer of Bowman's capsule (namely the podocytes). As a result, factors affecting blood pressure in the afferent arterioles, tuft of capillaries, or efferent arteriole, have the capacity to influence GFR and potentially damage renal vessels. Due to its effect on afferent arteriole pressure, it was established early on, that DN could be hemodynamically affected and perhaps generated by systemic hypertension. Unlike in type 1 diabetes, where systemic hypertension typically occurs in patients with established renal damage [53], it was observed that hypertension preceded DN in some patients with T2D [54, 55]. Studies in the Pima Indian population revealed that hypertension was in fact a predictor of diabetic nephropathy, five years after the onset of diabetes [56]. This association may be explained in part, by the common risk factor for hypertension and diabetes; obesity [57]. The

presence of systemic hypertension prior to the manifestation of DN offered a plausible causative link between the two, which was explored and characterized. The mechanisms associated to the development of systemic hypertension and the later manifestation of DN in T2D are complex. While still being incompletely understood, they include: excess sodium retention, sympathetic nervous system and RAAS activation, endothelial cell dysfunction, and increased oxidative stress [57]. The pathogenic effects of systemic hypertension on the kidney occur when blood pressure elevation is transmitted to the renal microvasculature [58]. If sustained, this can initiate a vicious cycle of hypertension, causing benign nephrosclerosis at first, followed by malignant nephrosclerosis if hypertension is not controlled [58]. Initially, and below a certain systolic threshold, increase in systemic blood pressure can be prevented from reaching renal blood flow and affecting glomerular hydrostatic pressure [59, 60]. This protection mechanism is guaranteed by the renal autoregulatory vasoconstrictive response [59, 60]. In this instance, and when blood pressure remains within this autoregulatory range, only benign nephrosclerosis will be observed [60]. If systemic blood pressure remains elevated and goes beyond that threshold, renal autoregulatory mechanism begin to fail and acute disruptive injury (malignant nephrosclerosis) will occur [61]. Once the development of renal injury initiated, the autoregulatory response can be secondarily compromised resulting in the vicious cycle of hypertension mentioned above and further renal damage [58].

Throughout the 1980s and 1990s, in parallel to our growing understanding of the hemodynamic effects of systemic hypertension on DN, a growing body of evidence began to shine light on the impact of intraglomerular hypertension as an important hemodynamic modulator of DN. A sizable proportion of our knowledge of the negative effects of intraglomerular hypertension in accelerating renal injury in diabetes have been discovered with experimental rodent models of diabetic nephropathy [62-65] and use of cultured cells [66-68]. A landmark study in the field is that conducted by Brenner's group [65], which showed the capacity of inhibitors of the RAAS - specifically ACE inhibitors (ACEi) - to confer renoprotective effects in streptozotocin-induced diabetic rats, thus highlighting the importance of renal specific hemodynamic factors in DN. This study revealed, by means of micropuncture procedures, the range of intrarenal

hemodynamic abnormalities in nephrons of rats with diabetes. These included increased intraglomerular pressure, increased single nephron GFR and preferential afferent arteriole vasodilation, and were shown to be attenuated by ACEi treatment. The results of this study contributed to the recognition of intraglomerular hypertension as a central actor of renal injury in DN, thus demonstrating that the determinants of DN progression included not only systemic hypertension but also intrarenal hemodynamic changes [65, 69].

Hemodynamic factors: systemic and local effects of RAAS: The study conducted by Brenner's group was followed by further understanding of the role of RAAS mediated renal injury due to several investigations conducted with the Ren-2 rat model [69]. This model introduced the murine renin Ren-2 gene in the rat genome and led to an increased expression of various components of the RAAS leading to the development of systemic hypertension by means of a known monogenic model (specifically, the additional renin gene) [70, 71]. Ren-2 rats offered the opportunity to study the controversial role of the RAAS in diabetes and enabled various components of this system to be measured within the kidney [34, 72]. These studies contributed to an important conceptual change regarding our understanding of the RAAS [73]. While the systemic RAAS was known for decades, the discovery of local or tissue RAAS in the 1970s demonstrated that RAAS components – AGT, processing enzymes, angiotensins (Ang I and Ang II), and specific receptors at tissue level - could be produced locally in several organs and tissues [73, 74]. The observation that local tissue RAAS could be regulated independently from circulatory RAAS, while still interacting with it, supported the idea that changes in the distribution of local RAAS could be important mediators of progressive renal injury [73]. The effects of local RAAS were therefore shown to occur in cells that produce the peptides (intracrine and autocrine functions), in adjacent cells (paracrine function) or through the bloodstream to a specific organ or tissue (endocrine function) [73, 75, 76]. These studies revealed that tissue RAAS affected the local regulatory mechanism that contribute to numerous homeostatic pathways such as cellular growth, extracellular matrix formation, vascular proliferation and endothelial apoptosis, thus contributing to renal damage [73, 75, 76]. Furthermore, it was demonstrated that diabetic kidneys could present increased sensitivity to

local and systemic RAAS elements [77], thus further amplifying the capacity of local RAAS elements to cause renal damage. The extent to which the diabetic milieu leads to abnormal concentrations of RAAS elements, the hemodynamic effects of these elements, and the means by which they contribute to systemic or renal hypertension, ultimately causing renal damage, are discussed below.

Hemodynamic factors: Angiotensinogen (AGT): AGT is a glycoprotein of 452 amino acids mainly produced in the liver but also in the heart, vessels, kidneys, and adipose tissue [73]. As explained previously, by the action of renin, AGT is transformed to Ang I (the precursor of the classical RAAS cascade). In the presence of ACE, Ang I is then transformed to Ang II (the effector peptide of the RAAS responsible for activating pro-hypertensive mechanisms). Due to its upstream position in the RAAS cascade, an increase in AGT levels can result in downstream increases in the concentrations of Ang I and Ang II, leading to increased hypertension and potential renal damage. Several studies evaluated if AGT was expressed locally in the kidney, to determine potential effects on local RAAS. In the late 1980s, in situ hybridization and immunohistochemical studies revealed that the AGT gene is specifically present in the proximal tubules of the kidney and that the renal AGT protein is specifically located in the proximal convoluted tubules (PCT) [78-80]. The localization of AGT in the PCT is of interest due to various experiments reporting high pro-renin (the precursor of renin) and renin concentrations in the kidney tubules during diabetes (see paragraph on renin for further details) [81, 82]. As a result, and under diabetic conditions, both the substrate and an increased concentration of the enzyme required to produce Ang I (and consequently Ang II later), are present in the kidney. The diabetic renal environment, where local AGT expression is combined to increased renin concentrations in the tubules, is therefore favorable to RAAS activation and hypertension-induced kidney damage. In addition to these findings, various groups have demonstrated that the M235T polymorphism in the AGT gene was associated to circulating and tissue levels of AGT [83-85]. This genetic variation, responsible for an increase in RAAS activity, is caused by the T235 allele of the AGT gene, which is associated with greater formation of Ang II in the kidneys leading to DN and CKD [83-85]. However, some contradicting data has been presented on this

topic and questions the relation of the AGT gene polymorphism with DN [86, 87], thus requiring further evidence to consolidate the role of this polymorphism in DN.

Hemodynamic factors: Renin: Renin is an aspartyl protease enzyme produced by the juxtaglomerular apparatus, which cleaves AGT to produce Ang I. As discussed, AGT is specifically present in the proximal convoluted tubule. Once combined to high renin activity caused by diabetes [81, 82], all upstream elements of RAAS are locally present in the kidney. This results in increased Ang II concentrations, and therefore increased blood pressure. In addition, the conventional role of renin was expanded in recent years following the discovery of the renin/prorenin receptors [73, 88]. Renin was shown to have an agonist function on the RAAS by inducing signaling through its own receptor [88]. Binding of renin to its receptor is thought to increase its catalytic effect on AGT, which in turn is thought to be implicated in target-organ lesion in the kidney [89]. In addition, it was demonstrated that Ang II activates collecting duct pro-renin production in diabetes [81], thus promoting additional flux through the RAAS. Lastly, significant levels of *de novo* renin synthesis were identified in the connecting segment and collecting duct [90], revealing yet another source of pro-hypertensive activity.

Hemodynamic factors: Angiotensin-Converting Enzyme (ACE): The key role of ACE within the RAAS, as the main Ang II-synthesizing enzyme was established in the work of Skeggs and colleagues [91]. In addition, ACE acts outside the RAAS by degrading the vasodilating compound bradykinin (BK). By its combined actions of generating Ang II and degrading bradykinin, ACE acts a strong pro-hypertensive hormone. ACE was found to be localized on endothelial cells of the renal microvasculature, and its mRNA was found to be abundant on the brush border of proximal tubules [92, 93]. Levels of ACE activity have been shown to be increased in diabetic patients, with an additional significant increase in ACE activity levels in diabetic patients with DN [94], thus suggesting an essential role for ACE in the development of DN. In addition to elevated levels of ACE during diabetes, genetic associations linked to these increased levels have be uncovered. More than 160 ACE gene polymorphisms are known [95], but Rigat et al. were the first to report the ACE insertion/deletion (I/D) involving the insertion or absence of a 287-bp sequence in intron 16 of the gene [96]. This insertion/deletion became the most studied

polymorphism in DN, leading to the discovery of a statistically significant relationship between the D allele, DD genotype of the ACE polymorphism and DN with consistent effect in various ethnic groups [97, 98].

Hemodynamic factors: Angiotensin II (Ang II): It was rapidly understood that Ang II had both hemodynamic effects on the diabetic kidney [77], as well as non-hemodynamic effects [68]. Both of these effects are typically mediated via the action of Ang II on the Angiotensin II receptor type 1 [68, 77, 99]. The hemodynamic effects of Ang II on the diabetic kidney, include renal vasoconstriction, increased capillary pressure (leading to increase capillary permeability), and mesangial cell contraction resulting in reduction in filtration surface area [77]. Due to its capacity to induce systemic and glomerular blood pressure increase, Ang II also has the potential to trigger hypertensive renal damage [100]. In terms of non-hemodynamic effects, Ang II was shown to contribute to extracellular matrix accumulation through stimulation of pro-sclerotic cytokine TGF-β [68], inhibition of extracellular matrix degradation [77], and activation of intracellular mediators involved in progressive renal injury such as protein kinase C (PKC) [101], and nuclear transcription factor kappa B (NF-κB) [102]. In addition, Ang II was shown to have the capacity to induce vascular, glomerular, and tubulo-interstitial injuries by inducing cell proliferation, as well as leukocyte recruitment and interstitial fibrosis [103], factors which all contribute to further kidney injury and worsening of DN. These deleterious effects of Ang II are further amplified by the increase concentration of upstream RAAS elements in the diabetic kidney which were described in previous paragraphs.

Hemodynamic factors: Modulators of glomerular vasomotor tone – Other vasoactive compounds beside RAAS actors have been studied for their involvement in progressive renal injury during DN. These investigations were supported by the fact that intraglomerular pressure is affected by compounds affecting vasodilation or vasoconstriction via their action on the afferent or efferent arterioles respectively. As a result, opposing actions on intraglomerular pressure can occur in the setting of DN [69]. In addition to Ang II, compounds such as endothelins and vasopressin also lead to vasoconstriction, while compounds such as bradykinin and atrial natriuretic factor lead to vasodilation thus affecting glomerular vasomotor tone in

disparate ways. These compounds will be briefly reviewed below.

Discovered in 1988 by Yanagisawa and colleagues [104], endothelins are part of a three-member family of 21-amino acid peptides: ET-1, ET-2, and ET-3. The ET-1 isoform stands as the most biologically active one, and exerts its vasoconstrictor effect via two receptor subtypes ET_A and ET_B[2] [105]. With close to 27,000 publications on this topic, ET-1 is now well recognized as a peptide with the capacity to accelerate hypertension and atherosclerosis-induced vascular disease. Its main and even larger role however, is in regulating renal function and injury [105, 106]. With the highest level of ET-1 found in the kidney (specifically the renal medulla), the role of this peptide as a regulator of renal functions and mediator of renal injury progression is well established [107], and this despite an incomplete understanding of the basic mechanisms by which ET-1 causes DN [105].

Vasopressin, also referred to as antidiuretic hormone, is a peptide hormone secreted by the posterior pituitary gland which acts to retain water and to constrict blood vessels via its action on receptors AVPR1A, and AVPR2 [108]. As proven in animal models with streptozotocin-induced diabetes, and in patients with T2D; circulating levels of vasopressin are increased during diabetes [109]. While reduction of extracellular volume caused by glycosuria (excess glucose in urine) alone or in combination with increased sensitivity of hypothalamic osmoreceptors to plasma osmolality are thought to be involved, the exact cause of increased vasopressin concentration in T2D are not full elucidated [110]. Nonetheless, prolonged and persistent high-levels of vasopressin have been shown to have deleterious effects on the kidneys leading to albuminuria and CKD [108, 111, 112].

Bradykinin is a 9-amino acid peptide chain part of the larger kinin – kallikrein system. In brief, this system involves substrates (kininogens) which are transformed by plasma and tissue activators (kallikreins) resulting in the production of two vasoactive peptides: bradykinin and kallidin [113]. Kinins act via G protein coupled receptors B_1 and B_2. The former is known to have low levels under physiological conditions but is upregulated by numerous pathological stimuli, while the latter is involved in vasodilation [113, 114]. Bradykinin is now known to be a pro-inflammatory mediator and regulator of vascular and renal functions [113]. While there is still debate in the field regarding the potential benefit of bradykinin receptor modulation in various

regions of the kidney [114], it is now well understood that in normal physiological conditions, bradykinin has a renal protective effect [115]. This effect was shown to be true in disease as well, in a study demonstrating that the absence of bradykinin B1 and B2 receptors enhances nephropathy in diabetic mice [115]. The metabolism of bradykinins to its inactive compound is catalyzed by ACE [113]. Due to the higher affinity of ACE for bradykinin rather than Ang I, concentrations of bradykinin can be severely decreased in situations of increased ACE levels [113, 114], as in the case in diabetes [95]. Decreased levels of bradykinin will result in diminution of vasodilation in combination to increased vasoconstriction as promoted by the conversion of Ang I to Ang II by ACE.

Hemodynamic factors: complexification of the RAAS: The association of RAAS with DN was further extended following the discoveries of other RAAS active elements, with the capacity to increase Ang II concentration, or mimic the action of Ang II [73]. The identification of angiotensin-(1-7); a biological active metabolites of the RAAS [116, 117], of the Ang IV receptor; a transmembrane enzyme referred to as insulin-regulated aminopeptidase (IRAP) [118], and the ACE homologue ACE2; an angiotensin peptide processing enzyme [119], all contributed to our current understanding of the RAAS. This system is now seen as a complex cascade with multiple mediators, multiple receptors and multi-functional enzymes. While the synthesis of Ang II usually results from a chain of reactions involving liver, juxtaglomerular cells and lung produced enzymes, diabetes can increase local kidney concentration of RAAS actors. This modulation of local RAAS by diabetes results in hemodynamic and non-hemodynamic stresses to the kidneys, which promote CKD progression and DN worsening.

Hemodynamic factors: Nitric Oxide (NO): Up to this point our discussion of hemodynamic factors associated with the pathogenesis of DN has been focused on actors of the RAAS and vasoactive hormones. This discussion would not be complete; however, without exploring the role of NO, one of the several oxides of nitrogen classified as a free radical (due to its chemical structure including unpaired electrons) [120]. NO has been demonstrated to be a potent vasodilator, which influences glomerular vasomotor tone [121]. Due to the wealth of publication on the role of NO in the pathogenesis of DN, which sometimes present contradictory evidences,

defining an exact role for NO in DN is complex [69, 122]. The contrasting results can be explained, in part, by the techniques used to study NO levels in the diabetic kidney (RT-PCR vs. histo-chemistry [123, 124]), the study of endothelial NO synthase (eNOS) vs. inducible NO synthase (iNOS) [125, 126], and the duration of diabetes [122]. Despite this, and as meticulously detailed by Prabhakar, S.S. [122], some clarity vis-à-vis of the role of NO can be derived from an exhaustive and meticulous literature review. First, it must be noted that the metabolic milieu in DN can enhance or suppress NO production. Second, one must understand that it is the balance of enhanced and decreased NO production, which varies depending on diabetes duration, that ultimately determines the role of NO at that period [122]. To correctly understand the implication of NO in DN pathogenesis, one must therefore observe its effect at the onset of disease and several years following that event. In early DN, increased NO production occurs and is thought to result from eNOS activity caused by diabetes generated hyperglycemia [125, 127]. This increased NO in the kidney is believed to contribute to glomerular hypertension, hyperfiltration and microalbuminuria. The scenario is different in later stages of DN however. At that period, a decline in renal NO is observed and has been associated to increase oxidative stress, inhibition of eNOS and iNOS by TGF-β, and advance glycated end products (AGEs) [128-130]. This decline in NO may contribute to glomerulosclerosis and renal failure. As a result, the disease duration-dependent increased and decreased of NO levels have both been shown to be associated with kidney damage and DN worsening [122]. Moreover, it was demonstrated that the kidney was one of the best-known targets of the atrial natriuretic peptide (ANP), which coordinates efferent arteriolar vasoconstriction and afferent arteriolar vasodilatation via the activity of natriuretic peptide receptor A (NPRA) and its second messenger: cyclic guanosine monophosphate (cGMP) [131]. The ANP-dependent vasoconstriction of vascular smooth muscle cells requires cGMP and Protein Kinase G I (PKG-I), as PKGI deficient mice do not answer to NO [132].

Metabolic Factors: Key concept: A key metabolic characteristic of T2D is insulin resistance [133]. Clinically, this is defined as the impaired capacity of insulin to promote glucose uptake into tissues that express the glucose transporter GLUT4 (skeletal muscles, heart, and adipocytes)

[134]. This thesis will not present an exhaustive discussion of the mechanisms and cascades involved in insulin resistance (which can be reviewed in publications of Saltiel et al. [135, 136]). Nonetheless, the following paragraphs will present several pathways, which contribute to DN pathogenesis due to the hyperglycemia-generated metabolic state of "glucose toxicity" present in T2D.

Metabolic factors: Advanced glycated end products (AGEs): AGEs are heterogeneous and complex compounds, which have been associated to many diabetic complications [137]. In short, the production of AGEs occurs through the Maillard reaction where reducing sugars react non-enzymatically with amino groups in proteins, lipids, and nucleic acid [137]. Through a series of reactions, these reducing sugars form Schiff bases and Amadori products, which then produce AGEs. Readers who wish to acquire further details on the reaction pathways and chemical processes involved in the production of AGEs, are invited to consult the work of R. Singh et al. (specifically Figure 1) [137]. The process of advanced glycation being lengthy, its effects are typically observed on long-lived proteins such as collagen, myelin, tubulin, complement C3, plasminogen activator, and fibrinogen. Due to hyperglycemia, the concentration of AGEs is increased in diabetes [138]. The deleterious effects of AGEs and their implications in the pathogenesis of DN are numerous. These are believed to be caused by AGEs-induced apoptosis and subsequent tubular damage [139], interaction with receptors for advance glycation end products (RAGE) [140], increased synthesis of glomerular mesangium and tubulointerstitium extracellular matrix [141], podocyte DNA damage, podocyte detachment via stimulation of Ang II type 1 receptor [142], and alteration of glomerular basement membrane integrity [143]. Lastly, it is important to note that the deleterious effects of AGEs presented above are known to be amplified in DN due to diabetes-induced increase of podocyte receptor for advanced glycation end products (RAGE) [144]. Lastly, it was demonstrated in ADVANCE, a randomized trial conducted in 11,140 patients with T2D, that sRAGE levels were associated with new or worsening nephropathy (HR 1.20 for a 1-SD increase of log sRAGE [95% CI 1.02–1.41]; P = 0.032), and that circulating AGE levels were also independently associated with new or worsening nephropathy (HR 1.21 for a 1-SD increase

[95% CI 1.08–1.36]; P = 0.001) [145], thus providing strong evidence to support the deleterious role of AGE and RAGE in DN.

Metabolic factors: Polyol pathway: Hyperglycemia caused by diabetes results in elevated cytosolic glucose and alters rates of glucose metabolism; a condition referred to as hyperglysolia [146]. The exact cause behind hyperglysolia remains to be elucidated. Nonetheless, and due to its role in reducing glucose to sorbitol as part of the polyol pathway, a body of evidence indicates a potential implication of aldose reductase (AR) in the process leading to hyperglysolia [69, 146]. The role of polyols in activating pathways associated to DN has been demonstrated through the capacity of AR inhibitors to decrease PKC and TFG-β1 production (two mediators of renal cell damage) [147]. In addition, more recent results reported increased tubuloglomerular changes in diabetic mice overexpressing AR, thus further demonstrating the implication of the polyol pathway in early tubular cell changes [148].

Metabolic factors: Hexosamine pathway: It has been demonstrated that the hexosamine pathway is involved in the development of diabetic nephropathy and other diabetic complications [149-152]. In this pathway, which represents a minor branch of glycolysis [153], glucose is first phosphorylated to glucose-6-phosphate by the enzyme hexokinase. Following this step, glucose-6-phosphate is converted to fructose-6-phosphate by the enzyme phosphoglucose isomerase [149]. The conversion of fructose-6-phosphate to glucosamine-6-phosphate is then catalyzed by the rate-limiting enzyme glutamine: fructose-6-phosphate amidotransferase (GFAT), and uses glutamine as an amino donor [69]. The end product of this chain of reaction is UDP-N-acetylglucosamine, which along with other aminosugars generated by the hexosamine pathway, provides essential building blocks for glycosyl side chains of proteins and lipids [149]. The implication of this pathway in diabetic nephropathy has been revealed in experiments exploring the effect of overexpression of GFAT [150, 151] or its inhibition by enzymes in renal mesangial cells[152]. Increased activity of the hexosamine pathway is associated with PKC activation and increased TGF-β expression (respectively involved in progressive renal injury and extracellular matrix accumulation) [150, 151]. Inversely, inhibition of GFAT was shown to decrease extracellular matrix production due to the reduction

of glucose-induced TGF-β1 [152]. This data clearly indicates that the hexosamine pathway is not only a biosynthetic pathway for amino sugars, but also acts as a glucose-sensing pathway where increased flux through the pathway damages the kidney [152].

Metabolic factors: Glucose transporters: It was identified early on that several glucose transporters isoforms (GLUT 1, 3 and 4) as well as sodium-glucose cotransporters (specifically SGLT1) were expressed in cells of the renal glomerulus [154]. Of particular interest and starting in early diabetes, it was reported that a positive-feedback regulation mechanism existed in mesangial cells, where glucose induced expression of GLUT1 [155]. This increase in GLUT1 mesangial cell expression was shown to have a pathogenic role associated to the development of extracellular matrix expansion [154]. This pathogenic effect is thought to be mediated by the increased glucose metabolic flux in mesangial cells. This additional flux triggers the polyol pathway, activates PKC, increases TGF-β1 expression, and increases production of extracellular matrix protein fibronectin [69, 154, 155]. Furthermore, several studies have indicated that polymorphism in the GLUT1 gene were associated with DN and severity of diabetes in patients with T1D and T2D [156, 157]. It is currently believed that these polymorphisms are responsible for an increase in the rate of glucose transport and exacerbate the pathogenic factors mentioned above, thus placing patients at higher risk of DN. Lastly, it was reported that expression of the sodium/glucose cotransporter 2 (SGLT2), involved in glucose re-absorption in the kidney [158], was reduced in whole renal tissue of patients with diabetes as compared to well-matched people without diabetes [159], which could impact the benefit of SGLT2-inhibitor treatments on the tubules.

Metabolic factors: Oxidative stress and reactive oxygen species (ROS): Simply defined, oxidative stress corresponds to oxidant-derived tissue injury. This type of damage has the potential to occur in any particular tissue where the production of oxidants or ROS exceeds the amount of local antioxidants [160]. ROS include free radicals ($^{\cdot}O_2^-$, $^{\cdot}OH$, $^{\cdot}RO_2$), non-radical species (H_2O_2 and HOCl) as well as reactive nitrogen species ($^{\cdot}NO$, $^{\cdot}NO_2^-$, $ONOO^-$, HNO_2, RONOO) [160]. The topic of reactive oxygen specifies in the pathogenesis of DN is complex however. On one hand, several pathways proven to be implicated in DN pathogenesis generate ROS [161-163]. These include

auto-oxidation of glucose, transition metal-catalyzed Fenton reactions, glycolysis, polyol pathway flux, uncoupling of nitric oxide synthase, mitochondrial respiratory chain deficiencies, xanthine oxidase activity, NAD(P)H oxidase activity, and advance glycation [162]. On the other hand, clinical studies using antioxidants (such as vitamin E or β-carotene) do not delay DN pathogenesis or DN progression [164-166]. As a result, while the generation of ROS occurs in many pathogenic mechanisms involved in DN, their reduction by therapeutic agents does not seem to improve CKD outcomes. Recent reports have pointed out; however, that more targeted and rationally designed antioxidant approaches could be more successful in decreasing the deleterious effects of ROS in the diabetic kidney [69, 162].

Nephrin: a protein affected by hemodynamic and metabolic factors: the glomerular protein nephrin was first cloned in 1998 [167] and was then discovered to have a key role in the development and function of the glomerular filtration barrier (through its implication in the podocyte filtration slits) [168]. Following its discovery and characterization, it was demonstrated that nephrin expression was reduced in kidneys of rats and patients with diabetes [169]. This process is believed to occur via the action of glycated albumin on RAGE, and Ang II-generated cytoskeleton re-distribution, leading to shedding of nephrin [170]. As a result, nephrin stands as a clear example of a renal structural element that is disturbed by both hemodynamic and metabolic factors associated to diabetes, and can lead to increased proteinuria.

Interaction between hemodynamic and metabolic factors: As presented in the previous paragraphs, hemodynamic and metabolic factors can independently cause renal damage. This is based on their mutual capacity to activate intracellular signaling molecules within renal structures. As mentioned above, increased flux in the polyol pathway and increased concentration of AGE have the capacity to increase the activity of PKC, and NF-κB; two important mediators of renal damage [171, 172]. This capacity is also shared by Ang II whose concentration is heavily modulated in diabetes due to numerous changes in upstream elements of the RAAS. Based on their downstream capacity to modulate growth factors and cytokines, in addition to exerting structural changes in the kidney via extracellular accumulation, PKC and

NF-κB stand as the most likely site of interaction between hemodynamic and glucose dependent pathways [69]. The pathological pathways of DN, their numerous actors, and their areas of interaction are schematically represented in Figure 2.

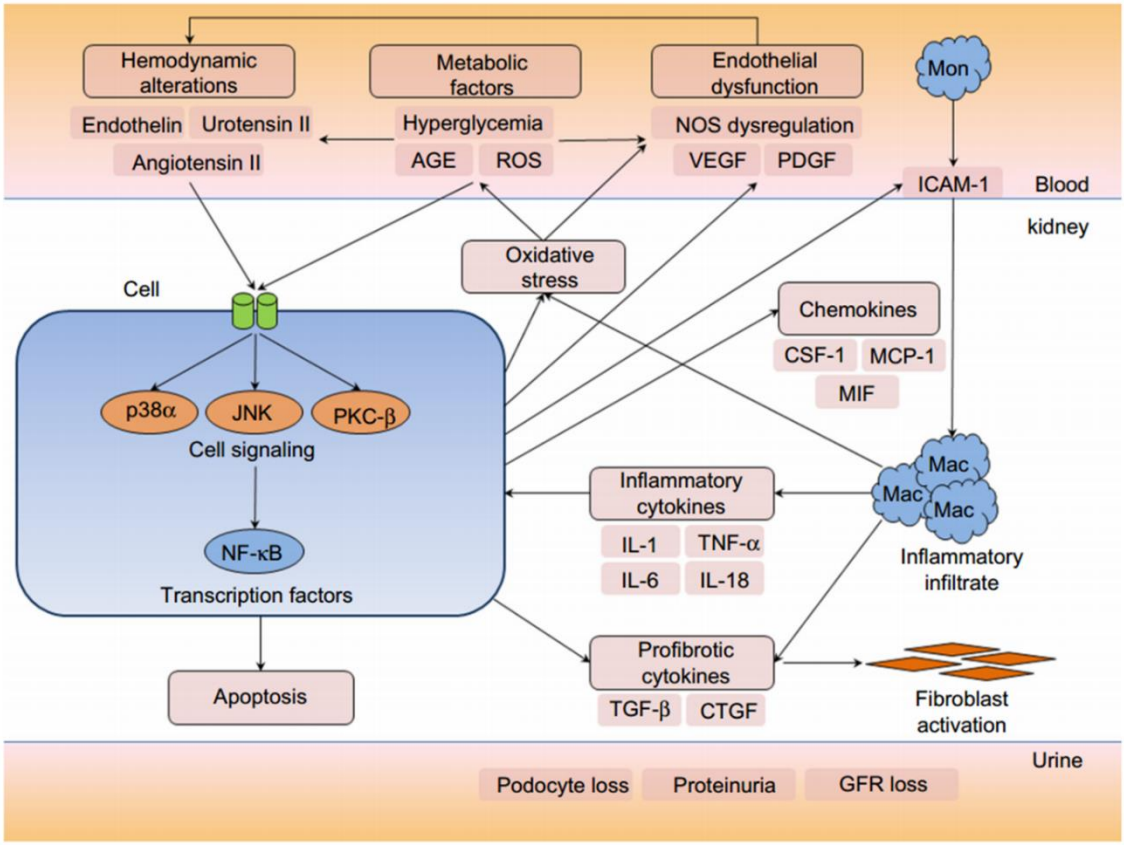

Figure 2: Overview of the pathological pathways in diabetic nephropathy

Notes: In the diabetic milieu, metabolic derangements and hemodynamic alterations, particularly activation of the renin–angiotensin system, trigger a number of cell signaling cascades, including the MAPKs (p38 and JNK) and PKC-β, which mediate a cellular response through activation of key transcription factors such as NF-kB. In response to such signals, renal cells such as tubular epithelial cells, podocytes, and mesangial cells can produce chemokines, growth factors, and profibrotic cytokines. CSF-1 and MCP-1 function as chemotactic molecules and promote the recruitment of monocytes from the circulation. Upregulation of ICAM-1 on endothelial cells – a key leukocyte adhesion molecule – facilitates infiltration of circulating mononuclear cells into the kidney. CSF-1 also promotes monocyte/macrophage differentiation, proliferation, and activation. MIF functions to retain macrophages at sites of inflammation and has counter-regulatory functions against the anti-inflammatory actions of glucocorticoids. Activated macrophages can produce proinflammatory and profibrotic cytokines, reactive oxygen species, and antiangiogenic factors and contribute to a cycle of inflammation, oxidative stress, cellular injury, progressive fibrosis, and loss of glomerular filtration rate. Podocyte loss, endothelial dysfunction, alterations in the GBM, and tubular injury contribute to increasing proteinuria during the development and progression of diabetic nephropathy.

Abbreviations: AGE, advanced glycation end-products; GBM, glomerular basement membrane; GFR, glomerular filtration rate; Mac, macrophages; Mon, monocyte; NOS, nitric oxide synthase; ROS, reactive oxygen species.

The two hypotheses for the pathogenesis of DN: Our discussion regarding the factors associated to DN pathogenesis, revealed that this process is caused by glomerular and tubular damages, which result from the combined action of hemodynamic and metabolic stresses. It is therefore useful to conclude this section by presenting two prevalent hypotheses/paradigms regarding the pathogenesis of DN and how these are supported by evidences presented in previous paragraphs.

The first, supports the role of glomerular hyperfiltration as an initiating event in the pathogenesis of DN [173]. Several mechanisms are thought to be involved in this process, such as primary abnormalities in vascular control, as well as greater afferent than efferent glomerular arteriole dilation [174, 175]. These result in renal vasodilation, increase renal blood flow, GFR, intraglomerular pressure, and filtration fraction, which ultimately lead to glomerulopathy, benign and then malignant nephrosclerosis [64]. While this hypothesis has not been refuted, the lack of glomerular hyperfiltration in a proportion of type two diabetic patients presenting high albuminuria, lead to further investigations to understand the causes of DN pathogenesis in these patients [48, 173].

Throughout the years, the results of these investigations have brought forward a second hypothesis to DN pathogenesis, which is now referred to as the "tubular hypothesis" [176, 177]. This hypothesis, based on several observations, supports that damages to the tubules, and subsequent disturbance of the renal environment, could be responsible for the onset of DN. As we have seen, this hypothesis is backed by evidence which implicates a tubular renin-AGT stimulation of the RAAS, leading to kidney lesions and hypertension. In addition, the tubular hypothesis is further supported by evidence which indicates early hypoxic damage suffered by the tubules as a result of chronic hyperglycemia [176].

It is important to note that the exact relationship between these two hypotheses is complex and incompletely resolved. While albuminuria increase and eGFR decline were initially thought to be closely linked, studies reporting cases of T2D patients with CKD stage 3, but without albuminuria [178], have questioned that notion [179]. As of today, and following an active debate in the field of DN that lasted over two decades, the notion that glomerular and tubular damages are independent but additive components of DN has gained support [180]. As a result,

the capacity of the two hypothesis of DN pathogenesis to manifest themselves to different extents, or in combination depending on the duration of diabetes and the metabolic profiles of each patient now stands a plausible paradigm [48].

Overall the pathogenesis of DN remains a convoluted topic. While concise details of the molecular mechanisms involved in this process have not been fully elucidated, it is now understood that renal injuries in the framework of T2D are caused by the lasting insults that hemodynamic and metabolic factors bring upon the kidneys. Furthermore, the manifestation of glomerular and/or tubular injuries depends on the metabolic profile and history of each patient, which further complicates the creation of a unifying theory for the pathogenesis of DN. The multi-factorial basis of DN pathogenesis, as well as its close relation with other risk factors of MS (namely hypertension, hyperglycemia, and obesity), requires that therapeutic management of DN be multi-factorial and focused on controlling the main drivers of this complication of T2D.

A.d. Management of DN: current approaches and treatments

When discussing the clinical and therapeutic management of DN, it is important to keep two key factors in mind. First, and as of today, one must note that no treatment regimen has been able to consistently and systematically achieve complete reversal of DN in patients with T2D. While successes in the prevention and regression of DN have been reported [181-183] as well as cases of reversal of DN in animal models [184], current standards of care do not enable a complete elimination of this complication of T2D. Second, and as with most metabolic diseases, the time at which treatment onset occurs has a considerable impact on the treatment's capacity to prevent disease progression. Thus, medications present a higher success rate when they are given at early states of the disease [185, 186].

Since complete reversal of DN remains a complicated objective to achieve, the management of DN in T2D patients is based on preventing renal disease onset in patients who do not present

renal deterioration when diagnosed with T2D, and preventing disease progression in patients who already have renal disease at the time of T2D diagnosis. The management of DN is based on controlling the numerous factors that contribute to this complication. Due to their deleterious effects on the kidneys, hypertension and hyperglycemia must be controlled to manage DN. Similarly, and due to its close association with diabetes and its complications, the management of obesity must be included in treatment strategies for DN, thus placing dyslipidemia as a therapeutic target. Lastly, lifestyle modifications which can decrease the severity of the above risk factors must be considered for inclusion in management strategies for DN [187]. Smoking cessation, diet modifications, regular exercise, and reduction of alcohol consumption are therefore key elements to include in treatment strategies of DN. In this section, we will explore the common therapeutic agents used in the treatment and management of DN and discuss some novel treatment therapies currently investigated.

A.d.i. Blood Pressure Control

As we have discussed, the effects of systemic and glomerular hypertension are deleterious to the kidneys and are associated to DN onset and progression [54, 55]. Reducing blood pressure to an optimal range of 130/80mmHg in hypertensive T2D patients has therefore become a core element of clinical guidelines for the management of T2D and DN [188, 189]. The implication of many RAAS elements, amongst other hemodynamic factors, in the pathogenesis of hypertension offers numerous targets for the reduction of blood pressure within the management of DN progression. As a result, blockade of the RAAS stands as a common first-line treatment for hypertension in patients with T2D. This blockade is commonly achieved with ACEi or Angiotensin II Receptor Blockers (ARBs). The use of RAAS blockade now being incorporated in international guidelines [188, 189], the benefit, efficacy, and safety of combining RAAS blocking agents with one another or with second-line agents was assessed. To validate the usefulness of such combinations in reducing incidence of major macro- and microvascular events, clinical trials (ONTARGET, ADVANCE, ACCOMPLISH, and ASCOT) were conducted. The ONTARGET study reported that no cardiovascular benefit resulted from a dual RAAS blockade combination using an ACEi (ramipril) and an ARB (telmisartan), and that this combination lead

to an increase in acute renal failure and hyperkaliemia [190, 191]. As a result, the dual combination of RAAS blocking elements ceased to be recommended in the exception of patients with progressive macroalbuminuria and refractory/resistant hypertension [2]. In terms of combining RAAS blockade treatments with second-line agents, the ADVANCE trial demonstrated the capacity of a combination using an ACEi (perindopril) with a thiazide-like diuretic (indapamide) to reduce new onset of microalbuminuria and the progression from micro- to macroalbuminuria in T2D patients [183]. Later, the ACCOMPLISH [181, 192] and ASCOT [193] trials revealed that the use of an ACEi (benazepril and perindopril respectively) with a calcium channel blocker (amlodipine) was superior to that of an ACEi with a thiazide diuretic in reducing renal events. It is important to note however, that the ACCOMPLISH trial reported that the reduction of albuminuria was better in the patient group receiving benazepril and the thiazide [194], thus demonstrating that the benefit of calcium channel blockers is seen only on kidney function type events and not albuminuria type events. While the ACCOMPLISH and ASCOT trials were not conducted in all diabetic population, patients with diabetes mellitus responded in similar fashion to non-diabetic patients. In parallel, and since several plausible options exist to block the RAAS, the effect of medications affecting upstream elements of the RAAS (such as renin) or downstream effectors (such as aldosterone) were investigated. While the AVOID trial initially reported beneficial effects of the renin inhibitor aliskiren in a population of T2D patients receiving an ARB [195], the larger ALTITUDE trial which was ceased prematurely due to adverse outcomes, mitigated these results [196]. As for aldosterone inhibitors (spironolactone or elperenone), the benefit of combining them with an ACEi was shown to reduce proteinuria in addition to presenting anti-inflammatory benefits but was mitigated by an increased risk of hyperkalemia [197, 198]. These scenarios echoed the one of the ONTARGET trial, and further reinforced the notion that double RAAS blockade combinations should be restricted to patients with difficult hypertension and proteinuria.

In addition, it is important to highlight that intensive blood pressure control (using all major classes of antihypertensive agents) in hypertensive patients at high-risk of cardiovascular disease (with or without CKD), beyond a systolic target of 120mmHg, does not significantly reduce the incidence of renal outcomes [199]. As demonstrated in the SPRINT trial, such

patients with CKD, experience an equivalent rate of renal events whether receiving an intensive blood pressure control regimen or a standard one [199]. In comparison, patients with the same profile but without CKD, and receiving the intensive treatment regimen, experienced more renal outcomes than patients receiving the standard treatment [199]. As a result, blood pressure control, as achieved with a combination of first-line and second-line agents, stands a central pillar of DN management. The choice of antihypertensive medication as well as the intensity of the treatment should be based on the metabolic and hypertensive profiles of patients, as well as their CKD stage.

A.d.ii. Glycaemia control

Hyperglycemia represents one of the major risk factors present in T2D. As we saw, hyperglycemia can lead to renal damage via activation/flux of several pathways (namely the polyol and hexosamine pathways) thereby increasing the generation of AGEs, and the concentration of inflammatory and pro-fibrotic molecular elements [138, 147, 150]. As a result, most current guidelines recommend achieving an optimal glycemic control by reducing glycated hemoglobin levels to less than 7% [188, 189]. Clinical trials such as the UKPDS, the Kumamoto study, and the ADVANCE trial all demonstrated the benefit of intensive glucose control. With a fasting plasma glucose target of under 6 mmol/l for the UKPDS trial, an Hba1c targets <7.0% for the Kumamoto study, and a Hba1c target ≤6.5% for ADVANCE, these studies reported a reduction of new onset albuminuria between 10 and 30% and an increase in the regression of albuminuria by 15% [200-202]. It is important to note, that failure to reach Hba1c levels below 7.0% can be associated with detrimental events [203, 204]. This was demonstrated in the ACCORD trial, where T2D patients who failed to reach such levels of glycemic control presented excess mortality [203, 204]. Lastly, it was observed that specific drugs used for the control of blood glucose (specifically PPAR-γ inhibitors and dipeptidyl peptidase-4) confer a protective effect on the kidney independently of their hypoglycemic action. This action is thought to result from their anti-inflammatory and anti-fibrotic actions [205, 206]. Renal data from ongoing clinical studies will provide additional information regarding the potential reno-protection conferred by these agents [207].

A.d.iii. Lipid Control

While some evidence exists, and suggests that lipid control provides an added value to the therapeutic management of DN, the benefit of anti-lipid agents remains controversial. The *post-hoc* analysis of the ADVANCE study revealed that low concentrations of HDL cholesterol were associated with significantly greater risk of micro- and macroalbuminuria [208]. Similarly, the Casale Monferrato study demonstrated that apolipoprotein and high-density lipoprotein cholesterol levels were independent risk factors for progression of overt nephropathy [209]. In addition, experiments in streptozotocin-induced diabetic rats revealed that statins could reduce NF-κB activation and AGE-mediated ROS activation [139, 210]. On the other hand, the CARDS study conducted in 2,838 patients with T2D and randomized to atorvastatin or a placebo, demonstrated that this lipid lowering agent did not influence the incidence of albuminuria [211]. In addition, no significant regression to normoalbuminuria, and only modest improvement in annual change in eGFR were observed [211]. Overall, and despite the aforementioned evidence, there remains limited data from intervention studies with regards to renal outcomes and use of lipid lowering agents. Despite these facts, lipid-lowering agents (specifically statins) are recommended for T2D patients with DN and aged 40 years or over. This recommendation is based on the cardiovascular benefit brought by statins rather than their renal protective effects per se [212].

A.d.iv. Multifactorial Approaches

While the downfalls of combining RAAS blocking agents in the framework of blood pressure control were discussed earlier, it is crucial to highlight the usefulness and impact of intensive multifactorial approaches, which simultaneously target hypertension, hyperglycemia, dyslipidemia, and lifestyle factors. The impact of such intervention on DN has been well demonstrated in the STENO-2 study, which evaluated the effect in 160 microalbuminuric patients with T2D, of setting the following intensive treatment targets: behaviour modification, blood pressure < 130/80mmHg, Hba1c < 6.5%, fasting cholesterol < 4.5mmol/L, and fasting triglyceride < 1,7mmol/L [213]. Patients were randomized to a dietary intervention (fat and saturated fatty acid intake respectively 30% and 10% less of total daily energy intake), light to

moderate exercise (defined as three to five 30min sessions per week), cessation of smoking, an ACEi (or ARB if side effect occurred with ACEi – to which thiazides, calcium channel blockers or beta blockers could be added if blood pressure targets were not reached), vitamin C and E, aspirin, hypoglycemic agent (in the event that Hba1c did not fall below 6.5% with diet intervention alone - using gliclazide for lean patients and metformin in obese patients), and statins (in addition to fibrates for patients with hypertriglyceridemia). The STENO-2 study reported that patients under the intensive treatment regimen experienced significantly lower rates of progression of nephropathy with a 60% decrease in overt proteinuria [213]. The benefit of intensive multifactorial intervention was echoed in a Japanese study conducted in 216 patients with T2D [214]. After receiving treatments for the combined management of diabetes, hypertension and hyperlipidemia, a resulting 57% regression of microalbuminuria onset was observed in these patients [214].

A.d.v. Novel potential therapeutic targets:

As we have seen in the section of this thesis pertaining to DN pathogenesis, the diabetic renal milieu is a complex environment. As a result, numerous pathological processes and pathways could potentially be targeted to reduce the progression of DN. Novel strategies are therefore under investigation to assess how to complement existing interventions.

AGE inhibitors: One of these potential target is the glucose-dependent advanced glycation reaction. This pathway has the potential to be targeted by AGE inhibitors, which reduce AGE formation, enhance degradation, or break AGE crosslinks [207]. Two AGE inhibitors, aminoguanidine or pyridoxamine have been investigated for their capacity to interrupt the AGE/RAGE axis. Aminoguanidine was initially tested in streptozotocin-induced diabetic rats and was shown to have the capacity to retard development of albuminuria and mesangial cell expansion [215]. Despite being initially promising, due to its capacity to decrease proteinuria, when given to patients with T2D, aminoguanidine was associated with adverse effects such as glomerulonephritis [216]. Pyridoxamine; however, continues to be investigated in phase II and III trials in patients with DN [217], with some initial safety concerns requiring meticulous

investigation of adverse effect incidence [218].

Serum Uric Acid control: Several studies have reported an association of serum uric acid (SUA) levels with the development of micro- and macroalbuminuria in patients with T1D and T2D [219-221]. Based on these observations, a *post-hoc* analysis of the RENAAL trial set off to test the hypothesis of whether reduction in SUA with the Ang II receptor antagonist losartan was associated with renoprotection [222]. This analysis reported that the risk of renal events (defined here as doubling of serum creatinine or end-stage renal disease) was decreased by 6% per 0.5mg/dL reduction in SUA, and that up to one fifth of losartan's renoprotective effect could result from its capacity to decrease SUA [222]. Other experiments on diabetic mice have revealed that allopurinol (a xanthine oxidase inhibitor aimed at reducing the production of SUA) reduces albuminuria and tubulointerstitial injury by reducing urinary TGF-β and endothelial dysfunction [223, 224]. The capacity of allopurinol (versus a placebo) to reduce GFR loss among subjects with T1D is currently being investigated in the pilot of a randomized trial conducted by the PERL consortium (PERL trial) [225].

Endothelin inhibitors: due to its implication in the pathogenesis of DN [105, 106], endothelin stands as a valid target to consider for the management of DN. In diabetic rats, blockade of the endothelin receptor ET_A with the ET_A antagonists atrasentan and avosentan lead to the reduction of albuminuria and renal fibrosis [226, 227]. The encouraging experimental results using endothelin receptor antagonists lead to the investigation of their capacity to reduce renal outcomes in humans. As of today, clinical studies assessing the effects of avosentan and atrasentan in the management of DN present problematic results. In the ASCEND trial [228], where avosentan was used to delay time to doubling of serum creatinine and ESRD in patients with T2D, a reduction of albuminuria was achieved but an increased in edema, and congestive heart failure lead to the early cessation of the trial. Two other studies conducted in diabetic patients with nephropathy and assessing the effects of avosentan and atrasentan [229, 230], also reported successes in reducing albuminuria but noted a similar increase in edema and congestive heart failure. As a result, the use of endothelin inhibitors remains dangerous for

patient safety up to this day. Nonetheless, the SONAR trial (NCT01858532) is currently studying the capacity of atrasentan along with maximum doses of RAAS inhibitor to decrease incidence of renal events in patients with T2D [231].

Glycosaminoglycan degradation control: The antithrombotic and profibrinolytic drug sulodexide has also been investigated as a potential treatment for DN. This mixture of 80% heparan sulfate and 20% dermatan sulfate may reduce the enhanced heparan sulfate degradation which takes place in the basement membrane of glomerular cells during DN [207]. Sulodexide has been shown to have anti-inflammatory properties, and the capacity to inhibit hyperglycemia-induced production of ROS, as well as cytokines MCP-1 and IL-6 in endothelial cells [232]. While promising results were initially reported on the capacity of sulodexide to reduce albuminuria at high doses in patients with T1D and T2D of the DiNAS trial [233], the later Sun-MICRO and Sun-MACRO trials mitigated those results by showing no significant difference in doubling of serum creatinine, or ESRD in T2D patients receiving sulodexide [234, 235].

Vitamin D: As we have seen when discussing the Steno-2 trial [213], vitamin D has been considered and used in treatment regimens for DN management. The use of vitamin D is based on its antioxidant capacity to attenuate oxidative stress. By restoring nuclear factor (erythroid-derived 2)-like 2 (Nrf2) levels, which result in reduced NF-κB activation, vitamin D has been shown to reduce albuminuria [236]. Furthermore, the association of CKD with low levels of vitamin D, and the capacity of vitamin D analogues to protect podocytes from injuries during DN [237], provided additional support for the inclusion of vitamin D in the management of DN. The capacity of vitamin D agonist (such as paricalcitol) to reduce albuminuria was evaluated in the VITAL study, which reported lower albuminuria in T2D patients randomized to that treatment after 24 weeks [238]. Further evidence on the effect of vitamin D receptor agonist to lower RAAS activity will be provided by the results of the VALIDATE-D trial, which follows forty subjects with T2D and microalbuminuria receiving calcitriol (the hormonally active metabolite of vitamin-D) or a placebo [239].

Oxidative stress control: as explained above, vitamin D has been considered for the management of DN due to its antioxidant capacities. Based on the positive results of vitamin D, other antioxidant agents have been considered for DN management. One such agent is the experimental semi-synthetic triterpenoid compound bardoxolone, which specifically targets ROS generation, to prevent oxidative stress. Similarly, as vitamin D, bardoxolone prevents ROS mediated oxidative stress by activating Nrf2 and inhibiting NF-κB [240]. Clinical trials using bardoxolone have reported contrasting results however. On one hand, the randomized BEAM trial, during which patients with T2D were treated for 52 weeks with bardoxolone, reported an improvement of eGFR in the treatment group [241]. On the other hand, the larger BEACON trial, where CKD stage 4 T2D patients were randomized to bardoxolone or a placebo, had to be halted after 9 months due to increased cardiovascular events in the treatment group [242]. In addition, a study reported side effects occurring in rats treated with analogues of bardoxolone [243]. These combined results generated concerns regarding the safety of bardoxolone and its analogues, thus questioning their inclusion in DN management regimens.

PKC inhibitors: As discussed, due to its effect on the polyol and hexosamine pathway, hyperglycemia can lead to the activation of PKC [147, 150]. As a result, the PKC family of enzymes, and more specifically PCKα and PKCβ, have been extensively studied for their involvement in DN pathogenesis [244]. While experiments evaluating the renoprotective effect of ruboxistaurin (a PKCβ inhibitor) in diabetic rats were promising [245], these results did not translate well in human studies [246]. In parallel, a preclinical study has evaluated the role of PCKα in onset of nephropathy [247]. The authors reported an absence of albuminuria in streptozotocin-diabetic induced 129/SV PCKα-/- mice as compared to wild type 129/SV mice. In addition, they assessed that glucose-induced albuminuria was mediated by PKCα downregulation of proteoglycans in the glomerular basement membrane and regulation of vascular endothelial growth factor expression [247]. As a result, approaches using a combination of PCKα and PCKβ inhibitors have been considered for their capacity to decrease albuminuria by means of their anti-fibrotic actions, and are currently being investigated [248].

Anti-fibrotic and anti-sclerotic agents: As observed earlier, and as illustrated in Figure 1, the downstream consequences of hemodynamic and metabolic disorders associated to T2D, lead to the increase of renal pro-sclerotic and pro-fibrotic cytokines (namely TGF-β and connective tissue growth factor, CTGF) [68, 127, 143, 147]. These evidences therefore support a rational for the inclusion of anti-fibrotic and anti-sclerotic agents in the management of DN. One such agent is pirfenidone, an anti-inflammatory drug, which was shown to reduce fibroblast proliferation [249], and inhibit TGF- β production [250]. Although the mechanism of action of pirfenidone remains to be fully elucidated, a study evaluated its effect in MMCs and HEK293 cell lines as well as homozygous obese KSJ db/db mice [251]. In this study, the authors reported a reduction in matrix expansion and renal matrix genes, but no effect on albuminuria [251]. Recently, a randomized trial conducted in diabetic patients with albuminuria and reduced GFR reported that pirfenidone could prevent the decline in GFR at low dose, but that high doses were associated with serious adverse effects [252].

While results of pirfenidone in humans are mitigated, other studies assessing the effect of different antifibrotic agents are still ongoing. The FG-3019 human monoclonal anti-body to CTGF is currently investigated in microalbuminuric patients with T1D or T2D, and has been reported to be well tolerated for at least 6 weeks [253].

In addition, experimental studies were conducted in streptozotocin-induced diabetic rat using the anti-fibrotic drug 3-methoxy-4-propargyloxycinnamoyl anthranilate (FT011) [254]. The authors, reported the capacity of this compound to inhibit TGF-β and platelet-derived growth factor (PDGF), leading to an attenuated decline in GFR, proteinuria and glomerulosclerosis [254].

Lastly, phosphodiesterase inhibitors such as cilostazol and pentoxifylline have been investigated for their inclusion in DN management strategies due to their capacity to reduce TGF-β and TNF-α expression in diabetic rats [255, 256]. In human studies, cilostazol and pentoxifylline have been observed to reduce albuminuria but with modest results on kidney function [257-260]. It is important to note, that while most of these anti-fibrotic agents are not specific to the kidney, their capacity to provide renoprotection is of interest in the context of DN management.

In conclusion, the strategies to manage DN are numerous and require to be tailored to the specific metabolic profile of patients with T2D. While a considerable number of treatment combinations can be considered, the abundance of experimental and clinical evidence must be meticulously examined in order to devise the optimal treatment strategy. The complexity of the diabetic milieu results in no single treatment being successful and favors the use of multifactorial approaches [2, 207]. A summary of possible pathways, which can be targeted to efficiently manage DN with current and potential treatments is indicated in Figure 3.

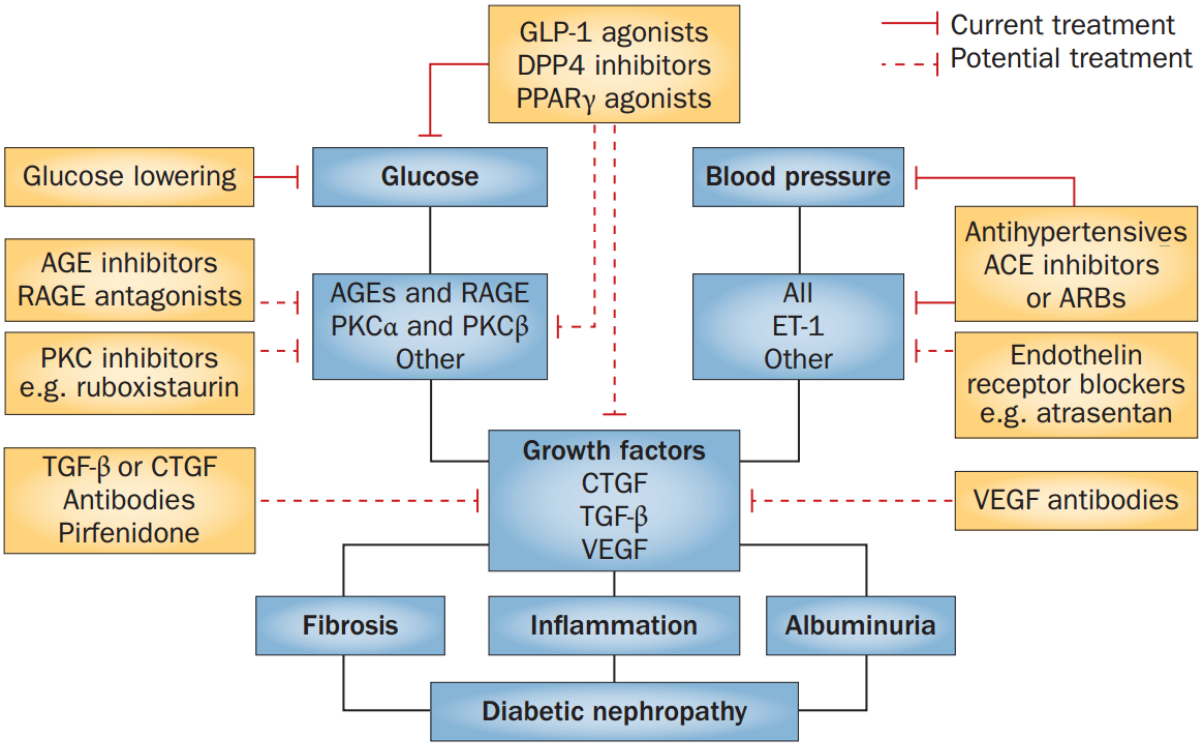

Figure 3: Summary of pathways and compounds, which can be targeted in the management of DN with current and potential treatments. Putative pathways implicated in the pathogenesis of diabetic nephropathy, including established and potential new treatment strategies. Raised glucose and blood pressure activate various pathways that can be specifically targeted in order to reduce the classic pathological hallmarks of diabetic nephropathy, fibrosis, inflammation and albuminuria. Abbreviations: ACE, angiotensin I converting enzyme; AGE, advanced glycation end product; ARB, angiotensin II receptor blocker; CTGF, connective tissue growth factor; DPP4 inhibitor, dipeptidyl peptidase-4 inhibitor; ET-1, endothelin 1; GLP-1, glucagon-like peptide 1; PKC, protein kinase C; PPARγ, peroxisome proliferator-activated receptor γ; RAGE, receptor for AGE; TGF-β, transforming growth factor β; VEGF, vascular endothelial growth factor.

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A.e. Diabetic nephropathy, why should we care?

Up to this point, we have discussed various aspects pertaining to DN. From the T2D pandemic at the basis of the increasing worldwide DN prevalence, to the complex diabetic environment, which promotes DN pathogenesis, as well as the various therapeutic agents used for DN management. These various elements, while providing some essential information on key characteristics of DN, do not fully answer the question: why should life-science professionals care about DN? In this section, we will discuss the four reasons why this complication of T2D should be a leading concern of healthcare professionals and clinicians alike.

A.e.i. The road to end stage renal disease

As discussed previously, world prevalence of diabetes is expected to increase in the coming decades [15, 17]. It is projected that 642 million individuals will be affected by this metabolic disorder by 2040 [15], with the majority being affected by T2D [15]. Due to the continuous stress brought upon the kidney during T2D, a considerable proportion of patients who are diagnosed with this metabolic condition are at risk of developing DN over time (either under the form of albuminuria, kidney function decline, or a combination of both). This fact was well illustrated in the UKPDS study, which revealed that within a 10-year window, 25% of patients affected with T2D will develop microalbuminuria [261].

The section of this thesis pertaining to management of DN enabled us to understand that the complete reversal, and elimination of DN is rarely achieved. Due to the lack of a permanent cure for DN, worsening of DN can only be delayed in patients with T2D [2, 207]. While successes in achieving regression of DN have been reported [182, 183, 193, 194], complete and lasting elimination of the risk of progressing to more dire stages of DN over time, is not systematically achieved as of today. As a result, and due to the difficulties met when attempting to eliminate this complication of T2D, all patients affected by DN are at risk of ultimately progressing to the final stages of the disease. Such stages include ESRD, for which the only viable treatments are dialysis or kidney transplant [262], or death from renal failure. The severity of the final stages of DN, as well as the limited capacity of current therapeutic agents to completely alleviate the risk of progressing to such stages, makes this complication of T2D one to be concerned with. For

those reasons, DN stands as a topic which requires immediate and prolonged attention from researchers and clinicians. It is crucial that investigators continue to seek a better understanding of the molecular basis of DN pathogenesis and progression, in order to design new agents or combinations of existing agents to optimally manage and ultimately eliminate DN.

A.e.ii. Association of DN with cardiovascular complications

As we have just observed, DN is a worrying complication of T2D, due to the possibility of its final stages being fatal, and the difficulty in completely alleviating the risk of progressing to these stages. In addition to this renal burden, one must consider the associations of DN with macrovascular complications to fully understand why this complication of T2D is one to be concerned with.

The FinnDiane study, conducted in 4,083 Finnish patients with type 1 diabetes, evaluated the impact of DN and severe retinopathy on the incidence of stroke, cerebral infarction and hemorrhage, as well as lacunar infarction [263]. The results of this study revealed that in analyses adjusted for conventional clinical risk factors, microalbuminuria, macroalbuminuria and end stage renal disease all increased the risk of cerebrovascular events [263]. Furthermore, the increase in risk was proportional to the severity of DN; where microalbuminuria increased the risk of stroke with a hazard ratio (HR) of 3.2 (95% CI 1.9 – 5.6), macroalbuminuria with a HR of 4.9 (95% CI 2.9 – 8.2), and end stage renal disease with a HR of 7.5 (95% CI 4.2 – 13.3) [263]. The risks for other cerebrovascular events assessed in this study were increased by the stages of DN in an analogous manner to that of stroke [263].

Comparable results to those reported in the FinnDiane study have been presented in patients with T2D. Within the framework of the ADVANCE study, Nimoniya, T. et al. [180], demonstrated that in 11,140 T2D patients, albuminuria and kidney function decline independently and additively increased the risk of cardiovascular events and cardiovascular death. In this study, cardiovascular events were defined as cardiovascular death, nonfatal myocardial infarction, or nonfatal stroke. Following adjustments for conventional clinical variables, higher UACR, and lower eGFR at baseline were log-linearly associated with cardiovascular events and

cardiovascular death. The authors reported that every ten-fold increase in UACR (which corresponds to the passage of one albuminuria class to another) increased the multivariable-adjusted risk of cardiovascular events by 1.6-fold (95% CI 1.3 – 1.9) and that of cardiovascular death by 2-fold (95% CI 1.8 – 2.3). Comparable results were reported for eGFR, where every halving of baseline eGFR increased the multivariable-adjusted risk of cardiovascular events and cardiovascular death by 1.5-fold (95% CI 1.1 – 2.1) and 1.9-fold (95% CI 1.7 – 3.5) respectively. These results were echoed by several other studies [264, 265], which reported associations between kidney function decline and cardiovascular events. In addition, a meta-analysis gathering 140,231 participants from 10 published studies, demonstrated a clear and independent associations between proteinuria and stroke [266]. Lastly, a meta-analysis gathering 160,949 patients from 26 cohorts confirmed the relationship between proteinuria and coronary risk [267].

Overall, these studies clearly highlight the strong association of DN with cerebrovascular and cardiovascular events. Based on this data, as well as the one presented in the previous sub-section of this thesis, one can understand the extent to which DN is problematic. Patients affected by T2D who develop this complication are not only at risk of progressing to ESRD and dying due of renal failure, but also present elevated risks for a broad range of cerebrovascular and cardiovascular complications. For that reason, DN unquestionably stands as a complication of T2D to be concerned with.

A.e.iii. Genetic predisposition

The risks that DN represents at a microvascular and macrovascular level have been presented in the prior sections. It is important to note; however, that not all patients with T2D are equally likely to develop this renal complication. In addition, within the patients who do develop this complication, not all individuals will be affected with the same magnitude. For patients affected with DN, and as with most metabolic conditions, numerous factors can explain the variations in disease severity from one patient to another (presence or absence of comorbid risk factors, such as obesity or hypertension [268], adherence to medication [269], and lifestyle factors [270]). Once those factors adjusted for, one remaining distinguishing feature can explain

differences in disease prevalence and severity: genetics. As we have observed, specific genetic traits, such as the M235T polymorphism in the AGT gene [83, 85], or the deletion of a 287-bp sequence in intron 16 of the ACE gene [95], have been associated to DN pathogenesis. As a result, the likeliness of developing DN for patients affected by T2D is impacted by their genetic makeup. Patients who possess these genetic signatures are therefore not only at greater risk of developing DN, but also at greater risk of developing complications associated with DN (namely ESRD and macrovascular events).

The effect of genetic determinants on DN are not limited to an increase in the likeliness of developing this complication of T2D, but also have the potential to affect the severity and magnitude of the disease. This notion has been particularly well illustrated in a series of recent publications from the CKDGen consortium, which identified specific single nucleotide polymorphisms (SNPs) associated with albuminuria increase and kidney function decline [271-273]. By means of Genome Wide Association Studies (GWAS) meta-analysis, conducted in a discovery population of 133,413 patients and a replication population of 42,166, Pattaro et al. (including our genomic data from ADVANCE Caucasian subjects) identified 24 new loci associated with eGFR, and confirmed the association to eGFR of 29 previously identified loci [271]. Of interest, in the context of this thesis, 19 of these identified variants were associated to eGFR decrease in diabetic individuals. By combining GWAS results to epigenetic analyses (namely chromatin state mapping and DNase I hypersensitivity analyses) the authors demonstrated that the identified genetic variants were preferentially located in kidney and extra-renal tissues. Using a similar methodology in 67,542 patients (of which 7,787 had diabetes), Teumer et al. recently confirmed previously reported associations of the *CUBN* loci with albuminuria [272] (again, including our ADVANCE data). In addition, this study of the CKDGen consortium reported newly discovered gene-by-diabetes interactions for genetic variants at the *HS6ST1* and *RAB38/CTSC* loci. For these loci, the effect of these variants on albuminuria was only seen in patients with diabetes. Similar studies, conducted in various ethnic groups, have confirmed associations of previously reported loci as well as reported associations of novel loci to eGFR and albuminuria [274, 275].

Beyond the significant and valuable molecular insights that these recent publications brought

to their field, it is important to highlight their common message: patients who possess the risk alleles for these genetic variants are at greater risk of having a decline of their eGFR, and an increase of their albuminuria. These findings have two key implications. The first is that even before the onset of T2D, a specific patient population presents a genetic makeup, which places them at greater risk of developing DN. The second consists of the fact, that for two patient populations presenting equivalent clinical and environmental risk factors, the population with risk alleles for these specific genetic variants will have a greater risk of developing a more severe form DN. These notions, when combined to the macrovascular risk to which DN is associated, and the severity of its final stages, make DN a complication of T2D requiring continued attention from clinicians and researchers alike.

A.e.iv. Patient and healthcare burden

Our discussion, pertaining to the reasons that justify a continued focus towards improving our understanding of DN pathogenesis, its management and its early detection, would not be complete without reviewing the burden that DN imposes on patients and healthcare systems alike.

As briefly touched upon in the previous sub-section, patient perspective, due to its capacity to impact behaviours such as medication adherence or lifestyle choices, is a vital component to consider in DN management. As explained by Braun, L. et al. [276], in most cases of chronic diseases without a cure, patient perspective must be considered as a reliable means to understand illness experience, treatment expectations, and unmet needs with current treatments. Understanding these factors could offer a window of opportunity to improve patient perspective. A better patient perspective could in turn, positively affect patient-driven behaviours such as medication adherence, treatment expectations, and lifestyle choices. Regrettably, few studies exploring patients' perspective following DN onset and during DN management have been conducted to this day [276]. Some findings pertaining to the effects of CKD on various aspects of patients' lives have been reported however [277-281]. These results, to some extent, are applicable to DN since T2D is the leading cause of CKD in most developed and developing countries [11, 14, 282]. These studies reported that patients affected by CKD

commonly experienced burdensome conditions such as cognitive impairment, dementia, sleep disturbance, pain, as well as emotional and physical discomfort. In these studies [277-281], patient health-related quality of life (HRQOL) was measured with the Short Form (36) Health Survey (SF-36)[283], and the Kidney Disease Quality of Life Short Form (KDQOL - 36)[284]. Chin, H.J. et al. [279] reported that SF-36 scores were low amongst all patients with reduced eGFR, with mental health component scores similarly low across all groups, while physical component scores decreased in patient groups with significantly reduced eGFR (CKD stage 3 and beyond). These results were echoed in studies making using of the KDQOL to measure HRQOL [278]. Overall, these studies demonstrated a strong association between CKD and low HRQOL. Combined to the fact that reduced HRQOL has also been reported in patients affected by T2D [285], HRQOL can be expected to reach even lower scores in T2D patients with DN. The burdensome conditions experienced by patients affected by DN impact their lives in many ways [286]. From lost productivity, to disability and absenteeism from the workplace, multiple elements of the daily lives of patients with DN are negatively affected by the condition [287]. The impact of these hindrances on normal daily life has financial consequences for both the patients, who often experience reduced professional productivity, as well as healthcare systems for which inpatient, outpatient and drug costs are experiencing a significant increase due to the T2D and DN epidemics [287] [288].

While the humanistic burden of DN stands as a factor of paramount importance when considering elements which should direct our attention towards this complication of T2D, the economic burden that DN bares on healthcare system stands close behind. The American Diabetes Association published a report detailing the economic costs of diabetes in the U.S. in 2012 [289]. This study follows a similar methodology to the ones used in earlier publications of the American Diabetes Association assessing the costs of diabetes in 2002, and 2007 [290, 291]. Previous studies made use of a prevalence based approach to estimate the medical costs by demographic group, health service category, and medical condition. The 2012 study improved upon this methodology by including race/ethnicity as a demographic dimension. Numerous major U.S. medical data sources were used to estimate the size of the population with diabetes, frequency of health services used, as well as direct and indirect medical costs attributed to

diabetes. The authors report an estimated 22.3 million people affected by diabetes in 2012, with an estimated national cost of diabetes reaching $245 billion. This national cost is composed of $176 billion (72%) for direct health care expenditures attributed to diabetes and $69 billion (28%) for lost productivity from work-related absenteeism, reduced productivity at work and at home, unemployment from chronic disability and premature mortality. While these costs represent the overall economic burden of diabetes it is important to note that the health care expenditures attributed to DN represent 5% of the overall hospital inpatient costs, 7% of physician office related costs, 5% of emergency department costs, and 3% of hospital outpatient [289]. These percentages of health care expenditures attributed to DN seem modest in comparison to those attributed to cardiovascular complications of diabetes (26%, 14%, 12%, and 13% respectively for hospital inpatient, physician office, emergency department, and hospital outpatient costs). It is important to remember; however, that due to its association with cardiovascular complications, DN indirectly contributes to the healthcare expenditures attributed to cardiovascular complications [289]. The data presented in this estimation of the 2012 U.S. costs attributed to Diabetes must be complemented with further information to appropriately understand the economic impact of DN. Like several incurable chronic diseases, the medical costs associated with DN increase as the disease progresses [292]. Nichols, G.A. and colleagues conducted a studied in 7,758 T2D patients followed for up to 8 years. In this study, they compared the annualized inpatient, outpatient, pharmaceutical, and total medical costs between patients who progressed to higher stages of nephropathy from those who did not [292]. They reported that patients who progressed from normo- to microalbuminuria experienced an annualized change in baseline costs that was $396 higher ($P<0.001$) than patients who remained within the normoalbuminuria range. Similarly, for patients progressing within the microalbuminuria class, progression was associated with at $747 difference ($P<0.001$) as compared to patient who did not progress. Overall, within patients who had a progression of their albuminuria, costs were 37% higher following a progression from normo- to microalbuminuria ($10,188 vs. $7,424; $P<0.001$), and 41% higher following a progression from micro- to macroalbuminuria ($12,371 vs. $8,753; $P<0.001$). These results are not limited to the U.S. healthcare as similar costs and increase in costs of treating DN have been reported for

India by Dasgupta, I. [293] and Germany by Happich, M. et al. [294]. Overall, and based on the above evidences, DN stands as a complication of T2D which is responsible for a sizable proportion of healthcare expenditures, and for which these expenditures increase in magnitude as patients progress to more severe stages of the disease.

In summary, this section has explored four key factors, which highlight the overall clinical, humanistic and economic impact of DN. Considering the weight that DN bares on patients and healthcare systems across the world, as well as its expected increasing prevalence in the coming decades; efforts should be dedicated to improving the understanding of DN and optimizing its management. Due to the complex nature of DN, attempts to optimize its management through various approaches should be considered. One such approach, which will be discussed in the following section of this thesis, is the identification and understanding of unmet needs in the context of DN treatment and management.

B. The unmet needs of Diabetic Nephropathy

The first main section of this thesis focused on providing various information and metrics to provide a clear picture of the status of the DN epidemic, the source of its pathogenesis, the methods employed for its management, and its need for continued research and clinical attention. This section will focus on a key thematic element of this thesis: the concept of unmet needs in T2D patients. We will begin by defining unmet needs, and more specifically unmet renal needs, to then review the factors causing them, and conclude by quantifying their prevalence in several clinical trials. These elements of discussion will facilitate a transition to the topic of precision medicine, and explore how the consideration of unmet renal needs in the context of precision medicine could improve DN management.

B.a. Definition of unmet needs, and focus on unmet renal needs

To understand the notion of unmet needs, one must begin with the definition of a medical need from the perspective of the patient. Simply said, a medical need arises when a patient

develops a condition, and requires a medical intervention, without which the condition would persist and possibly worsen. With this definition in hand, one can understand that when attempting to answer a patient's medical need, one of two outcomes will arise. The first, and desired outcome occurs when the medical intervention effectively meets the patient's need and resolves the condition. The second, and more problematic outcome, takes place if the medical intervention partially or totally fails to meet the patient's medical need. In this instance, the condition has not been resolved, leaving the patient with an "unmet medical need", which will require a different or novel medical intervention if it is available.

Due to the fact that T2D is a condition rarely seen in isolation, but rather as a component of MS [21], one can understand that T2D patients have a constellation of medical needs (such as needs for hypertension, glycemic, and lipid control among others), and therefore have a high potential to develop unmet medical needs. Of interest to this thesis, are unmet renal needs (URN) – i.e., renal medical needs of patients affected by DN, which are not met by current medical interventions. As mentioned previously, successes in the management of DN exist and have been reported with various combinations of evidence-based recommended medications [182, 183, 193, 194]. Nonetheless and as we discussed, no current medication or combination of medications have been reported to achieve reversal of DN or elimination of the risks of DN onset and worsening in all subjects. Medical renal needs are therefore incompletely met in a portion of population. As a result, URN have become a known and challenging feature of DN management and are increasingly being recognized as a factor justifying continued research in the field of drug development as well as fundamental research in DN pathogenesis [2, 276, 295].

To refine the definition of URN, it is important to review two notions that are associated with them. The first, is treatment non-response, which consists of a patient's incomplete response to evidence-based recommended recommendations for DN (for any of several potential reasons – see paragraph below), thus failing to meet his medical need, resulting in the consequential development of URN [207]. The second, are residual microvascular risks, and more specifically residual renal risks [295]. Residual renal risks correspond to the persistent risk of developing a detrimental renal event or having a progression of established renal damage, despite receiving

evidence-based recommended medications even if effective in a majority of subjects [296]. The above notions can be synthesized as follows: treatment non-response is responsible for the incapacity of current evidence-based recommended medications to meet renal needs of T2D patients affected by DN, resulting in the development of residual renal risks, which are the basis of URN in that segment of population.

Lastly, and to complete the definition of URN, it is essential to mention an element of granularity, which is imposed by renal anatomy and physiology. As we have discussed in several sections of this thesis, it is appropriate to think of the kidney's nephrons as having two distinct parts: the glomerulus and the tubules [32]. We reviewed, that for these two areas of the kidney, DN progression is monitored with different metrics [47], that damages can manifest themselves independently from one another [178], that disease stages independently predict cardiovascular outcomes [180], and that genetic determinants of disease progression are different [271, 272]. These anatomical, clinical, and genetic evidences, therefore support that URN should be further classified as two distinct elements: unmet renal needs for eGFR (URN_{eGFR}) and unmet renal needs for UACR (URN_{UACR}).

B.b. Factors leading to unmet renal needs

Having a good understanding of URN and renal residual risks in hand, it is now useful to review the four main reasons, which can explain the presence of URN in patients with T2D. First, and as briefly touched upon previously, patient behaviour, by its capacity to affect factors such as medication adherence and lifestyle choices, can impact the capacity of evidence-based medications to meet patient renal needs [269, 270, 297, 298]. Second, patients who present more aggressive forms of DN, as caused by factors such as resistant hypertension, resistant hyperglycemia [299], or high tubular concentrations of TGF-β1 [300, 301], are more likely to have poor responses to medications and to develop URN. Third, patients who possess specific pharmacodynamic profiles, which alter their response to evidence based recommended medications, will respond less favourably to medications and will be at greater risk of developing URN. One such scenario has been reported in Caucasian patients carrying the

previously mentioned deletion of 287bp Alu sequence in intron 16 of the ACE gene locus and who respond less favourably to ACEi [98]. Lastly, specific genetic or epigenetic signatures, by their association with any of the three above mentioned factors, or their capacity to influence key hemodynamic and metabolic pathways, can increase a patient's risk of developing URN.

B.c. Quantification of unmet renal needs

To complete our discussion pertaining to URN and to accurately measure the percentage of patients concerned by this problem, one must quantify the prevalence of URN. While URN and renal residual risks are increasingly being recognized as topics to be concerned with from a public health or patient perspective [2, 276, 295], there has been little work done to quantify the number of patients with URN across studies and ethnicities. The objective of this section is to determine the percentage of patients affected by URN across various clinical trials, which took place in the past twenty years. Since the study populations of each trial are not identical, the investigated medications different and the renal events defined differently, the quantification of URN will not be specific to any one patient population, to any one class of drug (or drug combinations), or to any one type of URN. Rather, the objective is to apply a systematic methodology across each trial to determine the percentage of patients within that specific trial who present URN_{eGFR}, or URN_{UACR}. The methodology is based on measuring the percentage of patients who develop kidney function decline or albuminuria increase within the treatment groups of each trial. These patients, who progress along the path of renal disease despite receiving evidence-based recommended medications and for whom therapeutic adherence is well ascertained correspond to individuals with URN.

We will begin with the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT) [302]. In this study, 41,814 high-risk stage 1 or 2 hypertensive patients, 55 years or older, with at least one other coronary heart disease risk factor, were randomized to receive the diuretic chlorthalidone (n=15,255), the calcium channel blocker amlodipine (n=9,048), the ACEi lisinopril (9,054), or the α_1-selective alpha blocker doxazosine (n=8,460) [302]. The renal

outcomes investigated in this study were the incidence of ESRD or a decrement in GFR of 50% or more from baseline, both severe manifestation of kidney function decline. The incidence of these events is reported for patients with and without T2D receiving chlorthalidone, amlodipine, and lisinopril in a study published in 2005 [303]. The percentage of patients per treatment group who developed the event of interest over 4.9 years of follow up: were 5.0, 4.9, and 5.2% for chlorthalidone, amlodipine, and lisinopril respectively (amlodipine – chlorthalidone RR: 0.98, 95% CI: 0.80 – 1.19, P=0.82; lisinopril – chlorthalidone RR: 1.04, 95% CI: 0.85-1.26, P=0.71) [303]. The results being similar in diabetic and non-diabetic patients and due to the lack of significant differences in the development of renal events in between treatment groups, the authors reported that amlodipine and lisinopril were not superior to chlorthalidone in preventing renal outcomes. Overall, the percentages of patients with T2D and severe URN_{eGFR} ranges from 5.0 to 5.2% across treatment groups, with the greatest prevalence of severe URN_{eGFR} in the group receiving the ACEi. Severe URN_{eGFR} are therefore present on average, in 5.1% of patients with T2D, regardless of the treatment these patients receive.

The next multicentre randomised controlled trial to be reviewed is the Anglo-Scandinavian Cardiac Outcomes Trial-Blood Pressure Lowering Arm (ASCOT-BPLA) [193]. This trial gathered 19,257 Caucasian European patients, aged 40-79 with untreated hypertension or treated hypertension but systolic blood pressure ≥140mmHg, and diastolic blood pressure ≥90mmHg. In addition, and to be included in the trial, patients were required to have three or more cardiovascular risk factors. From the overall trial population, 9,649 patients were randomized to the calcium channel blocker amlodipine (adding the ACEi perindopril as required), while 9,618 patients were randomized to the β1 receptor antagonist atenolol (adding the thiazide diuretic bendroflumethiazide or potassium as required). Of these patients, 27% were diabetic in both treatment groups. The focus of ASCOT-BPLA was to compare the long-term effects of the above medications on the combined endpoints of non-fatal myocardial infarction and fatal coronary heart disease. In addition, renal impairment was measured as a tertiary endpoint. After 5.5 years of follow up, 4% of patients treated with amlodipine/perindopril developed the renal event, while 5% of patients treated with atenolol developed the event (HR: 0.85, 95% CI: 0.75 –

0.97, P=0.0187) [193]. In ASCOT-BLPA, the percentage of patients with severe URN_{eGFR} was therefore significantly greater in patients receiving the β1 receptor antagonist.

The next study of interest is a post-hoc analysis of the Incipient to Overt: Angiotensin II Blocker, Telmisartan, Investigation on Type 2 Diabetic Nephropathy (INNOVATION) [304]. In this study, 163 normotensive microalbuminuric Japanese patients with T2D, aged 30-74 were randomized to 40mg (n=54), or 80mg (n=58) of the angiotensin II receptor antagonist telmisartan, or a placebo (n=51). Similarly, 351 hypertensive T2D patients within the same age range were randomized to these same medications (n=120, n=114, n= 117 for 40mg, and 80mg telmisartan, and placebo respectively). Eligibility criteria required the absence of cardiovascular accidents in the past 6 months, and no history of heart failure. Unlike the two previous studies, the renal outcome of interest was the transition from micro- to macroalbuminuria. Within normotensive patients, 33.3, 12.1, and 9.8% developed the event over a period of 52 weeks within the placebo, 40mg, and 80mg telmisartan groups respectively (P<0.01 for placebo – 40mg telmisartan, as well as placebo – 80mg telmisartan). Similarly, within hypertensive patients, 34.2, 14.9, and 11.1% of patients developed the event within the different treatment groups (P<0.01 for placebo – 40mg telmisartan, as well as placebo – 80mg telmisartan) [304]. Despite, a significant decrease of the percentage of URN_{UACR} in patients receiving the angiotensin II receptor antagonist, up to 11.1% of patients receiving the highest dose of the medication continued to progress along the path of renal disease [304].

Next, we will revisit the results of the STEN0-2 trial [182], which we previously discussed within the framework of DN management using multifactorial approaches to prevent DN progression. In this study, 160 Danish patients with T2D and persistent microalbuminuria were randomized to conventional multifactorial treatment (n=80), or intensified, target-driven therapy involving medications and behaviour modifications (n=80). Details of the intensified therapy can be found in section *A.c.iv.* of this thesis. The renal outcome of the Steno-2 trial was the development of overt nephropathy, defined here as the transition from micro- to macroalbuminuria. During the entire observation period (13 years), 25% of patients in the

intensified therapy group developed the renal outcome of interest, as compared to 46.3% in the conventional therapy group [182]. While the rate of events is significantly different between groups receiving the standard vs. intensive-therapy (RR: 0.44, 95% CI: 0.25 – 0.77, P=0.004) [182], the percentage of URN_{UACR} within the intensive and conventional therapy groups is sizeable.

To continue our analysis, we will focus on the Action in Diabetes and Vascular Disease: preterax and Diamicron MR Controlled Evaluation (ADVANCE) [305]. In ADVANCE, 11,140 T2D patients, ≥55 years, with a history of major macrovascular or microvascular disease, were randomized to a blood pressure control arm, as well as to a glucose control arm in a 2 x 2 factorial design. The blood pressure arm compared the effect of the addition of Preterax (a combination of the ACEi perindopril, and the thiazide-like diuretic indapamide) to that of a placebo, while the blood glucose arm assessed the effect of Diamicron (gliclazide, a sulfonylurea anti-diabetic drug) against that of standard therapy. The renal events investigated in ADVANCE were a composite of primary and secondary renal outcomes. The primary renal outcome was defined as requirement for renal replacement therapy, death from renal disease, development of macroalbuminuria or a doubling of serum creatinine to a level of at least 200µmol/L, all severe manifestations of kidney function decline. The secondary outcome was defined as new onset of microalbuminuria. The double-blind randomization process led to four distinct groups respectively receiving Preterax and Diamicron (n=2783), Preterax and the glycemia placebo (n=2786), the blood pressure placebo and Diamicron (n=2788), or standard therapy (n=2783). The incidence of combined renal events (primary and secondary renal end points) were respectively 21.2, 22.6, 24.6 and 27.9% for the four treatment groups mentioned above [305]. Despite the fact that when compared to neither active intervention, the combined treatment reduced the risk of all renal events by 54% (P<0.0001) [305], one can observe that URN (here defined as a composite of URN_{UACR} and URN_{eGFR}) are present in all treatment groups and can impact up to 27.9% of patients who do not receive any intensive intervention. It is particularly interesting to note that patients who did not receive any of the trial interventions were nonetheless treated according to the standards of care of their country (see Table 2 in Patel, et

al. for full details [183]). As a result, the percentage of URN in these patient groups indicates the presence of renal non-response to a wealth of different medications (blood pressure, glycemia, and lipid control).

Next, we will focus on a pre-specified secondary analysis of the Avoiding Cardiovascular Events through Combination Therapy in Patients Living with Systolic Hypertension (ACCOMPLISH) trial, pertaining to renal outcomes [194]. This double-blind randomised trial recruited 11,506 patients with hypertension and at high-risk of cardiovascular events from the U.S., Sweden, Norway, Denmark and Finland. 5,744 patients were randomized to receive the ACEi benazepril plus the calcium channel blocker amlodipine, while 5,762 patients were randomized to receive benazepril and the diuretic hydrochlorothiazide. The pre-specified renal outcome of ACCOMPLISH was a severe deterioration of kidney function defined as the first event of doubling serum creatinine concentration or end-stage renal disease as defined by an eGFR ≤15mL/min/1.73m^2. It is important to note that the ACCOMPLISH trial was terminated early (after 2.9 years of follow-up) because of superior efficacy of benazepril and amlodipine in reducing cardiovascular morbidity and mortality. Regarding renal events, 113 (2.0%) patients receiving benazepril and amlodipine developed the renal outcome, whereas 215 (3.7%) in the benazepril plus hydrochlorothiazide group developed the outcome over 2.9 years (HR: 0.52, 95% CI: 0.41 – 0.65, P<0.0001) [194]. Within patients with CKD (defined here as eGFR≤46mL/min/1.73m^2 in women and eGFR≤55mL/min/1.73m^2 in men) who had diabetic nephropathy, no difference in progression of chronic kidney disease was observed between the treatment groups (4.8% for benazepril and amlodipine vs. 5.5% for benazepril and hydrochlorothiazide – HR: 0.78, 95% CI: 0.38 – 1.56, P=0.48) [194]. Overall, the percentage of patients with severe URN_{eGFR} varies from 2.0 to 4.8% in patients receiving benazepril and amlodipine and from 3.7 to 5.5% for patients receiving benazepril and hydrochlorothiazide, with the highest percentages observed in patients with CKD and DN. The topic of unmet renal needs within the ACCOMPLISH trial is complex however. As described above, the benazepril and amlodipine regimen showed greater efficacy in reducing eGFR based events, and lead to fewer patients with URN_{eGFR} This scenario differed when considering albuminuria based events

and URN_{UACR}. As shown in Table 8 of Weber M.A. et al. 2010 publication [192], patients receiving benazepril and amlodipine had an increase in albuminuria as compared to the decrease observed in patients receiving benazepril and hydrocholorothiazide (92.2mg/dl vs. -20.1mg/dl respectively, $p<0.001$); thus, revealing the benefice of the benazepril and hydrocholorothiazide combination for decreasing albuminuria. The opposite capacities of both combinations in preventing worsening of one type of renal event but not the other, reveals once again the complex nature of DN, and highlights the notion that the incidence of URN_{eGFR} and URN_{UACR} are affected differently by the same drug.

The last study that we will consider in the present analysis is the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial [306]. Overall the ACCORD trial recruited 10,251 patients with T2D and at high-risk of cardiovascular events, from the U.S. and Canada. All participants were randomized to intensive or standard glycemic control. From the overall patient population, 4,733 participants were further randomized to intensive (n=2362), or standard (n=2371) blood pressure therapy to respectively reach systolic blood pressure targets of <120mmHg and <140mmHg. The classes of agents provided in the study for both treatment groups to achieve systolic blood pressure targets were: ACEi, diuretics, β-blockers, dihydropyridine and non-dihydropyridine calcium channel blockers, α-blockers, angiotensin II receptor blockers (ARBs), sympatholytics, α-/β-blockers, and the following combinations: a thiazide diuretic and a potassium-sparing diuretic, a β-blocker and a diuretic, an ACEi and a diuretic, an ARB and a diuretic, as well as a dihydropyridine calcium channel blocker and an ACEi. The renal end points evaluated in ACCORD were the incidence of renal failure, ESRD or need for dialysis, as well as micro- or macroalbuminuria onset. For ESRD or need for dialysis, no significant difference in event rate over 4.7 years of mean follow up was seen in between intensive therapy and standard therapy (2.5 vs. 2.4% respectively, P=0.93). Over the same follow up duration, the incidence of macroalbuminria was different between groups however (6.6% for the intensive group as compared to 8.7% for the standard group, P=0.009). It therefore appears that depending on the agents used, intensive and standard blood pressure

therapy do not change the percentage of patients developing severe URN_{eGFR}, but that the intensive blood pressure therapy significantly decreases the amount of URN_{UACR}.

The present analysis, conducted across seven studies and gathering a total of 88,773 patients (of which more than 25% have T2D) revealed three interesting insights.
First, it is important to note, that the difference in the proportions of patients quantified as having URN at the end of follow up, is mainly driven by the different nature of renal events under consideration. The proportion of URN_{eGFR} depends on the incidence of severe events such as ESRD, doubling of serum creatinine events, or need for dialysis. Those events represent the most severe stages of kidney function decline and their incidences are low since few patients reach those final stages over the duration of follow up. The proportion of URN_{UACR} is based on the incidence of new onset of microalbuminuria or macroalbuminuria. While the latter is associated with overt nephropathy, the former corresponds to the onset of albuminuria. This event being less severe, a greater proportion of patients is likely to develop it. Nonetheless, the significance of albuminuria onset should not be neglected due to its association with increased risk of cerebrovascular and macrovascular event [180] [263]. As a result, the key notion to remember from this discussion is not a comparison of the severity of one renal event versus that of another, or that the proportion of URN_{eGFR} more accurately depicts URN than that of URN_{UACR}. Rather, it is essential to understand that to appropriately capture patients who present URN one must consider both type of renal events. Such an approach will enable the identification of patients with URN_{eGFR} and/or URN_{UACR}. As we have seen, when combining these renal events, as is the case in the ADVANCE study described previously [305], one can observe that the percentage of Caucasian T2D patients with URN varies from 21.2 up to 27.9% depending on the use of intensive therapies or not (combined treatment reduced the risk of all renal events by 28% (95% CI 19–35%, $P < 0.0001$). Such figures clearly indicate that URN can affect up to a quarter of patients with T2D. As a concluding comment, and in light of the results presented in the *Manuscript* of this thesis, it must be stated that the URN quantification (which results from a novel classification algorithm – see Methods of *Manuscript*) identifies patients as having URN_{UACR} or URN_{eGFR} based on their individual renal progression slopes rather than the

incidence of renal events during follow up. The percentages of patients with URN can therefore not be directly compared with the ones from the current analysis.

Second, the present quantification of URN has revealed that their incidence is greater in patients with already existing renal condition at baseline. This is supported by the results from the INNOVATION, Steno-2, ACCOMPLISH and ADVANCE trials [182, 194, 201, 304]. These four trials demonstrated that for patients who already had an onset of renal disease at baseline (reduced eGFR or microalbuminuria), the incidence of renal outcomes was greater than in patients free of DN at baseline. These evidences support, that the later along the path of renal disease the medical interventions are provided, the more likely patients are to develop URN.

Third, the various clinical trials reviewed in this section have revealed clear statistical differences, regarding the incidence of renal outcomes in patients treated with different blood pressure treatment regimens. It is important to note; however, that even in the treatment arms presenting the lowest incidence of renal events, a small fraction of patients nonetheless develops URN. This indicates, that as of today, and despite the use of the best evidence-based recommended medications, certain patients are incompletely protected from developing URN over time. This encourages us to follow the path of precision medicine, a tool that could enable the early identification of those patients at risk of developing URN, and the possibility to better satisfy their medical needs.

C. Precision medicine to manage DN in a larger portion of subjects

The objective of this section is to provide background information on precision medicine and on its potential use in DN management. As we have seen, due to its limits in preventing the onset or progression of DN, and the manifestation of URN, the management of diabetic nephropathy is currently facing some barriers. Progress in fundamental research focused on refining our understanding of DN pathogenesis, and the development of novel therapeutic agents to optimize DN management are viable solutions to the current problem. While being promising, such investigations and research are often lengthy processes. The inclusion of precision

medicine in strategies to manage DN is a promising avenue to explore, and its applications could enter the realm of public health in the near future.

C.a. Precision medicine: definition

In the context of defining "precision medicine" it is useful to mention "personalized medicine", as these two terms have overlapping meanings. According to the U.S. National Research Council Committee on a Framework for Developing New Taxonomy of Disease [307], "personalized medicine" is an older term similar to "precision medicine". A distinction was made between the two terms however, to appropriately distinguish the notions they respectively carry. On one hand, "personalized medicine", as defined by the U.S. National Research Council, refers to a situation where therapeutic agents are tailored for specific individuals [307]. On the other hand, and as Euan A. Ashley describes it in a recent review on this topic [308], "precision medicine" aims to understand diseases at a deeper level by means of genomic tools in order to develop more targeted therapy. This definition is further refined in the Precision Medicine Initiative of the National Institute of Health where it is defined as an emerging approach for disease treatment and prevention that takes into account individual variability in genes, environment, and lifestyle for each person. Overall, and as Eaun A. Ashley summarizes [308], precision medicine is increasingly understood as being a technology-driven and participant-centred approach for disease treatment and prevention.

C.b. Precision medicine in the management of diabetic nephropathy: how and when

With the above definition of precision medicine in hand, we can now assess how this approach to disease treatment and prevention can be included in the framework of DN management. To better understand how and when the use of precision medicine can have the most impact for DN treatment, we must review four recurring elements, which have been discussed in this thesis.

First, and as we have seen, DN pathogenesis can occur via the individual or combined action of tubular and glomerular lesions. While some studies have presented cases of T2D patients with high albuminuria but no glomerular hyperfiltration [48, 173], other instances of patients with T2D, who reach CKD stage 3 while remaining normoalbuminuric have been reported [178]. As a result, not all patients with T2D and DN present equivalent forms of renal lesions with similar extent of glomerular and tubular damages. The introduction of precision medicine tools, that can characterize individual specificities in DN pathogenesis, could offer a means to better understand the specific component of DN that affects each patient. Individualized DN profiles for T2D patients could in turn offer the means to tailor management strategies to target more efficiently patient-specific sources of renal insults.

Second, and in the context of DN management, we reviewed the impact that lifestyle modifications can have on DN pathogenesis [187], on medication adherence [269], and as a result on the likeliness of developing URN. For that reason, lifestyle modifications, targeting diet, physical exercise, as well as consumption of alcohol and tobacco, are often included in DN management strategies [182, 187]. Lifestyle habits, choices, and behaviours, can be influenced by the socio-economic [309] and cultural environments [310] in which patients evolve. Since such environments can significantly affect patient behaviour, and their likeliness to adopt recommended lifestyle modifications [309, 310], it is crucial that individual variations in such factors be taken into consideration in DN management strategies. As a result, and to achieve a successful participant-centred approach to the treatment of DN, precision medicine tools should include individual variations in patient specific environments and lifestyle habits.

Third, we observed through the results of clinical trials, that the incidence of renal outcomes is greater in patients for whom DN onset already occurred. The proportion of patients experiencing a transition from micro- to macroalbuminuria in the Steno-2 trial (25% in intensive group vs. 46.3% in the regular group), demonstrates that while the intensive treatment significantly decreases renal outcome incidence, the rate of progression to macroalbuminuria remains elevated within microalbuminuric patients [182]. Such results were echoed by the ACCOMPLISH trial, which demonstrated a higher rate of ESRD and doubling of serum creatinine within patients with CKD stage 3 at the beginning of the study (2.0% for non-CKD stage 3

patients vs. 4.8% for CKD stage 3 patients). These results highlight the importance of achieving primary prevention in patients at risk of DN. Once disease onset occurs, not only are the chances for achieving complete reversal rare, but the risks of progressing to more advanced stages of the disease are increased. The inclusion of precision medicine tools, with the capacity to identify patients likely to develop DN before disease onset, would offer a window of opportunity for primary prevention and thus higher success rate for DN management treatments.

Moreover, it has been reported that genetic variability in genes directly involved in DN pathogenesis such as the ACE gene [96-98], or in loci which have been associated to eGFR decline [273], and presence of albuminuria [272], can affect the likeness to develop DN as well as the severity of DN. The existence of such genetic variants and their association with DN pathogenesis or traits associated with DN worsening, implies that specific genetic modifications can increase the risk of developing URN. Moreover, it is important to highlight that genetic factors can impact points one through three of the current discussion, due to their associations with resistant hypertension or glycemia [311] (which both affect DN pathogenesis), and lifestyle behaviours [312]. Using precision medicine tools to identify whether T2D patients, at risk of developing DN, present such genetic variations, could enable an early identification of patients at high-risk of DN onset, thus contributing to the objective of increasing primary prevention.

Based on the above considerations, DN stands as a complication of T2D which can benefit from precision medicine tools. Treatment strategies, which include data pertaining to individual genetic and lifestyle variabilities, could improve treatment outcomes in two major ways. First, early screening of T2D patients with genomic tools, prior to the manifestation of DN clinical symptoms, could allow detecting patients at risk of developing risk factors associated to DN, of developing actual DN, or at risk of having severe forms of DN. Such patients could begin treatment strategies and intensive life style modifications at earlier stages, with hopes of preventing the onset of DN. Achieving a greater proportion of primary prevention in DN would represent a key milestone, due to the current limited capacities of treatments to achieve disease reversal following its onset. Next, identifying the precise genetic makeup of each

patient could enable a better understanding of individual risk of progressing along the path of kidney function decline and/or albuminuria. Achieving this degree of precision in the characterization of renal risk of each patient is of interest as this could offer a means to tailor treatment therapies to the specific individual needs of each patient. As a result, the early use of precision medicine tools, based on a combination of genomic information and participant-centered approaches, could benefit DN management by increasing primary prevention and providing patient specific DN profiles enabling targeted therapy.

D. ADVANCE study

This section of thesis will provide further information on the ADVANCE clinical trial. This will enable reader to grasp a deeper understanding of the patient population from which are derived the results exposed in this thesis.

D.a. Background information

As mentioned in section *B.c.* of this thesis, the ADVANCE trial population was composed of 11,140 T2D patients, ≥55 years, with a history of major macrovascular or microvascular disease followed for a median duration of 4.4 years. Participants were randomized to a blood pressure control arm, as well as to a glucose control arm of which the details have been exposed previously. The main results of the ADVANCE trial demonstrated the efficacy of a treatment regimen based on an ACEi and a thiazide-like diuretic to reduce the combined incidence of macrovascular and microvascular events in a diabetic population [183]. In addition, the benefits of intensive glucose control based on a sulfonylurea anti-diabetic drug, on the reduction of vascular outcome was demonstrated [313]. Regarding renal events, the benefit of intensive glucose control alone or when combined with intensive blood pressure control were respectively proven [201, 305]. Following the conclusion of the ADVANCE follow-up period, 8,494 participants were followed for a median of 5.4 additional years in the ADVANCE-ON

study, to assess the long-term benefit or potential risks of intensive blood pressure and glucose control. The results of this post-trial follow up were presented in two publications. First, the long-term benefits of blood pressure lowering were shown by an attenuated, yet lasting difference, in the rate of death from all cause and from cardiovascular causes between the two treatment groups (HR 0.91, 95% CI: 0.84 to 0.99; P=0.03 and HR 0.88, 95% CI: 0.77 to 0.99; P=0.04 respectively) [314]. No such long-term benefits for these two events were shown for intensive blood glucose control [314]. Regarding renal outcomes, the ADVANCE-ON study revealed a lasting benefit of intensive glucose control on the rate of incidence of ESRD in patients randomized to gliclazide during the initial ADVANCE trial (HR 0.54, P<0.01) [315]. Interestingly, this lasting benefit was observed despite converging levels of Hba1c in patients from both treatment groups during post-trial follow up [315]. Furthermore, it is important to highlight that the lasting benefit of intensive glucose treatment were increased in participants with earlier stages of CKD at baseline (P=0.04) [315], thus demonstrating once again the benefit of starting DN management strategies before renal disease onset or progression. Overall, the ADVANCE and ADVANCE-ON studies have revealed valuable insights on the efficacy, safety, and lasting benefits of blood pressure control with an ACEi and diuretic and of intensive glucose control with a sulfonylurea on high risk of T2D in populations of diverse origin.

D.b. Genetic data in ADVANCE

In addition to clinical data, DNA samples were collected from ADVANCE participants at baseline. As of today, 4,089 Caucasian participants of the ADVANCE trial have been genotyped in our laboratory on Affymetrix Genome-Wide Human SNP Arrays 5.0 or 6.0 (Affymetrix, Santa Clara, California, USA), or with the UK biobank Axiom Array (UK Biobank, England). This data was reported as part of the CKDGen consortium in several publications [271-273]. This genetic data is also the basis of two studies for which the results are presented in later sections of this thesis.

E. Hypothesis

E.a. Genetic determinants of unmet renal needs in type 2 diabetic patients

The main objective of this thesis was to assess if T2D patients who experience a worsening of their renal condition over a period of five years, despite receiving evidence-based recommended medication, can be genetically distinguished from patients who experience an improvement of their renal condition. The type 2 diabetic population of this project is composed of the genotyped participants of the ADVANCE study. Within the context of this project, "worsening of renal condition" corresponds to one of the two following scenarios: a progression along the path of kidney disease as experienced through kidney function decline or albuminuria increase, or a stabilization at the highest CKD stages or albuminuria classes. Similarly, the opposite notion, "improvement of renal condition", corresponds to one of two scenarios: a regression along the path of kidney disease experienced through kidney function increase or decrease of albuminuria, or a stabilization at the lowest CKD stages and albuminuria classes. We therefore hypothesized that patients with and without renal non-response, as identified with a classification algorithm, present distinct genetic architectures identifiable by GWAS.

The secondary objectives of this thesis were based on the outcome of the main objective. If existent, the genetic determinants of renal non-response would be used to create a genetic risk score (GRS) of renal non-response. The capacity of this GRS to identify patients who benefit the most from ADVANCE trial treatments would then be tested. In addition, the capacity of this GRS to identify patients who do not experience an onset of albuminuria or kidney function decline would be evaluated. As a result, our secondary hypothesis was that genetic determinants of renal non-response identified by GWAS, can be used in a GRS to stratify T2D patients in groups most and least at risk of developing unmet renal needs.

Following the discovery of distinct genetic architectures between Caucasian ADVANCE participants, enabling their separation in groups with Slavic and Celtic geo-ethnic origins (see *Results*, *Publication 2*), the main objective of this thesis as well as its associated hypothesis

were refined. The presence of geo-ethnic variations in the genetic determinants of renal non-response between patients of Slavic and Celtic geo-ethnic origins were to be assessed. If existent, the capacity of geo-ethnic specific genetic determinants of renal non-response to stratify patients in groups with or without unmet renal needs, would be compared to that of non-geo-ethnic specific genetic determinants.

Overall, this thesis aims to evaluate the potential value of including genetic determinants of renal non-response in management strategies for DN.

Lastly, and in light of this research project being focused around the topic of genetics, it was appropriate to review and discuss the topic of gene-environment interactions. As detailed in *Publication 1* of the result part of this thesis, gene-environment interactions have the potential to considerably modulate results of genetic studies and must therefore be carefully considered when evaluating results of such studies. As a result, publication 1 key presents the key methods and approaches to explore such interactions.

Results

A. Publication 1

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Key Considerations and Methods in the Study of Gene-Environment Interactions

Paul H. G. Simon[1], Marie-Pierre Sylvestre[1], Johanne Tremblay[1], Pavel Hamet[1†]

1: CHUM Research Center, Centre hospitalier de l'Université de Montréal, Montréal, Québec

†: Correspondence to Dr. Pavel Hamet

Paul H G Simon: CRCHUM 900, rue St-Denis, 14ème étage, bureau R14.412, Montréal, Québec, Canada H2X0A9. Tel: +1 514 996 6663; email: paul.simon@mail.mcgill.ca

Marie-Pierre Sylvestre: CRCHUM, 850, rue St-Denis, 2ème étage, bureau S02.430, Montréal, Québec, Canada H2X0A9. Tel: +1 514 890 9000 poste 31473; email: marie-pierre.sylvestre@umontreal.ca

Johanne Tremblay: CR CHUM 900, rue St-Denis, 8ème étage, bureau R08.456, Montréal, Québec, Canada H2X 0A9. Tel: +1 514 890 8247; email: johanne.tremblay@umontreal.ca

Pavel Hamet: CRCHUM 900, rue St-Denis, 14ème étage, bureau R14.404, Montréal, Québec, Canada H2X0A9. Tel: +1 514 890 8246; email: pavel.hamet@umontreal.ca

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Abstract:

With increased involvement of genetic data in most epidemiological investigations, gene–environment (G × E) interactions now stand as a topic, which must be meticulously assessed and thoroughly understood. The level, mode, and outcomes of interactions between environmental factors and genetic traits have the capacity to modulate disease risk. These must, therefore, be carefully evaluated as they have the potential to offer novel insights on the "missing heritability problem", reaching beyond our current limitations. First, we review a definition of G × E interactions. We then explore how concepts such as the early manifestation of the genetic components of a disease, the heterogeneity of complex traits, the clear definition of epidemiological strata, and the effect of varying physiological conditions can affect our capacity to detect (or miss) G × E interactions. Lastly, we discuss the shortfalls of regression models to study G × E interactions and how other methods such as the ReliefF algorithm, pattern recognition methods, or the LASSO (Least Absolute Shrinkage and Selection Operator) method can enable us to more adequately model G × E interactions. Overall, we present the elements to consider and a path to follow when studying genetic determinants of disease in order to uncover potential G × E interactions.

Introduction:

In recent years, the advent of Genome-Wide Association Studies (GWAS) and of their meta-analyses has enabled the discovery of many genetic loci associated with complex diseases[1]. Combined to familial studies, these results have broadened the understanding of the genetics of complex diseases such as type 2 diabetes (T2D) and hypertension[2,3]. While offering novel insights on incremental risks for individuals bearing those genetic traits, these remarkable progresses have repeatedly been faced with the concept of "missing heritability"[4,5]. New challenges therefore lie in our capacity to understand the underlying factors responsible for this missing heritability[6]. Unlike monogenic diseases, complex traits are to a much greater degree a function of environment. The existence of gene–environment (G × E) interactions, therefore, stands as a plausible way to expand our current understanding of heritability. In this review, we will explore some key findings that reveal how the environment can modulate genetic impact, and we will summarize methods pertinent to the study and measure of G × E interactions. For simplicity and general comprehension, G × E interactions will be considered in the broad sense of the term throughout this review, with the objective of demonstrating that the effect of a gene depends on the level of an environmental factor. For a more in-depth description, we advise readers to review the work of Thomas[7], which presents a tutorial for epidemiological study design and explores specific genetic and environmental interactions in the paradigm of current GWAS studies.

Gene-environment interaction: definition and importance

In its simplest term, a G × E interaction refers to a relationship between a genetic variant present in a given population and an environmental factor to which this population is exposed[8]. From this relationship, and as described by Ottman[9], four risk strata can be defined when assuming a categorical outcome of yes/no:

1. The stratum most at risk (r_{11}) where both the environmental factor and genetic variant are present.

2. Two intermediate risk strata—one where the environmental factor is present but the genetic variant absent (r_{10}).
3. The other where the environmental factor is absent but the genetic variant present (r_{01}).
4. The stratum least at risk (r_{00}) where neither the environmental factor nor genetic risk is present.

The importance of G × E interactions is best highlighted when one considers the capacity of an environmental factor to exacerbate the risk of a population bearing a deleterious genetic variant. Schulz et al.[10] perfectly exposed the importance of such an interaction when they reported the increased prevalence of T2D in Pima Indians living in the United States as compared to Pima Indians living in Mexico. The high prevalence of T2D in the Pima Indians has been reported in 1971[11], thus suggesting a genetic predisposition for this population to develop T2D. By demonstrating that a same population presented varying prevalence of T2D when living in different environments, Schulz et al. revealed the basis for a G × E interaction at work within the Pima Indian population.

When considering the context of G × E interactions, it is important to note that the notion of "environmental factor" refers to any external environmental pressure or stress affecting an individual with the genetic variant thought to interact with the said pressure or stress. Such environmental factors also include factors such as the gestational environment[12], the intestinal microbiota[13,14], medication[15], occupational exposure, and socioeconomic factors. In the current review, we extend the traditional definition of G × E to include nongenetic internal factors (such as lifestyle behaviors).

Lifestyle behaviours and their importance in G x E interactions

Being a resultant of both genomic and environmental factors, the phenotypic manifestation of most complex traits must be appropriately deconstructed if G × E interactions are to be correctly identified. In this section, we will consider the study of G × E interactions in the framework of cardiovascular disease. When evaluating if modifiable risk factors (such as diet, smoking, physical activity, or other lifestyle behaviors) predict/explain the risk for

cardiovascular events, one must consider the possible impact of interactions between these modifiable risk factors and a genetic trait beyond and above the sum of each of their independent effects. This is well illustrated in the PRIME study[16], conducted in different socioeconomic groups of middle-aged men in France and Northern Ireland, which investigated the capacity of lifestyle behaviors (smoking, physical activity, alcohol consumption, and diet) to explain total mortality and cardiovascular incidence when considered in isolation or combined to other cardiovascular risk factors. This study, while demonstrating that the median residual contribution of lifestyle behaviors could explain 28% of total mortality and 41% of cardiovascular incidence (when considered alone) and up to a maximum of 38% and 61%, respectively (when considered along with other cardiovascular risks), could not explain a substantial proportion of these differential risks. The question of a missing genomic contribution associated with the lifestyle behaviors assessed in this study and potentially explaining the missing part of this risk gradient by itself, through a gene–environment addition or through a G × E can therefore be raised (i.e., G + E as distinct from G × E). Cardiovascular disease, like most other complex diseases, is associated with the comorbidity of many complications. These complications have the potential to represent a significant polygenic contribution to the pathogenesis of complex diseases, therefore, reinforcing the importance of assessing which risk factors may have genomic traits associated with the disease (hence offering the potential for G × E interactions) versus risk factors that are purely environmental.

To put the above example in perspective, we shall consider 2 recent studies that go one step further in their assessment of G × E interactions. Whereas Woodside et al.[16] categorized smoking as a lifestyle behavior and stratified the patients of the PRIME study in various risk strata based on their cigarette consumption to assess variations in risk, Young et al.[17] decided to measure the extent to which body mass index (BMI)-related single nucleotide polymorphisms (SNPs) interacted with smoking by means of logistic regression models. In their study evaluating the interacting effects of smoking and genetics on obesity in adolescents, they identified 2 SNPs—rs2112347 (POC5P for interaction term P=0.04) and rs57312 (MC4R P = 0.05) in Americans of European descent—as well as 1 SNP rs151417 (TNNI3K P = 5.9E-05) in

Americans of Hispanic origin. Similarly, in our recent study[18], we have observed a strong interaction between smoking and rs1799963 (polymorphism in 3'UTR of prothrombin gene) in subjects of the "undetermined" ischemic stroke category by making use of a regression model. This evidence suggests that at least in some situations, genetic screening should be considered for inherited thrombophilia in ischemic stroke patients. These examples illustrate how without the notion of interaction one would assume that smoking is responsible for an additive effect in risk and would be unable to explain a significant proportion of the differential risk between patients. The two previous studies highlight that it is in fact the interacting nature of specific genetic traits with environmental factors that are the cause of variations in risk and that when adequately identified improve our understanding of differential risk. We will observe in a subsequent section of this review how the genetic associated with certain lifestyle behaviors can also be the source of a G × E interaction

Heterogenicity of complex traits: one disease can hide the genes of another

One of our earlier studies[19] illustrates the extent to which a phenotypic deconstruction of comorbid traits can enable the identification of novel genetic loci. In short, we extended sequential oligogenic linkage analysis routine-corrected logarithm of the odds score through density analysis and permutations to rank multigenerational families from this study's population according to their distinct contributions to several traits. This methodology enabled us to remove the hindrance of genetic heterogeneity and uncover causal genomic factors contributing to a trait variance that would have been missed by sequential oligogenic linkage analysis routine analysis. As a result, we were able to identify numerous and previously unreported quantitative trait loci associated with various traits present in patients with many comorbidities affecting metabolic phenotypes of hypertension as discussed further below.

We can illustrate the importance of deconstructing complex diseases into their comorbid components in order to identify their genetic determinants by analyzing two additional studies where stratification by BMI enabled such findings. We identified and confirmed the increased importance of a hypertension locus for which the logarithm of the

odds signal was greater in obese hypertensive individuals from extended French–Canadian families as compared to non-obese hypertensive individuals[20]. Similarly, Perry et al.[21] were able to identify two previously unreported loci for T2D in European populations by conducting discovery and replication GWAS analyses in patients stratified on their BMI.

The genetic component of a disease can penetrate earlier than the environmental impact

While we previously highlighted the importance of including the genetic determinants of lifestyle behaviors and modifiable risk factors, this section will be the first of several to expose the fact that they are not the sole explanation to the missing heritability problem. It is important to note that due to the fact that genetic factors have more impact at a younger age, G × E interactions are not static and will vary through the lifespan of an individual. Our studies in French–Canadian families[19,20] demonstrated that the penetrance of hypertension, in families ascertained for their genetic predisposition, appears at younger age than in the general population living in the same geographical area, as illustrated in Figure 1. We attribute this to the fact that genetic contribution (enriched in ascertained families) leads to an early penetrance of the disease, while in the general population, environmental exposure requires a prolonged period for hypertension to appear. In the context of the ADVANCE clinical trial, Zoungas et al.[22] reported a series of noteworthy findings, which highlight the importance of considering the impact of age at diagnosis and duration of disease to reveal age-specific trends. Among several findings that demonstrated the association of diabetes duration with the risk of macro- and microvascular events as well as death, this study revealed an interaction between the earlier age of onset as a specific risk of microvascular events. Similarly, in the context of youth with T2D, the TODAY Study Group[23] reported an increased prevalence of hypertension and microalbuminuria among subjects aged between 10 and 17 years compared to older subjects. These two studies highlight the fact that the genetic components of specific phenotypes are at work early on in the life of patients, resulting in the need for investigators to consider possible G × E interactions at an early stage. Moreover, one must consider that

environmental factors have the capacity to vary considerably with time and as a result affect interactions throughout the life of patients. As mentioned previously, the microbiota can be considered as an extension of the environment as it is heavily modulated by external factors such as diet and medication. The state of the microbiota changes considerably throughout life[24], offering potential insights as to why the prevalence of disease can differ between age groups and thus re-emphasizes the importance of considering varying degrees of G × E interaction for different age groups.

Environment as age and sex: the importance of distinguishing epidemiological strata to reveal gene-age and gene-sex interactions

Further to preceding discussion, one must note that it is not always age that matters but time elapsed since exposure to environment as well. This is correlated with age, but age only stands as a marker of the period of latency that is under consideration.

In their study on gene–age interaction, Simino et al.[25] set off to explore the impact of genetic architecture on blood pressure (BP) in 9 different studies from well-established consortia (CHARGE, GBPgen, and ICBP). They used a systematic and large-scale approach to identify age-dependent effects of 20 novel loci on BP and revealed a large age interaction with an opposite effect in young and old. Thus, an index SNP in gene CASZ1, rs880315 was significantly associated with an increase of systolic BP in young and its decline in elderly, a counterintuitive finding. The authors report that the failure of previous studies to detect a change in genetic effects over time lied in the methodology of these studies (which relied on meta-analyses of GWAS that included age as a continuous covariate) due to the fact that they adjusted for age instead of stratifying by age or including it in an interaction term to allow the effect of the gene to differ by age strata that would have otherwise been missed.

Through a comparable approach but this time focused on stratification by sex to reveal gene–sex interactions, we previously demonstrated the importance of considering sex-specific gene effects[26]. In our study, we reported the importance of the association of rs575121 (on chr12) with the highest systolic pressure in men bearing the GG genotype as compared to an

association with the lowest systolic pressure in women bearing the same GG genotype within the same families (the highest systolic pressure being associated with women carrying the AA genotype). This scenario illustrates how a SNP has the potential to be deleterious in one sex while being protective in another one. Lastly, and in a similar fashion as in the gene–age interaction described above, which did not reveal any interaction when the population was not stratified by age, it is of paramount importance to note that in our study, the association signal in both sex combined was not significant after adjustment. Moreover, the association between rs575121 and systolic pressure would have been missed if sex-specific analysis had not been conducted.

The study of Winkler et al.[27] further emphasizes the importance of considering gene–age (G × Age) and gene– sex (G × Sex) interactions in the framework of meta-analysis studies. In this study, the authors meta-analyzed 114 studies with the goal of identifying age- and sex-specific effects of genetic variants on BMI and waist-to-hip ratio adjusted for BMI (WHRadjBMI). A meta-analysis of 320,485 individuals of European descent screened specifically for age-specific, sex-specific, and age-sex–specific (G × Age × Sex) effects that vary between men and women were completed in this study via the use of 4 sex–age strata (men ≤ 50 years, men > 50 years, women ≤ 50 years, and women > 50 years). It revealed 15 loci for BMI (11 previously established and 4 new ones) and 44 loci for WHRadjBMI (27 established and 17 new ones). From the 15 BMI loci, 11 showed larger effects in individuals of less than 50 years while no sex-dependent effects were identified for BMI. From the 44 loci reported for WHRadjBMI, 28 showed a larger effect size in women, whereas 5 showed a larger effect size in men and 11 showed opposite effects between sexes. This highlights the capital importance of not only conducting meta-analyses stratified by age but also by sex in order to reveal sex-age–specific G × E, which have the potential of remaining hidden when study populations are investigated using only age and sex as covariates.

Medication as an environment: the importance of distinguishing epidemiological strata to reveal drug-gene interactions

Between 2005 and 2009, we described several genetic loci on chromosome 16 associated with metabolic syndrome phenotypes[19,28]. This locus was later discovered to contain the FTO gene, which in addition to being confirmed by a large number of GWAS was shown to have polymorphism rs9939608 of which the impact on fat mass is modulated by physical activity[29]. Nevertheless, these studies associated the FTO gene with obesity, and only our group reported its association with BP[28]. We believe that the lack of association of FTO to BP as reported in previous studies was linked to the study populations, in which medication had not been withdrawn. In our study[15], we discontinued medication in our population of French–Canadians for 4 weeks. We reported the significant association of rs7196791 (on chr16) with high systolic and diastolic BP in patients not receiving medication for hypertension as compared to nonsignificant association in same subjects when receiving antihypertensive medication (Figure 2). This study reveals the importance of considering medication as a major environmental factor capable of overshadowing existing associations.

The basis for drug–gene interactions is based on individual- or population-specific mutations in the CYP gene family, pertinent to the pharmacokinetic modulation of drug metabolism principally in the liver. A recent study from Verbeurgt et al.[30] set off to identify the proportion of drug interactions due to drug–drug (D × D), drug–gene (D × G), and drug– drug–gene (D × D × G) interactions in 1,053 patients of known CYP genotypes. The vast majority of reported interactions were D × D (66.1%), but a considerable 33.9% were D × G and D × D × G, thus highlighting the importance of such interactions. Furthermore, this study reported that when compared with D × D alone, D × G and D × D × G had the capacity to increase the total number of potentially clinically significant interactions by 51.3%. This study reveals the high potential for scenarios, such as the one we reported[15], to be present in many patient populations thus demonstrating the extent to which D × G interactions should be a concern in the study of G × E interactions. As indicated by the two studies described above, medication, due to its capacity to interact with genetic traits in patient populations, or to make these interactions inaccessible to evaluation, stands as an important environmental barrier to

consider in the framework of studies aiming to explain the missing heritability problem by means of exploring G × E interaction.

Exploring the extent to which physiological conditions can dynamically modulate significance of genomic determinants

In order to illustrate the importance of the link between genetic traits and physiological modulation of specific phenotypes, we assessed BP variations in 384 sib-pairs from a founder population of French–Canadian families ascertained by early onset hypertension and dyslipidemia[31]. The purpose of this study was to measure BP, as well as 3 hemodynamic and 7 neuroendocrine phenotypes in these subjects when lying in a supine position as well as when standing upright in order to assess the acute influence of change in posture on the dynamics of genetic linkage. This led us to identify a set of SNPs for which the effect of posture had considerable effects on their linkage signals. For instance, the linkage of rs842873 with stroke volume was significant in the supine position ($P = 3.5 \times 10^{-3}$) while not significant during the standing position ($P = 0.22$). In contrast, the level of linkage of rs10494478 with BP was barely significant in the supine position ($P = 0.04$) but revealed to be significant in the standing position ($P = 4.0 \times 10^{-4}$). The strength of this study lies in its capacity to present the dynamic nature of the genetic architecture of BP and intermediate phenotypes during orthostatic stress. As a result, this study highlights the importance of considering physiological environment of specific phenotypes when investigating genetic linkage for these phenotypes.

It is important to specify that modulations of genetic linkages between genetic traits and specific phenotypes, when generated by mental stress, have far more complex ramifications than when generated by physical stress. We explore here the importance of considering the genomic components of lifestyle behaviors due to their capacity to modulate the stress response and several other complex phenotypes due to the over-representation of the genetic components of these phenotypes in the neural synapse. The following study presents the results of an investigation, which set off to assess the extent to which substance use habits can affect the heart rate and BP following a mental stress. We submitted members

of our French–Canadian families to a math mental stress while measuring their heart rate and BP measures pretest, during the test and post-test[32]. The stress response was defined as the difference in values of BP math test and baseline which correlated with BMI, BP, and alcohol and tobacco habits. This study reported that the neural synapse and its plasticity is a shared interface behind the study traits (substance use, obesity and responses to mental and physical stress, and hemodynamic traits).

Genetic contribution of complications requires the presence of disease

We will briefly discuss here the important notion that G × E interactions within the framework of one disease are in certain cases conditional on the presence of another disease. In order to illustrate this point, we will turn to the recent study of Teumer et al.[33] that presents new results from the CKDGen consortium. In addition to providing confirmation that the gene CUBN is associated to the urinary albumin to-creatinine ratio in an overall sample of 51,886 individuals of European ancestry, this study was able to identify gene-by-diabetes interactions by analyzing separately the 5,825 individuals with diabetes and the 46,061 individuals without diabetes from its overall sample. Through this method, variants in HS6ST1 and near RAB38/CTSC were shown to demonstrate a genetic effect on urine albumin creatinine ratio in individuals with but not without diabetes. These results were further ascertained in a biological knockout model of the protector gene RAB38, which on its own has no consequence on glucose level nor albuminuria, but when present in animals made diabetic by streptozotocin, results in a rapid and massive appearance of albuminuria. This study puts forward the crucial notion of gene-by-disease interaction in the presence of specific environmental factors, whereby the association of a genetic locus with a phenotypic trait is conditional on the presence of a disease.

Methods to study gene-environment interactions and their limitations

To be successful, genetic discovery needs to rely on statistical strategies that embrace, rather than ignore, the complexity of the genotype to phenotype relationship, including G + G and G × E interactions[34]. This is a tremendous challenge because the complex nature of genetic architecture calls for statistical methods that considers a very large number of SNPs (and their

interactions) simultaneously, in many cases exceeding the number of observations (the "curse of dimensionality"[35,36]). Conventionally, G + E and G × G interactions are detected using regression modeling[37]. A single model is estimated for each SNP and its interaction with another factor. Hence, discovery of G + E and G × G interactions usually requires large datasets because the statistical power needed to detect an interaction is usually low and is further reduced by the severity of the multiple testing corrections, which need to be applied due to the considerable number of tests performed. The methodological challenge is further exacerbated when higher-order interactions (interactions between multiple SNPs) are considered and the number of combinations of SNPs to consider grows exponentially[38]. The case-only study was designed specifically to identify G × E and gene–gene interactions[39,40] that can be applied to GWAS[41]. While the case-only design has a clear statistical power advantage over case–control designs, its validity hinges on the independence assumption between the genes and environment factors at the population level, which is difficult to assess[37,39].

Novel methods to identify G × E and G × G interactions can be classified into 3 strategies: (i) filtering, (ii) data mining, and (iii) regression-based approaches to detect interactions[42]. The aim of filtering methods is to reduce the computational burden of an exhaustive testing of interactions by screening the candidate SNPs and environmental factors to identify informative or promising variables to be subsequently tested or modeled[42,43]. Most widely used examples of such methods include the ReliefF algorithm[44] and its extensions[45–47] and entropy-based[48] and synergy-based methods[49]. Data mining approaches "let the data speak" by detecting patterns or selecting the best model without making a priori assumptions about the functional form of the models[42,50]. While these methods usually avoid the issue of multiple testing by using a global null hypothesis, they are often coupled with a follow-up analysis to estimate the optimal model and produce statistical inference[42]. Examples of data mining methods include pattern recognition methods[51,52], decision-tree methods[53,54], multidimensional reduction methods[55,56], and partitioning methods[57–59]. Regression-based approaches extend the conventional regression-based strategies to reduce their parametric assumptions and improve their computational efficiency. An advantage of such methods is their

explicit modeling and testing of the interaction terms[42]. Examples of such methods include exhaustive search methods such as penalized regression[60,61] or the LASSO (Least Absolute Shrinkage and Selection Operator) methods[62,63].

While in-depth discussion of the methods[7,37,40,42] and comparisons between selected techniques based on simulations exist[64–67], there is currently no consensus on the optimal method to detect interactions, nor a set of criteria on how to choose the best method given the data at hand.

Future directions

While the genetic determinants of disease are useful to study, due to their presence in an early life, the modification of phenotypic variants and penetrance of disease as generated by the environment are worth considering. As we have seen, this can be achieved by numerous methods reaching from stratification of populations (by age, gender, or medication status) all the way to sophisticated statistical models. Our future directions should be focused not only on elucidating the problem of missing heritability but also solving the situations of hidden heritability for which we are proposing some of the solutions here.

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Tables and Figures

Figure 1:

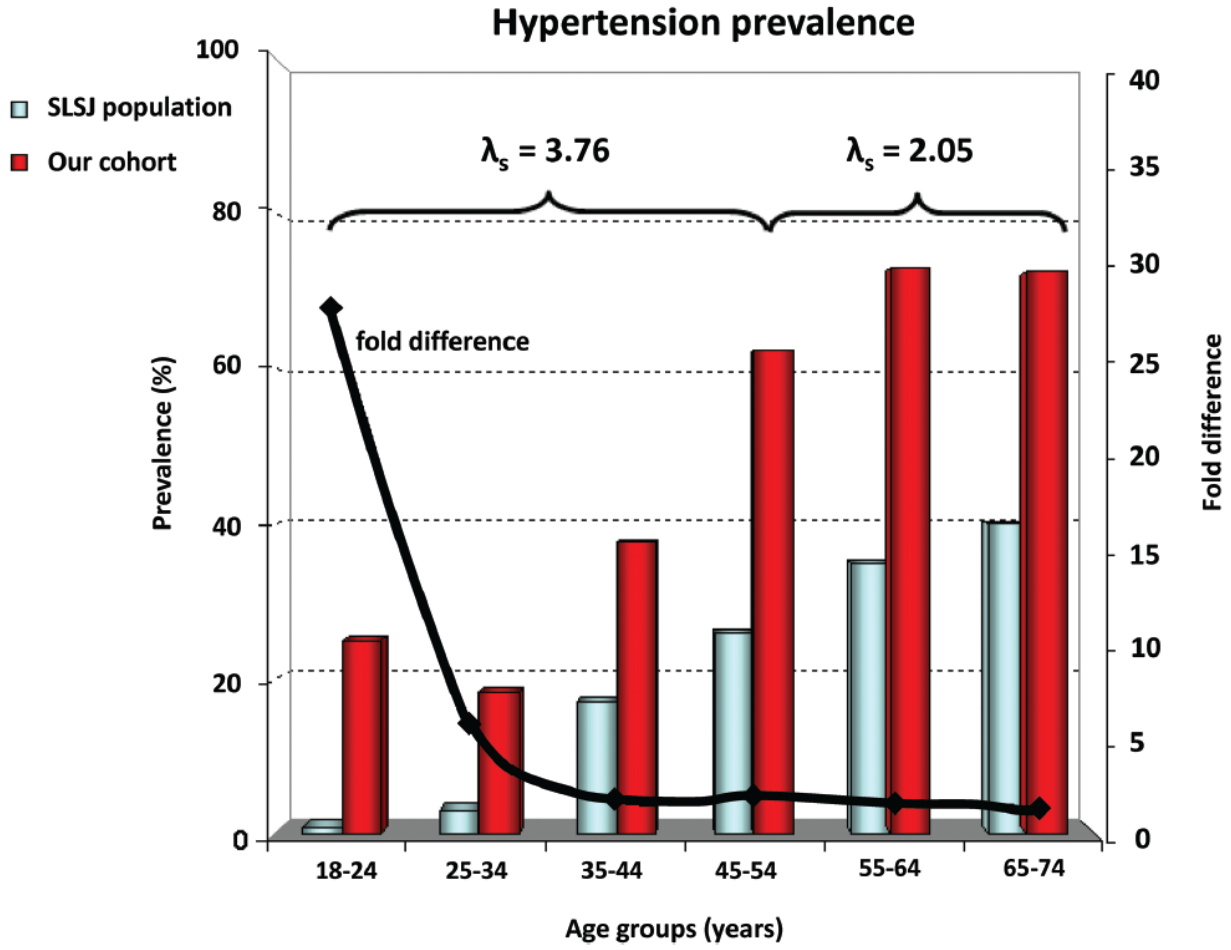
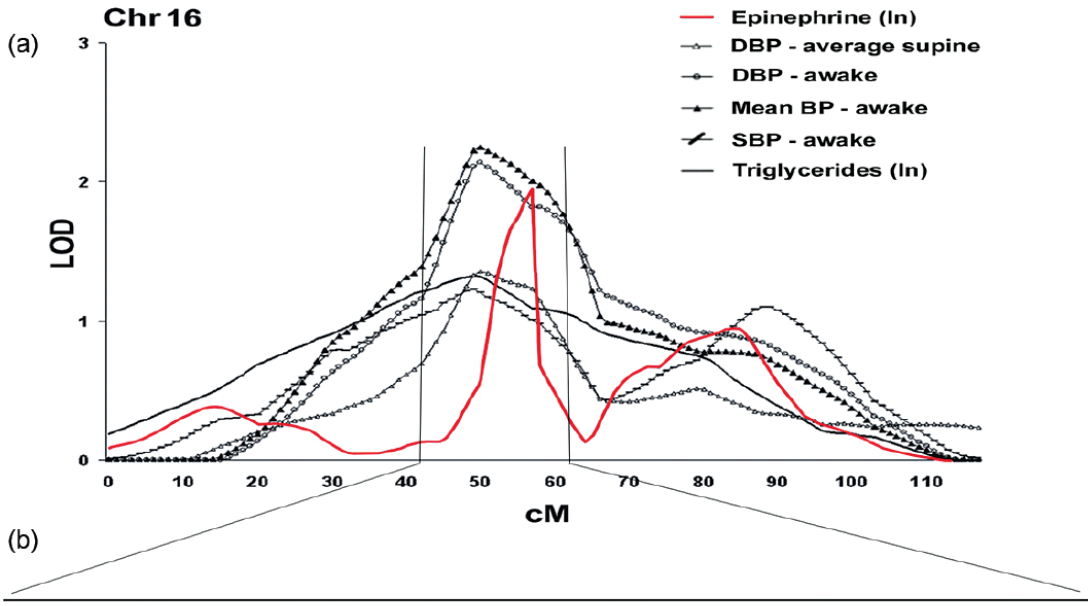


Figure 1: The sibling risk ratio (λs) of hypertension per age group in French–Canadians. We observed a strong familial clustering in younger individuals, the highest λs being for cases occurring between 18 and 24 years, ~27. Augmented λs for hypertension reflects increased genetic susceptibilities of our cohort, compared to the general population of Saguenay–Lac-St-Jean (SLSJ). However, the siblings may differ by environmental risk factors for hypertension, thus increasing the risk of hypertension independently of genetic effects (unpublished data from ref. Hamet et al.[19]).

Figure 2:



Phenotype	rs10492790	rs7196791	rs7202468	rs8047316	rs10521237
SBP without medication	0.01	0.009	0.02	0.01	0.02
DBP without medication	0.003	0.04	0.01	0.02	0.007
SBP with medication	0.04	0.7	0.04	0.9	0.06
DBP with medication	0.2	0.3	0.2	0.7	0.2

Figure 2: (a) Linkage analysis of chromosome 16 region shows significant and suggestive LOD scores only for phenotypes in the absence of anti-HT medication. (b) FBAT association analyses of SNPs within the peak of linkage on chromosome 16 for systolic and diastolic blood pressures with anti-HT medication and in the absence of anti-HT medication. Majority of SNPs within this region show a significant association with blood pressures only in the absence of anti-HT medication (data from ref. Noël et al.[15]).
Abbreviations: BP, blood pressure; DBP, diastolic blood pressure; FBAT, family-based association test; HT, hypertension; LOD, logarithm of the odds; SBP, systolic blood pressure; SNP, single nucleotide polymorphism.

Thematic link between publication 1 and 2

Publication 1 offered a detailed explanation of the basis for gene-environment interactions, reviewed their potential impact on GWAS studies as well as their capacity to explain the missing heritability paradigm and provided insights on novel methods used to investigate such interactions. In the next section of this thesis dedicated to Publication 2, novel findings from our team regarding the capacity of principal component analysis to stratify Caucasian ADVANCE participants according to their geo-ethnic origin and the impact of specific loci on key phenotypes will be presented. The identification of a distinct genetic architecture between patients of different geo-ethnic backgrounds provides explanations to phenotypic variations observed in Caucasian patients of the ADVANCE study. Specifically, geo-ethnic-specific loci were found to be associated to age of onset of diabetes. Of these geo-ethnic-specific loci, PROX1/PROX1-AS1 genes (rs340841) had the highest impact, with the CC genotype being associated with a 4.4 year earlier onset of T2D in ADVANCE patients of Slavic geo-ethnic origins living or not in countries with predominant Slavic populations. As a result, Publication 2 offers a concrete example of the importance of accounting for geo-ethnic variations in study populations categorized under a common ethnic background as a mean to identify genetic variants with geo-ethnic specific effects.

B. Publication 2

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Prox1 gene CC genotype as a major determinant of early onset of type diabetes in Slavic study participants from Action in Diabetes and Vascular Disease: Preterax and Diamicron MR Controlled Evaluation study

Pavel Hamet[a,b,†], Mounsif Haloui[b], François Harvey[b], François-Christophe Marois-Blanchet[b], Marie-Pierre Sylvestre[b], Muhammad-Ramzan Tahir[b], Paul H.G. Simon[b], Beatriz Sonja Kanzki[d], John Raelson[b], Carole Long[b], John Chalmers[e], Mark Woodward[e], Michel Marre[f], Stephen Harrap[g], and Johanne Tremblay[a,b,†]

a: Department of Medicine, Gene Medicine Services, Université de Montréal, CRCHUM,

b: Department of Medicine, Université de Montréal, CRCHUM,

c: Department of Social and Preventive Medicine, Université de Montréal, CRCHUM,

d: Department of Software Engineering and the Information Technology, École de Technologie Supérieure, Montréal, Québec, Canada,

e: The George Institute for Global Health, University of Sydney, Sydney, New South Wales, Australia,

f: Hôpital Bichat, Claude Bernard, Université Paris 7, Paris, France and

g: Royal Melbourne Hospital, University of Melbourne, Melbourne, Victoria, Australia

Correspondence to: Pavel Hamet, OQ, MD, PhD, FRCPC, Centre hospitalier de l'Université de Montréal, 900 Saint-Denis, roomR14.404, Montre´ al, Que´ bec, Canada, H2X 0A9. Tel: +1 514 890 8246; e-mail: pavel.hamet@umontreal.ca

*Pavel Hamet and Johanne Tremblay are members of College International de Recherche Servier and Johanne Tremblay received honoraria for presentations of ADVANCE at scientific meetings.

†: Pavel Hamet and Johanne Tremblay contributed equally to this work.

Abstract

Background: The prevalence of diabetic nephropathy varies according to ethnicity. Environmental as well as genetic factors contribute to the heterogeneity in the presentation of diabetic nephropathy. Our objective was to evaluate this heterogeneity within the Caucasian population.

Methods: The geo-ethnic origin of the 3,409 genotyped Caucasian type 2 diabetes (T2D) patients of Action in Diabetes and Vascular Disease: Preterax and Diamicron MR Controlled Evaluation was determined using principal component analysis. Genome-wide association studies analyses of age of onset of T2D were performed for geo-ethnic groups separately and combined.

Results: The first principal component separated the Caucasian study participants into Slavic and Celtic ethnic origins. Age of onset of diabetes was significantly lower in Slavic patients ($P=7.3x10^{-20}$), whereas the prevalence of hypertension ($P=4.9x10^{-31}$) and albuminuria ($P=5.1x10^{-9}$) were significantly higher. Age of onset of T2D and albuminuria appear to have an important genetic component as the values of these traits were also different between Slavic and Celtic individuals living in the same countries. Common and geo-ethnic-specific loci were found to be associated to age of onset of diabetes. Among the latter, the PROX1/PROX1-AS1 genes (rs340841) had the highest impact. Single-nucleotide polymorphism rs340841 CC genotype was associated with a 4.4 year earlier onset of T2D in Slavic patients living or not in countries with predominant Slavic populations.

Conclusion: These results reveal the presence of distinct genetic architectures between Caucasian ethnic groups that likely have clinical relevance, among them PROX1 gene is a strong candidate of early onset of diabetes with variations depending on ethnicity.

Keywords: albuminuria, diabetic kidney disease, environment, ethnic groups, genetics

Abbreviations: ADVANCE, Action in Diabetes and Vascular Disease Preterax and Diamicron MR Controlled Evaluation; CKD, chronic kidney disease; CKD-EPI, Chronic Kidney Disease

Epidemiology Collaboration; eGFR, estimated glomerular filtration rate; GWAS, genome-wide association studies; MAF, minor allele frequency; PCA, principal component analysis; SNP, single-nucleotide polymorphism; T2D, type 2 diabetes; UACR, urinary albumin–creatinine ratio

Introduction

The incidence of type 2 diabetes (T2D) is increasing even in younger study participants in both industrialized and economic transition countries totaling 415 million study participants worldwide in 2015[1]. The major increased risk of mortality associated with both type 1 diabetes and T2D arises from diabetic nephropathy[2-4], which is estimated to affect about one-third of individuals with diabetes.

Different genetic architectures, such as variations in allele frequencies and linkage disequilibrium structure have long been noted between populations of different racial origins and the ability of this hidden population structure to confound genome-wide association studies (GWAS) findings has been well documented[5-8]. Moreover, it is known that GWAS using populations of differing racial backgrounds may help identify different sets of associated genes for complex diseases and drug responses[9-11]. Differences in genetic risks have been shown among Caucasians, Africans, and Asians for T2D[12-16] and for chronic kidney disease (CKD)[17-19]. Evidence exists for population substructure within Caucasian samples as well[20-21].

It is also well established that both environmental and genetic factors contribute to the occurrence of hypertension, diabetes, and CKD[22-25]. To distinguish the effects of environmental and lifestyle factors from genetic effects in explaining phenotypic differences in the development of renal complications of T2D, we studied T2D study participants of Caucasian origin and European descent from the Action in Diabetes and Vascular Disease: Preterax and Diamicron MR Controlled Evaluation trial (ADVANCE)[26]. Study participants were recruited from a range of European countries as well as from countries of European settlements such as Canada, Australia, and New Zealand. Using principal component analysis (PCA), we identified two main ethnic genetic profiles (Celtic and Slavic) within the ADVANCE Caucasian study participants. To assess the relative effects of genetic and environmental factors, we compared study participants with a Slavic genetic profile living in countries with predominantly Celtic populations with individuals with a Slavic genetic profile living in predominantly Slavic countries of Europe. Significant differences between Slavs living in Slavic and Celtic countries would

suggest an environmental/lifestyle effect, whereas no differences between Slavs living in Celtic or Slavic countries would support an impact of genetic influence.

As age of onset of T2D appears to be more dependent on genetic than environmental factors, we performed GWAS for 'age of onset of T2D' within the two ethnic groups separately and for the combined sample.

Methods

Sample

In total, 11 140 participants recruited from 215 centers in 20 countries who were 55 years or older and had T2D since the age of 30 years or older were enrolled in ADVANCE, a factorial randomized controlled clinical trial of blood pressure (BP) lowering and intensive glucose control. All participants were ascertained for high outcome risk according to one of the following criteria: a history of major macrovascular or microvascular disease or diagnosis of T2D 10 years prior to entry in study or presence of another major risk factor for vascular disease, including smoking, dyslipidemia or microalbuminuria, or being 65 years or older. Detailed study methods have been published elsewhere[26].

Approval to conduct the trial was obtained from the ethics committee of each study center, and all participants provided written informed consent for the study conduct and a specific, separate consent for genetic sub-study. Genotyping was performed only in patients who consented to the genetic sub-study.

Complication phenotypes

Several phenotypes associated with diabetes and its complications were determined at baseline in each study participants by the ADVANCE study team. These included age at baseline, age at diagnosis of T2D, duration of diabetes at baseline, BMI, blood glucose, glycated hemoglobin, treatment for hypertension, heart rate, and SBP and DBP. Renal phenotypes included estimated glomerular filtration rate (eGFR), in ml/min per 1.73m2, estimated from

serum creatinine levels using the CKD-Epidemiology Collaboration formula[27] and albuminuria, expressed as a ratio of urinary albumin and creatinine in mg/mg [urinary albumin–creatinine ratio (UACR)].

Genotyping

In this study, we have genotyped 3,629 Caucasian study participants using the Affymetrix Genome-Wide Human SNP Arrays 5.0 or 6.0 (Affymetrix, Santa Clara, California, USA) following standard protocols recommended by the manufacturer. A quality control filtering step was applied to the genotype calls. The microarray data was analyzed using the Affymetrix power tools and individuals with a quality control call rate lower than 86% were filtered out. Additional quality control steps included coarse-grain stratification to ensure a Caucasian population ratio more than 0.8 (STRUCTURE software[28]), a genetic relatedness check to ensure independent samples (PLINK) and a sex check to ensure genetic accuracy and database integrity[29]. Quality control was also performed on the final genotypes to remove any single-nucleotide polymorphism (SNPs) with more than 4% of missing values across the entire cohort and any sample with more than 2% of missing SNP genotypes. A more stringent threshold was used for any SNPs with between 1 and 5% minor allele frequencies (MAF). Any of these low MAF SNPs with more than 1% of missing values was removed prior to the imputation; nonetheless, only SNPs with MAF higher than 5% were retained after imputation for use in the GWAS. After completion of the quality control process, a total of 3,409 genotyped individuals remained available for analysis.

Principal component analysis

A subset of 139 186 independent SNPs was selected from the set of common genotyped SNPs from 5.0 and 6.0 arrays using the linkage disequilibrium pruning application subroutine from PLINK. This set of SNPs was used to perform a PCA for the ADVANCE study participants of Caucasian origin using the EIGENSOFT 3.0 package[7]. The first principal component (PC1) was used to characterize the ethnic profiles of individuals form this Caucasian population that was

sampled in European countries ranging east to west from Russia to Ireland and from countries with populations of European descent, including Canada, Australia, and New Zealand.

Imputation

Two sets of imputation were performed separately for the individuals genotyped on Affymetrix arrays 5.0 and 6.0 using SHAPEIT[30] and IMPUTE2 software[31] and the 1000 genome project[32] phased 3 data set as reference. Only those SNPs with a MAF greater than or equal to 5% and with an imputation quality score greater than or equal to 0.80 were kept as has been proposed in previous studies[33].

Statistical analysis

Analyses of the differences in phenotype values (mean values for quantitative traits and numbers of individuals affected for qualitative traits) between groups (between individuals with Celtic and Slavic genetic profiles; between individuals with Slavic profiles living in predominantly Germano–Celtic European or European descent countries (Celtic region) and individuals with Slavic profiles living in predominantly Slavic European countries (Slavic region); and between individuals with Slavic genetic profiles living in predominantly Germano–Celtic European countries and individuals with Celtic profiles living in those countries were performed using general linear models included in the R software[34].

Differences in age of onset of diabetes and duration of diabetes were tested using sex as a covariate. All other phenotype differences were tested using age and sex as covariates so that the significance of these differences are age and sex adjusted. Mean phenotype values were also adjusted for sex and age where appropriate using the epicalc library[35] of the R statistical software[34]. When appropriate, adjustment for treatment for such traits as SBP, UACR, and eGFR were done using nonparametric adjustment as described[36].

Genome-wide association studies

GWAS were performed for age of onset of T2D separately for individuals with a Celtic or Slavic genetic profile as determined by their value for PC1 and for the combined Celtic and Slavic sample using linear regression with an additive genetic model and sex as well as the two respective first principal components of population stratification as covariates.

Association analyses were performed separately on the two imputed datasets for individuals that were genotyped on the different arrays (5 986 672 SNPs for 1015 individuals genotyped on chip 5.0 and 6 442 695 SNPs for 2394 individuals genotyped on chip 6.0) and results were merged using a fixed effects meta-analysis routine in the PLINK software[29] to avoid the possibility of any bias that might have arisen from uneven phenotype distributions across different genotyping chip technologies. The combined meta-analysis data set contained a total of 5 045 527 SNPs that passed all previous quality control steps in both data sets and passed a combined test for Hardy–Weinberg equilibrium using a critical P value of $1x10^{-3}$ ($P<10^{-3}$).

Effect size

The relative effect sizes (Υ_j) for each SNP, weighted by the size of the b coefficient of regression (β_j), the standard error of β_j (Seβ_j), and the MAF of the SNP (MAF_j) were estimated by the following equation[37]:

$$\Upsilon_j = SQRT\ [2MAF_j – (1\text{-}MAF_j)]\ (\beta_j / Se\ \beta_j)$$

Results

PCA of 3,409 genotyped ADVANCE study participants using EIGENSOFT 3.0 package identified two major principal components. The first PC1 divided the individuals of Europe along an east–west region, whereas principal component 2 separated individuals of Europe into a north–south gradient (Fig. 1a). PC1 clearly separated countries with populations of predominantly Germano– Celtic ethnic background ('Celtic', PC1<0) from those with a Balto-Slavic ethnicity ('Slavic', PC1≥0) with Germany aligned in the center of the distribution (Fig. 1b and c). When recruitment centers of the ADVANCE trial were ordered by values of PC1, we noted a pivot point between PC1 threshold values of 0.0 and 0.01 that separated Germany into Celtic (Munich) and Slavic (Dresden) origins (Fig. 1d).

Table 1 shows the main demographic and clinical characteristics of the two geo-ethnic groups at the entry of ADVANCE trial. The most striking difference between the two ethnic groups was the mean age of onset of diabetes. Individuals with Slavic profiles had T2D at a younger age ($P=7.3x10^{-20}$), had higher SBP and DBP ($P=4.5x10^{-22}$ and $P=5.3x10^{-29}$, respectively) despite the fact that a larger number of them were treated for hypertension and that they had a higher UACR at baseline ($P=5.1x10^{-9}$) even after adjusting for age, sex, and medication.

We then determined the effect of ethnic origin (Celtic vs. Slavic) and environment (Celtic region vs. Slavic region) on the most divergent phenotypes between Slavic and Celtic patients namely age of onset of T2D, BP, and renal function.

To assess the relative effects of genetic and environmental factors on the differences between individuals with Celtic and Slavic profiles, we compared study participants with a Slavic genetic profile living in countries with predominantly Germano–Celtic populations with individuals with a Slavic genetic profile living in predominantly Slavic countries of Europe. Significant differences between Slavs living in Slavic and Celtic countries would suggest an environmental/lifestyle effect, whereas no differences between Slavs living in Celtic or Slavic countries would support an impact of genetic influence. As shown in Fig. 2a, the highly significant earlier age of onset of T2D observed in individuals of Slavic origin was also present

among the 175 Slavic study participants living in countries of predominantly Celtic populations, suggesting a genetic drive for this trait. Similarly, UACR was higher in Slavic individuals living in either Slavic or Celtic regions (Fig. 2c). This contrasted with eGFR that was higher in Celtic than Slavic individuals after adjustment of age, sex, and medication but was not different between Celtic and Slavic individuals living in the same environment (Fig. 2d). SBP is another good example of environmental effect as SBP was higher in Slavic than Celtic individuals but not different between Slavic and Celtic individuals living in Celtic countries (Fig. 2b).

As age of onset of T2D showed strong genetic differences between Slavic and Celtic geo-ethnic groups, we performed GWAS of this phenotype in Slavic, Celtic, and the two combined populations. All SNPs with association of nominal significance of P values below 10^{-5} from GWAS analysis are presented in Table 2. Associations that are nominally significant in each of the two independent Celtic and Slavic GWAS and that increase in significance in the combined Celtic and Slavic GWAS are considered to be replicated in two independent sub-cohorts, that is, Celtic and Slavic genetic profile populations. These SNPs are indicated in bold type for combined sample in Table 2. Seven independent SNPs (not in linkage disequilibrium with each other) were found to be associated with age of onset of diabetes at $P<10^{-5}$ having the most significant P value for the combined Celtic and Slavic cohorts, and thus considered replicated by the above criteria. Other SNPs were associated specifically to one or the other ethnic group. Nine independent SNPs were significant only for the Celtic group and a different set of nine independent SNPs were significant only for the Slavic group. Two SNPs within the same locus were associated with age of onset of diabetes in the two different groups. SNP, rs35372009, near the CLEC14A gene was the most significantly associated for the combined Celtic and Slavic cohort ($P = 3.3 \times 10^{-6}$; Fig. 3a) and SNP, rs1754680, was the most significantly associated for the Slavic only group ($P = 8.3 \times 10^{-6}$). The SNPs are 65 662 bp apart on chromosome 14q21.1 and are in high linkage disequilibrium. These two SNPs, which lie within a region that is 5' of the CLEC14A gene, are representing the same association (Fig. 3a and b).

The most interesting association is within the PROX1/PROX1-AS1 gene locus (rs340841) that is characterized by one of the highest effect sizes for age of onset of T2D. The homozygous

CC genotype for rs340841 is associated with 4.4 years earlier onset of T2D in Slavic patients living either in Slavic countries or in Celtic countries (Fig. 4). Furthermore, the C allele is the major allele in Slavic individuals (Table 2). This locus is also associated with eGFR decline in Slavics, with macroalbuminuria and hypertension in all ADVANCE study participants of Caucasian origin and with IL-6 levels at baseline (data not shown). A literature search indicated that the PROX1 gene has been associated with abnormalities of glucose metabolism and risk of diabetes with ethnically specific individual polymorphisms[38-41].

Discussion

The global burden of cardio-metabolic risk factors adjusted for age and sex has been shown to be greater in Eastern than in Western European countries[42]. We have recently reviewed the importance of lifestyle behavior in gene–environment interactions analysis[24]. The current study is adding the notion that analysis of migration of a population within a distinct population even before its admixture may help dissect environmental from genetic contributions.

There is some debate as to the original homeland of the Balto-Slavs. One hypothesis holds that modern Baltic and Slavic populations descend from a proto-Slavonic parental group most likely located in a homeland roughly corresponding to the modern western Ukraine and then expanded by the sixth and seventh centuries A.D. as the Prague–Penkov–Kolochin complex of cultures to an area defined by the Baltic Sea in the north, approximately the Volga river in the east, the area defined by the modern day Czech Republic and the Elbe River in modern Germany in the west and the Danube basin in the south[43].

The area of north eastern Germany between the Oder and Elbe rivers was occupied by the Polabian Slavic ethnic group by the sixth century. By the ninth century conflicts between Christian Germanic people and these western pagan Slavs began. The conflicts eventually resulted in the incorporation of the area into the Holy Roman Empire by the thirteenth century and the linguistic germanification of the populations[44]. Therefore, it is reasonable to hypothesize the surviving presence of genetic evidence for an ethnic divide between a Germano–Celtic group (here referred to as simply ‘Celtic’) and a Balto-Slavic group (here referred to simply as ‘Slavic’) centered roughly along the Elbe river and the border of Czech Republic in northern Europe. We have demonstrated that evidence of this ancestral Celtic–Slavic genetic divide still exists in the modern European population and that it is reflected in differences in genetic–phenotype correlations.

Noticeably, the Caucasian populations of the non-European countries involved in ADVANCE have founding populations that are principally of Germano–Celtic origin as a result of British

Empire expansion. Slavic migration is more recent in these countries and therefore represents a minor ethnic component.

The principal difference between the Celtic and Slavic ethnic groups is age of onset of T2D, which is also correlated with other phenotypes such as albuminuria. We have previously introduced the concept of accelerated aging as being a primary cause of many complex genetic diseases[45]. It is a strong possibility that individuals with a Slavic genetic profile, despite their environment, are genetically more susceptible to accelerated aging resulting in earlier onset of T2D and associated albuminuria. In addition, our results from ADVANCE demonstrated that in contrast to macrovascular complications of diabetes that are strongly age dependent with an added risk conferred by duration of diabetes, the adverse effects of duration of diabetes on microvascular events were observed in the youngest age group[46], which is also compatible with observations of the Treatment Options for type 2 Diabetes in Adolescents and Youth trial[47]. Although the ADVANCE trial amply demonstrated, the decrease of renal events and total mortality by intensification of BP as well as of blood glucose control[48], a finding that is confirmed for glycemic control in the Veteran's Affairs Diabetes Trial and Action to Control Cardiovascular Risk in Diabetes trials[49], the current study suggests that further specific functional benefits on eGFR and UACR should be analyzed with respect to geo-ethnicity.

PROX1 encodes the prospero homeobox 1 protein, a human homologue of the Drosophila prospero gene. This protein is a homeobox transcription factor involved in developmental processes such as cell fate determination, gene transcriptional regulation, and progenitor cell regulation in a number of organs. It plays a critical role in embryonic development. PROX1 has been shown to be associated with diabetes and its complications in a number of studies[38-41.50-52]. Here, we present evidence that the genetic influence of PROX1 on age of onset of diabetes is different within Caucasian ethnic groups. It is of interest that several polymorphisms at this locus are associated with insulin levels and its control in adolescence, selected for a lesser impact of environmental determinants at this age by Lecompte et al.[40]. As we have mentioned, the ADVANCE trial demonstrated that earlier onset of diabetes has more impact on micro than macrovascular complications[46]. We propose that earlier onset of T2D in context of genetic x

environmental influences and ethnicity deserves further attention as a potential new target for early detection and intervention in T2D.

In conclusion, genetic analyses have to consider geo-ethnic characteristics even within Caucasians, demonstrated here for cardinal features of T2D. Our data suggest that understanding of distinct genomic architectures is important to ascertain clinical utility.

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Conflicts of interest

There are no conflicts of interest

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Tables and Figures

Figure 1:

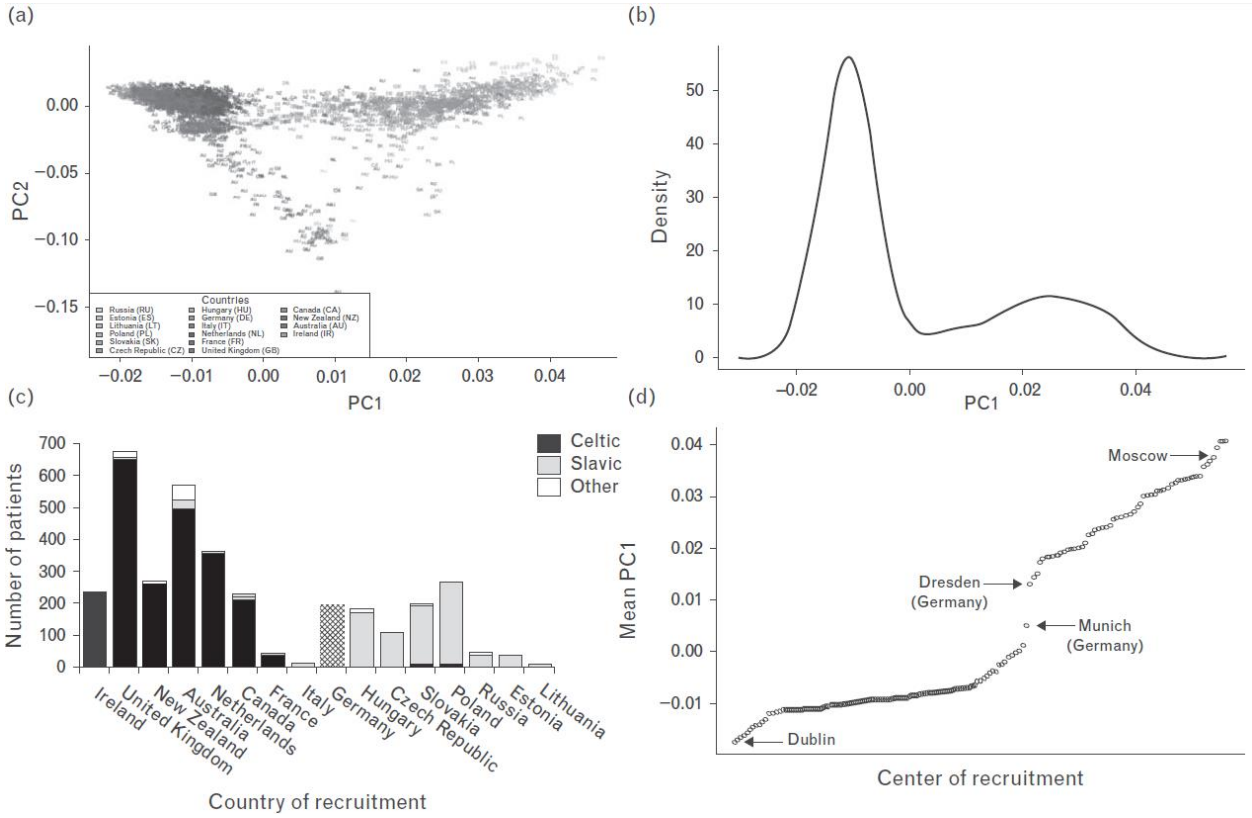

Figure 1: Distribution of genotyped ADVANCE study participants (n¼3409) according to principal components of genotype structure using EIGENSOFT 3.0 package. (a) ADVANCE individuals are plotted against the first two principal components PC1 (west–east gradient) and PC2 (north–south gradient), (b) frequency distribution of study participants by value of PC1. (c) Distribution of principal component values by countries of recruitment of patients in ADVANCE. (d) ADVANCE recruitment centers ordered by mean value of PC1.

Trait	All ($n = 3409$) Mean (SD) or %	Celtic ($n = 2307$) Mean (SD) or %	Slavic ($n = 1102$) Mean (SD) or %	*P* Value
Age (years)	67.3 (6.6)	68.0 (6.6)	65.9 (6.6)	1.9×10^{-16}
Men (sex)	64.7	69.8	54.0	5.8×10^{-19}
Age at diagnosis of diabetes (years)	60.1 (8.5)	61.0 (6.1)	58.2 (6.1)	7.3×10^{-20}
Diabetes duration (years)	6.7 (6.1)	6.4 (6.1)	7.4 (6.1)	2.9×10^{-6}
BMI	30.1 (5.1)	30.1 (5.0)	30.0 (5.0)	7.6×10^{-1}
Blood glucose assessment				
HbA1c (%)	13.4 (2.7)	13.4 (2.8)	13.4 (2.8)	7.5×10^{-1}
Glucose (mmol/l)	18.8 (4.6)	18.8 (4.6)	18.8 (4.7)	7.2×10^{-1}
Blood pressure assessment				
SBP (mmHg)	185.5 (30.3)	182.0 (30.0)	192.8 (30.2)	4.5×10^{-22}
DBP (mmHg)	103.6 (16.7)	101.4 (16.5)	108.3 (16.7)	5.3×10^{-29}
Heart rate (beats/min)	94 (16)	92 (16)	98 (16)	3.0×10^{-21}
Currently treated hypertension[c]	60.0	53.0	74.6	4.9×10^{-31}
Renal function assessment				
$eGFR_{CKD\text{-}EPI}$ (ml/min per 1.73 m^2)	69.6 (17.9)	70.9 (15.8)	66.8 (15.9)	1.2×10^{-11}
UACR (μg/mg)	78.1 (155)	63.5 (152)	96.7 (153)	5.1×10^{-9}
Microalbuminuria[a]	25.4	23.7	28.9	1.7×10^{-3}
Macroalbuminuria[b]	5.4	4.0	7.6	3.6×10^{-5}

Table 1: Demographic and clinical characteristics at baseline of Caucasian Advance genotyped participants stratified by ethnic origin. Age and age at diagnosis of diabetes are adjusted for sex; diabetes duration, BMI, currently treated hypertension and micro and macroalbuminuria are adjusted for age and sex; all other traits are adjusted for age, sex, and respective treatments. $eGFR_{CKD\text{-}EPI}$, estimated glomerular filtration rate calculated using Chronic Kidney Disease Epidemiology Collaboration equation; HbA1c, serum glycated hemoglobin; UACR, urinary albumin–creatinine ratio. [a]Urinary albumin–creatinine ratio between 30 and 300mg/mg. [b]Urinary albumin–creatinine ratio >300mg/mg.

[c]Blood pressure >140/90mmHg or receiving antihypertensive treatment

Figure 2:

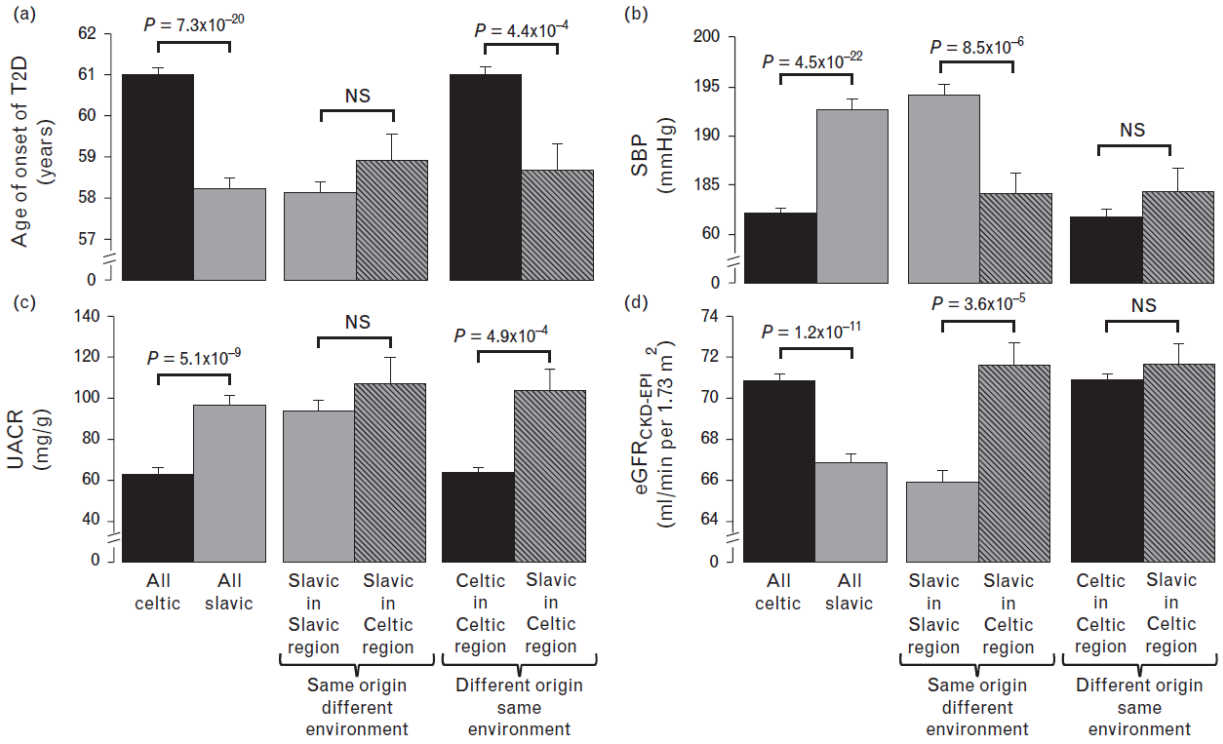

Figure 2: Effect of ethnic origin (Celtic vs. Slavic) and environment (Celtic region vs. Slavic region) on age of onset of T2D, SBP, UACR, and $eGFR_{CKD\text{-}EPI}$. Age of onset of T2D (a) and UACR (c) are more linked to genetics whereas SBP (b) and $eGFR_{CKD\text{-}EPI}$ (d) are more impacted by environmental factors. Age of onset of T2D is adjusted for sex whereas all other traits are adjusted for sex, age, and treatment (nonparametric adjustment[34]). $eGFR_{CKD\text{-}EPI}$, estimated glomerular filtration rate calculated using Chronic Kidney Disease Epidemiology Collaboration equation; PC, principal component; UACR, urinary albumin–creatinine ratio; T2D, type 2 diabetes.

Table 2:

SNP ID	Chr	Position (bp, hg19)	RA	Locus[a]	Celtic			Slavic			Combined[c]		
					P value	RAF%	Effect size[b]	*P* value	RAF%	Effect size[b]	*P* value	RAF%	Effect size[b]
SNPs that are significantly associated in both Celtic and Slavic genetic profiles and that increase in significance in combined group[c]													
rs34428389	3	52 894 142	A	**TMEM110**, MIR8064, SFMBT1	3.3×10^{-4}	19.1	−2.00	8.9×10^{-3}	18.4	−1.43	$\mathbf{8.1\times10^{-6}}$	18.9	−2.47
rs17447640	4	42 555 811	G	**ATP8A1**, SHISA3, GRXCR1	1.1×10^{-5}	13.9	−2.15	5.6×10^{-2}	15.7	−0.98	$\mathbf{1.5\times10^{-6}}$	14.5	−2.39
rs11298745	7	18 548 252	Del	**HDAC9**, MIR1302–6, TWIST1	2.3×10^{-3}	90.5	−1.26	4.6×10^{-5}	90.0	−1.73	$\mathbf{1.6\times10^{-6}}$	90.3	−2.01
rs34620785	8	103 452 308	A	UBR5, ODF1	1.6×10^{-4}	88.4	−1.71	1.7×10^{-3}	84.4	−1.61	$\mathbf{1.6\times10^{-6}}$	87.1	−2.27
rs76703216	10	9 512 026	G	LOC101928272	3.8×10^{-5}	94.1	−1.37	3.5×10^{-2}	96.2	−0.57	$\mathbf{3.6\times10^{-6}}$	94.7	−1.46
rs35372009	14	38 782 341	Del	.CLEC14A, LINC00639	1.1×10^{-2}	42.5	−1.78	9.0×10^{-6}	39.5	−3.07	$\mathbf{3.3\times10^{-6}}$	41.6	−3.24
rs148077446	16	77 310 608	Del	.SYCE1L, ADAMTS18	1.6×10^{-4}	28.7	−2.42	5.0×10^{-3}	31.9	−1.85	$\mathbf{5.7\times10^{-6}}$	29.7	−2.93
SNPs that are only significantly associated in Celtic profile						**RAF%**			**AF%**			**AF%**	
rs12743974	1	67 708 357	A	**IL23R**, C1ORF141, IL12RB2	$\mathbf{5.3\times10^{-6}}$	41.9	−3.18	3.8×10^{-1}	41.8	0.62	1.2×10^{-3}	41.8	−2.25
rs12736701	1	77 815 425	C	**AK5**, PIGK, ZZZ3	$\mathbf{7.6\times10^{-6}}$	93.4	−1.57	8.4×10^{-1}	93.9	0.07	3.2×10^{-4}	93.6	−1.24
rs7412314	1	171 287 252	T	**FMO4**, FMO1, TOP1P1	$\mathbf{4.3\times10^{-6}}$	73.3	−2.88	6.5×10^{-1}	69.8	−0.30	6.3×10^{-5}	72.1	−2.53
rs11099942	4	155 132 707	T	SFRP2, DCHS2	$\mathbf{4.3\times10^{-6}}$	6.4	−1.59	6.7×10^{-1}	5.1	0.13	1.7×10^{-4}	5.9	−1.26
rs9502478	6	6 593 695	T	**LY86-AS1**, F13A1	$\mathbf{4.3\times10^{-6}}$	17.3	−2.46	6.3×10^{-1}	16.0	−0.25	3.2×10^{-5}	16.9	−2.20
rs2107167	7	109 590 911	A	EIF3IP1	$\mathbf{8.0\times10^{-6}}$	13.7	−2.17	9.6×10^{-1}	9.6	−0.02	4.1×10^{-5}	12.4	−1.91
rs10973627	9	37 971 389	C	**SHB**, SLC25A51, ALDH1B1	$\mathbf{4.1\times10^{-6}}$	87.7	−2.14	6.4×10^{-1}	89.2	−0.21	9.9×10^{-6}	88.2	−2.02
rs113932007	11	115 491 436	Del	CADM1, LINC00900	$\mathbf{5.9\times10^{-7}}$	65.1	−3.37	5.1×10^{-1}	64.5	−0.45	8.8×10^{-6}	64.9	−3.00
rs10512488	17	40 963 904	G	**BECN1**, CNTD1, MIR6781	$\mathbf{3.1\times10^{-6}}$	74.0	−2.90	2.9×10^{-1}	72.2	−0.67	9.5×10^{-6}	73.4	−2.77
SNPs that are only significantly associated in Slavic profile						**AF%**			**RAF%**			**AF%**	
rs340841	1	214 124 470	C	**PROX1-AS1**, LINC00538, PROX1	7.9×10^{-1}	45.9	0.19	$\mathbf{8.6\times10^{-6}}$	52.8	−3.14	2.5×10^{2}	48.1	−1.59
rs12714314	2	1 943 617	C	**MYT1L**, PXDN, MYT1L-AS1	9.3×10^{-1}	75.8	0.05	$\mathbf{3.4\times10^{-6}}$	72.8	−2.92	1.9×10^{-2}	74.9	−1.44
rs58383906	3	29 246 181	C	LINC00693, RBMS3-AS3	3.3×10^{-1}	51.5	−0.68	$\mathbf{2.8\times10^{-6}}$	50.3	−3.32	6.9×10^{-4}	51.1	−2.40
rs13166103	5	57 742 202	C	LOC101928569, PLK2	3.5×10^{-1}	81.3	0.51	$\mathbf{4.1\times10^{-6}}$	79.8	−2.61	9.4×10^{-2}	80.8	−0.93
rs12188216	5	166 425 736	G	CTB-7E3.1, TENM2	2.9×10^{-1}	10.7	−0.46	$\mathbf{9.7\times10^{-6}}$	9.7	−1.85	1.7×10^{-3}	10.3	−1.35
rs9384193	6	154 554 249	C	**OPRM1**, CNKSR3	8.7×10^{-1}	36.3	0.11	$\mathbf{8.8\times10^{-6}}$	36.3	−3.02	2.7×10^{-2}	36.3	−1.50
rs11060464	12	130 097 850	G	**TMEM132D**, LOC101927735, LOC100190940	5.4×10^{-1}	70.9	0.39	$\mathbf{3.7\times10^{-6}}$	73.1	−2.90	6.0×10^{-2}	71.6	−1.20
rs1754680	14	38848 003	A	CLEC14A, LINC00639	2.3×10^{-2}	59.4	−1.54	$\mathbf{8.3\times10^{-6}}$	57.4	−3.17	4.8×10^{-5}	58.7	−2.85
rs13043901	20	52 170 783	A	**LOC101927770**, TSHZ2, ZNF217	3.3×10^{-1}	75.3	−0.60	$\mathbf{5.0\times10^{-6}}$	76.5	−2.74	4.2×10^{-4}	75.7	−2.14

Table 2: Summary statistics for association of age of onset of T2D for 25 independent single-nucleotide polymorphisms in Caucasians study participants from ADVANCE. Genome-wide association studies were done for all study participants (n=3409 combined group) and separately for Celtic (n=2307) and Slavic (n=1102) individuals stratified by ethnic origin, which is determined by principal component analysis. Selected single-nucleotide polymorphisms for all groups are associated to age at diagnosis of diabetes with P value less than $1x10^{-5}$

RA, risk allele; RAF, risk allele frequency; SNP, single-nucleotide polymorphism.

[a]If gene is in bold, SNP lies within the gene.

[b]The effect size (ES) is determined by the b, MAF, and standard error using the equation described in literatur[37].

[c]Association is considered replicated in two independent samples when P values are nominally significant for each of Celtic and Slavic samples and are more significant for combined sample.

Figure 3:

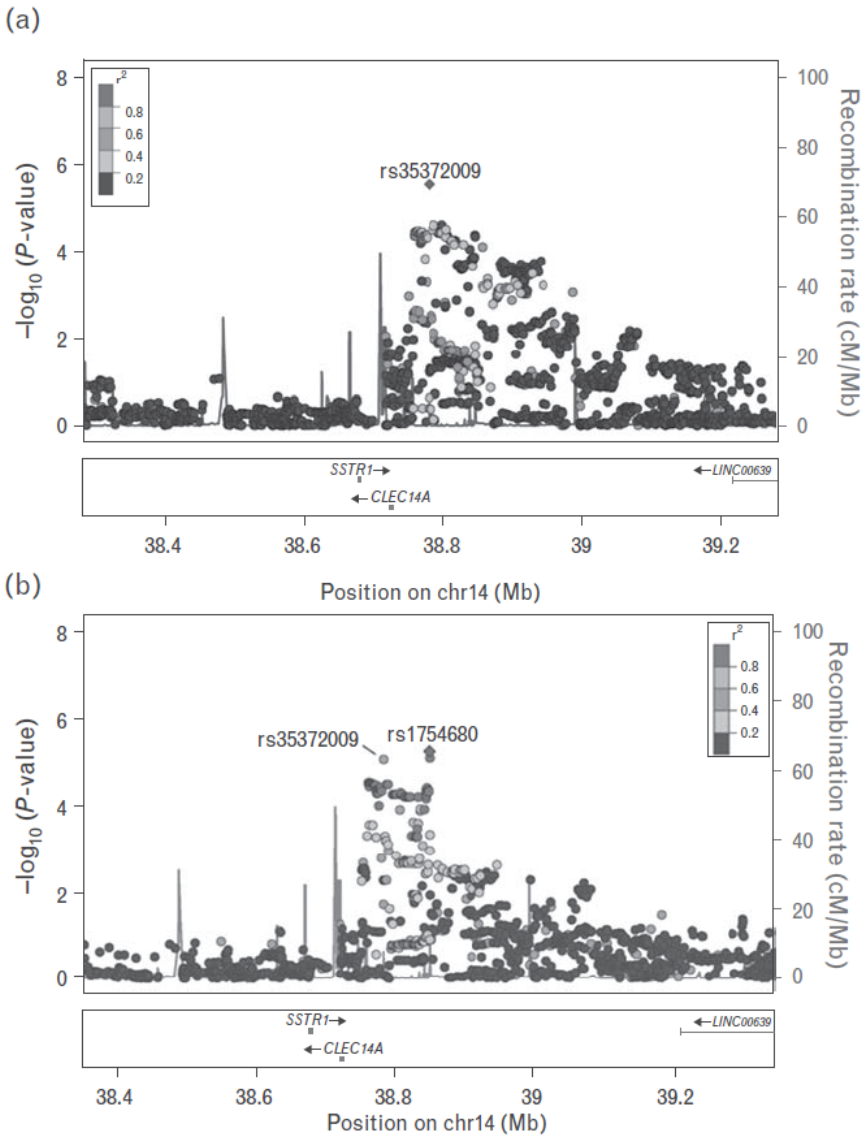

Figure 3: Regional association plots (1Mb window) of the CLEC14A/LINC00639 locus (chromosome 14) identified by GWAS of age of onset of T2D in the combined (a) and Slavic only cohorts (b), respectively. -log10 (P values) are plotted against genomic position (build 37, hg19). The lead SNPs (rs35372009 and rs1754680) are indicated in purple diamonds. The SNPs surrounding the lead SNPs are color coded based upon their linkage disequilibrium with the lead SNPs (taken from pairwise r2 values from the 1000 Genome EUR Database): red (r2 with lead SNP 0.8–1.0), orange (0.6–0.8), green (0.4–0.6), light blue (0.2–0.4), and dark blue (<0.2). The recombination rates (cM/Mb) are plotted in blue to reflect local linkage disequilibrium structure. Genes, exons, and direction of transcription from UCSC genome browser (genome.ucsc.edu) are noted. Plots are generated using LocusZoom (http://csg.sph.umich.edu/locuszoom). GWAS, genome-wide association studies; SNP, single-nucleotide polymorphism; T2D, type 2 diabetes.

Figure 4:

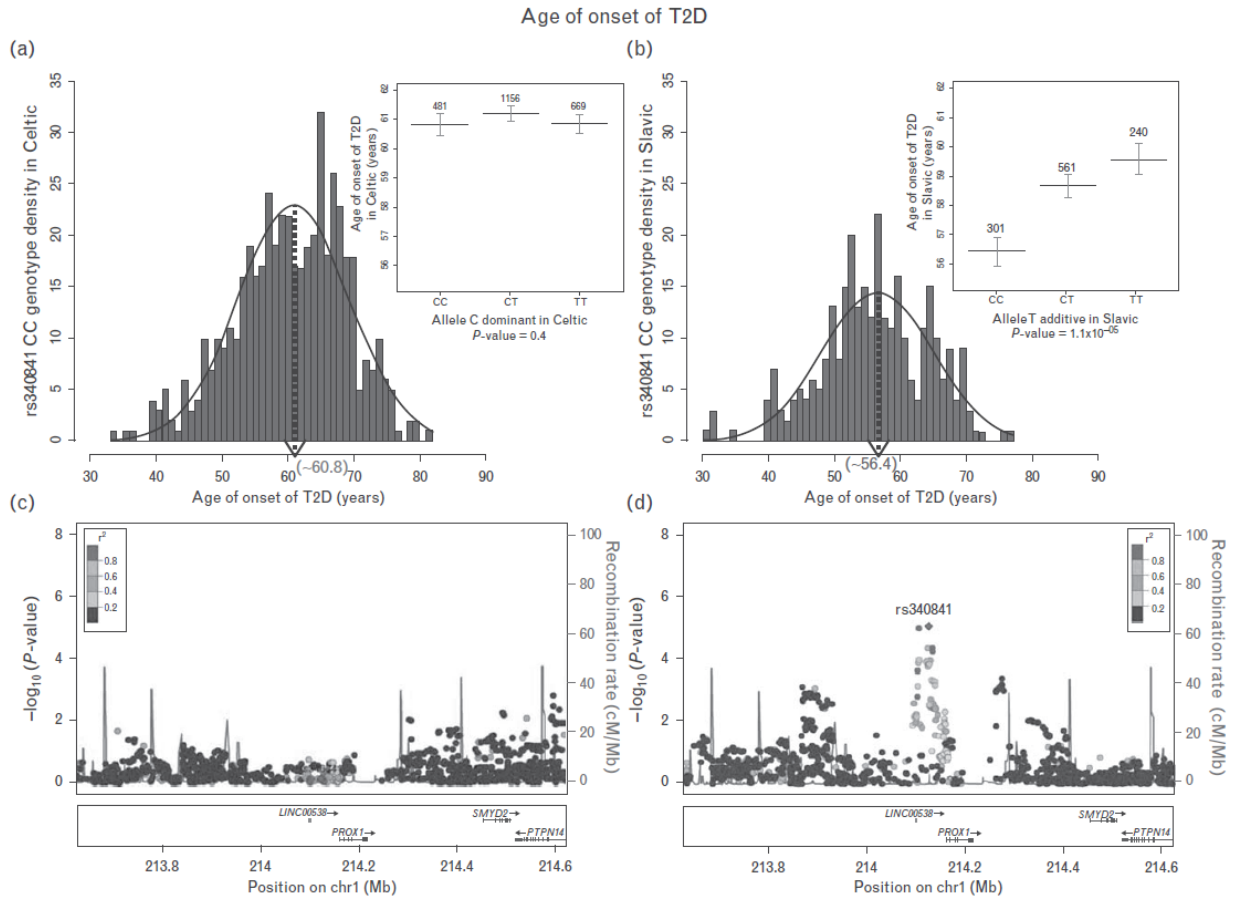

Figure 4: Distribution of the CC genotype frequencies for rs340841 located at the PROX1 locus in Celtic (a) and Slavic (b) study participants separately. Histograms of means of age of onset of T2D vs. genotype of rs340841 (inserts). Regional association plots (1Mb window) of the PROX1 locus (chromosome 1) identified by GWAS of age of onset of T2D in Celtic (c) and Slavic (d) study participants, respectively.

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log10 (P values) are plotted against genomic position (build 37, hg19). The lead SNP (rs340841) is indicated in purple diamond. The SNPs surrounding rs340841 are color coded based on their linkage disequilibrium with the lead SNP (taken from pairwise r2 values from the 1000 Genome EUR Database): red (r2 with lead SNP 0.8–1.0), orange (0.6–0.8), green (0.4–0.6), light blue (0.2–0.4), and dark blue (<0.2). The recombination rates (cM/Mb) are plotted in blue to reflect local linkage disequilibrium structure. Genes, exons, and direction of transcription from UCSC genome browser (genome.ucsc.edu) are noted. Plots are generated using LocusZoom (http://csg.sph.umich.edu/locuszoom). GWAS, genome-wide association studies; SNP, single-nucleotide polymorphism; T2D, type 2 diabetes.

Thematic link between Publication 2 and Manuscript

Publication 2 presented the capacity of principal component analysis to stratify Caucasian ADVANCE participants according to their geo-ethnic origins. In addition, GWAS studies conducted in separate populations (according to principal component 1 values) revealed the capacity to identify specific geo-ethnic genetic variants associated with T2D age of onset. The next section of this thesis will be focused on one of the core themes of this thesis: renal non-response. The methodology of the Manuscript leverages a clear definition of the renal non-response phenotype as well as a novel algorithm to classify patients according to the presence or absence of this phenotype to then conduct GWAS to identify genetic markers associated to renal non-response. Furthermore, findings presented in Publication 2 were considered in the analysis plan of the Manuscript, as GWAS were conducted in patients stratified according to their geo-ethnic origins to detect potential geo-ethnic variations in renal non-response. The results of the Manuscript present novel findings regarding the genetic basis of renal non-response, the clinical potential of a genetic risk score built with these genetic variants and the geo-ethnic variations of renal non-response.

C. Manuscript (In preparation)

Genetic Risk Scores Identify Patients with Unmet Renal Needs in ADVANCE

Paul H.G. Simon[1,2], Marie-Pierre Sylvestre[4], Alena *Krajčoviechová*[5], *Renata* Cífková[5], Mounsif Haloui[2], François Harvey[2], François-Christophe Marois-Blanchet[2], John Raelson[2], Carole Long[2], John Chalmers[6], Mark Woodward[6], Michel Marre[7], Stephen Harrap[8], Johanne Tremblay[2], Pavel Hamet[1,2,3]

[1]Department of Experimental Medicine, McGill University, Montreal Canada

[2]Department of Medicine, Université de Montréal, CRCHUM, Quebec, Canada

[3]Department of Medicine, Gene Medicine Services, CRCHUM, Université de Montréal, Canada

[4]Department of Social and Preventive Medicine, Université de Montréal, CRCHUM, Quebec, Canada

[5] Center for Cardiovascular Prevention, Charles University, First Faculty of Medicine and Thomayer Hospital, Prague, CZ

[6]The George Institute for Global Health, University of Sydney, Australia

[7]Hôpital Bichat, Claude Bernard, Université Paris 7, France

[8]Royal Melbourne Hospital, University of Melbourne, Australia

Correspondence to: Pavel Hamet, CRCHUM, Room R14.404, 900 Saint-Denis Street, Montréal, Québec, Canada H2X 0A9. Telephone: +1 514-890-8246, Fax: +1 514-412-7682; Email: pavel.hamet@umontreal.ca

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Abstract:

PURPOSE: A significant number of type 2 diabetic patients continue to lose their renal function despite receiving evidence-based medications. These unmet patient needs are caused by multiple risk factors including genetic ones. The goal of this study was to identify genetic determinants associated with renal non-response to currently available medications and to distinguish patients with different renal outcome profiles.

METHODS: Longitudinal eGFR and UACR measures for 3409 genotyped participants from the ADVANCE study were used to create UACR and eGFR non-response phenotypes and genome wide association studies (GWAS) were conducted to identify genetic markers associated with these phenotypes. These markers were used to construct two genetic risk scores, GRSUACR and GRSeGFR, and the capacity of these GRS to identify patients likely to present renal non-response was evaluated.

RESULTS: Genetic markers associated with renal non-response were found in genes involved in renal function. Despite equivalent responses to ADVANCE trial treatments with respect to blood pressure and glycaemia, patients with high GRSUACR or high GRSeGFR respectively presented worse progression of their levels of UACR and eGFR compared to patients with low GRSUACR or low GRSeGFR (P=3.1x10-29 and P=5.1x10-8). The performance of the GRS was validated in an internal replication using an independent set of ADVANCE patients. Both GRS were significantly associated with time to development of renal events in adjusted Cox proportional hazard models. T2D patients with low GRS benefited from primary prevention of renal disease with currently available drugs, including perindopril and indapamide combination.

CONCLUSIONS: This study revealed the independent nature of renal response with that of blood pressure and glucose response to ADVANCE trial treatments. High GRS of renal non-response can identify patients who present renal non-response to ADVANCE trial treatments. These patients cannot be distinguished from patients who present renal response based on phenotypic comparisons. GRS of renal non-response offers the possibility to stratify patients according to their renal non-response to ADVANCE trial treatments.

Condensed abstract:

Many type 2 diabetic patients continue to lose their renal function despite receiving recommended medications. This study aimed to identify genetic determinants associated with

renal non-response to currently available medication in patients with T2D and to leverage these findings to distinguish patients with different renal outcome profiles. The results of this study demonstrate an independent and dissociated component of renal response from that of blood pressure and glucose response. Genetic risk scores of renal non-response can identify patients who will not respond to current medication, as well as patients who respond to these treatments and who can be targeted for primary prevention of renal disease onset prior to development of hypertension and other clinical markers of renal impairment.

Keywords:

T2D, diabetic nephropathy, renal non-response, genetic risk score primary prevention

Introduction:

Type 2 diabetes (T2D) is one of the four most prevalent non-communicable chronic conditions [1], affecting an increasing number of individuals worldwide, from 108 million in 1980 to 422 million in 2014 [2], with a projection of 642 million by 2040 [3]. T2D and its complications bear a significant burden on patients, healthcare systems, and the global economy [2]. Diabetic nephropathy (DN) is one of the main complications of T2D and the leading cause of renal complications, chronic kidney disease (CKD), and end-stage renal disease in the U.S. and Europe [4]. If poorly managed, DN leads to kidney transplant or dialysis [5].

While glycemic control and blood pressure lowering treatments are successful in the majority of T2D patients [6-9], a significant proportion progresses towards DN. For instance, in the Action in Diabetes and Vascular Disease: PreterAx and DiamicroN Modified-Release Controlled Evaluation (ADVANCE) study, trial interventions with perindopril-indapamide and gliclazide MR led to a reduction of annual event rate of new or worsening nephropathy by 33%, and of new onset macroalbuminuria by 54% [10]. Despite the observed decrease in the number of renal complications in ADVANCE, 3% of patients receiving both trial medications reported a serious worsening of nephropathy event (doubling of plasma creatinine) [10], and 19% of patients developed new-onset microalbuminuria [10], which are predictors of macrovascular complications [11]. In the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT) patients were randomized to three blood pressure management medications, and regardless of the treatment arm, 5% of patients developed end-stage renal disease or a decrement in glomerular filtration rate of 50% or more from baseline [12]. In the Incipient to Overt: Angiotensin II Blocker, Telmisartan Investigation on Type 2 Diabetic

Nephropathy (INNOVATION), patients were randomized to the angiotensin II receptor antagonist telmisartan, or a placebo. Despite a significant decrease in the number of transitions from micro- to macroalbuminuria between treatment groups, 10% of patients reported this renal event [13].

The objective of this study is to assess whether genetics play a role in the development of DN in treated T2D patients, which would explain their observed renal non-response [14]. The use of genetics for the early detection of T2D patients at risk of renal non-response may help to detect patients requiring close monitoring and more likely to benefit from novel therapies, thus decreasing the burden of DN on patients and health care systems. We used longitudinal albuminuria and estimated glomerular filtration (eGFR) data collected during the 5 year follow-up period of the ADVANCE study and defined renal non-response as new or worsening nephropathy despite the use of evidence-based recommended medications [15]. We performed Genome-Wide Association Studies (GWAS) to identify Single Nucleotide Polymorphism (SNPs) associated with renal non-response. We constructed two Genetic Risk Score (GRS) based on associated SNPs and assessed their capacity to distinguish patients at high or low risk of developing renal complications.

Methods:

ADVANCE cohort

The ADVANCE study is a factorial randomised controlled trial assessing the impact of blood pressure lowering with perindopril-indapamide (Preterax) and intensive glucose control based on slow release gliclazide (Diamicron MR) on macrovascular and microvascular outcomes. Details of the ADVANCE trial methodology have been published elsewhere [16]. The ADVANCE protocol was approved by the Institutional Ethics Committee of each participating centre and all participants provided written informed consent before their enrolment in the trial, including specific consent to a genomic sub-study. A total of 11,140 participants 55 years or older were recruited in 20 countries from 215 centers. Participants had been diagnosed with T2D at the age of 30 or above and had an elevated risk of vascular disease. Participants were followed-up at 3, 4, and 6 months after randomization and subsequently every six months for five years. Clinical variables measured at each visit included blood pressure, glucose, HbA1C and lipid levels. Urine albumin: creatinine ratio (UACR) was measured twice at baseline, 24, and 48 months after randomization and at the end of follow-up. Serum creatinine was measured at baseline, at conclusion of the run-in period, 4 and 12 months after randomization, and at subsequent year intervals as well as at the end of follow up. For this study, eGFR measures were calculated from creatinine values using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [17] and UACR measures were log-transformed because their

distribution was skewed. Composite outcomes were defined as the combined incidence of major macrovascular events (stroke, myocardial infarction, and cardiovascular death), and major microvascular events (doubling of serum creatinine, overt nephropathy).

Renal non-response phenotype definition

We designed a classification algorithm to identify participants with evidence of renal non-response that accounted for both the baseline values of eGFR or UACR and their trends over time. The classification was performed irrespective of the treatment arms. Both baseline values and longitudinal measures of eGFR and UACR are essential to identify patients with renal non-response. For instance, in patients who enter the study with eGFR values in the normal range, stable eGFR patterns over time do not indicate presence of renal non-response, while stable eGFR patterns in patients with baseline eGFR values in a disease range suggests that they did not respond to the treatment and thus have renal non-response. The classification algorithm is illustrated in Supplemental Figures 1 and 2 and described below.

Step 1 stratified patients based on UACR and eGFR baseline values. Step 2 estimated eGFR and UACR individual time trends. Step 3 identified patients with renal non-response based on their eGFR and UACR trend values. Step 4 created overall groups of renal non-responders and responders (refer to online appendix for full details).

In order to have sufficient data to estimate individual renal time trends, participants with renal measurements at study registration and 2 years after randomization were eligible for inclusion into the current study, as was done in previous analyses of the ADVANCE consortium [18].

Genotyping and imputation

Details pertaining to genotyping and imputation of ADVANCE Caucasian participants have been reported [19]. In short, 3,629 Caucasian individuals of the ADVANCE study were genotyped using Affymetrix Genome-Wide Human SNP Arrays 5.0 or 6.0 (Affymetrix, Santa Clara, California, USA) with the standard protocols recommended by the manufacturer. Following genotyping, a quality control (QC) filtering step using Affymetrix power tools was applied to the genotype calls. To ensure a Caucasian population ratio superior to 0.8, independent samples, as well as genetic accuracy and database integrity, additional QC steps respectively included: coarse-grain stratification (STRUCTURE software [20]), a genetic relatedness check (PLINK) and a sex check [21]. As reported in detail elsewhere [19], genetic data from individuals genotyped on Affymetrix arrays 5.0 and 6.0 were imputed separately with software SHAPEIT [22] and IMPUTE2 [23]. 1000 genome project [24] and phased 3 data were respectively used as

references. A total of 3,409 genotyped individuals were available for analysis. 5,986,672 SNPs for 1,015 individuals genotyped on Affymetrix chip 5.0 and 6,442,695 SNPs for 2,304 individuals genotyped on Affymetrix 6.0).

Genome-wide association studies

GWAS were conducted separately on both the UACR and eGFR non-response phenotypes using logistic regressions with an additive genetic model. To account for the genetic heterogeneity in ADVANCE Caucasian participants [19] we conducted GWAS in Celtic and Slavic populations separately and then combined. Models were adjusted for age, sex and further adjusted for ethnicity using principal components [19] (Supplemental Figures 4 -7). Analyses were performed separately on the two imputed datasets and results were merged using a fixed effects meta-analysis [21].

Statistical analysis

Clinical characteristics and medication profiles of participants with and without renal non-response were compared using frequencies and chi-squared tests for categorical variables and means, and using t-tests and Wilcoxon tests for continuous variables with normal and asymmetric distributions respectively. Similar procedures were used to compare participants with low or high Genetic Risk Score of UACR non-response (GRSUACR) as well as low or high Genetic Risk Score of eGFR no-response (GRSeGFR). All calculations were performed with R version 3.4.1 [25].

SNP selection from GWAS and Genetic Risk Score calculation

We used a liberal approach [26-28] for the inclusion of SNPs in GRS. SNPs with the highest statistical association level in each peak - defined as regions of the GWAS where multiple SNPs in linkage disequilibrium showed an association signal with the phenotype of interest - were selected for inclusion in the GRS. A total of 19 and 9 SNPs were selected for the general Caucasian GRSUACR and GRSeGFR, respectively (Table 1). GRS of renal non-response for the two phenotypes were calculated by summing the risk alleles for each SNP. Specific Slavic and Celtic GRSUACR were composed of 11 SNPs which were statistically significant in the combined Caucasian population in addition to 8 Celtic/Slavic specific SNPs for Celtic and Slavic patients respectively (Supplemental Table 1). Similarly, the specific Slavic and Celtic GRSeGFR comprised

3 SNPs statistically significant in the combined Caucasian population in addition to 6 Celtic/Slavic specific SNPs for Celtic and Slavic patients respectively (Supplemental Table 2).

Assessing the discriminating ability of GRS of renal non-response

For both GRSUACR and GRSeGFR, patients were classified as having either a "high" or "low" GRS of renal non-response if their respective scores were either one standard deviation above or below the mean GRS value (Supplemental figure 3). Second, clinical characteristics and medication profiles at baseline for patients with high or low GRSUACR or GRSeGFR were compared to assess if these patients presented different clinical profiles at study entry. Third, clinical characteristics at the end of study, in addition to UACR and eGFR deltas (difference between end of study and baseline values) were compared to assess if patients with high or low GRSUACR or GRSeGFR present different clinical evolutions over time. Fourth, we evaluated the difference in incidence of renal primary prevention events between patients with high or low GRSUACR or GRSeGFR. These events were defined as remaining normoalbuminuric during follow-up (patients with UACR < 30µg/mg at baseline who remain so during follow up) for UACR, and remaining above CKD2 during follow up (patients with eGFR ≥ 60mL/1.72m2/min at baseline and who remain so during follow up) for eGFR. Lastly, Cox proportional hazards models were estimated to assess the association of GRS with the time to development of worsening renal events. For UACR, these were as defined as the progression from (i) normoalbuminuria to micro and/or macroalbuminuria or (ii) microalbuminuria to macroalbuminuria. For eGFR, these were defined as, (i) progression from CKD stage 1 to 2 or lower or (ii) CKD stage 2 to 3 or lower or (iii) CKD stage 3 to 4 or lower or (iv) CKD stage 4 to 5). Models were adjusted for conventional clinical variables and ADVANCE blood pressure and glycemia trial treatments.

Internal validation of renal non-response GRS in independent ADVANCE dataset

An independent set of 689 ADVANCE patients not previously included in analysis were genotyped on the UK BioBank Axiom Array (UK Biobank, England) following manufacturer protocol and using similar quality control steps as described above. These patients were then used to assess the predictive ability of the GRS in an independent sample.

Relevance of renal non-response loci in early stages of DN

We assessed the ability of the GRS to detect individuals at risk of developing DN by investigating the association between SNPs included in renal non-response GRS and phenotypes associated with pre-DN (uric acid, albumin creatinine ratio and creatinine levels) in the Czech post-MONICA study, a cross-sectional survey investigating the prevalence and treatment of cardiovascular risk factors in the general population of the Czech Republic. A total of 3,612 Caucasian individuals aged 25–64 years were examined from 2007–2009 [29, 30]. Specific details of the study were reported elsewhere [29, 30] and are available in the appendix. In addition, we evaluated the association of SNPs included in renal non-response GRS with phenotypes of worsening nephropathy in studies of the CKDGen consortium. Similarly, UACR non-response loci were evaluated in a recent meta-analysis conducted in 67,542 patients (of which 7,787 had diabetes) for UACR and microalbuminuria [31]. Lastly, eGFR non-response loci were evaluated in a recent meta-analysis conducted in a discovery population of 133,413 patients and a replication population of 42,166 for eGFR based on serum creatinine [32].

Results:

Differences in clinical characteristics of patients with and without renal non-response

For UACR, our algorithm identified 38.3% of patients with renal non-response, 52.0% patients with renal response, and removed 9.7% patients. Similarly, for eGFR 43.1% of patients were identified with renal non-response, 41.5% with renal response, and 15.4% were removed. For UACR, non-responder patients were older, had longer duration of diabetes, more history of major macrovascular disease, a lower percentage of microalbuminuria at baseline, and lower levels of eGFR as compared to responders (Supplemental Table 3). For eGFR, non-responder patients were older, had a longer duration of diabetes, more history of major macro and micro vascular events, were less frequently of Slavic ethnicity, had more severe hypertension, obesity and albuminuria than responders (Supplemental Table 4). Renal non-responders received on average more medications than responders (Supplemental Table 5). Lastly, 17.9% of patients were identified as being both UACR and eGFR non-responders, while 23.3% of patients were identified as both UACR and eGFR responders.

Ability of GRS to predict renal events

Respectively 19 and 9 SNPs were selected for GRSUACR and GRSeGFR in the Caucasian population (Table 1). At baseline, patients with high or low GRSUACR or high or low GRSeGFR had similar clinical measures, except for the low GRSUACR group who had higher UACR levels (P=1.4x10-8) and a higher percentage of microalbuminuria (P=6.5x10-4) at baseline (Table 2). Patients with low GRSeGFR had lower UACR (P=1.4x10-2) (Table 3). There were no differences

in medication profiles between patients with high or low GRSUACR or high or low GRSeGFR (Supplemental Table 6).

At the end of study, patients with high GRSUACR had a significantly higher UACR and lower eGFR (P=2.1x10-8 and P=6.5x10-3 respectively), had a shallower decrease in their five-year UACR slopes and a highly positive UACR delta (P=3.1x10-29 and P=1.1x10-8, respectively), as well as a higher percentage of composite risk events (P=4.7x10-2). Similarly, patients with high GRSeGFR presented lower eGFR values at the end of the study (P=1.7x10-11), and a five-year eGFR slope and eGFR deltas which were respectively five and three times higher than patients with a low GRSeGFR (P=5.1x10-8 and P=3.5x10-19, respectively). Over the duration of the study, patients with high GRSUACR presented a significant increase between their first and last measure of UACR (P=1.9x10-9), whereas patients with low GRSUACR presented a significant decrease (P=2.9x10-7). Similarly, patients with a high GRSeGFR presented a significant decrease between their first and last measure of eGFR (P=4.8x10-20) as compared to a slight decrease for patients with low GRSeGFR (P=3.9x10-2).

Patients with high or low GRSUACR or high or low GRSeGFR, presented no significant differences in their systolic blood pressure and glycated hemoglobin levels (Table 4), suggesting equivalent blood pressure lowering and glycemic control treatment responses in patients with high or low GRSUACR or GRSeGFR. This in turn suggests a genetic basis for renal non-response that is independent of high blood pressure or high glucose. Indeed, when separated in groups receiving both ADVANCE trial medications (Preterax and Diamicron MR) or no ADVANCE trial medication (Table 5), patients with a high GRSUACR receiving both trial treatments presented comparable blood pressure and glycated hemoglobin levels as patients with low GRSUACR but significantly different renal measures for UACR and eGFR. In patients with high GRS, renal response is therefore distinct and dissociated from blood pressure and glucose response.

Association of GRS with worsening renal events and primary prevention

Patients with low GRSUACR or low GRSeGFR who were in the lowest stage of the disease at baseline (i.e normoalbuminuric for albuminuria and CKD stage 1-2 for kidney function decline) presented a higher incidence of primary prevention events than patients with high GRSUACR or high GRSeGFR (P=7.3x10-7 and P=1.6x10-7 respectively for GRSUACR and GRSeGFR) (Figure 1). The absolute risk difference for UACR and eGFR worsening events between patients with low or high GRSUACR as well as patients with low or high GRSeGFR, were respectively 19% and 16% (Figure 1). Moreover, GRSUACR and GRSeGFR were respectivly associated with time to development of UACR and eGFR worsening events in fully adjusted proportional Cox hazard

model (P=8.7x10-7 and P=1.4x10-7 respectively for GRSUACR and GRSeGFR)(Supplemental Table 7 and 8).

Comparison of clinical and genetic identification of renal non-response

General Caucasian based GRSUACR had a positive predictive value of 69.3% and a negative predictive value of 79.2% to identify UACR renal non-responders (Supplementary Table 9). Sensitivity and specificity of this GRSUACR were 79.2% and 71.6% respectively. General Caucasian based GRSeGFR had a positive predictive value of 69.0%, a negative predictive value of 63.8%, and a sensitivity as well as a specificity of 66.1% and 66.8% respectively (Supplementary Table 9). The performance of specific Slavic & Celtic based GRS was improved in the case of GRSUACR but not in the case of GRSeGFR (Supplementary Table 9). The sensitivity of specific Slavic & Celtic based GRSUACR increased to 85.2% and its specificity increased to 72.7%.

Relevance of ADVANCE renal non-response loci

Several loci identified in ADVANCE participants and associated to UACR or eGFR renal non-response were confirmed in GWAS of renal phenotypes completed in patients of the Czech-post Monica study, the CKDGen consortium, and in the literature (Supplementary Table 10 - 11). For UACR non-response, 12 loci out the 18 identified in ADVANCE and used in GRSUACR were associated to eGFR, UACR and uric acid in patients of the Czech-post MONICA study with p-values ranging from 7.4x10-4 to 8.0x10-5. Furthermore, three loci (CHN2, HS6ST1, PTPRT, and RAB38) associated to UACR non-response were reported as associated to UACR in diabetic patients and microalbuminuria in non-diabetic patients in a study of the CKDGen consortium [31]. For eGFR non-response, 7 of the 9 identified loci in ADVANCE and used in GRSeGFR present associations with these same renal phenotypes in the Czech-post MONICA study, with p-values ranging from 8.8x10-4 to 2.7x10-5. In addition, three loci (TFDP2, TSPAN9, and UMOD) associated with eGFR non-response were reported as associated to eGFR based on serum creatinine in a study of the CKDGen consortium [32].

Replication of renal non-response GRS performance

We evaluated the performance of renal non-response GRS in an additional set of 689 Caucasian patients from ADVANCE genotyped using the UK BioBank array and not included in the 3409-initial dataset (Supplemental Table 12). The 689 patients with high or low GRSUACR or GRSeGFR had similar clinical measures at baseline, over the duration of the study, patients with high GRSUACR had a greater increase of their level of UACR than patients with low GRSUACR

(P=2.3x10-3). Similarly, patients with high GRSeGFR presented a greater decrease in their levels of eGFR than patients with low GRSeGFR (P=2.2x10-3). These differences in renal response were observed despite equivalent response to blood pressure lowering and glycemic control treatments. This initial replication thus confirmed the capacity of GRSUACR and GRSeGFR to respectively distinguish patients with significant increase of their UACR and decrease of their eGFR from patients who benefit at the renal level of blood pressure lowering and glycemic control treatments.

Discussion:

The present study presents three key takeaways. First, the existence of a genetic basis to renal non-response in patients with T2D. Second, the dissociated nature of renal non-response in relation to blood pressure and glucose response. Third, the capacity of GRS of renal non-response to identify patients who will not answer to current treatments from those who will. Patients with high or low GRSUACR or GRSeGFR respond to blood pressure lowering and glucose control arms of the ADVANCE clinical trial. Interestingly, and despite the decrease of their systolic blood pressure and reduction of Hba1c level, patients with a high GRSUACR or GRSeGFR experience a renal deterioration (as seen by a significant increase of their UACR and a significant decrease of their eGFR) while patients with a low genetic risk present renal improvement and/or stabilization of their renal condition, which is further accentuated when they receive trial medication. Levels of UACR at the end of follow-up in high GRSUACR patients were significantly higher than those in low GRSUACR patients, regardless of whether they received both ADVANCE trial treatments or not. Patients with low GRSUACR at the end of follow up, however, presented levels of UACR which were further decreased when receiving both ADVANCE treatments. It therefore appears, that not all patient benefit equally at the renal level from a reduction in their blood pressure and glucose, and that genetic determinants of renal non-response have the capacity to identify patients who will benefit at the renal level from these treatments.

Renal non-response in patients with T2D can be caused by several factors acting in combination or alone. In cases of non-adherence to medical treatment [33], and unhealthy lifestyle choices [34], renal non-response has the potential to arise due to patient behaviour. In other instances, such as those where patients present persistent hyperglycaemia [35], high tubular concentrations of TGF-β1 [36, 37], or resistant hypertension [38], renal non-response can be caused by an aggressive form of DN which results from uncontrolled T2D risk factors (namely hypertension and hyperglycemia). Lastly, renal non-response can arise in cases where patients present specific pharmacodynamic profiles which alter their response to evidence based

recommended medications, as in the case of Caucasian patients carrying a deletion of 287bp Alu sequence in intron 16 of the ACE gene locus and who respond less favourably to ACE inhibitors [39]. The genetic determinants of renal non-response uncovered in the present study suggests that the manifestation of renal non-response in ADVANCE participants is associated to the severity of risk factors leading to DN or to dysfunctional kidney. For UACR (Sup Figure 4 and 5), identification of loci such as PAX8 - associated with blood urea nitrogen [40], PALLD - associated with advanced glycation end product levels [41], ARHGAP24 - associated to T2D-attributed ESRD in African Americans [42], SLC35B3 - associated with glucose homeostatic traits [43] provide pathophysiological relevant evidence to support the implication of dysfunctional glucose homeostasis and advanced glycated end products in the incidence of UACR renal non-response. For eGFR (Sup Figure 6 and 7), identification of UMOD – associated with kidney function decline in European patients and for which the effects are mediated within the kidney [32, 44], supports the implication of an abnormal kidney in the manifestation of eGFR non-response. These genetic determinants therefore support the implication of more severe risk factors in UACR non-response and of dysfunctional kidneys in eGFR non-response.

In parallel to the above observation, Cox proportional hazards models presented in this study and adjusted for conventional clinical measures, revealed a significant association of genetic risk scores with time to development of renal worsening events, but did not reveal significant interactions between SNPs associated to renal non-response and ADVANCE trial medication (data not shown). While the lack of significant interactions could stem from insufficient power, our results do not currently point to a pharmacodynamic basis for the manifestation of renal non-response in patients with T2D. Rather, severity of DN as driven by glucose pathways and abnormal kidneys, despite normal response to blood pressure and glucose control treatments seem to be involved in renal non-response.

The genetic determinants of renal non-response identified in the current study show an association with worsening renal events (progression of albuminuria classes and CKD stages). As a result, patients with low GRSUACR or GRSeGFR presented a greater proportion of renal primary prevention event, whereas patients with high GRSUACR or GRSeGFR presented a greater proportion of renal worsening events. Using genetic determinants as a clinical screening tool could allow the early detection of patients unlikely to respond to current evidence based medications and enable early initiation of interventions to prevent onset or worsening of renal complications. Furthermore, the inclusion of specific Slavic/Celtic SNPs to create specific Slavic & Celtic GRSUACR and GRSeGFR improved the performance of GRSUACR but not that of GRSeGFR. These results are in agreement with our recent study, where we reported a greater genetic basis to UACR than to eGFR in Caucasian ADVANCE participants [19]; thus, providing a rational to consider ethnically based therapeutic choices in DN management.

Studies using repeated measures from the same individuals are prone to the regression to the mean statistical phenomenon, which corresponds to the tendency for individuals with extreme baseline values to have subsequent values closer to the mean [45]. To assess whether there are differences between responders and non-responders beyond differences expected from the regression to the mean, we computed the variances of eGFR and logUACR at baseline and endline and tested their equality using Pitman's test (equality rejected, $p<0.001$) [46]. Under the assumption of a biological effect explaining change in repeated measures over and above the regression to the mean phenomenon, one expects measures to converge to the mean and to reduce in variance [46]. This suggests that the slopes from which non-response is derived is not a pure result of regression to the mean.

Our study demonstrated that despite all patients (with high or low GRS of renal non-response) responding to ADVANCE trial treatments at a blood pressure and glycemia level, patients genetically identified as renal responders benefit from those treatments at a renal level and improve their condition whereas patients genetically identified as renal non-responders present a deterioration of their renal profile. High GRS of renal non-response can identify patients who do not respond at a renal level and can benefit from novel therapeutic approaches, while subjects with low GRS UACR can be targeted for primary prevention of renal disease onset - prior to development of hypertension and other clinical markers of renal impairment.

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Table 1: SNPs identified in the study of UACR and eGFR non-response and included in GRS_{UACR} and GRS_{eGFR}

SNP ID	Chr	Position (b37; h19)	Locus	Location in relation to the gene	Flanking genes (up to 500kb)	Risk allele	Non-risk allele	RAF	β	p-value
Trait of interests: development of UACR non-response										
rs1493992	1	34 038 006	CSMD2	Intronic	ZSCAN20 - HMGB4	T	C	0.53	0.26	3.5×10^{-6}
rs67237254	2	114 012 928	PAX8	Intronic	PSD4 - CBWD2	T	A	0.21	0.36	9.2×10^{-8}
rs11918427	3	74 882 210	Intergenic	NA	CNTN3 - FAM86DP	G	C	0.12	0.38	1.1×10^{-5}
rs13140153	4	45 930 292	Intergenic	NA	- GABRG1	G	A	0.9	0.44	2.8×10^{-6}
rs149213511	4	86 707 802	ARHGAP24	Intronic	WDFY3-AS2 - MAPK10	A	AAC	0.69	0.26	7.1×10^{-6}
rs61603300	4	169 527 905	PALLD	Intronic	DDX60L - CBR4	G	T	0.91	0.55	1.4×10^{-7}
rs150233516	5	132 011 621	IL4	Intronic	IL13 - KIF34	ATGTG	A	0.82	0.38	1.9×10^{-6}
rs34104013	5	171 939 378	Intergenic	NA	SH3PXD2B - NEURL1B	C	CA	0.52	0.25	5.1×10^{-6}
rs10533367	6	8 145 014	Intergenic	NA	EEF1E1 - SLC35B3	A	G	0.87	0.38	9.1×10^{-6}
rs34656786	6	35 742 752	Intergenic	NA	ARMC12 - CLPSL2	T	C	0.33	0.6	5.3×10^{-6}
rs929506	7	122 197 763	CADPS2	Intronic	FEZF1 - TAS2R16	A	G	0.11	0.41	1.0×10^{-5}
rs68030383	12	26 577 551	ITPR2	Intronic	SSPN - ASUN	T	C	0.35	0.26	6.2×10^{-6}
rs61948880	13	25 064 748	PARP4	Intronic	C1QTNF9 - TPTE2P6	T	C	0.17	0.33	5.4×10^{-6}
rs970817	13	55 235 608	Intergenic	NA	MIR1297 -	C	T	0.18	0.31	9.7×10^{-6}
rs6573040	14	60 876 186	Intergenic	NA	TBPL2 - KTN1-AS1	C	T	0.19	0.35	4.3×10^{-7}
rs17758297	14	79 913 167	NRXN3	Intronic	ADCK1 - DIO2	A	T	0.29	0.27	3.9×10^{-6}
rs7157963	14	96 008 866	GLRX5	Intronic	SNHG10 - TCL6	A	T	0.22	0.31	3.7×10^{-6}
rs7212486	17	77 096 177	RBFOX3	Intronic	ENGASE - ENPP7	C	T	0.35	0.27	1.8×10^{-6}
rs35101292	21	33 641 106	MIS18A	Exonic	HUNK - MRAP	G	GT	0.74	0.28	9.7×10^{-6}
Trait of interests: development of eGFR non-response										
rs3123025	1	156 267 004	Intergenic	NA	C1orf85 - VHLL	A	T	0.61	0.29	1.0×10^{-6}
rs201500153	2	201 624 094	AOX2P	Intronic	AOX1 - BZW1	C	CATAA	0.41	0.29	2.0×10^{-6}
rs66916463	4	37 235 118	Intergenic	NA	- MIR4801	A	G	0.08	0.49	8.1×10^{-6}
rs6451864	5	20 599 383	Intergenic	NA	CDH18 -	T	G	0.58	0.25	1.0×10^{-5}
rs66801926	6	157 161 915	ARID1B	Intronic	- TMEM242	GC	G	0.66	0.27	9.8×10^{-6}
rs636554	12	10 140 184	Intergenic	NA	CLEC12A - CLEC1B	T	A	0.46	0.26	4.9×10^{-6}
rs9668721	12	130 515 821	Intergenic	NA	TMEM132D - FZD10-AS1	T	C	0.14	0.38	7.6×10^{-6}
rs9540222	13	65 368 490	Intergenic	NA	Gene desert	C	T	0.80	0.33	4.7×10^{-6}
rs6497475	16	20 354 282	UMOD	Intronic	GP2 - PDILT	C	T	0.75	0.31	6.6×10^{-6}

Table 1: *Locus* is based on build 37, h19 and refers to the gene in which the identified SNP is located. *Location in relation to the gene* provides information regarding the location of the SNP in a gene's intron or exon (intergenic SNPs are therefore indicated as NA). *Flanking genes* are indicated for a 500kb window, with hyphens symbolizing the location of the SNP. *RAF* indicates risk allele frequency.

Table 2*:* Comparison of baseline characteristics between ADVANCE genotyped population, and patients with a high and low GRS_{UACR}.

Traits at baseline	Genotyped dataset (n = 3409)	Patients with high GRS_{UACR} (n = 547)	Patients with low GRS_{UACR} (n = 531)	p-value
Women, n (%)	1203 (35.3)	193 (35.3)	196 (36.9)	6.2×10^{-1}
Age, mean y (SD)	67.3 (6.6)	67.1 (7.0)	67.0 (6.4)	8.4×10^{-1}
Age at T2D diagnosis, mean y (SD)	60.1 (8.5)	59.8 (8.9)	60.0 (8.2)	6.6×10^{-1}
Duration of diabetes mellitus, mean y (SD)	6.7 (6.05)	6.8 (5.9)	6.5 (5.8)	2.9×10^{-1}
Slavic ethnicity, n (%)	1102 (32.3)	181 (33.1)	155 (29.2)	1.9×10^{-1}
History of major macrovascular disease, n (%)	1314 (38.5)	205 (37.5)	217 (40.9)	2.8×10^{-1}
History of major microvascular disease, n (%)	313 (9.2)	44 (8.0)	50 (9.4)	4.9×10^{-1}
Metabolic syndrome, n (%)	2126 (62.4)	342 (62.5)	324 (61.0)	6.1×10^{-1}
Blood pressure control				
SBP, mean mmHg (SD)	146.9 (20.8)	145.5 (21.1)	148.3 (20.7)	**2.8×10^{-2}**
DBP, mean mmHg (SD)	81.4 (10.6)	80.9 (10.5)	82.0 (10.6)	9.8×10^{-2}
Pulse blood pressure, mean mmHg (SD)	65.5 (16.5)	64.5 (16.5)	66.3 (15.9)	7.7×10^{-2}
History of treated hypertension, n (%)	2407 (70.6)	384 (70.2)	387 (73.0)	3.4×10^{-1}
Glucose control				
Fasting blood glucose, mean mmol/L (SD)	8.4 (2.6)	8.3 (2.5)	8.4 (2.7)	6.2×10^{-1}
Hba1c, mean % (SD)	7.3 (1.3)	7.3 (1.3)	7.2 (1.4)	5.7×10^{-1}
Renal factors				
Microalbuminuria, n (%)	882 (25.4)	107 (20.7)	149 (29.2)	**2.8×10^{-3}**
Macroalbuminuria, n (%)	173 (5.3)	24 (4.7)	28 (5.5)	5.5×10^{-1}
UACR, median µg/mg (IQR)	13.3 (6.2 - 40.7)	9.72 (4.6 - 30.9)	15.9 (7.9 - 46.8)	**1.4×10^{-8}**
eGFR, median mL/min per $1.73m^2$ (IQR)	72.6 (61.1 - 85.2)	71.9 (60.7 - 84.8)	73.9 (63 - 85.8)	1.9×10^{-1}
Lipid control				
Total cholesterol, mean mmol/L (SD)	5.1 (1.1)	5.2 (1.1)	5.1 (1.1)	3.1×10^{-1}
LDL-cholesterol, mean mmol/L (SD)	3.0 (1.0)	3.1 (1.0)	3.0 (1.1)	5.0×10^{-1}
HDL-cholesterol, mean mmol/L (SD)	1.2 (0.3)	1.2 (0.3)	1.2 (0.3)	5.1×10^{-1}
Triglycerides, median mmol/L (IQR)	1.7 (1.2 - 2.4)	1.7 (1.2 - 2.36)	1.7 (1.2 - 2.32)	8.4×10^{-1}
Other risk factors				
Heart rate, mean beats/min (SD)	72 (12.3)	71.7 (12.3)	72.4 (12.0)	3.0×10^{-1}
Current smoking, n (%)	556 (15.5)	90 (16.5)	84 (15.8)	8.4×10^{-1}
Waist circumference, mean cm (SD)	104 (12.6)	104.9 (12.0)	103.1 (12.6)	**1.8×10^{-2}**
BMI, mean kg/m^2 (SD)	30.1 (5.1)	30.3 (5.2)	29.8 (5.2)	1.8×10^{-1}
Diabetes family history, n (%)	1617 (47.4)	257 (47.0)	252 (47.5)	9.2×10^{-1}

Table 2*:* Comparison of baseline characteristics between ADVANCE genotyped population, and patients with a high and low GRS_{UACR}. Slavic ethnicity based of PC1 value ("Celtic ethnicity", PC1 <0; "Slavic ethnicity", PC1 ≥0). Hba1c: glycated hemoglobin, UACR: urine albumin: creatinine ratio, eGFR: estimated glomerular filtration rate, BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure. P-values are obtained from chi-square tests for binomial variables, two-sided student t-test for normally distributed continuous variables and Wilcoxon test for non-normally distributed continuous variables. Median and inter-quartile range (IQR) are indicated for triglycerides, UACR and eGFR clinical variables.

Table 3*:* Comparison of baseline characteristics between ADVANCE genotyped population, and patients with a high and low GRS_{eGFR}.

Traits at baseline	Genotyped dataset (n = 3409)	Patients with high GRS_{eGFR} (n=634)	Patients with low GRS_{eGFR} (n=638)	p-value
Women, n (%)	1203 (35.3)	233 (36.8)	219 (34.3)	4.0×10^{-1}
Age, mean y (SD)	67.3 (6.6)	67.2 (6.4)	67.3 (6.5)	8.2×10^{-1}
Age at T2D diagnosis, mean y (SD)	60.1 (8.5)	60.2 (8.4)	60,5 (8.4)	5.5×10^{-1}
Duration of diabetes mellitus, mean y (SD)	6.7 (6.05)	6.6 (5.9)	6.4 (5.8)	5.5×10^{-1}
Slavic ethnicity, n (%)	1102 (32.3)	230 (36.3)	189 (29.6)	**1.4×10^{-2}**
History of major macrovascular disease, n (%)	1314 (38.5)	237 (37.4)	232 (36.4)	7.5×10^{-1}
History of major microvascular disease, n (%)	313 (9.2)	54 (8.5)	55 (8.6)	1.0
Metabolic syndrome, n (%)	2126 (62.4)	414 (65,3)	388 (60,8)	9.7×10^{-2}
<u>Blood pressure control</u>				
SBP, mean mmHg (SD)	146.9 (20.8)	147.1 (20.6)	146.3 (20.4)	5.1×10^{-1}
DBP, mean mmHg (SD)	81.4 (10.6)	81.6 (10.4)	81.4 (10.6)	7.4×10^{-1}
Pulse blood pressure, mean mmHg (SD)	65.5 (16.5)	65.5 (16.7)	64.9 (16.1)	5.4×10^{-1}
History of treated hypertension, n (%)	2407 (70.6)	458 (72.2)	434 (68.0)	1.1×10^{-1}
<u>Glucose control</u>				
Fasting blood glucose, mean mmol/L (SD)	8.4 (2.6)	8.5 (2.7)	8.3 (2.4)	2.4×10^{-1}
Hba1c, mean % (SD)	7.3 (1.3)	7.3 (1.4)	7.2 (1.2)	1.5×10^{-1}
<u>Renal factors</u>				
Microalbuminuria, n (%)	882 (25.4)	163 (27.2)	138 (22.7)	7.5×10^{-2}
Macroalbuminuria, n (%)	173 (5.3)	34 (5.7)	24 (4.0)	1.6×10^{-1}
UACR, median µg/mg (IQR)	13.3 (6.2 - 40.7)	13.6 (6.2 - 47.7)	11.5 (5.3 - 32.7)	**1.4×10^{-2}**
eGFR, median mL/min per $1.73m^2$ (IQR)	72.6 (61.1 - 85.2)	72.4 (62.2 - 86.2)	73.1 (60.7 - 85.8)	9.9×10^{-1}
<u>Lipid control</u>				
Total cholesterol, mean mmol/L (SD)	5.1 (1.1)	5.2 (1.1)	5.0 (1.1)	6.3×10^{-2}
LDL-cholesterol, mean mmol/L (SD)	3.0 (1.0)	3.1 (1.1)	3.0 (0.9)	**4.3×10^{-2}**
HDL-cholesterol, mean mmol/L (SD)	1.2 (0.3)	1.2 (0.3)	1.2 (0.3)	8.3×10^{-1}
Triglycerides, median mmol/L (IQR)	1.7 (1.2 - 2.4)	1.7 (1.2 - 2.3)	1.6 (1.1 - 2.2)	3.0×10^{-1}
<u>Other risk factors</u>				
Heart rate, mean beats/min (SD)	72 (12.3)	71.8 (12.2)	72.6 (12.4)	2.8×10^{-1}
Current smoking, n (%)	556 (15.5)	100 (15.8)	105 (56.7)	8.0×10^{-1}
Waist circumference, mean cm (SD)	104 (12.6)	104.5 (12.2)	103.4 (12.9)	1.1×10^{-1}
BMI, mean kg/m^2 (SD)	30.1 (5.1)	30.3 (4.9)	30.0 (5.1)	8.5×10^{-2}
Diabetes family history, n (%)	1617 (47.4)	314 (49.5)	298 (46.7)	3.4×10^{-1}

Table 3*:* Slavic ethnicity based of PC1 value ("Celtic ethnicity", PC1 <0; "Slavic ethnicity", PC1 ≥0). Hba1c: glycated hemoglobin, UACR: urine albumin: creatinine ratio, eGFR: estimated glomerular filtration rate, BMI: body mass index, systolic blood pressure, DBP: diastolic blood pressure. P-values are obtained from chi-square tests for binomial variables, two-sided student t-test for normally distributed continuous variables and Wilcoxon test for non-normally distributed continuous variables. Median and inter-quartile range (IQR) are indicated for triglycerides, UACR and eGFR clinical variables.

Table 4*:* Comparison of baseline and end of study characteristics between patients with a high or low GRS_{UACR}, and patients with a high or low GRS_{eGFR}.

Baseline	**Patients with high GRS_{UACR} (n = 547)**	**Patients with low GRS_{UACR} (n = 531)**	**p-value**	**Patients with high GRS_{eGFR} (n = 634)**	**Patients with low GRS_{eGFR} (n = 638)**	**p-value**
SBP, mean mmHg (SD)	145.5 (21.1)	148.3 (20.7)	**2.8×10^{-2}**	147.1 (20.6)	146.3 (20.4)	5.1×10^{-1}
Hba1c, mean % (SD)	7.3 (1.3)	7.2 (1.4)	5.7×10^{-1}	7.3 (1.4)	7.2 (1.2)	1.5×10^{-1}
UACR, median µg/mg (IQR)	9.7 (4.6 - 30.9)	15.9 (7.9 - 46.8)	**1.4×10^{-8}**	13.6 (6.2 - 47.7)	11.5 (5.3 - 32.7)	**1.4×10^{-2}**
eGFR, median mL/min per 1.73m^2 (IQR)	71.9 (60.7 - 84.8)	73.9 (63 - 85.8)	1.9×10^{-1}	72.4 (62.2 - 86.2)	73.1 (60.7 - 85.8)	9.9×10^{-1}
4.4y median follow-up						
SBP, mean mmHg (SD)	138.7 (18.7) *	138.6 (18.9) *	9.4×10^{-1}	137.8 (18.0) *	139.4 (18.1) *	1.3×10^{-1}
Hba1c, mean % (SD)	7.0 (1.1) *	6.9 (1.1) *	**4.8×10^{-2}**	6.8 (1.1) *	6.9 (1.1) *	7.5×10^{-2}
UACR, median µg/mg (IQR)	18.1 (7.1 - 54.5) *	9.7 (5.3 - 24.8) *	**2.1×10^{-8}**	12.4 (6.2 - 36.2)	11.6 (5.3-31.0)	1.3×10^{-1}
UACR 5y progression slope, mean (SD)	0.06 (0.1)	-0.04 (0.1)	**3.1×10^{-29}**	0.004 (0.2)	0.006 (0.1)	8.0×10^{-1}
UACR delta, mean µg/mg (SD)	25.9 (117.2)	-17,0 (109.3)	**1.1×10^{-8}**	-5.2 (129.2)	-4.7 (113.6)	9.5×10^{-1}
eGFR, median mL/min per 1.73m^2 (IQR)	64.7 (51.2 - 79.4) *	68.9 (57.0 - 80.9) *	**6.5×10^{-3}**	62.0 (49.8 - 74.6) *	71.9 (58.4 - 83.9) *	**1.7×10^{-11}**
eGFR 5y progression slope, mean (SD)	-1.3 (6.2)	-1.6 (7.8)	4.5×10^{-1}	-2.5 (7.7)	-0.5 (4.6)	**5.1×10^{-8}**
eGFR, delta, mean mL/min per 1.73m^2 (SD)	-7.3 (16.6)	-5.5 (14.4)	**6.2×10^{-2}**	-10.5 (15.6)	-3.0 (13.2)	**3.5×10^{-19}**
Composite outcomes, n (%)	129 (23.6)	99 (18.6)	**4.7×10^{-2}**	153 (24.1)	144 (22.6)	5.1×10^{-1}

Table 4*:* P-values are obtained from two-sided student t-test for normally distributed continuous variables and Wilcoxon test for non-normally distributed continuous variables. Hba1c: glycated hemoglobin, UACR: urine albumin: creatinine ratio, eGFR: estimated glomerular filtration rate, composite outcomes: combined incidence of major macrovascular events (stroke, myocardial infarction, and cardiovascular death), and major microvascular events (doubling of serum creatinine, overt nephropathy), SBP: systolic blood pressure. Median and inter-quartile range (IQR) are indicated for triglycerides, UACR and eGFR clinical variables). UACR and eGFR deltas indicate average of individual patient differences between last and first measures of UACR and eGFR respectively. * Indicates statistically significant differences between the baseline and end of study measures of a given patient group (P<0.1).

Table 5*:* Baseline study characteristics between patients with a high or low GRS_{UACR}, compared to end of study characteristics between patients with high or low GRS_{UACR} receiving both ADVANCE trial treatments or no ADVANCE trial assignments.

Baseline	**Patients with high GRS_{UACR} (n = 547)**			**Patients with low GRS_{UACR} (n = 531)**		**p-value**
Systolic blood pressure, mean mmHg (SD)	145.5 (21.1)			148.3 (20.7)		**2.8×10^{-2}**
Hba1c, mean % (SD)	7.3 (1.3)			7.2 (1.4)		5.7×10^{-1}
UACR, median µg/mg (IQR)	9.7 (4.6 - 30.9)			15.9 (7.9 - 46.8)		**1.4×10^{-8}**
eGFR, median mL/min per 1.73m^2 (IQR)	71.9 (60.7 - 84.8)			73.9 (63.0 - 85.8)		1.9×10^{-1}
4.4y median follow-up	**Receiving both ADVANCE medications (n = 132)**	**Receiving no ADVANCE medications (n = 133)**	**p-value**	**Receiving both ADVANCE medications (n = 139)**	**Receiving no ADVANCE medications (n = 134)**	
SBP, mean mmHg (SD)	137.4 (16.1)	140.3 (19.0)	$1{,}8 \times 10^{-1}$	136.3 (18.8)	137.6 (17.7)	5.5×10^{-1}
Hba1c, mean % (SD)	6.7 (0.8)	7.2 (1.0)	**$3{,}9 \times 10^{-6}$**	6.6 (0.9)	7.1 (1.3)	**1.8×10^{-4}**
UACR, median µg/mg (IQR)	18.6 (6.2-58.7) *	18.1 (8.6 - 53.3)	$8{,}5 \times 10^{-1}$	7.4 (4.4 - 18.1) *	11.95 (4.4 - 35.7)	6.4×10^{-2}
UACR 5y progression slope, mean (SD)	0.06 (0.1) *	0.06 (0.1) †	$6{,}6 \times 10^{-1}$	-0.04 (0.1) *	-0.02 (0.1) †	2.1×10^{-1}
UACR delta, mean µg/mg (SD)	32.0 (125.8) *	33.9 (137.5) †	$9{,}2 \times 10^{-1}$	-12.8 (102.4) *	0,1 (118.6) †	3.8×10^{-1}
eGFR, median mL/min per 1.73m^2 (IQR)	60.7 (51.2-74.1) *	69.3.0 (56.4 - 83.6)	**$1{,}3 \times 10^{-2}$**	67.0 (56.5 - 81.1) *	66.8 (52.8 - 83.4)	5.6×10^{-1}
eGFR 5y progression slope, mean (SD)	-1.7 (8.1)	-0.6 (4.9)	$1{,}2 \times 10^{-1}$	-3.2 (13.3)	-1.0 (3.6)	7.3×10^{-2}
eGFR, delta, mean mL/min per 1.73m^2 (SD)	-10.8 (16.5)	-4.2 (15.8)	$1{,}1 \times 10^{-3}$	-7.0 (14.7)	-4.6 (14.2)	1.8×10^{-1}
Composite outcomes, n (%)	33 (25.0)	29 (21.8)	$5{,}4 \times 10^{-1}$	26 (18.7)	25 (18.7)	9.9×10^{-1}

Table 5*:* Both ADVANCE trial treatments: Preterax and Diamicron MR. No ADVANCE trial assignments: blood pressure arm placebo and glucose control arm placebo. P-values are obtained from two-sided student t-test for normally distributed continuous variables and Wilcoxon test for non-normally distributed continuous variables. UACR: urine albumin: creatinine ratio, eGFR: estimated glomerular filtration rate, composite outcomes: combined incidence of major macrovascular events (stroke, myocardial infarction, and cardiovascular death), and major microvascular events (doubling of serum creatinine, overt nephropathy), SBP: systolic blood pressure. Median and inter-quartile range (IQR) are indicated for UACR, and eGFR clinical variables. UACR and eGFR deltas indicate average of individual patient differences between last and first measures of UACR and eGFR respectively. * Indicates statistically significant differences between values of patients with high or low GRS_{UACR} receiving both ADVANCE medication (P<0.1), † Indicates statistically significant differences between values of patients with high and low GRS_{UACR} receiving no ADVANCE medication (P<0.1).

Figure 1*:* Comparison of renal event incidence between patients with high or low GRS_{UACR} and patients with high or low GRS_{eGFR}.

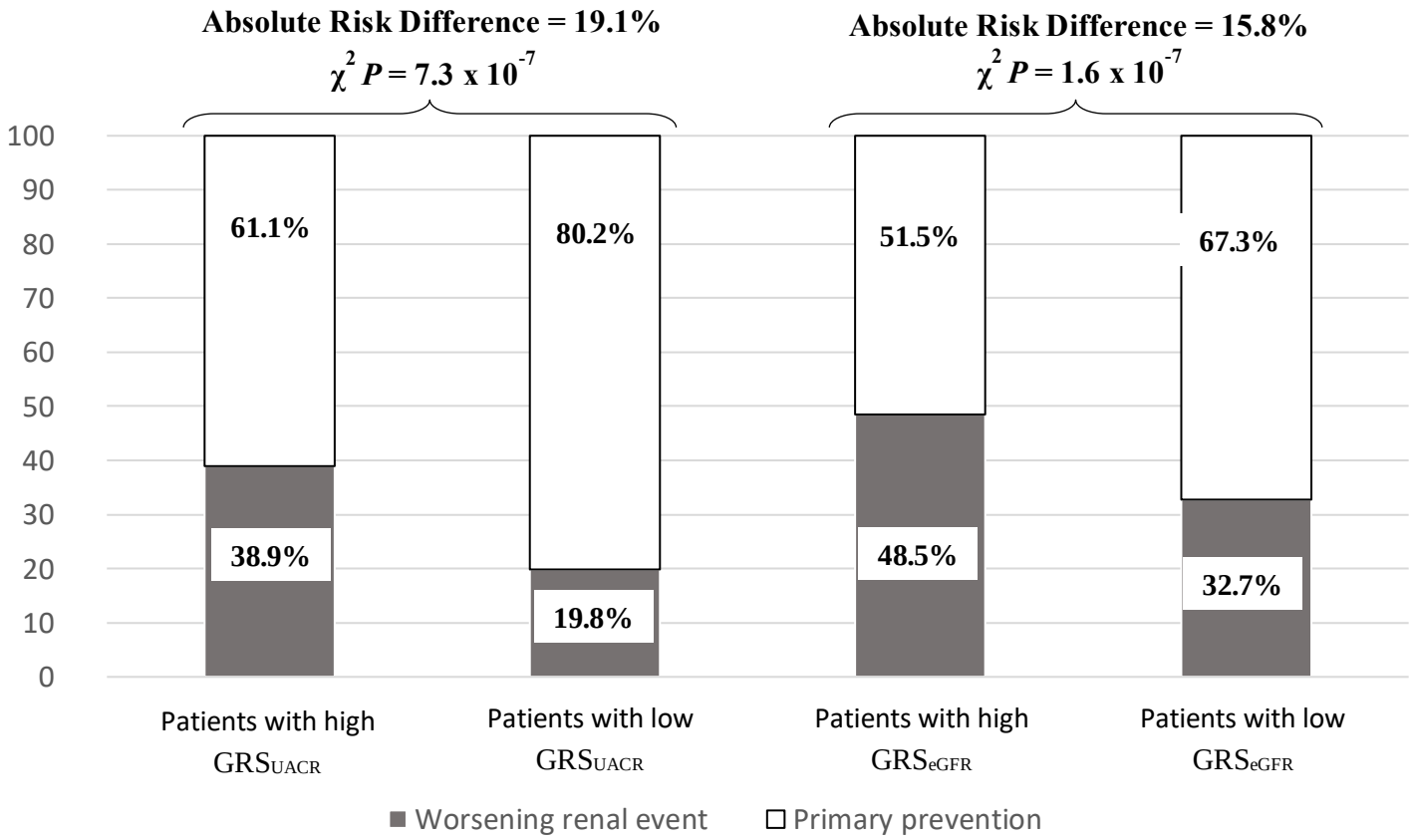
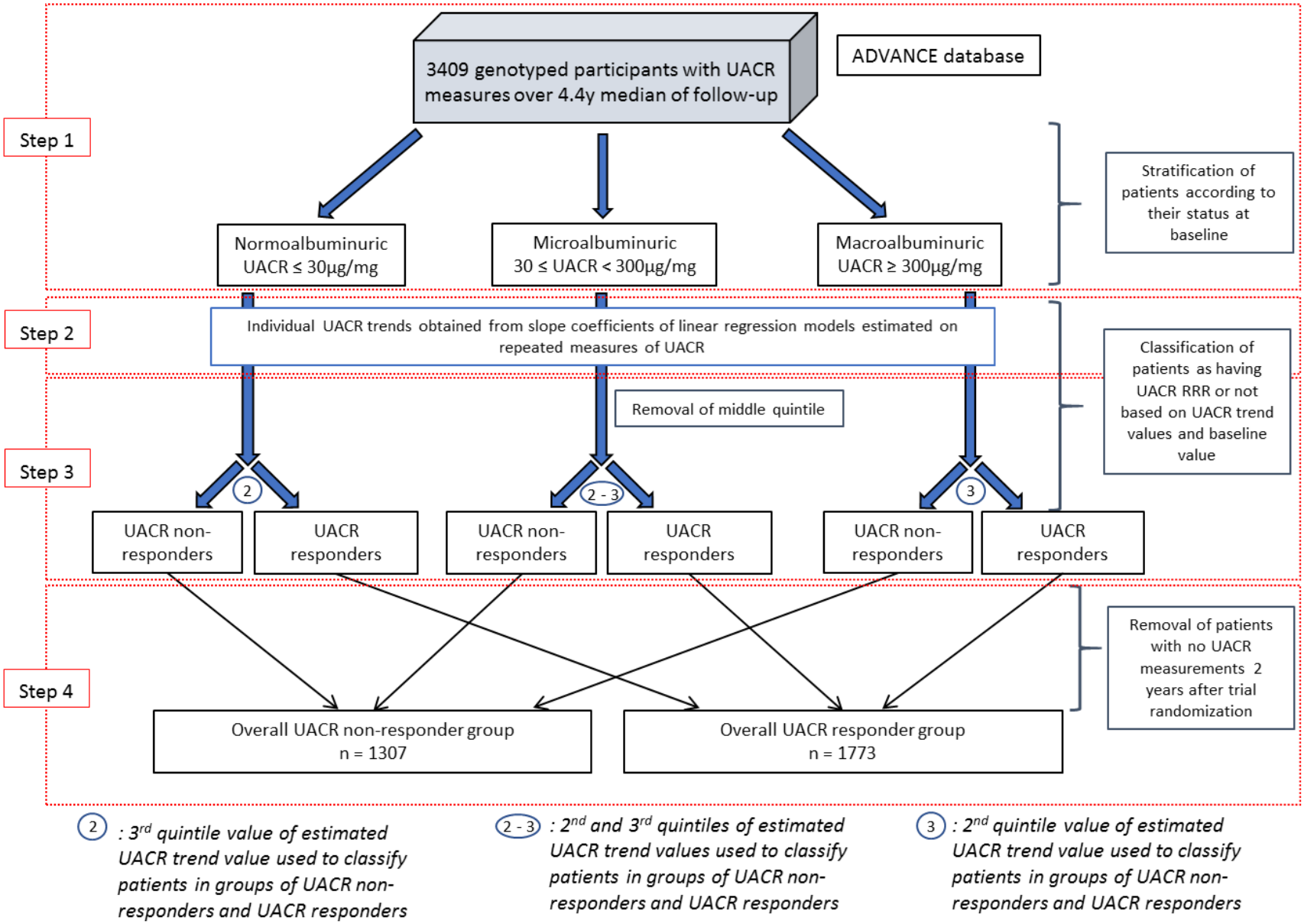


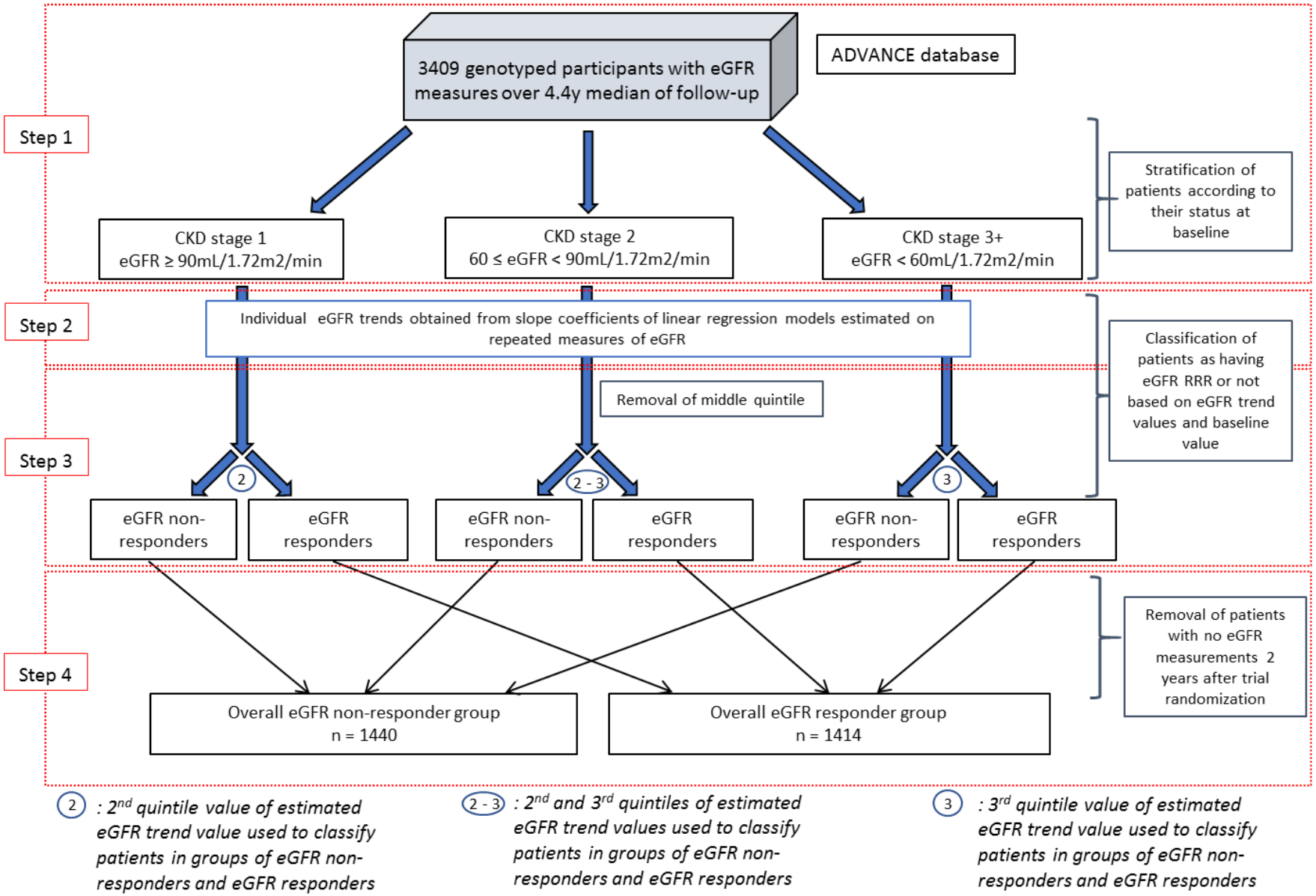
Figure 1*:* Comparison of renal event incidence between patients with high or low GRS_{UACR} and patients with high or low GRS_{eGFR}. Comparison of UACR worsening event (from normoalbuminuria to microalbuminuria) with UACR primary prevention event (normoalbuminuria status conserved throughout the trial) between normoalbuminuric patients with a high or low GRS_{UACR}. Comparison of eGFR worsening event (from CKD1-2 - eGFR ≥ 60mL/1.72m^2/min to CKD3+ eGFR < 60mL/1.72m^2/min) and eGFR prevention event (CKD1-2 status conserved throughout the trial) between CKD1-2 patients at baseline with a high or low $GRS_{eGFR.}$ The absolute risk difference indicates the difference of risk of renal worsening events between patients with high or low GRS. The indicated p-values is from a chi-square test of a 2 by 2 table for renal worsening and primary prevent event between patients with high or low GRS_{UACR} and GRS_{eGFR} respectively.

Supplemental Figure 1: Step by step process of the UACR non-response classification algorithm



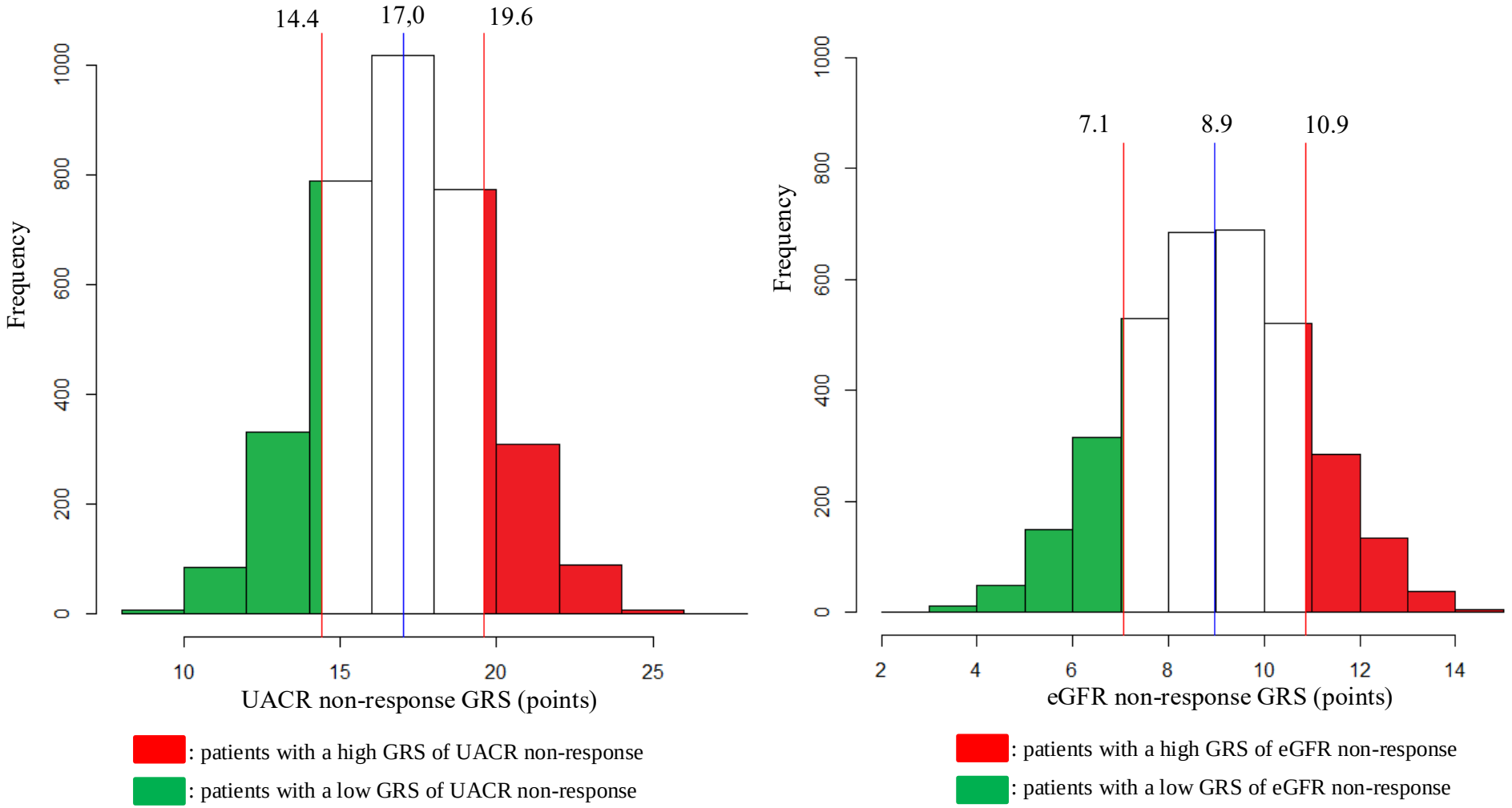
Supplemental Figure 1: Individual UACR progression trend values were estimated from repeated UACR measures collected during the ADVANCE study for each genotyped ADVANCE patients. Patients were stratified according to their albuminuria status at study entry and then classified in groups of UACR non-responders or UACR responders based on their UACR trend values. Overall UACR non-responder or UACR responder groups were then generated by regrouping stratum specific groups. Patients who died during the study were removed from the analysis.

Supplemental Figure 2: Step by step process of the eGFR non-response classification algorithm



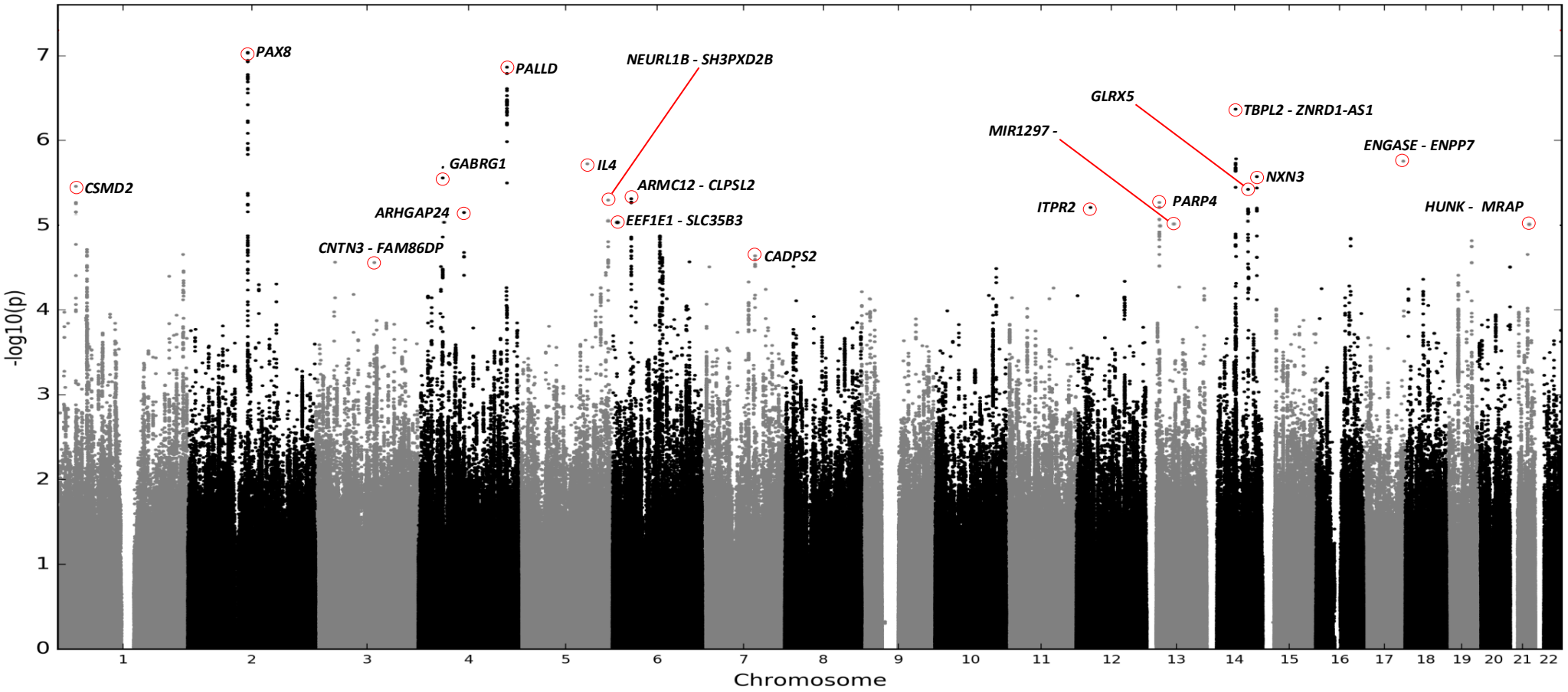
Supplemental Figure 2: Individual eGFR progression trend values were estimated from repeated eGFR measures collected during the ADVANCE study for each genotyped ADVANCE patients. Patients were stratified according to their CKD status at study entry and then classified in groups of eGFR non-responders or eGFR responders based on their eGFR trend values. Overall eGFR non-responder or eGFR responder groups were then generated by regrouping stratum specific groups. Patients who died during the study were removed from the analysis.

Supplemental Figure 3: Histogram distribution of UACR and eGFR non-response GRS values for 3409 genotyped Caucasian ADVANCE participants



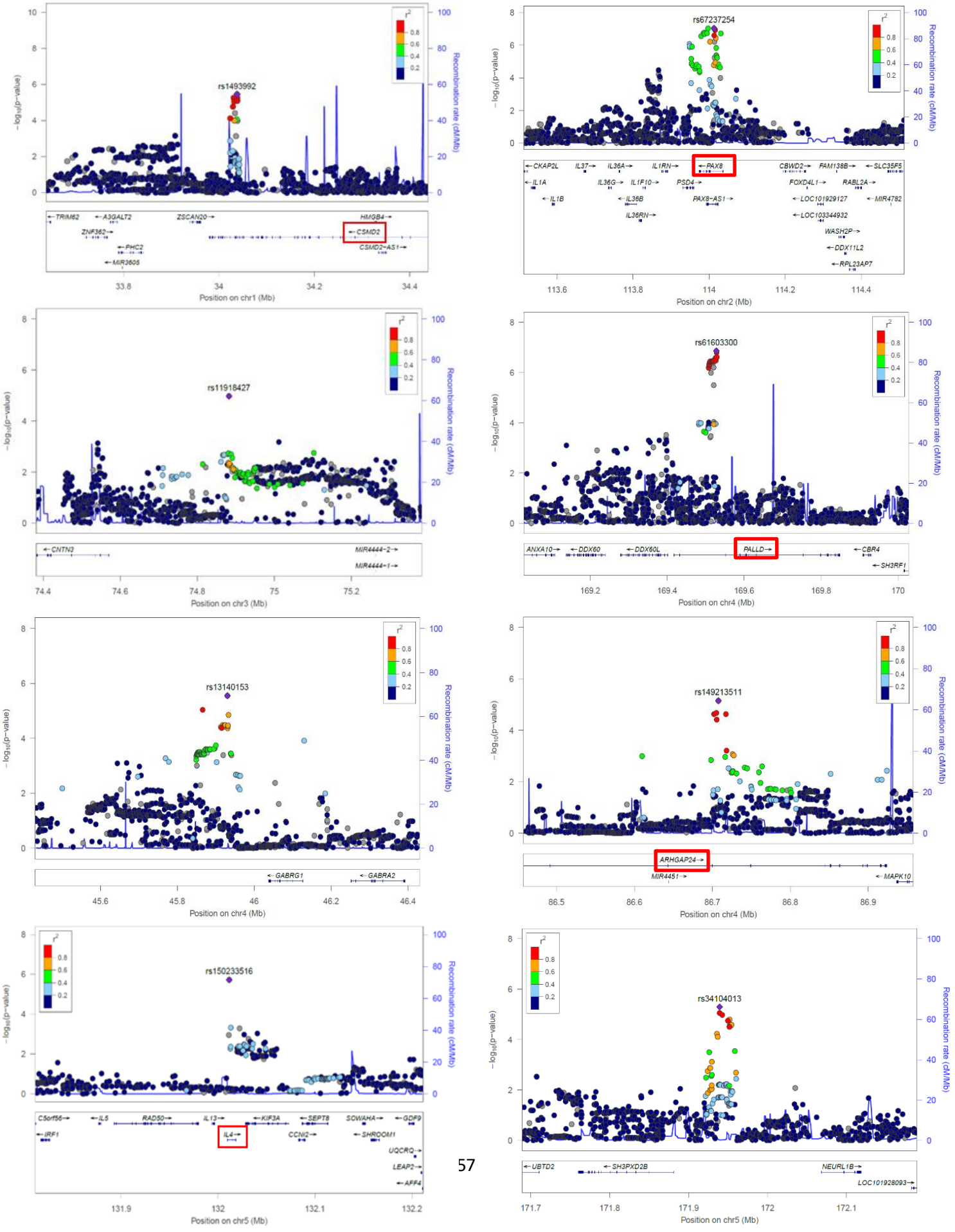
Supplemental Figure 3: Left: histogram of GRS_{UACR} values. Right: histogram of GRS_{eGFR} values. Mean value indicated in blue, mean value +/- standard deviation respectively indicated on the right and the left of the mean by red lines. Patients with a high GRS are identified as patients who possess a GRS one standard deviation above the mean. Patients with a low GRS are identified as patients who possess a GRS one standard deviation below the mean.

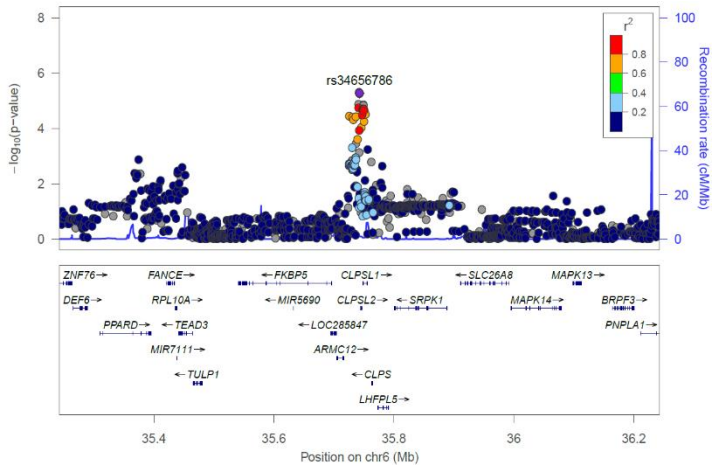
Supplemental Figure 4: Genome-wide $\log_{10}$ P value plot UACR non-response

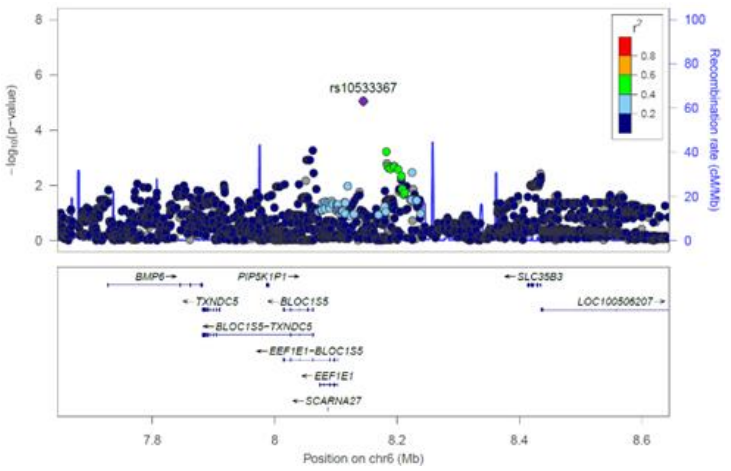


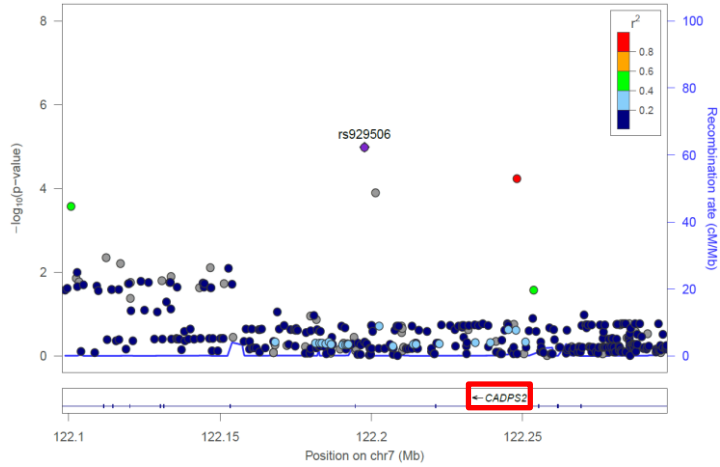
Supplemental Figure 4: Genome-wide $\log_{10}$ P value plot of UACR non-response. Loci associated to each SNP are indicated in bold, intergenic SNPs are represented by hyphens with upstream and downstream flanking genes within a 500kb window positioned accordingly.

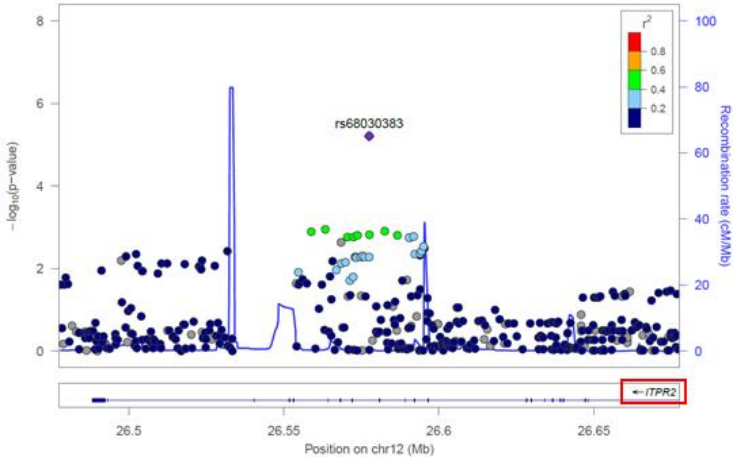
Supplemental Figure 5: Locus zoom of most significant SNPs from the GWAS for the UACR non-response

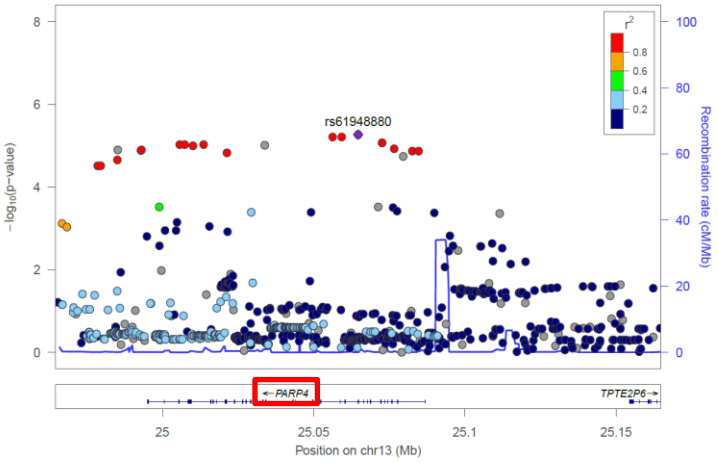


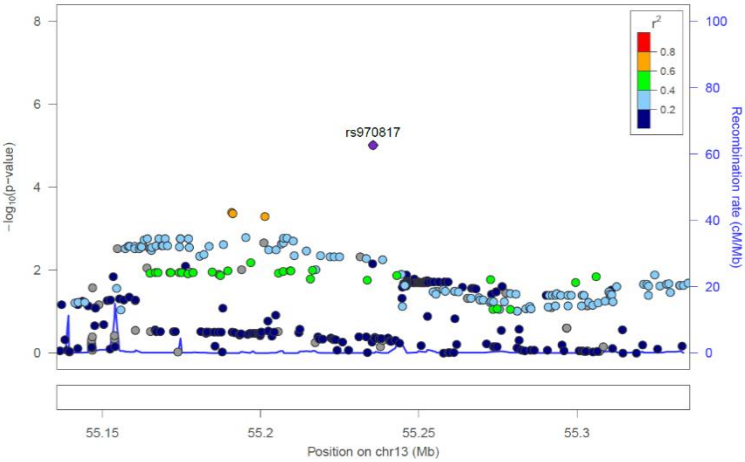

rs34656786
ZNF76→ FANCE→ ←FKBP5 CLPSL1→ ←SLC26A8 MAPK13→
DEF6→ RPL10A→ ←MIR5690 CLPSL2→ ←SRPK1 MAPK14→ BRPF3→
PPARD→ ←TEAD3 ←LOC285847 PNPLA1→
MIR7111→ ARMC12→
←TULP1 ←CLPS
LHFPL5→
35.4 35.6 35.8 36 36.2
Position on chr6 (Mb)
−log10(p−value)
Recombination rate (cM/Mb)
r²

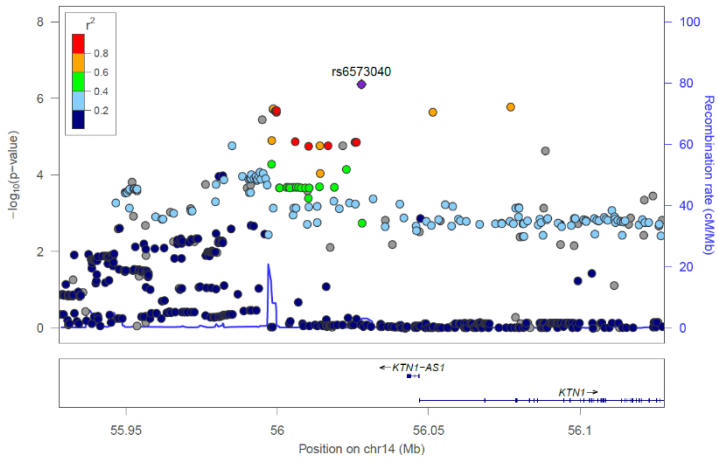

rs10533367
BMP6→ PIP5K1P1→ ←SLC35B3
←TXNDC5 ←BLOC1S5 LOC100506207→
←BLOC1S5-TXNDC5
←EEF1E1-BLOC1S5
←EEF1E1
←SCARNA27
7.8 8 8.2 8.4 8.6
Position on chr6 (Mb)
−log10(p−value)
Recombination rate (cM/Mb)
r²

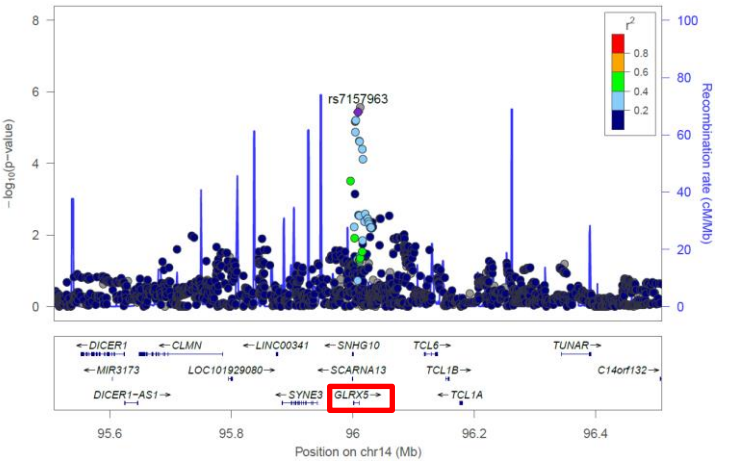

rs929506
←CADPS2
122.1 122.15 122.2 122.25
Position on chr7 (Mb)
−log10(p−value)
Recombination rate (cM/Mb)
r²

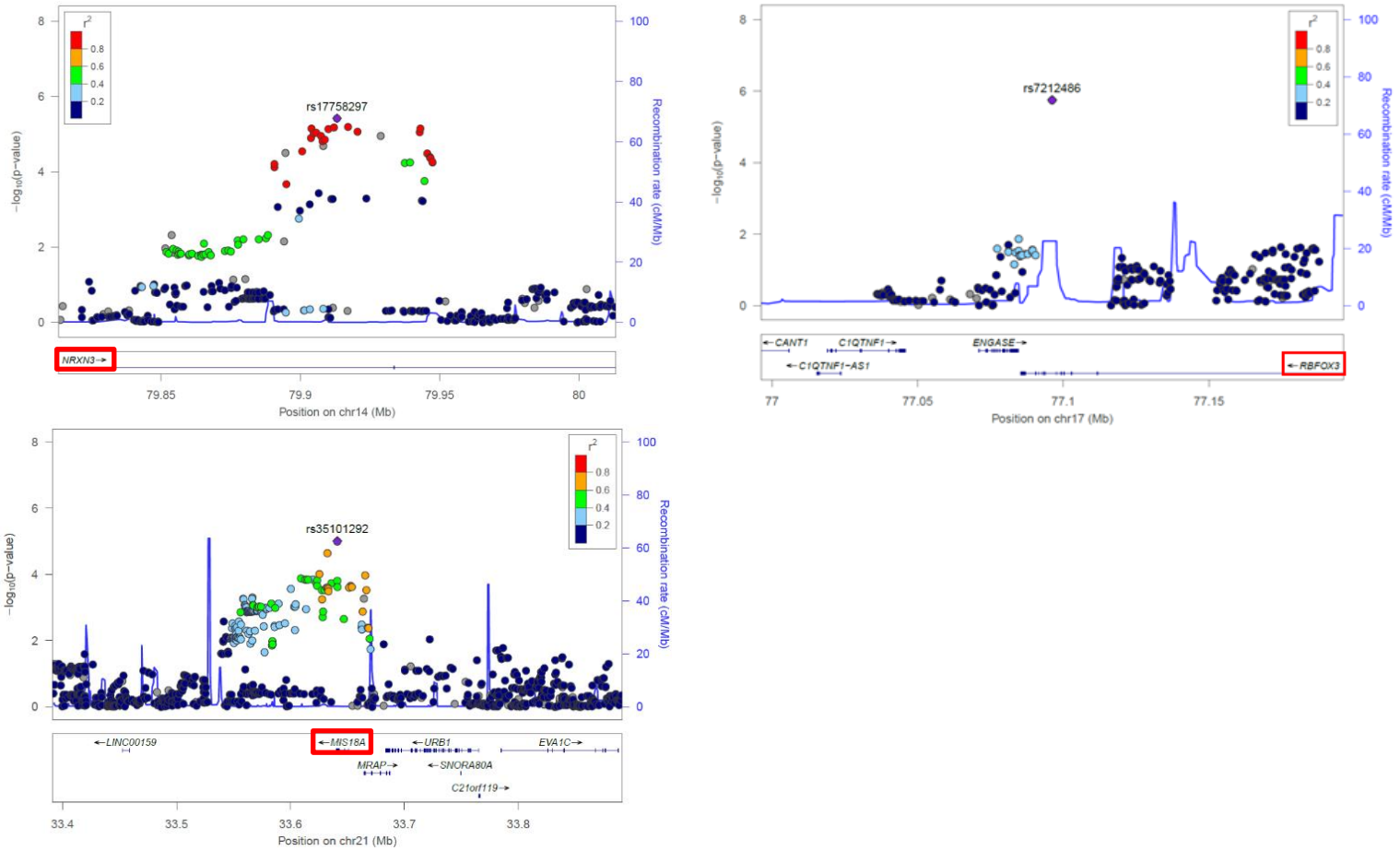

rs68030383
←ITPR2
26.5 26.55 26.6 26.65
Position on chr12 (Mb)
−log10(p−value)
Recombination rate (cM/Mb)
r²


rs61948880
←PARP4
TPTE2P6→
25 25.05 25.1 25.15
Position on chr13 (Mb)
−log10(p−value)
Recombination rate (cM/Mb)
r²


rs970817
55.15 55.2 55.25 55.3
Position on chr13 (Mb)
−log10(p−value)
Recombination rate (cM/Mb)
r²

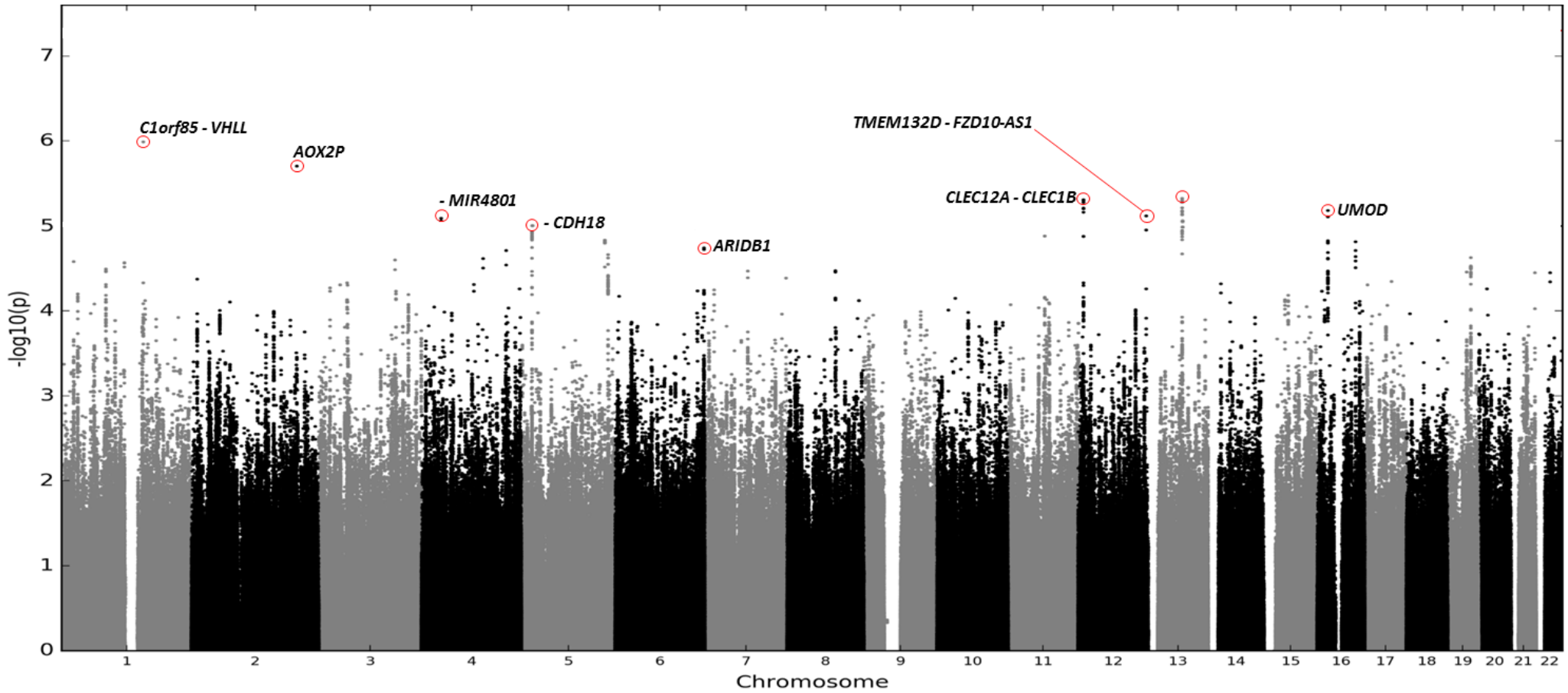

rs6573040
←KTN1-AS1
KTN1→
55.95 56 56.05 56.1
Position on chr14 (Mb)
−log10(p−value)
Recombination rate (cM/Mb)
r²


rs7157963
←DICER1 ←CLMN ←LINC00341 ←SNHG10 TCL6→ TUNAR→
←MIR3173 LOC101929080→ ←SCARNA13 TCL1B→ C14orf132→
DICER1-AS1→ ←SYNE3 GLRX5→ ←TCL1A
95.6 95.8 96 96.2 96.4
Position on chr14 (Mb)
−log10(p−value)
Recombination rate (cM/Mb)
r²



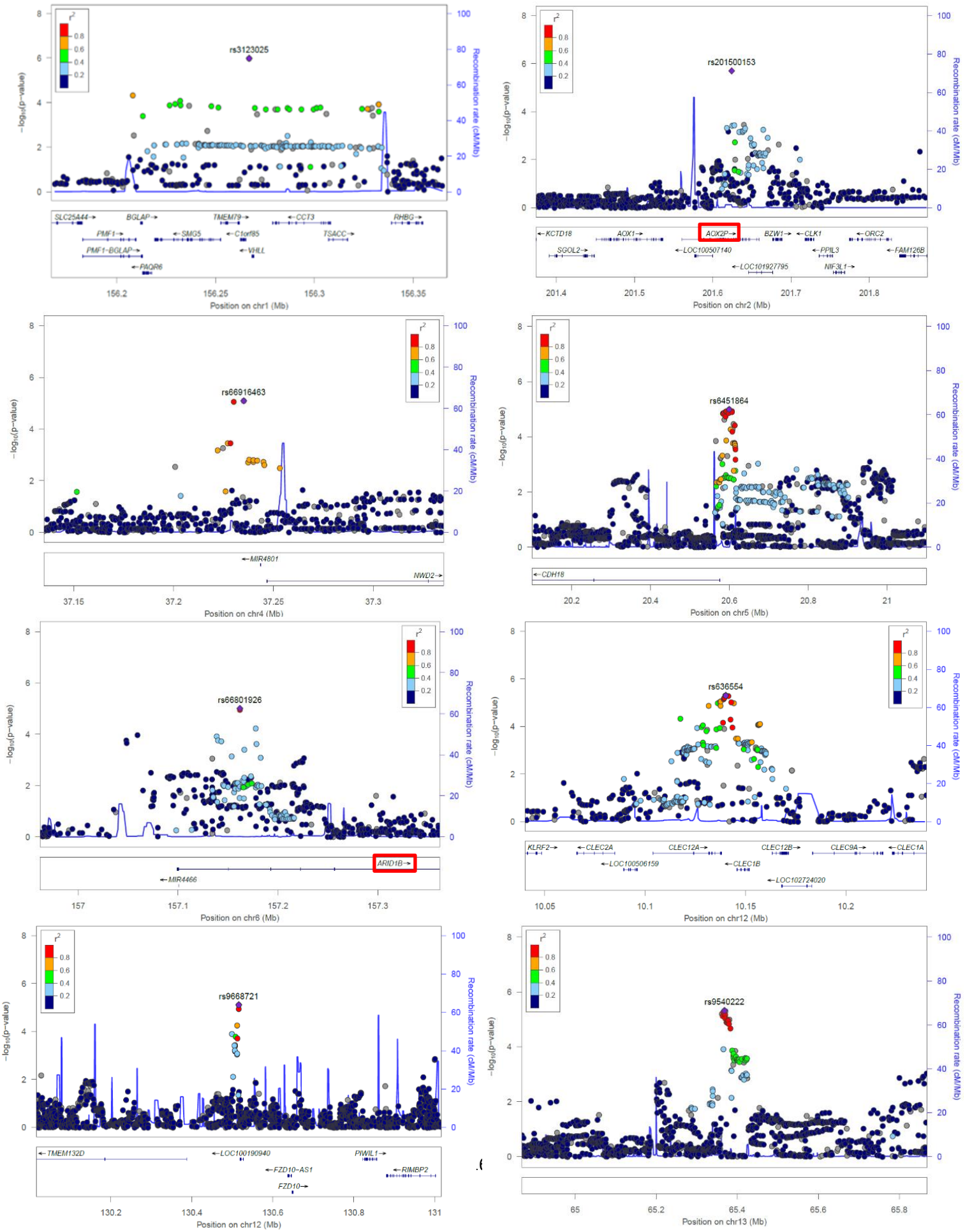
Supplemental Figure 5: Locus zoom of most significant SNPs from the GWAS of UACR non-response, included in the GRS_{UACR}. Red boxes indicate the gene in which a specific SNP is located. Locuszoom plots with no red boxes represent SNPs located in intergenic regions.

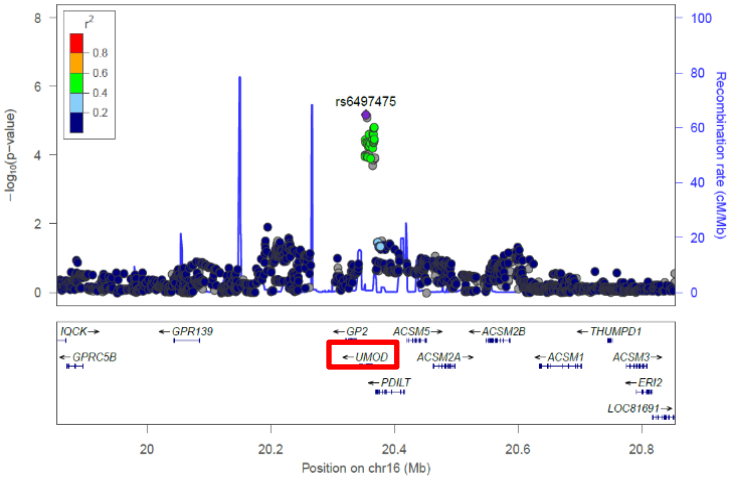
Supplemental Figure 6: Genome-wide $\log_{10}$ P value plot eGFR non-response



Supplemental Figure 6: Genome-wide $\log_{10}$ P value plot of eGFR non-response. Loci associated to each SNP are indicated in bold, intergenic SNPs are represented by hyphens with upstream and downstream flanking genes within a 500kb window positioned accordingly.

Supplemental Figure 7: Locus zoom of most significant SNPs of the GWAS for the eGFR non-response





Supplemental Figure 7: Locus zoom of most significant SNPs of the GWAS of eGFR non-response, included in the GRS_{eGFR}. Red boxes indicate the gene in which a specific SNP is located. Locuszoom plots with no red boxes represent SNPs which located in intergenic regions.

Supplemental Table 1: Genetic association results of SNPs identified in the study of UACR non-response and respectively included in geo-ethnic GRS_{UACR}

						Celtic			Slavic			Combined		
SNP ID	**Chr**	**Position (b37; h19)**	**Locus**	**Risk allele**	**Non-risk allele**	**AF risk allele**	**β**	**association p-value**	**AF risk allele**	**β**	**association p-value**	**AF risk allele**	**β**	**association p-value**
rs1493992	1	34 038 006	CSMD2	T	C	0.53	0.25	1.7×10^{-4}	0.53	0.25	1.1×10^{-2}	0.53	0.26	**3.5×10^{-6}**
rs11918427	3	74 882 210	CNTN3 - FAM86DP	G	C	012	0.43	4.6×10^{-5}	0.13	0.29	5.6×10^{-2}	0.12	0.38	**1.1×10^{-5}**
rs13140153	4	45 930 292	GABRA2 - GABRG1	G	A	0.89	0.40	2.0×10^{-4}	0.92	0.61	1.6×10^{-3}	0.90	0.44	**2.9×10^{-6}**
rs61603300	4	169 527 905	PALLD	G	T	0.92	0.37	2.5×10^{-3}	0.90	0.93	2.1×10^{-6}	0.91	0.55	**1.4×10^{-7}**
rs150233516	5	132 011 621	IL4	ATGTG	A	0.83	0.44	8.7×10^{-6}	0.80	0.27	4.9×10^{-2}	0.82	0.38	**1.9×10^{-6}**
rs34104013	5	171 939 378	NEURL1B - SH3PXD2B	C	CA	0.52	0.26	9.4×10^{-5}	0.51	0.23	1.8×10^{-2}	0.52	0.25	**5.1×10^{-6}**
rs34656786	6	35 742 752	ARMC12 - CLPSL2	T	C	0.34	0.24	3.8×10^{-4}	0.30	0.29	5.4×10^{-3}	0.33	0.6	**5.3×10^{-6}**
rs61948880	13	25 064 748	PARP4	T	C	0.17	0.23	1.2×10^{-2}	0.17	0.54	2.0×10^{-5}	0.17	0.33	**5.4×10^{-6}**
rs6573040	14	60 876 186	TBPL2 - KTN1-AS1	C	T	0.18	0.38	6.7×10^{-5}	0.20	0.31	1.0×10^{-2}	0.19	0.35	**4.3×10^{-7}**
rs7157963	14	96 008 866	GLRX5	A	T	0.21	0.34	2.7×10^{-5}	0.22	0.24	4.0×10^{-2}	0.22	0.31	**3.7×10^{-6}**
rs17758297	18	79 913 167	NRXN3	A	T	0.28	0.27	1.9×10^{-4}	0.31	0.27	6.9×10^{-3}	0.29	0.27	**3.9×10^{-6}**
SNPs only significant in Celtics														
rs600540	6	141 177 675	MIR4465 – NMBR	T	C	0.33	0.33	**2.0×10^{-6}**	0.68*	0.04	7.3×10^{-1}	0.32	0.22	1.5×10^{-4}
rs10533367	4	8 145 014	EEF1E1 - SLC35B3	A	G	0.86	0.50	**1.0×10^{-6}**	0.88	0.11	4.6×10^{-1}	0.87	0.38	9.1×10^{-6}
rs149213511	4	867 07 802	ARHGAP24	A	AAC	0.67	0.34	**3.4×10^{-6}**	0.72	0.12	2.5×10^{-1}	0.69	0.26	7.1×10^{-6}
rs2529206	7	101 421 979	MYL10 - CUX1	G	T	0.87	0.50	**1.7×10^{-6}**	0.84	0.06	6.6×10^{-1}	0.86	0.35	2.3×10^{-5}
rs929506	7	122 197 763	CADPS2	A	G	0.12	0.49	**9.7×10^{-6}**	0.10	0.19	2.6×10^{-1}	0.11	0.41	1.0×10^{-5}
rs259172	7	896 334 554	ZNF804B - DPY19L2P4	A	G	0.78	0.39	**4.1×10^{-6}**	0.75	0.12	3.1×10^{-1}	0.77	0.30	1.2×10^{-5}
rs1502003	16	55 601 388	LPCAT2	G	A	0.46	0.30	**7.5×10^{-6}**	0.47	0.002	9.8×10^{-1}	0.46	0.20	1.7×10^{-4}
rs7212486	17	77 096 177	RBFOX3	C	T	0.36	0.33	**3.0×10^{-6}**	0.34	0.15	1.4×10^{-1}	0.35	0.27	1.8×10^{-6}
SNPs only significant in Slavics														
rs150208974	2	42 366 651	PKDCC - EML4	CT	C	0.25*	0.14	5.2×10^{-2}	0.74	0.57	**1.8×10^{-6}**	0.74	0.07	2.4×10^{-1}
rs9342077	6	87 219 320	NT5E - HTR1E	C	T	0.14	0.04	6.4×10^{-1}	0.18	0.57	**7.6×10^{-6}**	0.15	0.22	3.4×10^{-3}
rs2876674	6	148 287 078	SMD5 - SASH1	T	A	0.31	0.03	6.0×10^{-1}	0.33	0.49	**2.7×10^{-6}**	0.31	0.17	2.5×10^{-3}
rs784684	9	109 899 527	MIR548Q - RAD23B	G	A	0.7	0.08	2.8×10^{-1}	0.71	0.50	**9.5×10^{-6}**	0.7	0.20	7.0×10^{-4}
rs10846991	12	126 321 728	TMEM132B - LINC00939	A	G	0.12	0.02	8.2×10^{-1}	0.10	0.74	**5.2×10^{-6}**	0.11	0.24	5.9×10^{-3}
rs17632271	15	54 084 457	WDR72 - UNC13C	T	C	0.60	0.04	5.8×10^{-1}	0.59	0.47	**2.7×10^{-6}**	0.60	0.18	1.5×10^{-3}
rs2601895	15	70 208 092	KIF23 - TLE3	C	T	0.22	0.03	7.1×10^{-1}	0.21	0.55	**7.9×10^{-6}**	0.22	0.19	5.8×10^{-3}
rs4889197	16	73 394 615	ZFHX3 - PSMD7	A	G	0.46	0.06	3.3×10^{-1}	0.51	0.47	**1.1×10^{-6}**	0.47	0.18	6.2×10^{-4}

Supplemental Table 1: Geo ethnic GRS_{UACR} composed of 11 SNPs significantly associated to UACR non-response in the combined Caucasian population in addition to 8 SNPs for Celtic patients or 9 SNPs for Slavic patients. (*) indicates instances where the risk allele of a specific SNP changes between Slavic and Celtic populations. *Locus* reports the genes in which a SNP is located or flanking genes within a 500kb window for intergenic SNPs, with hyphens symbolizing the location of the SNP.

Supplemental Table 2: Genetic association results of SNPs identified in the study of eGFR non-response and respectively included in geo-ethnic GRS_{eGFR}

						Celtic			Slavic			Combined		
SNP ID	**Chr**	**Position (b37; h19)**	**Locus**	**Risk allele**	**Non-risk allele**	**AF risk allele**	**β**	**association p-value**	**AF risk allele**	**β**	**association p-value**	**AF risk allele**	**β**	**association p-value**
rs3123025	1	15 626 7004	GLMP – VHLL	A	T	0.61	0.25	6.3×10^{-4}	0.62	0.38	4.4×10^{-4}	0.61	0.29	**1.0×10^{-6}**
rs201500153	2	201 624 094	AOX2P	C	CATAA	0.39	0.32	1.3×10^{-5}	0.45	0.19	6.6×10^{-2}	0.41	0.29	**2.0×10^{-6}**
rs6451864	5	20 599 383	CDH18	T	G	0.58	0.22	1.5×10^{-3}	0.58	0.34	1.2×10^{-3}	0.58	0.25	**1.0×10^{-5}**
SNPs only significant in Celtics														
rs62138595	2	53 543 006	- ASB3	C	T	0.81	0.41	**3.8×10^{-6}**	0.77	0.02	8.4×10^{-1}	0.80	0.27	1.4×10^{-4}
rs6820038	4	12 213 480	MIR572 -	G	A	0.48	0.35	**3.6×10^{-6}**	0.52*	0.05	6.5×10^{-1}	0.48	0.22	3.7×10^{-4}
rs4698735	4	13 567 131	RAB28 - BOD1L1	G	A	0.82	0.44	**4.2×10^{-6}**	0.14*	0.04	7.9×10^{-1}	0.83	0.31	1.5×10^{-4}
rs66801926	6	157 161 915	ARID1B	GC	G	0.66	0.35	**1.7×10^{-6}**	0.68	0.09	3.8×10^{-1}	0.66	0.27	9.8×10^{-6}
rs112804364	11	101 915 848	KIAA1377 - C11orf70	T	C	0.08	0.69	**2.6×10^{-6}**	0.90	0.07	6.7×10^{-1}	0.09	0.39	2.9×10^{-4}
rs6497475	16	20 354 282	UMOD	C	T	0.75	0.40	**1.8×10^{-6}**	0.86	0.11	3.8×10^{-1}	0.75	0.31	6.6×10^{-6}
SNPs only significant in Slavics														
rs79272126	8	79 752 228	- IL7	A	C	0.18*	0.02	8.1×10^{-1}	0.80	0.63	**1.1×10^{-5}**	0.81	0.19	1.2×10^{-2}
rs72107471	10	130 130 285	MKI67 – MGMT	CT	C	0.56	0.06	4.3×10^{-1}	0.50	0.56	**1.8×10^{-6}**	0.54	0.21	7.6×10^{-4}
rs7109015	11	1 679 977	MOB2	T	C	0.65*	0.15	5.3×10^{-2}	0.21	0.53	**4.6×10^{-6}**	0.33	0.11	2.4×10^{-1}
rs1359081	12	10 143 498	CLEC12A - CLEC1B	A	C	0.43	0.1	1.5×10^{-1}	0.43	0.49	**2.5×10^{-6}**	0.43	0.23	1.1×10^{-4}
rs11636403	15	51 548 744	CYP19A1	T	C	0.57*	0.007	9.2×10^{-1}	0.49	0.46	**8.6×10^{-6}**	0.45	0.14	1.7×10^{-2}
rs846744	20	49 334 703	FAM65C - PARD6B	G	A	0.79*	0.06	4.8×10^{-1}	0.21	0.62	**1.5×10^{-6}**	0.21	0.14	4.6×10^{-2}

Supplemental Table 2: Geo ethnic GRS_{eGFR} composed of 3 SNPs significantly associated to eGFR non-response in the combined Caucasian population in addition to 6 SNPs for Celtic patients or 6 SNPs for Slavic patients. (*) indicates instances where the risk allele of a specific SNP changes between Slavic and Celtic populations. *Locus* reports the genes in which a SNP is located, or flanking genes within a 500kb window for intergenic SNPS, with hyphens symbolizing the location of the SNP.

Supplemental Table 3*:* Baseline characteristics of ADVANCE genotyped population, UACR non-responders; and UACR responders

Traits at baseline	Genotyped dataset (n = 3409)	UACR non-responders n = 1307	UACR responders n = 1773	p-value
Women, n (%)	1203 (35.3)	433 (33.1)	665 (37.5)	**1.2×10^{-2}**
Age, mean y (SD)	67.3 (6.6)	67.4 (6.5)	66.8 (6.6)	**5.7×10^{-3}**
Age at T2D diagnosis, mean y (SD)	60.1 (8.5)	60.2 (8.5)	59.8 (8.4)	1.7×10^{-1}
Duration of diabetes mellitus, mean y (SD)	6.7 (6.05)	6.7 (5.8)	6.4 (6.0)	9.7×10^{-2}
Slavic ethnicity, n (%)	1102 (32.3)	407 (31.1)	600 (33.8)	1.2×10^{-1}
History of major macrovascular disease, n (%)	1314 (38.5)	534 (40.9)	631 (35.6)	**2.9×10^{-2}**
History of major microvascular disease, n (%)	313 (9.2)	83 (6.4)	181 (10.2)	**1.6×10^{-4}**
Metabolic syndrome, n (%)	2126 (62.4)	809 (61.9)	1097 (61.9)	9.8×10^{-1}
<u>Blood pressure control</u>				
SBP, mean mmHg (SD)	146.9 (20.8)	145.9 (21.0)	147.2 (20.4)	**3.6×10^{-2}**
DBP, mean mmHg (SD)	81.4 (10.6)	80.6 (10.5)	82.1 (10.4)	**3.3×10^{-5}**
Pulse blood pressure, mean mmHg (SD)	65.5 (16.5)	65.3 (16.8)	65.1 (16.0)	3.9×10^{-1}
History of treated hypertension, n (%)	2407 (70.6)	923 (70.6)	1205 (68.0)	1.1×10^{-1}
<u>Glucose control</u>				
Fasting blood glucose, mean mmol/L (SD)	8.4 (2.6)	8.3 (2.6)	8.4 (2.5)	9.8×10^{-2}
Hba1c, mean % (SD)	7.3 (1.3)	7.3 (1.3)	7.3 (1.4)	3.8×10^{-1}
<u>Renal factors</u>				
Microalbuminuria, n (%)	882 (25.4)	186 (15.3)	501 (31.5)	**2.4×10^{-20}**
Macroalbuminuria, n (%)	173 (5.3)	29 (2.7)	116 (9.6)	**2.2×10^{-8}**
UACR, median µg/mg (IQR)	13.3 (6.2 - 40.7)	7.5 (3.5 - 18.6)	17.9 (8.6 - 53.0)	**1.9×10^{-22}**
eGFR, median mL/min per $1.73m^2$ (IQR)	72.6 (61.1 - 85.2)	71.6 (59.9 - 84.6)	74.7 (63.0 - 86.6)	**2.6×10^{-5}**
<u>Lipid control</u>				
Total cholesterol, mean mmol/L (SD)	5.1 (1.1)	5.1 (1.1)	5.1 (1.1)	6.5×10^{-2}
LDL-cholesterol, mean mmol/L (SD)	3.0 (1.0)	3.0 (1.0)	3.0 (1.0)	1.5×10^{-1}
HDL-cholesterol, mean mmol/L (SD)	1.2 (0.3)	1.2 (0.3)	1.2 (0.3)	5.5×10^{-2}
Triglycerides, median mmol/L (IQR)	1.7 (1.2 - 2.4)	1.7 (1.2 - 2.4)	1.7 (1.2 - 2.3)	**$1,2 \times 10^{-2}$**
<u>Other risk factors</u>				
Heart rate, mean beats/min (SD)	72 (12.3)	71.3 (12.5)	72.4 (12.0)	**5.8×10^{-3}**
Current smoking, n (%)	556 (15.5)	217 (16.6)	272 (15.3)	3.4×10^{-1}
Waist circumference, mean cm (SD)	104 (12.6)	104.2 (11.7)	103.6 (12.8)	1.0×10^{-1}
BMI, mean kg/m^2 (SD)	30.1 (5.1)	30.1 (4.9)	30.1 (5.1)	4.9×10^{-1}
Diabetes family history, n (%)	1617 (47.4)	637 (48.7)	838 (47.3)	4.2×10^{-1}
ADVANCE blood glucose treatment, n (%)	1669 (48.9)	607 (46.4)	904 (51.0)	**1.3×10^{-2}**

Supplemental Table 3: Patient groups with or without UACR non-response at the end of follow as obtained from the classification algorithm for UACR non-response (Supp. Figure 1). Slavic ethnicity based of PC1 value ("Celtic ethnicity", PC1 <0; "Slavic ethnicity", PC1 ≥0). Hba1c: glycated hemoglobin, UACR: urine albumin: creatinine ratio, eGFR: estimated glomerular filtration rate, BMI: body mass index, systolic blood pressure, DBP: diastolic blood pressure. P-values are obtained from chi-square tests for binomial variables, two-sided student t-test for normally distributed continuous variables and Wilcoxon test for non-normally distributed continuous variables. Median and inter-quartile range (IQR) are indicated for triglycerides, urinary: albumin creatinine ratio and eGFR clinical variables.

Supplemental Table 4*:* Baseline characteristics of ADVANCE genotyped population, and patient with or without eGFR non-response

Traits at baseline	Genotyped dataset (n = 3409)	eGFR non-responders n = 1470	eGFR responders n = 1414	p-value
Women, n (%)	1203 (35.3)	525 (35.7)	521 (36.8)	5.3×10^{-1}
Age, mean y (SD)	67.3 (6.6)	67.7 (6.5)	66.6 (6.8)	**2.0×10^{-6}**
Age at T2D diagnosis, mean y (SD)	60.1 (8.5)	60.0 (8.8)	60.0 (8.3)	4.9×10^{-1}
Duration of diabetes mellitus, mean y (SD)	6.7 (6.05)	7.2 (6.3)	6.0 (5.5)	**5.9×10^{-8}**
Slavic ethnicity, n (%)	1102 (32.3)	407 (27.7)	548 (38.8)	**2.7×10^{-10}**
History of major macrovascular disease, n (%)	1314 (38.5)	602 (41.0)	515 (36.4)	**1.3×10^{-2}**
History of major microvascular disease, n (%)	313 (9.2)	163 (11.1)	108 (7.6)	**1.5×10^{-3}**
Metabolic syndrome, n (%)	2126 (62.4)	999 (67.9)	834 (59.0)	**5.5×10^{-7}**
<u>Blood pressure control</u>				
SBP, mean mmHg (SD)	146.9 (20.8)	149.1 (21.3)	144.6 (20.3)	**1.8×10^{-9}**
DBP, mean mmHg (SD)	81.4 (10.6)	81.7 (10.6)	81.2 (10.5)	8.4×10^{-2}
Pulse blood pressure, mean mmHg (SD)	65.5 (16.5)	67.3 (17.1)	63.4 (16.1)	**3.4×10^{11}**
History of treated hypertension, n (%)	2407 (70.6)	1095 (74.5)	942 (66.7)	**3.5×10^{-6}**
<u>Glucose control</u>				
Fasting blood glucose, mean mmol/L (SD)	8.4 (2.6)	8.5 (2.7)	8.3 (2.5)	**4.2×10^{-3}**
Hba1c, mean % (SD)	7.3 (1.3)	7.4 (1.4)	7.2 (1.3)	**3.6×10^{-4}**
<u>Renal factors</u>				
Microalbuminuria, n (%)	882 (25.4)	394 (30.5)	307 (23.8)	**1.4×10^{-3}**
Macroalbuminuria, n (%)	173 (5.3)	97 (9.7)	57 (5.5)	**2.2×10^{-3}**
UACR, median µg/mg (IQR)	13.3 (6.2 - 40.7)	16.8 (7.1 - 54.8)	11.5 (5.4 - 33.7)	**1.8×10^{-5}**
eGFR, median mL/min per $1.73m^2$ (IQR)	72.6 (61.1 - 85.2)	73.5 (59.8 - 86.4)	71.5 (59.5 – 84.7)	1.8×10^{-1}
<u>Lipid control</u>				
Total cholesterol, mean mmol/L (SD)	5.1 (1.1)	5.1 (1.1)	5.2 (1.1)	**4.1×10^{-3}**
LDL-cholesterol, mean mmol/L (SD)	3.0 (1.0)	3.0 (1.0)	3.1 (1.0)	**2.1×10^{-3}**
HDL-cholesterol, mean mmol/L (SD)	1.2 (0.3)	1.2 (0.3)	1.3 (0.4)	**6.7×10^{-7}**
Triglycerides, median mmol/L (IQR)	1.7 (1.2 - 2.4)	1.7 (1.3 - 2.5)	1.7 (1.2 - 2.3)	**2.1×10^{-4}**
<u>Other risk factors</u>				
Heart rate, mean beats/min (SD)	72 (12.3)	72.0 (12.4)	72.2 (12.3)	2.7×10^{-1}
Current smoking, n (%)	556 (15.5)	226 (15.4)	252 (17.8)	7.7×10^{-2}
Waist circumference, mean cm (SD)	104 (12.6)	105.2 (12.3)	103.3 (12.7)	**2.5×10^{-5}**
BMI, mean kg/m^2 (SD)	30.1 (5.1)	30.6 (5.1)	29.9 (5.0)	**4.5×10^{-4}**
Diabetes family history, n (%)	1617 (47.4)	706 (48.1)	665 (47.1)	5.9×10^{-1}
ADVANCE blood glucose treatment, n (%)	1669 (48.9)	729 (49.6)	682 (48.2)	4.7×10^{-1}
ADVANCE blood pressure treatment, n (%)	1726 (50.6)	776 (52.8)	704 (49.8)	1.1×10^{-1}

Supplemental Table 4: Patient groups with or without eGFR non-response at the end of follow as obtained from the phenotype identification process for eGFR non-response (Supp. Figure 2). Slavic ethnicity based of PC1 value ("Celtic ethnicity", PC1 <0; "Slavic ethnicity", PC1 ≥0). Hba1c: glycated hemoglobin, UACR: urine albumin: creatinine ratio, eGFR: estimated glomerular filtration rate, BMI: body mass index, systolic blood pressure, DBP: diastolic blood pressure. P-values are obtained from chi-square tests for binomial variables, two-sided student t-test for normally distributed continuous variables and Wilcoxon test for non-normally distributed continuous variables. Median and inter-quartile range (IQR) are indicated for triglycerides, urinary: albumin creatinine ratio and eGFR clinical variables.

Supplemental Table 5*:* Baseline medication of ADVANCE genotyped population, and patient with or without UACR non-response, and with or without eGFR non-response

Baseline medications	Genotyped dataset (n=3409)	UACR non-responders n=1307	UACR responders n=1773	p-value	eGFR non-responders n=1470	eGFR responders n=1414	p-value
HMG coa reductase, n (%)	1377 (40.4)	535 (40.9)	709 (40.0)	6.0×10^{-1}	631 (43.8)	560 (39.6)	7.0×10^{-2}
Thiazolidinedione, n (%)	157 (4.6)	52 (4.7)	93 (5.2)	1.0×10^{-1}	84 (5.7)	55 (3.9)	**2.2×10^{-2}**
Cholesterol lowering drugs other, n (%)	263 (7.7)	119 (9.1)	119 (6.7)	**1.4×10^{-2}**	128 (8.7)	102 (7.2)	1.4×10^{-1}
Gliclazide, n (%)	301 (8.8)	125 (9.6)	146 (8.2)	2.0×10^{-1}	130 (8.3)	123 (8.1)	8.9×10^{-1}
Sulphonylurea, n (%)	1890 (55.4)	776 (59.4)	904 (51.0)	**3.8×10^{-6}**	835 (56.8)	740 (52.3)	**1.6×10^{-2}**
Glinide, n (%)	76 (2.2)	20 (1.5)	50 (2.8)	**1.8×10^{-2}**	26 (1.8)	40 (2.8)	5.7×10^{-2}
Metformin, n (%)	1941 (56.9)	760 (58.1)	1035 (58.1)	9.0×10^{-1}	922 (62.7)	753 (53.2)	**2.6×10^{-7}**
Alpha glucosidase inhibitors, n (%)	160 (4.7)	47 (3.6)	83 (4.7)	1.4×10^{-1}	59 (4.0)	66 (4.7)	3.9×10^{-1}
Insulin day, n (%)	16 (0.5)	5 (0.4)	12 (0.7)	2.8×10^{-1}	15 (1.0)	9 (0.6)	2.6×10^{-1}
Thiazide, n (%)	544 (16.0)	217 (16.6)	280 (15.8)	6.3×10^{-1}	225 (15.3)	232 (16.4)	4.2×10^{-1}
Perindopril, n (%)	330 (9.7)	125 (9.6)	161 (9.1)	6.5×10^{-1}	165 (11.2)	130 (9.1)	7.2×10^{-2}
Angiotensin II receptor blocker, n (%)	237 (7.0)	91 (6.5)	125 (6.6)	9.2×10^{-1}	127 (8.6)	86 (6.0)	**8.7×10^{-3}**
Beta blocker, n (%)	988 (29.0)	399 (30.5)	480 (27.0)	**3.5×10^{-2}**	441 (30.0)	396 (28.0)	2.4×10^{-1}
Calcium channel blocker, n (%)	886 (26.0)	320 (24.4)	436 (25.0)	9.4×10^{-1}	404 (27.5)	339 (23.3)	**3.1×10^{-2}**
Diuretic other, n (%)	426 (12.5)	156 (11.9)	170 (9.6)	**3.6×10^{-2}**	189 (12.9)	143 (10.1)	**2.1×10^{-2}**
ACE inhibitors other, n (%)	1236 (48.8)	480 (36.7)	604 (34.0)	1.3×10^{-1}	544 (37.0)	488 (34.5)	1.6×10^{-1}
Antihypertensive other, n (%)	281 (8.2)	115 (8.8)	119 (6.7)	**3.1×10^{-2}**	130 (8.8)	84 (5.8)	**2.2×10^{-3}**
Aspirin, n (%)	1607 (47.1)	581 (44.4)	804 (45.3)	6.2×10^{-1}	690 (47.6)	622 (44.4)	1.1×10^{-1}
Antiplatelet other, n (%)	182 (5.3)	68 (5.2)	89 (5.0)	8.2×10^{-1}	76 (5.2)	75 (5.3)	1.1×10^{-1}
Anticoagulant other, n (%)	183 (5.4)	77 (5.9)	76 (4.3)	**4.3×10^{-2}**	79 (5.4)	61 (4.3)	1.9×10^{-1}
Nitrate, n (%)	492 (14.4)	239 (13.5)	163 (12.5)	4.1×10^{-1}	196 (13.3)	182 (12.9)	7.1×10^{-1}
Hormone Replacement Therapy, n (%)	148 (4.3)	61 (4.7)	78 (4.4)	7.2×10^{-1}	72 (4.4)	73 (4.6)	7.5×10^{-1}

Supplemental Table 5: Comparison of baseline medications between ADVANCE genotyped population, patients UACR non-responders and UACR responders, as well as, patients with eGFR non-responders and eGFR responders. All p-values are obtained from chi-square tests.

Supplemental Table 6*:* Baseline medication of ADVANCE genotyped population, and patient with high or low GRS_{UACR} and with high or low GRS_{eGFR}

Baseline medications	Genotyped dataset (n=3409)	Patients with a high GRS_{UACR} (n= 547)	Patients with a low GRS_{UACR} (n=531)	p-value	Patients with a high GRS_{eGFR} (n=634)	Patients with a low GRS_{eGFR} (n=638)	p-value
HMG coa reductase, n (%)	1377 (40.4)	216 (39.5)	213 (40.1)	8.8×10^{-1}	242 (38.2)	233 (36.5)	5.8×10^{-1}
Thiazolidinedione, n (%)	157 (4.6)	27 (4.9)	25 (4.7)	9.7×10^{-1}	28 (4.4)	24 (3.7)	6.5×10^{-1}
Cholesterol lowering drugs other, n (%)	263 (7.7)	37 (6,8)	40 (7.5)	7.1×10^{-1}	53 (8.4)	43 (6.7)	3.2×10^{-1}
Gliclazide, n (%)	301 (8.8)	41 (7.5)	41 (7.7)	9.8×10^{-1}	51 (8.0)	58 (9.0)	5.7×10^{-1}
Sulphonylurea, n (%)	1890 (55.4)	319 (58.3)	272 (51.2)	2.3×10^{-1}	361 (56.9)	342 (53.6)	2.5×10^{-1}
Glinide, n (%)	76 (2.2)	5 (0.9)	13 (2.4)	8.4×10^{-2}	9 (1.4)	7 (1.1)	7.9×10^{-1}
Metformin, n (%)	1941 (56.9)	310 (56.7)	300 (56.5)	1.0	354 (55.8)	352 (55.2)	8.6×10^{-1}
Alpha glucosidase inhibitors, n (%)	160 (4.7)	24 (4.3)	25 (4.7)	9.2×10^{-1}	30 (4.7)	32 (5.0)	9.2×10^{-1}
Insulin day, n (%)	16 (0.5)	2 (0.3)	4 (0.8)	6.6×10^{-1}	3 (0.5)	3 (0.5)	1.0
Thiazide, n (%)	544 (16.0)	89 (16.3)	87 (16.4)	1.0	107 (16.9)	106 (16.6)	9.6×10^{-1}
Perindopril, n (%)	330 (9.7)	52 (9.5)	39 (7.3)	2.4×10^{-1}	63 (9.9)	59 (9.2)	7.5×10^{-1}
Angiotensin II receptor blocker, n (%)	237 (7.0)	35 (6.4)	41 (7.7)	4.7×10^{-1}	42 (6.6)	41 (6.4)	9.8×10^{-1}
Beta blocker, n (%)	988 (29.0)	161 (29.4)	146 (27.4)	5.2×10^{-1}	181 (28.5)	180 (28.2)	9.4×10^{-1}
Calcium channel blocker, n (%)	886 (26.0)	146 (26.7)	155 (29.2)	4.0×10^{-1}	192 (30.3)	147 (23.0)	4.3×10^{-1}
Diuretic other, n (%)	426 (12.5)	70 (12.8)	60 (11.3)	5.1×10^{-1}	83 (13.1)	67 (10.5)	1.8×10^{-1}
ACE inhibitors other, n (%)	1236 (48.8)	196 (35.8)	201 (37.9)	5.3×10^{-1}	220 (34.7)	216 (33.9)	8.0×10^{-1}
Antihypertensive other, n (%)	281 (8.2)	45 (8.2)	41 (7.7)	8.5×10^{-1}	58 (9.1)	50 (7.8)	4.6×10^{-1}
Aspirin, n (%)	1607 (47.1)	254 (46.4)	256 (48.2)	6.0×10^{-1}	296 (46.2)	310 (48.6)	5.3×10^{-1}
Antiplatelet other, n (%)	182 (5.3)	25 (4.6)	30 (5.6)	5.1×10^{-1}	33 (5.2)	37 (5.8)	7.3×10^{-1}
Anticoagulant other, n (%)	183 (5.4)	29 (5.3)	25 (4.7)	7.6×10^{-1}	33 (5.2)	32 (5.0)	9.8×10^{-1}
Nitrate, n (%)	492 (14.4)	79 (14.4)	72 (13.6)	8.1×10^{-1}	100 (15.8)	94 (14.7)	6.6×10^{-1}
Hormone Replacement Therapy, n (%)	148 (4.3)	20 (3.7)	25 (4.7)	4.8×10^{-1}	36 (5.7)	19 (3.0)	$\mathbf{2.6 \times 10^{-2}}$

Supplemental Table 6: Comparison of baseline medications between ADVANCE genotyped population, patients with a high or low GRS_{UACR}, patients with a high or low GRS_{eGFR}. All p-values are obtained from chi-square tests.

Supplemental Table 7: Summary of Cox proportional hazard model for the prediction of worsening UACR events.

Variables	Coefficient	P-value	HR	Confidence Interval
Diabetes years	0.02	$2.3x10^{-2}$	1.02	1.0 - 1.04
bs(hba1c) 1 (three cubic splines)		2.5 to $4.1x10^{-2}$		
History of major microvascular disease	0.31	$1.3x10^{-3}$	1.36	1.13 - 1.65
UACR	-0.002	$3.2x10^{-2}$	0.99	0.996 - 0.999
eGFR epi	-0.01	$1.2x10^{-5}$	0.99	0.981 - 0.993
Pulse blood pressure	0.01	$4.8x10^{-2}$	1.01	1 - 1.02
Serum HDL cholesterol	-0.39	$2.5x10^{-2}$	0.67	0.47 - 0.95
Diabetes family history	0.25	$2.5x10^{-3}$	1.28	1.09 - 1.51
Retinopathy	0.33	$1.2x10^{-3}$	1.39	1.14 - 1.69
Hypertension drugs	0.30	$6.7x10^{-3}$	1.35	1.08 - 1.67
ADVANCE blood pressure treatment	-0.25	$2.6x10^{-3}$	0.78	0.66 - 0.92
GRS_{UACR}	0.07	$8.7x10^{-7}$	1.08	1.05 - 1.11

Supplemental Table 7: Summary of Cox proportional hazard model for the prediction of worsening UACR events. Worsening UACR events are defined as an increase of one or more albuminuria classes. *HR:* hazard ratio. GRS_{UACR}: genetic risk score of UACR non-response. Other co-variables included in the model but not significantly associated with time to development of worsening UACR events are sex, age at diagnostic, triglyceride, heart rate, waist circumference, history of major macrovascular disease, current smoker, systolic blood pressure, serum total cholesterol, serum LDL cholesterol, aspirin, hmg coa reductase, cholesterol lowering drugs, calcium antagonist, any hypoglycemic drug, and ADVANCE glucose lowering treatment.

Supplemental Table 8: Summary of Cox proportional hazard model for the prediction of eGFR events.

Variables	Coefficient	P-value	HR	Confidence Interval
Sex	-0.19	$3.4x10^{-3}$	0.82	0.72 - 0.94
Diabetes years	0.02	$9.5x10^{-3}$	1.02	1.01 - 1.03
Age at diagnostic	0.01	$6.2x10^{-3}$	1.01	1.00 - 1.03
Triglyceride	0.07	$3.9x10^{-2}$	1.07	1.00 - 1.14
UACR (transformed to the logarithmic scale)	0.08	$3.1x10^{-4}$	1.08	1.04 - 1.13
eGFR epi	0.03	$2.0x10^{-16}$	1.03	1.02 - 1.03
Current smoker	-0.17	$4.4x10^{-2}$	0.85	0.72 - 0.99
GRS_{eGFR}	0.05	$1.4x10^{-3}$	1.05	1.02 - 1.08

Supplemental Table 8: Summary of Cox proportional hazard model for the prediction of eGFR events. Worsening eGFR events are defined here as a decrease of one or more CKD stages. *HR:* hazard ratio. $GRS_{eGFR:}$ genetic risk score of eGFR non-response. Other co-variables included in the model but not significantly associated with time to development of worsening eGFR events are hba1c, heart rate, waist circumference, history of major microvascular disease, history of major macrovascular disease, systolic blood pressure, pulse blood pressure, serum total cholesterol, serum HDL cholesterol, serum LDL cholesterol, diabetes family history, retinopathy, hypertension drugs, aspirin, hmg coa reductase, any hypoglycemic drug, cholesterol lowering drugs, calcium antagonists, ADVANCE blood pressure treatment, and ADVANCE glucose treatment.

Supplemental Table 9: Test performance of non-geo-ethnic weighted GRS and geo-ethnic weighted GRS

Test performance metric	General Caucasian 19 SNP GRS_{UACR}	Specific Slavic & Celtic 19 SNPs GRS_{UACR}	General Caucasian 9 SNP GRS_{eGFR}	Specific Slavic & Celtic 9 SNPs GRS_{eGFR}
Positive predictive value	69.3	70.2	69.0	53.6
False discovery rate	30.7	29.7	31.0	46.4
Sensitivity	79.2	85.2	66.1	53.8
Negative predictive value	79.2	86.7	63.8	52.8
False omission rate	77.3	13.2	36.2	47.2
Specificity	71.6	72.7	66.8	50.9

Supplemental Table 9: Comparison of test performance between non-geo-ethnic weighted GRS (referred to as Caucasian GRS) and geo-ethnic weighted GRS (referred to as Geo-ethnic GRS). Positive predictive value, false discovery rate, sensitivity, negative predictive value, false omission rate and specificity are given in percentage.

Supplemental Table 10: Background information on loci associated to UACR non-response.

SNP (Locus Name)	Description of genes in each region
rs14393992 (CSMD2)	**CSMD2** (CUC AND Sushi Multiple Domains 2) is a protein Coding gene. Disease associated with CSMD2 include Intermediate Charcot-Marie-Tooth Neuropathy and Benign Adult Familial Myoclonic Epilepsy. An important paralog of this gene is CSMD1 (GeneCards®). CSMD2 has been associated to coronary artery aneurysm in Kawasaki disease, response to lithium treatment in bipolar disorder, response to anti-TNF therapy in rheumatoid arthritis, response to serotonin reuptake inhibitors in major depressive disorder, and depressive symptoms (GWAS Catalog).
rs67237254 (PAX8)	**PAX8** (Paired Box 8) encodes a member of the paired box (PAX) family of transcription factors. Members of this gene family typically encode proteins that contain a paired box domain, an octapeptide, and a paired-type homeodomain. This nuclear protein is involved in thyroid follicular cell development and expression of thyroid-specific genes. Mutations in this gene have been associated with thyroid dysgenesis, thyroid follicular carcinomas and atypical follicular thyroid adenomas. Alternatively spliced transcript variants encoding different isoforms have been described (RefSeq). PAX8 has been associated with kidney fucntion-related traits in east Asian populations (Okada, K. et al. 2012).
rs11918427 (CNTN3 – FAM86DP)	**CNTN3** (Contactin 3) is a Protein Coding gene. Diseases associated with CNTN3 include Plasmacytoma and Taylor's Syndrome. Among its related pathways are Metabolism of proteins and Post-translational modification- synthesis of GPI-anchored proteins. An important paralog of this gene is CNTN4 (GeneCards®). CNTN3 is associated to cadmium levels, mercury levels, smoking quantity and economic and political performance (GWAS catalog). **FAM86DP** (Family With Sequence Similarity 86 Member D, Pseudogene) (GeneCards®)
rs13140153 (GABRG1)	**GABRG1** (Gamma-Aminobutyric Acid Type A Receptor Gamma1 Subunit) encodes a protein which belongs to the ligand-gated ionic channel family. It is an integral membrane protein and plays an important role in inhibiting neurotransmission by binding to the benzodiazepine receptor and opening an integral chloride channel. This gene is clustered with three other family members on chromosome 4 (RefSeq). GABRG1 is associated to epilepsy (GWAS catalog).
rs149213511 (ARHGAP24)	**ARHGAP24** (Rho GTPase Activating Protein 24) encodes a Rho-GTPase activating protein, which is specific for the small GTPase family member Rac. Binding of the encoded protein by filamin A targets it to sites of membrane protrusion, where it antognizes Rac. This results in suppression of lamellae formation and promotion of retraction to regulate cell polarity. Alternative splicing results in multiple transcript variants (RefSeq). ARHGAP24 is associated to systolic blood pressure, obesity-related traits, PR segment and PR interval (GWAS catalog). Associated end stage kidney disease in African American patients with T2D (Guan, M. et al. 2016).
rs61603300 (PALLD)	**PALLD** (Palladin, Cytoskeletal Associated Protein) encodes a cytoskeletal protein that is required for organizing the actin cytoskeleton. The protein is a component of actin-containing microfilaments, and it is involved in the control of cell shape, adhesion, and contraction. Polymorphisms in this gene are associated with a susceptibility to pancreatic cancer type 1, and with a risk for myocardial infarction. Alternative splicing results in multiple transcript variants (RefSeq). PALLD is associated to bacteremia, advanced glycation end products, polychlorinated biphenyl levels, vein graft stenosis in coronary artery bypass surgery, and non-alcoholic fatty liver disease histology (GWAS catalog).

SNP (Locus Name)	Description of genes in each region
rs150233516 (IL4)	**IL4** (Interleukin 4) encodes a protein which is a pleiotropic cytokine produced by activated T cells. This cytokine is a ligand for interleukin 4 receptor. The interleukin 4 receptor also binds to IL13, which may contribute to many overlapping functions of this cytokine and IL13. STAT6, a signal transducer and activator of transcription, has been shown to play a central role in mediating the immune regulatory signal of this cytokine. This gene, IL3, IL5, IL13, and CSF2 form a cytokine gene cluster on chromosome 5q, with this gene particularly close to IL13. This gene, IL13 and IL5 are found to be regulated coordinately by several long-range regulatory elements in an over 120 kilobase range on the chromosome. Two alternatively spliced transcript variants of this gene encoding distinct isoforms have been reported (RefSeq). IL4 has been associated to inflammatory bowel disease and Hodgkin's lymphona (GWAS catalog).
rs34104013 (SH3PXD2B - NEURL1)	**SH3PXD2B** (Tyrosine Kinase Substrate With Four SH3 Domains) encodes an adapter protein that is characterized by a PX domain and four Src homology 3 domains. The encoded protein is required for podosome formation and is involved in cell adhesion and migration of numerous cell types. Mutations in this gene are the cause of Frank-ter Haar syndrome (FTHS), and Borrone Dermato-Cardio-Skeletal (BDCS) syndrome. Alternative splicing of this gene results in multiple transcript variants (RefSeq). SH3PXD2B is associated to body mass index change over time (GWAS catalog). **NEURL1** (Neuralized E3 Ubiquitin Protein Ligase 1) is a protein coding gene. Diseases associated with NEURL1 include Medulloblastoma. Among its related pathways are HIV Life Cycle and NOTCH2 Activation and Transmission of Signal to the Nucleus. GO annotations related to this gene include ligase activity and translation factor activity, non-nucleic acid binding. An important paralog of this gene is NEURL1B (GeneCards ®). NEURL1 is associated to schizophrenia, white matter hyperintensity burden, autism spectrum disorder and platelet aggregation (GWAS catalog).
rs10533367 (EEF1E1 – SLC35B3)	**EEF1E1** (Eukaryotic Translation Elongation Factor 1 Epsilon 1) encodes a multifunctional protein that localizes to both the cytoplasm and nucleus. In the cytoplasm, the encoded protein is an auxiliary component of the macromolecular aminoacyl-tRNA synthase complex. However, its mouse homolog has been shown to translocate to the nucleus in response to DNA damage, and it plays a positive role in ATM/ATR-mediated p53 activation. Alternative splicing results in multiple transcript variants. Read-through transcription also exists between this gene and the neighboring downstream MUTED (muted homolog) gene. An EEF1E1-related pseudogene has been identified on chromosome 2 (RefSeq). EEF1E1 has been associated to coronary artery calcification (GWAS catalog). **SLC35B3** (Solute Carrier Family 35 Member B3) encodes a protein involved in the transport of 3-prime phosphoadenosine 5-prime phosphosulfate (PAPS) from the nucleus or the cytosol to the Golgi lumen. This gene has been reported to be expressed preferentially in the human colon tissues. Alternative splicing results in multiple transcript variants (RefSeq). SLC35B3 has been associated to glucose homeostatic traits (GWAS catalog).
rs34656786 (ARMC12 . CLPSL2)	**ARMC12** (Amadillo Repeat Containing 12) is a protein coding gene. GO annotations related to this gene include binding (GeneCards ®). **CLPSL2** (Colipase Like 2) is a protein coding gene. GO annotations related to this gene include enzyme activator activity (GeneCards ®)

SNP (Locus Name)	Description of genes in each region
rs929506 (CADPS2)	**CADPS2** (Calcium Dependent Secretion Activator 2) encodes a member of the calcium-dependent activator of secretion (CAPS) protein family, which are calcium binding proteins that regulate the exocytosis of synaptic and dense-core vesicles in neurons and neuroendocrine cells. Mutations in this gene may contribute to autism susceptibility. Multiple transcript variants encoding different isoforms have been found for this gene (RefSeq). CADPS2 is associated to serum sulfate level, Alzheimer disease and age of onset, and 3-hydroxypropylmercapturi acid levels in smokers (GWAS catalog)
rs68030383 (ITPR2)	**ITPR2** (Inositol 1,4,5-Triphosphate Receptor Type 2) encodes a protein which belongs to the inositol 1,4,5-triphosphate receptor family, whose members are second messenger intracellular calcium release channels. These proteins mediate a rise in cytoplasmic calcium in response to receptor activated production of inositol triphosphate. Inositol triphosphate receptor-mediated signaling is involved in many processes including cell migration, cell division, smooth muscle contraction, and neuronal signaling. This protein is a type 2 receptor that consists of a cytoplasmic amino-terminus that binds inositol triphosphate, six membrane-spanning helices that contribute to the ion pore, and a short cytoplasmic carboxy-terminus. A mutation in this gene has been associated with anhidrosis, suggesting that intracellular calcium release mediated by this protein is required for eccrine sweat production (RefSeq). ITPR2 is associated to waist-to-hip ratio adjusted for body mass index, Kashin-Beck disease, age of smoking initiation, smooth-surface cells and renal cell carcinoma (GWAS catalog).
rs61948880 (PARP4)	**PARP4** (Poly(ADP-Ribose) Polymerase Family Member 4) encodes poly(ADP-ribosyl) transferase-like 1 protein, which can catalyze a poly(ADP-ribosyl)ation reaction. This protein has a catalytic domain which is homologous to that of poly (ADP-ribosyl) transferase, but lacks an N-terminal DNA binding domain which activates the C-terminal catalytic domain of poly (ADP-ribosyl) transferase. Since this protein is not capable of binding DNA directly, its transferase activity may be activated by other factors such as protein-protein interaction mediated by the extensive carboxyl terminus (RefSeq). PARP4 is associated to obesity-related traits and sudden cardiac arrest (GWAS catalog).
rs970817 (MIR1297)	**MIR1297** (MicroRNA 1297) is a short (20-24 nt) non-coding RNAs that are involved in post-transcriptional regulation of gene expression in multicellular organisms by affecting both the stability and translation of mRNAs. miRNAs are transcribed by RNA polymerase II as part of capped and polyadenylated primary transcripts (pri-miRNAs) that can be either protein-coding or non-coding. The primary transcript is cleaved by the Drosha ribonuclease III enzyme to produce an approximately 70-nt stem-loop precursor miRNA (pre-miRNA), which is further cleaved by the cytoplasmic Dicer ribonuclease to generate the mature miRNA and antisense miRNA star (miRNA*) products. The mature miRNA is incorporated into a RNA-induced silencing complex (RISC), which recognizes target mRNAs through imperfect base pairing with the miRNA and most commonly results in translational inhibition or destabilization of the target mRNA. The RefSeq represents the predicted microRNA stem-loop (RefSeq). MIR1297 is associated to amyotrophic lateral sclerosis (GWAS catalog).
rs6573040 (TBPL2 – KTN1-AS1)	**TBPL2** (TATA-Box Binding Protein Like 2) is a protein coding gene. Among its related pathways are HTLV-I infection and Influenza A. GO annotations related to this gene include transcription factor activity, sequence-specific DNA binding. An important paralog of this gene is TBP (GeneCards ®). **KTN1-AS1** (KTN1 Antisense RNA 1) is an RNA Gene and is affiliated with the non-coding RNA class (GeneCards ®).

SNP (Locus Name)	Description of genes in each region
rs17758297 (NRXN3)	**NRXN3** (Neurexin 3) encodes a member of a family of proteins that function in the nervous system as receptors and cell adhesion molecules. Extensive alternative splicing and the use of alternative promoters results in multiple transcript variants and protein isoforms for this gene, but the full-length nature of many of these variants has not been determined. Transcripts that initiate from an upstream promoter encode alpha isoforms, which contain epidermal growth factor-like (EGF-like) sequences and laminin G domains. Transcripts initiating from the downstream promoter encode beta isoforms, which lack EGF-like sequences. Genetic variation at this locus has been associated with a range of behavioral phenotypes, including alcohol dependence and autism spectrum disorder (RefSeq). NRXN3 is associated with body mass index, coronary artery, and aneurysm in Kawasaki disease (GWAS catalog). Associated to the causal role of obesity in diabetic kidney disease (Todd, J.N. et al. 2015).
rs7157963 (GLRX5)	**GLRX5** (Glutaredoxin 5) encodes a mitochondrial protein, which is evolutionarily conserved. It is involved in the biogenesis of iron-sulfur clusters, which are required for normal iron homeostasis. Mutations in this gene are associated with autosomal recessive pyridoxine-refractory sideroblastic anemia (RefSeq). GLRX5 is associated to obsesity-related traits and tuberculosis (GWAS catalog).
rs7212486 (RBFOX3)	**RBFOX3** (RNA Binding Protein, Fox-1 Homolog 3) is protein Coding gene. Diseases associated with RBFOX3 include Sella Turcica Neoplasm and Tuberculum Sellae Meningioma. Among its related pathways are Neuroscience. GO annotations related to this gene include nucleic acid binding and nucleotide binding. An important paralog of this gene is RBFOX1 (GeneCards ®). RBFOX3 is associated to 3-hydroxypropylmercapturic acid levels in smokers and urate levels (BMI interaction) (GWAS Catalog).
rs35101292 (MIS18A)	**MIS18A** (MIS18 Kinetochore Protein A) is a protein Coding gene. Diseases associated with MIS18A include Suppurative Periapical Periodontitis. Among its related pathways are Chromosome Maintenance and Cytoskeletal Signaling (GeneCards ®).

Supplemental Table 10: Annotations for intergenic SNPs are provided for flanking genes within a 500kb window. SNPs location in relation to flanking genes is indicated by a hyphen.

Supplemental Table 11: Background information on loci associated to eGFR non-response.

SNP (Locus Name)	Description of genes in each region
rs3123025 (C1orf85/GLMP – VHLL)	**C1orf85/GLMP** (Glycosylated Lysosomal Membrane Protein) is a Protein Coding gene. GO annotations related to this gene include transcription factor activity, sequence-specific DNA binding and ligand-dependent nuclear receptor transcription coactivator activity (GeneCards ®). C1orf85/GLMP has been associated to glycated hemoglobin levels (GWAS catalog). **VHLL** (Von Hippel-Lindau Tumor Suppressor Like) s a Protein Coding gene. Diseases associated with VHLL include Pancreatic Serous Cystadenoma. An important paralog of this gene is VHL (GeneCards ®). VHLL is associated with mean corpuscular hemoglobin cocentration (GWAS Catalog).
rs201500153 (AOX2P)	**AOX2P** (Aldehyde Oxidase 2 Pseudogene) is a Pseudogene. Among its related pathways are Nicotine Pathway, Pharmacokinetics (GeneCards ®). AOX2P is associated to alcohol dependence, response to citalopram treatment and intelligence (GWAS catalog).
rs66916463 (MIR4801 -)	**MIR4801** (MicroRNA 4801) is a short (20-24 nt) non-coding RNAs that are involved in post-transcriptional regulation of gene expression in multicellular organisms by affecting both the stability and translation of mRNAs. miRNAs are transcribed by RNA polymerase II as part of capped and polyadenylated primary transcripts (pri-miRNAs) that can be either protein-coding or non-coding. The primary transcript is cleaved by the Drosha ribonuclease III enzyme to produce an approximately 70-nt stem-loop precursor miRNA (pre-miRNA), which is further cleaved by the cytoplasmic Dicer ribonuclease to generate the mature miRNA and antisense miRNA star (miRNA*) products. The mature miRNA is incorporated into a RNA-induced silencing complex (RISC), which recognizes target mRNAs through imperfect base pairing with the miRNA and most commonly results in translational inhibition or destabilization of the target mRNA. The RefSeq represents the predicted microRNA stem-loop (RefSeq). MIR4801 has been associated to 3-hydroxypropylmercapturic acid levels in smokers (GWAS catalog).
rs6451864 (CDH18 -)	**CDH18** (Cadherin 18) encodes a type II classical cadherin from the cadherin superfamily of integral membrane proteins that mediate calcium-dependent cell-cell adhesion. Mature cadherin proteins are composed of a large N-terminal extracellular domain, a single membrane-spanning domain, and a small, highly conserved C-terminal cytoplasmic domain. Type II (atypical) cadherins are defined based on their lack of a HAV cell adhesion recognition sequence specific to type I cadherins. This particular cadherin is expressed specifically in the central nervous system and is putatively involved in synaptic adhesion, axon outgrowth and guidance. Alternatively spliced transcript variants encoding different isoforms have been found for this gene (RefSeq). CDH18 has been associated with response to anti-TNF therapy in rheumatoid arthritis, obesity in adult survivors of childhood cancer exposed to cranial radiation, leprosy, and mitochondrial DNA levels (GWAS catalog).
rs66801926 (ARID1B)	**ARID1B** (AT-Rich Interaction Domain 1B) encodes an AT-rich DNA interacting domain-containing protein. The encoded protein is a component of the SWI/SNF chromatin remodeling complex and may play a role in cell-cycle activation. The protein encoded by this locus is similar to AT-rich interactive domain-containing protein 1A. These two proteins function as alternative, mutually exclusive ARID-subunits of the SWI/SNF complex. The associated complexes play opposing roles. Alternative splicing results in multiple transcript variants (RefSeq). ARID1B is associated to multiple system atrophy, lipoprotein (a) -cholesterol levels, sitting height ratio, vein graft stenosis in coronary artery bypass grafting (GWAS catalog).

SNP (Locus Name)	Description of genes in each region
rs636554 (CLEC12A – CLEC1B)	**CLEC12A** (C-Type Lectin Domain Family 12 Member A) encodes a member of the C-type lectin/C-type lectin-like domain (CTL/CTLD) superfamily. Members of this family share a common protein fold and have diverse functions, such as cell adhesion, cell-cell signaling, glycoprotein turnover, and roles in inflammation and immune response. The protein encoded by this gene is a negative regulator of granulocyte and monocyte function. Several alternatively spliced transcript variants of this gene have been described, but the full-length nature of some of these variants has not been determined. This gene is closely linked to other CTL/CTLD superfamily members in the natural killer gene complex region on chromosome 12p13 (RefSeq). **CLEC1B** (C-Type Lectin Domain Family 1 Member B) is a Protein Coding gene. Among its related pathways are G beta-gamma signalling through PI3Kgamma and Response to elevated platelet cytosolic Ca2+. GO annotations related to this gene include transmembrane signaling receptor activity and carbohydrate binding. An important paralog of this gene is CLEC12B (GeneCards ®)
rs9668721 (TMEM132D – FZD10-AS1)	**TMEM132D** (Transmembrane Protein 132D) is a Protein Coding gene. Diseases associated with TMEM132D include Pthirus Pubis Infestation and Lice Infestation. An important paralog of this gene is TMEM132C (GeneCards ®). TMEM132D is associated to cognitive decline rate in late mild cognitive impairment, schizophrenia, diisocyanate-induced asthma, and anxiety disorder (GWAS catalog). **FZD10-AS1** (FZD10 Antisense RNA 1 (Head To Head)) is an RNA Gene, and is affiliated with the non-coding RNA class (GeneCards ®). FZD10-AS1 is associated to emphysema imaging phenotypes (GWAS catalog).
rs9540222 (Gene desert)	
rs6497475 (UMOD)	**UMOD** (Uromodulin) encodes a protein which is the most abundant protein in mammalian urine under physiological conditions. Its excretion in urine follows proteolytic cleavage of the ectodomain of its glycosyl phosphatidylinositol-anchored counterpart that is situated on the luminal cell surface of the loop of Henle. This protein may act as a constitutive inhibitor of calcium crystallization in renal fluids. Excretion of this protein in urine may provide defense against urinary tract infections caused by uropathogenic bacteria. Defects in this gene are associated with the renal disorders medullary cystic kidney disease-2 (MCKD2), glomerulocystic kidney disease with hyperuricemia and isosthenuria (GCKDHI), and familial juvenile hyperuricemic nephropathy (FJHN). Alternative splicing of this gene results in multiple transcript variants (RefSeq). UMOD is associated to glomerular filtration rate, chronic kidney disease, glomerular filtration rates in diabetics and non-diabetics (GWAS catalog).

Supplemental Table 11: Annotations for intergenic SNPs are provided for flanking genes within a 500kb window. SNPs location in relation to flanking genes is indicated by a hyphen.

Supplemental Table 12: Comparison of baseline and end of study renal measures ADAVANCE replication dataset (N=689)

Patient group category	Baseline UACR	End of study UACR	p-value
Patients with high GRS_{UACR} (N = 103)	14.4 (6.2 -30.0)	20.0 (11.5 – 52.5)	**2.3×10^{-3}**
Patients with low GRS_{UACR} (N = 102)	17.7 (7.1 – 30.3)	21.7 (11.3 – 50.6)	1.1×10^{-1}
	Baseline eGFR	**End of study eGFR**	
Patients with high GRS_{eGFR} (N = 134)	66.0 (53.5 – 81.5)	59,2 (49,0 – 74.4)	**2.2×10^{-3}**
Patients with low GRS_{eGFR} (N = 118)	71.8 (59.3 – 83.4)	69.7 (53.3 – 84.60	1.7×10^{-1}

Supplemental Table 12: *Patients with high GRS:* patients from the independent ADVANCE replication dataset identified as having a high GRS_{UACR} or GRS_{eGFR}. *Patients with low GRS:* patients from the independent ADVANCE replication dataset identified as having a low GRS_{UACR} or GRS_{eGFR} (see Supplemental Figure 4 for details). *Baseline and end of study UACR:* median µg/mg (IQR). *Baseline and end of study eGFR:* median mL/min per $1.73m^2$ (IQR). P-value obtained from Wilcoxon test for UACR and two-sided student t-test for eGFR.

Thematic link between Manuscript and Discussion

To the best of our knowledge, the Manuscript included in this thesis presents a novel methodology to identify patients who present phenotypic evidence of renal non-response, which offers novel insights on the genetic variants associated to renal non-response. In addition, this Manuscript reports the capacity of a GRS based on these variants to identify patients who benefit the most at a renal level from ADVANCE intensive trial medications as well as patients who do not respond at a renal level to this intervention. In addition, the results of this manuscript highlight the independent nature of renal non-response from that of blood pressure and glycemia response; as patients who present a renal non-response and a renal response possess equivalent blood pressure and glycemia response to ADVANCE intensive trial treatments. The implications of Publication 1 and 2 as well as the Manuscript will be discussed in the Discussion.

Discussion

As mentioned in the *Hypothesis* section of this thesis, the primary objective of this research project was to evaluate if patients with and without renal non-response possess distinct genetic architectures, and to assess whether these genetic determinants could allow for the early identification of patients likely to present URN. The results of this research project on DN management and precision medicine, which provides an answer to the objectives and hypotheses of this thesis will be reviewed in the following paragraphs.

A. Specificities of renal non-response and associated genetic determinants

The pathogenesis and progression of DN being complex, it is important to first discuss the approaches which were used to define renal non-response, and the implications of such definition. As we have seen in this thesis' *Introduction*, multiple factors can lead to DN onset and progression, which complicates the formulation of a clear paradigm for the pathogenesis and worsening of this complication of T2D. The notion of renal non-response and URN can also be interpreted in many ways, depending on which aspect of DN one focuses on and the clinical profile of the patients under consideration.

The characteristics and rational behind the renal non-response definition used in this project were influenced by the specificities of the ADVANCE cohort. Based on the fact that no ADVANCE participant inclusion or exclusion criteria were based on levels of eGFR, and that the presence of albuminuria was one of a number of potential eligibility criteria for inclusion, ADVANCE participants could enter the study at different stages of DN [180]. As a result, and to include all genotyped ADVANCE participants in our study of URN, our definition of renal non-response had to accommodate all potential stages of DN at study entry and assess which associated renal progression profiles over the duration of follow up were representative of renal non-response or renal response. For that reason, and as explained in the *Manuscript* of the *Results* section of this thesis, renal non-response was defined as the development of an

incident detrimental renal event or the progression of established renal damage, despite the use of evidence-based recommended medications. Such a definition allowed all patients to be classified in groups presenting evidence of renal non-response or not over the duration of ADVANCE.

A key factor to consider in the above definition is that of "evidence-based recommended medications". As explained in *Publication 1* of the *Results* of this thesis, medication is a strong environmental factor which can affect our capacity to detect genetic determinants associated with a specific phenotypic trait. It is important to realize that in the context of this study, and due to the design of ADVANCE, the evidence-based recommended medications to which participants can potentially be non-responders or responders are numerous and diversified. On one hand, ADVANCE participants were randomized to two trial treatments (namely preterax; an ACEi and diuretic combination, as well as gliclazide; a sulfonylurea anti-diabetic drug). On the other hand, and due to the clinical profiles of ADVANCE patients (55 years or older T2D patients at high risk of macrovascular complications), their medication was not limited to ADVANCE trial treatments, but included a wealth of other medications for blood pressure, glycemia, and lipid control (see Supplementary Table 5 and 6 of *Manuscript 1* from the *Results* section, and Table 2 in Patel, et al. [183] for details). To accommodate the diversity of medication profiles present in ADVANCE, and to capture genetic determinants associated with renal non-response rather than renal non-response associated to any one type of medication, we created a meticulous and medication-independent classification algorithm to separate patients in categories with or without evidence of renal non-response for kidney function decline or albuminuria increases.

Based on the above factors, the genetic determinants identified by GWAS analyses in this study are associated to renal non-response in T2D patients receiving several medication classes for the management of their blood pressure, glycemia, and dyslipidemia. The definition of renal non-response is therefore purely renal centric and aimed to identify loci associated with renal non-response affecting patients at all possible kidney disease stages and with diversified medication profiles.

B. Characteristics of renal non-response genetic determinants

As shown in *Manuscript 1* of the *Results* section of this thesis, patients presenting evidence of renal non-response can be genetically distinguished from patients presenting evidence of renal non-response. It is important to recall, that at a phenotypic level, albuminuria and kidney function decline were shown to have independent and additive effects on cardiovascular and renal outcomes [180]. In addition, large meta-analysis studies conducted in the CKDGen consortium demonstrated that different genetic loci were associated to eGFR and albuminuria [271, 272]. The results presented in this thesis continue to support the independent nature of kidney function and albuminuria in DN. We report, in the *Manuscript* that distinct genetic determinants of renal non-response exist for UACR non-response and eGFR non-response. Furthermore, and due to the nature of the genetic loci associated with each type of renal non-response in the context of DN, we believe that renal non-response is caused by variations in the severity of DN and in the mechanisms associated with DN progression. The association of UACR non-response loci to dysfunctional glucose homeostasis traits and advanced glycated end products, as well as the association of eGFR non-response loci to kidney function decline, support the notion that renal non-response is caused by differences in the pathways leading to DN pathogenesis and progression. In addition, interaction analyses in cox proportional hazards models between renal non-response loci and ADVANCE trial treatments and non-trial treatments did not reveal any significant interactions, thus failing to support the involvement of pharmacodynamic variations in renal non-response.

As detailed in *Manuscript 1*, we demonstrated that GRS based on SNPs associated to UACR and eGFR non-response have the capacity to identify patients most at risk of developing URN. In ADVANCE participants with varying stages of kidney disease at study entry, we presented the capacity of these GRS to stratify patients according to their risk of developing URN. Over the duration of the study, patients with high GRS of renal non-response experience a significant worsening of their renal condition (as seen by an increase of UACR and decrease of eGFR), whereas patients with the lowest GRS experience an improvement of their renal condition (as seen by a decrease of UACR and a decrease of eGFR less than the expected age-dependent

decrease [316]). In addition, in patients who do not present DN onset at study entry, we demonstrated the capacity of renal non-response GRS to stratify patients according to their risk of developing an onset of DN either by transitioning from normo- to microalbuminuria or by having a decline of their eGFR below 60 60mL/1.72m^2/min. Moreover, and as detailed in the *Manuscript*, a valuable characteristic of renal non-response genetic determinants and associated GRS, is their capacity to distinguish within a patient population the ones who respond appropriately to blood pressure and glycemic control treatments at a renal level, from the patients who will not benefit from these treatments at a renal level. As illustrated in Table 4 and 5 of the *Manuscript*, patients with high or low GRS of renal non-response respond equally well to ADVANCE trial treatments in terms of their blood pressure and glycemic control, but only patients at low GRS of renal non-response benefit at a renal level from these treatments.

In addition, an important feature of the genetic determinants of renal non-response and of the GRS based on them is their capacity to identify patients who are most likely to develop URN beyond the capacity of common clinical risk factors. As indicated in the *Manuscript*, at baseline, patients with high and low GRS of renal non-response cannot be efficiently distinguished based on clinical risk factors alone. The capacity to identify patients who will benefit from ADVANCE trial treatments on clinical indicators alone is therefore limited. The implications of such findings will be further discussed in the following paragraphs.

Overall, the hypotheses of this research project, which aimed to answer the question of whether patients who present phenotypic evidences of renal non-response can be genetically distinguished from those who do not, has been proven to be true. The secondary hypotheses of this project, which focused on evaluating the capacity of renal non-response genetic determinants to stratify patients according to their risk of developing URN was also proven to be true.

C. Implication of renal non-response genetic determinants for DN management strategies

Having demonstrated the capacity of genetic determinants of renal non-response to identify patients most at risk of developing URN, it is now important to review the implications of these findings for DN management.

Throughout the *Introduction* of this thesis, we observed the effects associated with treating patient with existing DN as compared to patients with no DN onset. On one hand, we observed that patients who entered clinical trials with already existing DN presented a greater rate of renal outcomes than patients with no onset of DN [182, 194, 304]. On the other hand, we reviewed the fact that annualized inpatient, outpatient, pharmaceutical, and total medical costs were 37% higher in patients who progressed from normo- to microalbuminuria as compared to patients who remain normoalbuminuric [292]. The combined patient and healthcare perspective of these results clearly highlight the value of achieving primary prevention and preventing DN onset.

In this context, the usefulness of genetic tests based on genetic determinants associated with renal non-response must be considered. The capacity to identify prior to DN onset, or at initial stages of the disease patients who will benefit the most from evidence-based recommended medication is valuable. Patients with low GRS of renal non-response could be given recommended-evidence based medications at an early stage knowing that their response to such medication can be expected to be positive and to result in DN prevention or delayed progression. Patients with high GRS of renal non-response could be targeted at earlier stages of the disease with hopes of preventing disease onset, or receive novel therapeutic agents in hopes of achieving greater degrees of renal response.

In light of the fact that achieving greater degrees of primary prevention could be beneficial to patients and healthcare systems alike, and in the context of genetic tools applied to precision medicine, it is essential to discuss a notion of timeline. In the field of kidney disease, a recent study reported the limited capacity (beyond common clinical risk factors) of a GRS based on 53 loci associated to reduced GFR to predict incident CKD stage 3 [317]. While the GRS was

associated to incident CKD stage 3, the authors reported its incapacity to improve prediction beyond clinical risk factors in models adjusted for age, sex, baseline eGFR, hypertension, type 2 diabetes, and proteinuria. While these results present a valuable insight, they do not appropriately discuss the potential value of genetic tools in the context of precision medicine. In a setting where achieving greater primary prevention stands as the goal, the value of genetic information should not necessarily be compared to that of clinical risk factors but rather its capacity to predict these clinical risk factors should be evaluated. To achieve primary prevention, precision medicine tools should be used before the manifestation of clinical risk factors. The ultimate purpose of such tools is to predict disease outcome before clinical risk factors arise, as the presence of such clinical risk factors is usually indicative of disease onset, and thus a missed window of opportunity for primary prevention.

We therefore suggest, that the genetic determinants of renal non-response identified in this study represent a valuable candidate to be included in precision medicine tools for the prediction of DN onset and worsening, as well as the identification of patients most likely to benefit at a renal level from evidence-based recommended medications. DN being a complex disease, such precision medicine tool could be improved with the inclusion of other genetic determinants associated to the development and progression of DN risk factors such as hypertension, hyperglycemia, and dyslipidemia, in addition to genetic components associated with renal traits.

D. Geo-ethnic factors in renal non-response

We reported in *Publication 2* of this thesis a heterogenic genetic architecture between Slavic and Celtic ADVANCE Caucasian participants. In the *Manuscript*, the effect of such heterogeneity on renal non-response was evaluated. While we observed that GRS based on geo-ethnic SNPs performed marginally better for UACR non-response but worse for eGFR non-response as compared to GRS based on non-geo-ethnic SNPs, the value of including geo-ethnic SNPs in GRS should not be disregarded. As indicated in *Publication 2*, analyses of Slavic patients living in

Slavic or Celtic regions revealed that UACR appears to have a greater genetic than environmental drive whereas eGFR appears to have a greater environmental driver. Such findings, could explain the increased performance of a geo-ethnic based GRS of UACR non-response and the decreased performance of a geo-ethnic based GRS of eGFR non-response. As a result, the usefulness of geo-ethnic based GRS cannot simply be disregarded based on test performance as environmental factors seem to have different effects of UACR and eGFR.

Conclusion

In conclusion, our studies reveal that a genetic basis for renal non-response exists and can be used to stratify patients according to their risk of developing URN. Like other studies have observed when studying renal traits or the genetics of such traits, we report the existence of distinct genetic determinants for UACR and eGFR non-response. Out study demonstrates the capacity of GRS based on these genetic determinants to identify patients most at risk of developing unmet renal needs within a patient population at varying stages of DN. In addition, and within a patient population with no DN onset, we report the capacity of GRS based on renal non-response genetic determinants to identify patients at risk of developing an onset of DN. Moreover, we report the capacity of GRS of renal non-response to identify patients who will benefit at a renal level from the positive effects of blood pressure and glycemia control treatments. Our studies report the existence of distinct genetic architectures between Slavic and Celtic Caucasian participants of ADVANCE as well as geo-ethnic variations in the genetic determinants of renal non-response. Due to UACR having a greater genetic than environmental drive in ADVANCE Caucasian patients, we report that a specific Slavic & Celtic GRS of UACR non-response performs marginally better than a general Caucasian GRS. In contrast, and because eGFR seems to be more affected by the environment in ADVANCE Caucasian patients, we report that a specific Slavic & Celtic GRS of eGFR non-response does not perform better than a general Caucasian GRS. Overall, this thesis suggests that genetic testing in the framework of DN management is valuable and that genetic determinants of renal non-response could be useful in the context of precision medicine to help achieve a greater proportion of renal met needs in patients with T2D, with greater effects observed for UACR.

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