UCLouvain

Faculté de médecine et médecine dentaire

Clinical Research Thesis

AQP7 Promoter Variant and Changes in Fat Distribution in Peritoneal Dialysis

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SUMMARY

Introduction. Peritoneal dialysis (PD) represents the main home-based kidney replacement therapy worldwide and is used by more than 300,000 patients with kidney failure (~10% of the dialysis population). The principle of PD relies on the use of the peritoneum as a dialysis membrane to remove metabolic waste products and excess of fluid by diffusion and osmotic water transport, from the organism of kidney failure patients. The use of glucose as an osmotic agent in the dialysis solution leads to systemic glucose absorption, which may in turn contribute to ectopic fat accumulation and to the high cardiovascular burden in this population. A common variant in the promoter of *AQP7* gene (rs2989924, A-953G SNP), coding for an aquaglyceroporin involved in fat metabolism, has been previously associated with obesity and metabolic complications in the non-dialysis population. The potential impact of this variant on fat distribution among PD patients – who are exposed to significant glucose and caloric loads – has not been investigated to date.

Objectives and methods. In a cohort of incident patients on PD, we combined clinical and genetic data, and detailed analysis of the body fat distribution using computed tomography and the Slice-O-Matic software (i) to investigate longitudinal changes in fat distribution over time on dialysis; and (ii) to test whether the common rs2989924 variant in the *AQP7* gene is associated with visceral fat accumulation in this population.

Results. The cohort included 108 incident patients on PD followed for a mean (±SD) time of 2.5 years (±1.8), for whom a total of 270 computed tomography scans were available. Mean (±SD) age at PD initiation was 54 years (±17), 37% were women, and mean yearly glucose exposure was 16.9 kg (±10.1). In the whole cohort, we observed a significant increase in visceral fat area after one year (+22%, P<0.001) and after two years (+36%, P<0.001) on PD. The minor allele frequency of the *AQP7* rs2989924 variant was 0.49, with 26 (24%), 57 (53%) and 25 (23%) patients carrying 0, 1 or 2 risk alleles (G), respectively. A significant increase in visceral fat area during time on PD was observed among patients carrying one or two alleles at risk (GA : +34% and +59%, GG : +20% and +66% after one and two years on PD), but not in those without any allele at risk (AA : -15% and -21%). The association was independent from age, glucose exposure and peritoneal solute transfer rate.

Conclusions. This study show that kidney failure patients treated with PD have a significant increase in visceral fat area over time on dialysis. The increase in visceral fat area was only observed among patients carrying the common risk variant A-953G of the *AQP7* gene.

RESUME

Introduction. La dialyse péritonéale (DP) constitue la principale technique de dialyse à domicile et est utilisée par plus de 300,000 patients dans le monde. La DP applique les principes de diffusion et d'osmose au travers du péritoine pour éliminer les déchets du métabolisme et l'excès d'eau. L'utilisation du glucose comme agent osmotique est cependant associée à une absorption systémique, qui pourrait contribuer à l'accumulation ectopique de graisses dans l'organisme, et au risque cardiovasculaire important dans cette population. Dans la population générale, un variant fréquent dans le promoteur du gène *AQP7* (rs2989924, A-953G), codant pour une aquaglycéroporine impliquée dans le métabolisme adipocytaire, a été associé à un risque d'obésité et de complications métaboliques. L'impact de ce variant *AQP7* sur la distribution des graisses dans l'organisme chez les patients en DP – exposés à des apports caloriques excessifs – n'est pas connu.

Objectifs et méthodes. Dans une cohorte de patients incidents en DP, nous combinons données cliniques, génétiques et analyse détaillée de la composition corporelle par tomographie computérisée et Slice-O-Matic afin (i) d'évaluer de manière longitudinale les modifications de composition corporelle au cours de la DP ; et (ii) d'investiguer l'association éventuelle entre le variant *AQP7* et accumulation de graisse viscérale.

Résultats. La cohorte a inclus 108 patients incidents en DP, suivis pendant une durée moyenne (±SD) de 2.5 ans (±1.8), et pour lesquels un total 270 images de tomographie étaient disponibles. L'âge moyen (±SD) à la mise en DP était de 54 ans (±17), 37% étaient des femmes, et l'exposition annuelle moyenne au glucose était de 16.9 kg (±10.1). Dans la cohorte globale, une augmentation de la graisse viscérale abdominale est observée après un an (+22%, P<0.001) et deux ans (+36%, P<0.001) de DP. La fréquence de l'allèle mineur du variant *AQP7* rs2989924 était de 0.49, avec 26 (24%), 57 (53%) et 25 (23%) patients porteurs de 0, 1 ou 2 allèles à risque (G), respectivement. Une augmentation significative de la graisse viscérale en cours de traitement était observée chez les patients porteurs d'un ou deux allèles à risque (GA : +34% et +59%, GG : +20% et +66% après un et deux ans de DP), mais pas chez ceux sans allèle à risque (AA : -15% et -21%). Cette association était independente de l'âge, de l'exposition au glucose ou des caractéristiques de transport peritoneal.

Conclusions. Cette étude met en évidence une accumulation de graisse viscérale chez les patients insuffisants rénaux traités par DP. Cette augmentation de graisse viscérale est exclusivement observée chez les patients porteurs du variant à risque A-953G du gène *AQP7*.

INTRODUCTION

Peritoneal dialysis : epidemiology and principles

Peritoneal dialysis (PD) is kidney replacement therapy used by more than 300,000 patients with kidney failure worldwide (~10% of the population on dialysis). PD can be used either as a temporary treatment while waiting for a kidney transplant, or as a definitive therapy for patients not eligible for transplantation. As compared with hemodialysis, PD offers better patient-reported outcomes, better preservation of residual renal function, increased flexibility and autonomy to patients, and is associated with lower societal costs **(1)**. These advantages explain why PD represents the main home-based dialysis technique worldwide, and a promising alternative to tackle disparities in the access to dialysis, including in developing countries **(2)**.

The principle of PD relies on the use of the peritoneum as a dialysis membrane to remove metabolic waste products and the excess of fluid in excess in the body of patients with kidney failure; to restore electrolyte homeostasis and to control blood pressure. During PD, a dialysis solution containing water, sodium and an osmotic agent, is instilled into the peritoneal cavity to generate diffusion of small solutes (e.g., urea, creatinine and electrolytes) and osmotic water transport. Glucose used in the dialysis solution creates a crystalloid osmotic gradient that drives water transport (also termed 'ultrafiltration') through interendothelial junctions and aquaporin (AQP) water channels.

The AQPs are a family of water membrane channels identified in the 1990s. Their function is to facilitate the diffusion of water molecules across biological membranes in response to an osmotic gradient. Currently, there are 13 known types of aquaporins in mammals (AQP 0-12). AQP1 is the archetypal member of this family of membrane water channels conserved in all living organisms **(3, 4, 5).** Initially identified in red blood cells and in renal proximal tubules, AQP1 is also constitutively expressed in endothelial cells lining peritoneal capillaries **(6, 7, 8)**. The development of mouse models of PD and their application to *Aqp1* knockout mice contributed to delineate the critical role of AQP1 water channels during PD **(9, 10)**. In these models, the targeted deletion of *Aqp1* resulted in a ~70% decrease in the initial, solute-free UF rate, and a ~50% decrease in cumulative UF **(11, 12).** The development of pharmacological modulators of AQP-mediated water transport offers perspectives to increase UF and restore fluid balance in PD patients **(12)**. The investigation of a common variant in *AQP1* among patients treated with PD was associated with decreased

ultrafiltration and an increased risk of death or technique failure **(13).** Thus far, AQP1 is the only identified molecule involved in microvascular water permeability and peritoneal transport **(14, 15)**.

Kidney failure, PD and risk of cardiovascular disease

Patients with chronic kidney disease are at higher risk for death and cardiovascular events compared to the general population as shown on Figure 1. In addition to traditional cardiovascular risk factors (age, diabetes, dyslipidemia, high blood pressure and tobacco consumption), patients with kidney failure also commonly have risk factors that are directly related to kidney disease and associated complications, such as abnormalities in the calcium and phosphorus metabolism, systemic inflammation, anemia or sodium and water retention **(16)**.

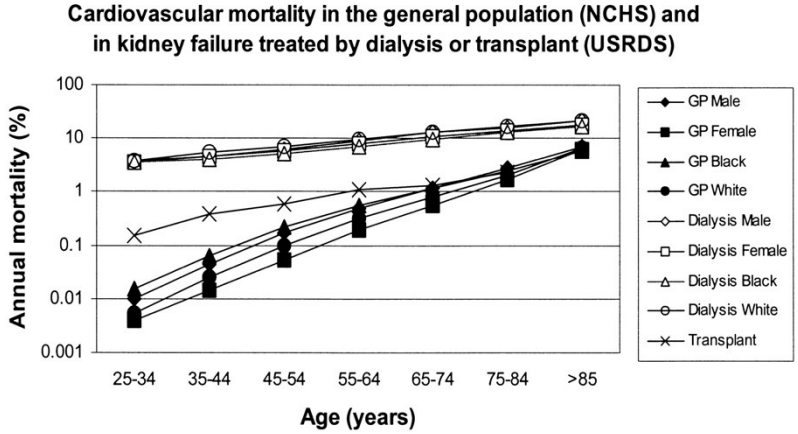


Figure 1. Prevalence of cardiovascular disease in the general population and chronic kidney disease. Ischemic heart disease, LVH (left ventricular hypertrophy – by echocardiogram), Heart failure, CKD (chronic kidney disease). Values are percentages. Sarnak et al. Hypertrension, 2003

Forty to 60 % of the mortality among patients under PD is associated to a cardiovascular event, regardless of age **(17)**. As compared to hemodialysis, PD has been associated with an increased risk of cardiovascular disease **(18)**. Although the reasons for this association remain unclear, several hypotheses have been suggested to account for the higher risk of cardiovascular morbidity and mortality in patients on PD, including loss of residual kidney function poor volume control, and exposure to glucose-based dialysis solutions **(19, 20, 21).**

Glucose absorption during PD

Glucose is the most widely used osmotic agent because it is highly effective, inexpensive and easily available **(21)**. However, glucose is a small molecule (molecular weight, 180 Da; hydrodynamic radius, 0.37 nm) rapidly absorbed across the peritoneal membrane, implying a transient effect on osmotic water transport and significant systemic glucose absorption **(22, 23)**.

During a 4-h dwell, approximately two thirds of the glycemic load is absorbed in the systemic circulation independently of its concentration in the dialysate **(24)**. The amount of glucose absorption is essentially determined by the numbers of glucose-based PD dwells; and by individual peritoneal transport characteristics **(25)** (Figure 2). Patients for whom small molecules such as creatinine diffuse rapidly across the peritoneal membrane also reabsorb glucose faster, from the dialysis solution towards the systemic circulation. Considering the two parameters, patients on PD absorb between 20 and 50 kg of glucose per year (i.e. 80,000 to 200,000 kcal per year), which represents up to 20% of the daily calorie intake **(26)**.

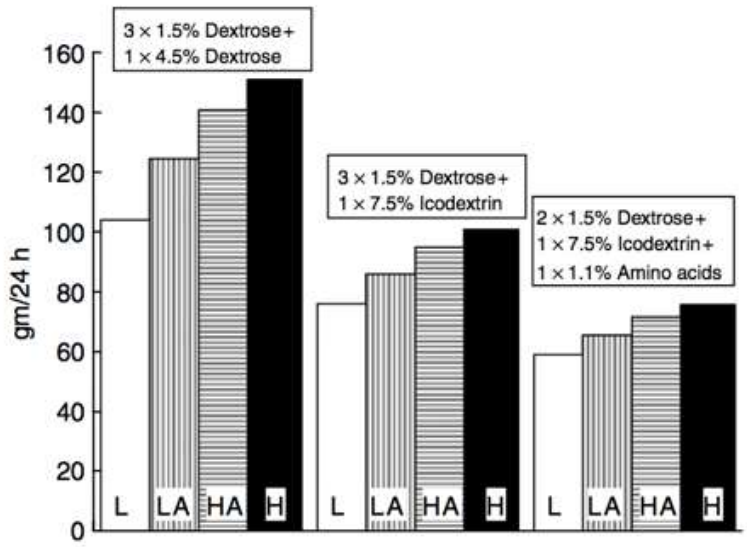


Figure 2. Mathematical modeling of glucose absorption in CAPD patients with various membrane transport characteristics. The model assumes three shorts dwells of 5 h and a long dwell of 9 h. Legend: L=low (MTAC 5.96 ml/min); LA=low-average (MTAC 8.35 ml/min); HA=high-average (MTAC 11.7 ml/min); H=high (MTAC 16.3 ml/min). Holmes et al, Kidney Int 2006 (3).

The excessive absorption of glucose and calories has been suggested to cause accumulation of ectopic fat (i.e. visceral fat and fatty muscle infiltration, or myosteatosis) **(26, 27, 28)**, which has been directly linked to the development of metabolic syndrome, and cardiovascular disease events in the non-dialysis population **(29).**

A crude population measure of obesity is the body mass index (BMI). A BMI of 30 kg/m^2 is currently being used to define obesity. However, the BMI does not distinguish the different localization of fat deposition on which cardiovascular risk depends. In fact, it has been proven that some people with a BMI ≥30 kg/m^2 could have a low CV risk and were qualified as metabolically healthy obese patients, while a good amount of overweight people (BMI between 25 and 30 kg/m^2) and 'non-obese' may have a higher cardiovascular risk, despite correction of the conventional risk factors (age, high blood pressure, tobacco, cholesterol) **(30).** The difference between these two groups remains in the localization of the fat : the patients with a high cardiovascular risk have the most important amount of abdominal visceral fat. This excess of abdominal fat can be estimated from the waist circumference or directly measure using imaging techniques. It has later been acknowledged that patients with accumulation of visceral fat are at risk for metabolic dysfunctions, such as prediabetes and type 2 diabetes, and for the development of metabolic syndrome **(31)**. The metabolic syndrome is defined by the National Cholesterol Education Program-Adult Treatment Panel III on the basis of 3 criteria out of the 5 following **(32)** : (i) abdominal obesity defined with an abdominal circumference ≥ 102 cm for men and ≥ 88 cm for women ; (ii) triglycerides ≥ 1,50 g/l (1,69 mmol/l) ; (iii) HDL-cholesterol <0,40 g/l (1,04 mmol/l) for men and <0,50 g/l (1,29 mmol/l) for women ; (iv) fasting blood glucose ≥1,00 g/l (6,1 mmol/l) – diabetes included ; (v) blood pressure ≥130/85 mmHg.

Role of the AQP7 in lipid metabolism and potential relevance for PD

After AQP1, the aquaglyceroporin AQP7 is the second most abundant member of the AQPs family expressed in the peritoneum **(10)** (Figure 3).

Located in the membrane of adipocytes, AQP7 facilitates the efflux of glycerol from adipocytes, thereby controls triglyceride accumulation and fat cell size **(33)**. Glycerol constitutes a key metabolite in the control of fat accumulation (as the carbon backbone of triglycerides) and glucose metabolism (as the major substrate for hepatic gluconeogenesis during fasting) (Figure 3). In mice, deletion of *Aqp7* leads to adipocyte hypertrophy, gain in visceral fat, adult-onset obesity and type 2 diabetes mellitus, suggesting that the glycerol channel plays a pivotal role in adipose tissue enlargement and function **(34, 35)**. In humans, a common variant in the promoter of the *AQP7* gene (rs2989924, A-953G, minor allele frequency 0.47-0.49) has been associated with decreased transcriptional activity of the *AQP7*

promoter, reduced AQP7 expression in adipose tissue, and increased risk of obesity and metabolic complications **(36).**

The potential association between *AQP7* promoter gene variant and fat distribution has not been investigated so far in patients treated with PD, who are at very high cardiovascular risk and exposed to huge amounts of exogenous glucose.

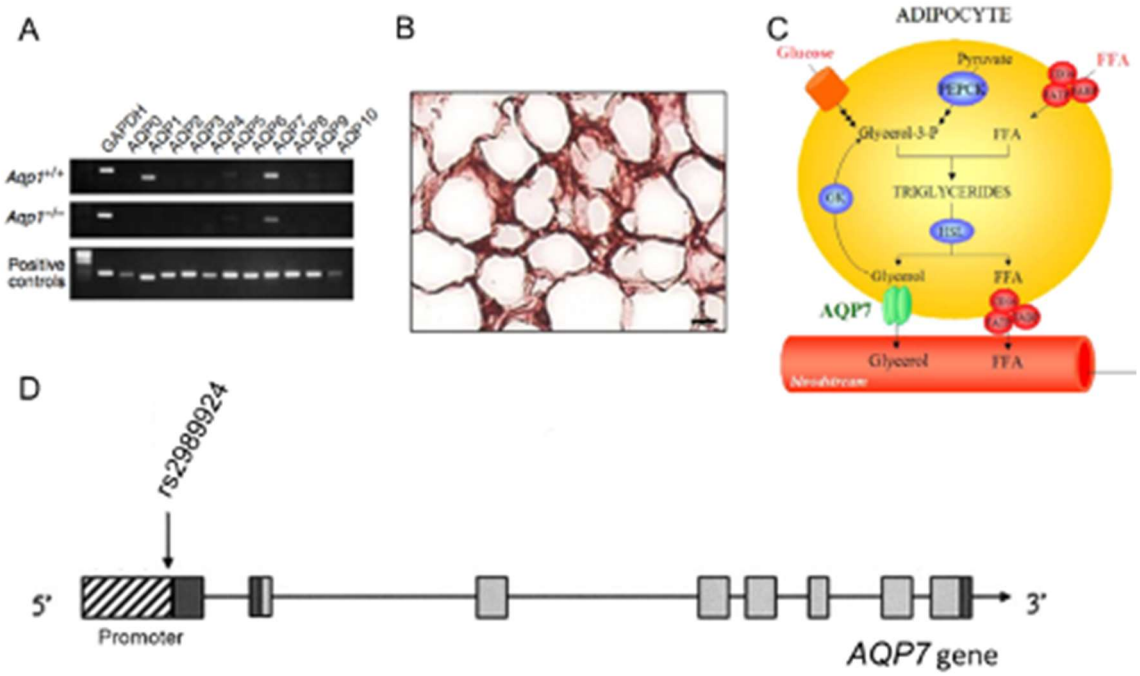


Figure 3. AQP7 in the peritoneal membrane and its role in adipocyte biology. AQP7 is the second most expressed AQP in murine visceral peritoneum (A), and is located in the membrane of adipocytes (B), where it facilitates the efflux of glycerol towards the circulation (C). The rs2989924 variant, located in the promoter of the human *AQP7* gene (D) has been associated with obesity and metabolic complications.

OBJECTIVES OF THE RESEARCH PROJECT

Taking advantage of a large and well-established cohort of incident patients starting PD between 2006 and 2019 at the home dialysis unit of the Cliniques universitaires Saint-Luc, we propose:

1. To assess the longitudinal changes in visceral fat area over time on PD based on abdominal computed tomography scans analysed using the Slice-O-Matic software, performed at the start of dialysis and then annually during treatment, as part of their clinical follow-up;
2. To test whether the common promoter variant in the *AQP7* gene is associated with changes in visceral fat in kidney failure patients treated with PD, who are exposed to a significant glucose and caloric load.

MATERIALS AND METHODS

Study population, demographic and clinical data

In order to achieve these goals, we included kidney failure patients starting PD between January 2006 and December 2019 at Saint-Luc Academic Hospital, Brussels, Belgium. Patients were required to have available DNA samples and available non-iodine contrast media CT scans. Of 158 consecutive incident PD patients during the inclusion period, 50 were not included as they had no CT scan (34), no DNA (9), or no CT scan and no DNA (7). Demographic and clinical characteristics were obtained from patient charts, and primary kidney diseases were classified according to the European Renal Association code. Residual urine volume was defined as a daily urine output of 200 ml or more. Time-independent variables were collected for each patient at study entry. Time-dependent variables, including parameters of fat distribution and body composition, were collected at dialysis initiation then yearly. Patients were followed until they died, were transferred to hemodialysis, underwent kidney transplantation, or were withdrawn from dialysis or until the end of the follow-up period on 31 December 2019. All patients provided written informed consent. Time-independent variables were collected for each patient at study entry.

The Charlson Comorbidity Index is one of the most worldwide used score **(37)** as a strong mortality predictor. It predicts 10-year survival in patients, based only on their comorbidities. The CCI assigns 1 point for history of myocardial infarction, congestive heart failure, peripheral vascular disease, cerebrovascular disease (transient ischemic attack or cerebrovascular accident), dementia, chronic pulmonary disease, connective tissue disorder, peptic ulcer disease, mild liver disease, and diabetes without end-organ damage; 2 points are assigned for hemiplegia, moderate to severe renal disease, diabetes with end-organ damage, tumor without metastases, leukemia, lymphoma, and myeloma; 3 points are assigned for moderate or severe liver disease; and 6 points are assigned for metastatic solid tumor or acquired immunodeficiency syndrome (AIDS). For every decade >40 years of age, 1 point is added to the score. Because all patients were on dialysis, the minimum Charlson score is 2.

The Davies score predicts survival in patients starting PD, based on the presence or absence of seven comorbidities **(38).** Briefly, the 7 comorbid domains are the following : malignancy (active and non-cutaneous), ischemic heart disease, peripheral vascular disease, left ventricular dysfunction, diabetes mellitus (type 1 or type 2), systemic collagen vascular disease (systemic vasculitis, rheumatoid arthritis and systemic sclerosis, either active or

requiring treatment), other significant non-treatable conditions or life threatening diseases (severe chronic obstructive pulmonary disease or severe COPD, cirrhosis, psychotic illness). The comorbid score for each patient is simply the number of these domains affected, giving a theoretical maximum of 7. The grade of comorbidity is derived directly from this score. Grade 0 (low risk) is a zero score, grade 1 (medium risk) is a score of 1 or 2, and grade 2 (high risk) a cumulative score of 3 or more.

The patients were followed until death, transplantation, transfer to hemodialysis or at the end of the study period. All the cardiovascular disease events were recorded, including : myocardial infarction or coronary revascularization; ischemic stroke, transient ischemic attack, or cerebrovascular revascularization; lower limb necrosis or peripheral arterial revascularization **(39)**.

Glucose and icodextrin exposures were quantified respecting the findings from previous studies **(39, 40)**. A greater use of hypertonic glucose (3.86%) and thus peritoneal glucose exposure precedes the rise in solute transport and continues to increase as solute transport increases **(41).** Besides, in terms of dialysis efficacy objectified by the aquaporin-mediated water transport, there is no difference between 1.36% and 3.86% glucose **(42)**. In order to figure out the amount of glucose the patient got exposed to, we use the dialysis prescriptions to calculate it from the number of litres of the dialysis fluid and its glucose content. For example, if a patient is using a 1.36% glucose concentration 4L volume exchange per day, he would be yearly exposed to 19 856g of glucose (4000/100 x 1.36 x 365 days). The possible quarterly adaptations of the dialysis prescription have been taken into account where appropriate.

A peritoneal equilibration test was performed yearly for most patients in order to assess the peritoneal membrane transport function. Briefly, it is determined by the ratio of creatinine concentration in dialysate and plasma (D/P ratio) at the end of a standard 4-h dwell, and the ratio of dialysate glucose 4-h after the beginning to the initial level in the dialysis solution (D4/D0 glucose) **(42)**. High rate or low rate of peritoneal transport can be estimated from the dialysate/plasma ratio of low molecular weight solutes crossing the peritoneum, almost independently of the fluid tonicity. A high rate transporter has a rapid absorption of glucose and thus loses the osmotic gradient early in the dialysis cycle which explains the poor ultrafiltration **(41)**. Moreover, a prolonged exposure of the peritoneal membrane to non-physiological dialysis solutions leads to loss of ultrafiltration capacity which precedes the peritoneal fibrosis **(40)**. A decrease of serum albumin concentration during PD attests the passage of albumin from blood to dialysate, through the peritoneum

which adds to the functional alterations of the peritoneum. Therefore, we also entered the serum albumin concentration as it is a surrogate marker for peritoneal fibrosis.

The intake of angiotensin converting enzyme (ACE) inhibitors, angiotensin II receptor antagonists, statins, betablockers, steroids, oral antidiabetic agents or hormone replacement treatment has also been included in this data base.

The study was in accordance with the World Medical Association's Declaration of Helsinki, the Belgian law related to experiments in human dated 7 May 2004, the General Data Protection Regulation 2016/679, and the Belgian law of 30 July 2018 regarding the protection of personal data. The Ethical Review Board of Cliniques universitaires Saint-Luc and UCLouvain approved the study (approval number: 2019/29OCT471). All patients provided written informed consent.

Application of the Slice-O-Matic software to the CT scans to evaluate body composition and fat distribution

The patients underwent a routine abdominal CT scan at baseline, then yearly (± 3 months). Importantly, CT scans were non-contrast enhanced, as iodine-contrast media interferes with MRA measurement **(43)**. CT scans were performed as part of routine clinical practice, in an attempt to detect severe structural changes in the peritoneal membrane exposed to PD, and to identify patients at risk for encapsulating peritoneal sclerosis, a rare but dramatic complication of long-term PD **(40).**

Abdominal CT scan is considered the gold standard imaging modality for measurement of visceral adipose tissue area. The advantages of the technique include **(44)**: fast image acquisition, as part of the clinical follow-up; accurate and sensitive detection of changes over time; high specificity; information of the tissues; excellent interobserver reproducibility. A single slice at the level of the third lumbar vertebra (L3) can be used as the standard landmark to accurately quantify fat distribution. Previous study showed that regional analysis of fat and fat-free mass at L3 with DXA or CT strongly predicted the whole-body fat and fat-free mass in cancer patients **(45)**. Another study presented L3 as reference to assess whole-body skeletal muscle and adipose tissue in both sexes **(46)**. Furthermore, other studies have been extracting L3 for analysis of skeletal muscle index, and subcutaneous and visceral adipose tissue areas **(47, 48, 49).**

The application of the Slice-O-Matic software (Tomovision, Canada) to these CT scans allowed a robust and validated assessment of the body composition, providing

information on i.e. visceral and subcutaneous fat areas and muscle density – a parameter that reflects ectopic fat deposition (Figure 4). This computer-assisted but manual method is widely used well validated. Briefly, the investigator must select manually the areas intended for analysis while the software operates based on Hounsfield unit (HU) thresholds. Adipose tissue and skeletal muscle are each assigned a specific threshold. Tissue density allows the distinction between subcutaneous fat and visceral fat, and muscle. We will evaluate the changes in body composition during treatment, their association with the metabolic syndrome and the risk of cardiovascular event.

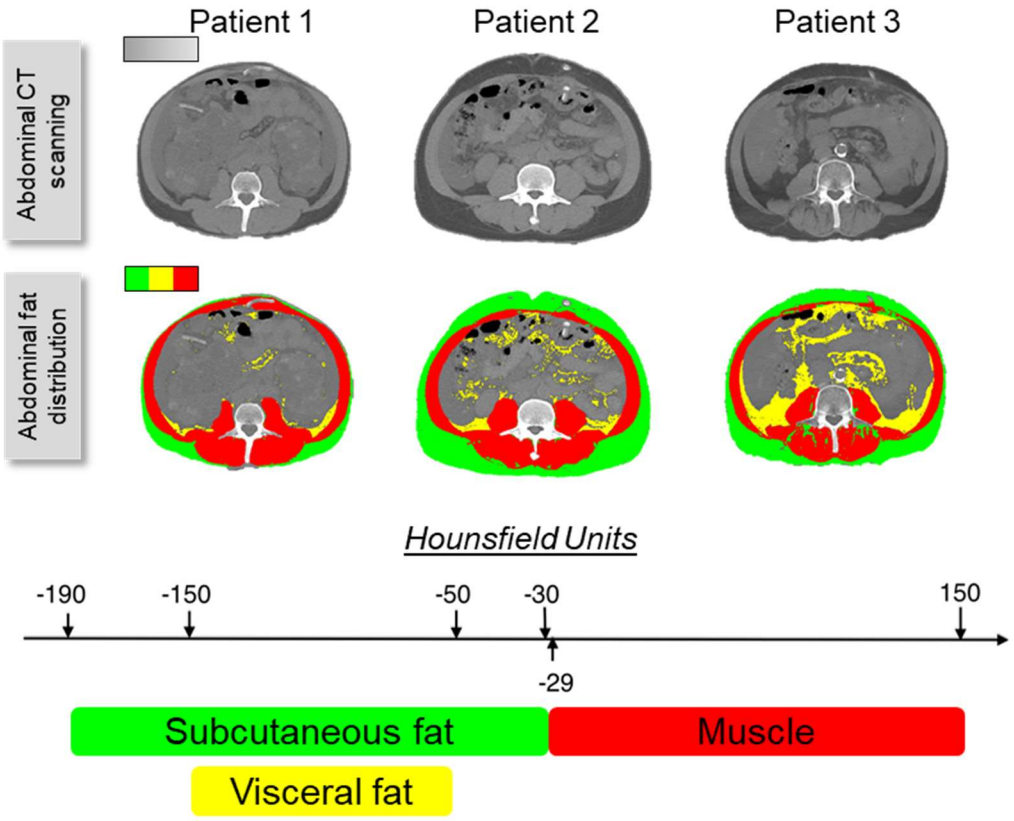


Figure 4. Evaluation of changes in body composition. Representative pictures generated by the Slice-O-Matic software on CT scans performed in PD patients – the gold-standard method for the evaluation of fat distribution – from three patients matched for gender (male), age (52, 49 and 52-year-old), body-mass index (20.0, 21.4 and 19.4 kg/m^2), and residual renal volume (>1000 ml/day in all three patients). Tissue density allows the distinction between intra-abdominal organs, muscles and adipose tissue. Subcutaneous fat is in green; visceral fat in yellow, and muscles in red.

Genotyping

Genomic DNA was extracted from peripheral-blood leukocytes (Puregene, Gentra Systems) and analyzed with a competitive allele specific polymerase-chain-reaction assay (LGC Group) for rs2989924.

Statistics

Results are presented as means ± SD or medians [interquartile range (IQR)] for continuous variables and as numbers and proportions for categorical variables. Age was defined at start of PD ; hypertension, diabetes, and history of coronary heart disease were constructed as binary presence/absence variables. Continuous variables were expressed in their natural units without standardization. Comparisons of means between groups that were defined according to genotype were performed with the use of unpaired t-tests, chis-square tests, or one-way analysis of variance followed by testing for multiple comparisons, as appropriate. Changes in fat distribution over time were assessed using mixed-effects models considering the availability of repeated measures in single individuals, and the Dunnett's test was applied to correct for multiple comparisons. Correlations were evaluated using the Spearman's coefficient. All analyses were performed with GraphPad Prism software, version 9.0.0 (Graph-Pad Software), or Stata software, version 17.0 (StataCorp). A P-value of less than 0.05 was considered to indicate significance.

RESULTS

Demographic characteristics, body composition at baseline and outcomes

The cohort included 108 patients with kidney failure who started peritoneal dialysis (PD) in our home dialysis unit. At initiation of dialysis, mean (±SD) age was 54 years (17); 68 (63%) patients were men; 28 (26%) had diabetes; 84 (78%), hypertension. Most patients (99, 92%) were European descent. Mean body-mass index was 25.0 kg/m^2 (±4.6) and mean Charlson index and Davies score were 5.1 (±2.5) and 1.4 (±1.2), respectively.

Regarding dialysis prescription, 53 (49%) and 55 (51%) patients were on continuous ambulatory or automated (cycler) PD, respectively; most patients (104, 96%) had significant residual kidney function; mean estimated yearly glucose exposure was 16.9 kg/year; and mean dialysate-over-plasma creatinine ratio at 4 hours was 0.74 (±0.12).

Baseline computed tomography scan of the abdomen, analyzed using the Slice-O-Matic software (Figure 4), showed a significant variability in body composition and fat distribution between the participants (Figure 5; Table 1).

Mean (±SD) time between the baseline CT and the start of PD was 65 days (±91). On those abdominal scanning, the median (IQR) visceral fat area was 92 cm^2 (67-119); visceral/subcutaneous fat ratio was 0.69 (0.60-0.87) and muscle density was 39 UH (35-42).

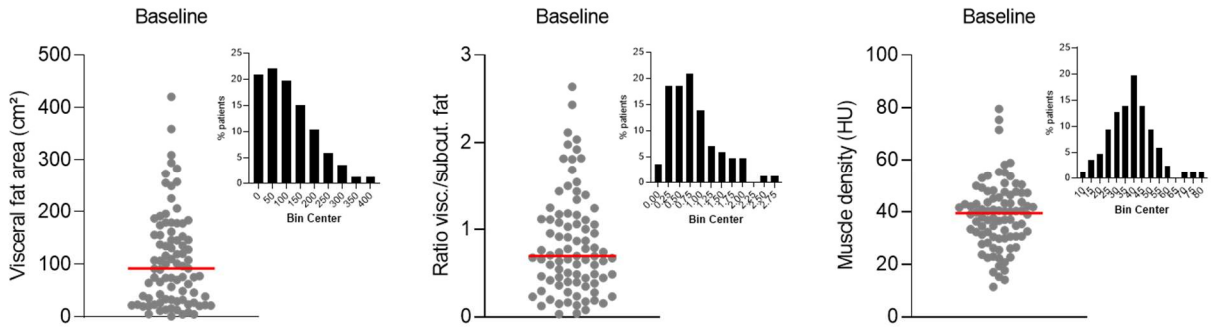


Figure 5. Distribution of body mass composition at start of dialysis, with focus on ectopic fat accumulation, in the cohort of kidney failure patients, including visceral fat area, ratio visceral/subcutaneous fat area and muscle density. Data are individual values and red lines medians. Inlet, histograms showing distribution of each parameter.

Table 1. Baseline characteristics of the study population by *AQP7* genotype.

Characteristic	Whole cohort n=108	*AQP7* genotype rs2989924 AA n=26	GA n=57	GG n=25	P
Age at PD start – years	54 ± 17	55 ± 12	53 ± 18	54 ± 20	0.84
Men – no. (%)	68 (63)	17 (65)	34 (60)	17 (68)	0.74
Ethnicity – no. (%)					0.37
White	99 (92)	22 (85)	54 (95)	23 (92)	
Sub Saharan African	3 (3)	2 (8)	1 (2)	0 (0)	
Asian	6 (6)	2 (8)	2 (4)	2 (8)	
Causes of kidney failure – no. (%)					0.32
Glomerular disease	38 (35)	7 (27)	24 (42)	7 (28)	
Interstitial nephritis	12 (11)	4 (15)	4 (7)	4 (16)	
Polycystic kidneys	11 (10)	4 (15)	4 (7)	3 (12)	
Hypertension	3 (3)	0 (0)	2 (4)	1 (4)	
Renal vascular disease	4 (4)	2 (8)	1 (2)	1 (4)	
Diabetes	20 (19)	1 (4)	13 (23)	6 (24)	
Miscellaneous	15 (14)	7 (27)	6 (11)	2 (8)	
Unknown	5 (5)	1 (4)	3 (5)	1 (4)	
Charlson index	5.1 ± 2.5	5.0 ± 1.6	5.2 ± 2.7	5.2 ± 2.8	0.98
Davies score	1.4 ± 1.2	1.2 ± 0.8	1.5 ± 1.3	1.3 ± 1.1	0.55
High BP – no. (%)	84 (78)	22 (85)	42 (74)	20 (80)	0.52
Diabetes – no. (%)	28 (26)	3 (12)	17 (30)	8 (32)	0.16
CHD – no. (%)	15 (14)	3 (12)	8 (14)	4 (16)	0.90
Long-term treatment – no. (%)					
Statin	62 (57)	16 (62)	30 (53)	16 (64)	0.56
ACE inhibitors	43 (40)	12 (46)	22 (39)	9 (36)	0.73
Beta blockers	42 (39)	7 (27)	24 (42)	11 (44)	0.35
ARB	35 (32)	10 (39)	15 (26)	10 (40)	0.36
Corticosteroids	21 (19)	6 (23)	12 (21)	3 (12)	0.55
BMI – kg/m^2	25.0 ± 4.6	25.1 ± 3.7	24.4 ± 4.6	26.3 ± 5.2	0.20
Systolic BP – mmHg	138 ± 21	139 ± 21	138 ± 19	136 ± 23	0.89
Diastolic BP – mmHg	83 ± 13	86 ± 11	83 ± 13	82 ± 16	0.56
Urine volume – mL/day	1469 ± 648	1568 ± 634	1507 ± 648	1274 ± 650	0.21
Serum albumin – g/L	37 ± 6	38 ± 5	36 ± 6	38 ± 4	0.25
CRP – mg/dL	3.7 ± 6.5	3.0 ± 8.3	4.1 ± 6.4	3.5 ± 4.2	0.42
Peritoneal dialysis					
CAPD – no. (%)	53 (49)	9 (35)	30 (53)	14 (56)	0.23
Gluc. exposure – kg/year	16.9 ± 10.1	18.2 ± 9.3	16.7 ± 8.4	16.1 ± 14.1	0.17
Icod. exposure – no. (%)	52 (48)	10 (39)	30 (53)	12 (48)	0.46

RKF – no. (%)	104 (96)	24 (92)	56 (98)	24 (96)	0.32
PSTR	0.74 ± 0.12	0.74 ± 0.12	0.74 ± 0.12	0.75 ± 0.13	0.84
Kidney transplant list	67 (62)	18 (69)	36 (63)	13 (52)	0.43

CHD, coronary heart disease ; ACE, angiotensin converting enzyme; ARB, angiotensin receptor blocker ; RKF, residual kidney function ; PSTR, peritoneal solute transfer rate, estimated from the dialysate-over-plasma creatinine ratio at 4h

During a median (IQR) follow-up of 28 months (13-47) on dialysis, 12 (11%) patients died, 44 (41%) were transferred to hemodialysis; and 39 (36%) received a kidney transplant. Among the remaining patients, 10 (9%) were still on PD at the end of the study, and 3 (3%) were lost to follow-up . During time on dialysis, 32 (30%) patients experienced a total of 33 major adverse cardiovascular events (MACE): including 21 (64%) nonfatal myocardial infarction, 9 (28%) nonfatal stroke and 3 (9%) cardiovascular death **(50)**.

Table 2. Outcomes in the whole cohort and by *AQP7* genotype.

	Whole cohort n=108	**AA n=26**	**GA n=57**	**GG n=25**
Dialysis outcomes				
Time to outcome – days	926 ± 650	994 ± 705	900 ± 625	915 ± 672
Outcomes – no. (%)				
Hemodialysis	44 (41)	10 (39)	27 (47)	7 (28)
Death	12 (11)	2 (8)	7 (12)	3 (12)
Transplantation	39 (36)	13 (50)	17 (30)	9 (36)
Peritoneal Dialysis	10 (9)	0 (0)	5 (9)	5 (20)
Lost to follow-up	3 (3)	1 (4)	1 (2)	1 (4)
CVD outcomes				
Patients with CVD events – no. (%)	37 (34)	10 (39)	19 (33)	8 (32)
CVD events – no. (%)	60	18	27	15
Myocardial infarction	22/60 (37)	5/18 (28)	11/27 (41)	6/15 (40)
Cerebrovascular accident	8/60 (13)	2/18 (11)	4/27 (15)	2/15 (13)
Transient ischemic attack	3/60 (5)	1/18 (6)	2/27 (7)	0/15 (0)
Lower limb Necrosis	23/60 (38)	9/18 (50)	7/27 (26)	7/15 (47)
Patients with MACE – no. (%)	32 (30)	8 (31)	17 (30)	7 (28)

MACE – no. (%)	33	8	17	8
Nonfatal myocardial infarction	21/33 (64)	5/8 (63)	11/17 (65)	5/8 (63)
Nonfatal stroke	9/33 (27)	3/8 (38)	5/17 (29)	1/8 (13)
Cardiovascular deaths	3/33 (9)	0/8 (0)	1/17 (6)	2/8 (25)

CVD, cardiovascular disease ; MACE, major adverse cardiovascular event.

Patients who developed MACE during follow-up were older (63 [56-70] vs 50 [38-64] years, P=0.001]) had higher BMI (26 [24-29] vs 24 [22-27] kg/m^2, P=0.004); were more likely to have diabetes (43% vs 19%, P=0.01) and hypertension (93% vs 72%, P=0.02). They also showed higher total abdominal fat area at baseline (266 [178-424] vs 183 [107-288] cm^2, P=0.02); higher visceral fat area at baseline (130 [76-192] vs 67 [24-149] cm^2, P=0.006); and lower muscle density at baseline (29 [23-40] vs 41 [36-48] UH, P<0.001) at baseline. There was no significant difference in subcutaneous fat area and in muscle area.

Altogether, these observations showed that participants were representative of the dialysis population and had a high CVD burden, with approximately one-third of them developing a MACE during a median follow-up of 2.5 years.

Changes in body mass composition over time on dialysis

We took advantage of this cohort of incident patients treated with PD to investigate the changes in body composition and fat distribution over time on dialysis, using the gold-standard method of computed tomography of the abdomen analyzed using the Slice-O-Matic software.

In our cohort, taking into account the availability of repeated measures in individual patients (mixed effects model), we observed a progressive and significant increase in visceral fat area after one (+22%, P<0.001) and two (+36%, P<0.001) years on PD (Figure 6; Table 3). This was paralleled by ectopic fat accumulation in skeletal muscle, with a decrease in muscle density (also referred to as muscle attenuation) **(51)** from 39 to 36 HU after two years on PD (P<0.05). In contrast, subcutaneous fat area remained unchanged over time on dialysis. As a result, the ratio of visceral-to-subcutaneous fat area – of which high baseline values are associated to MACE occurrence during FU – increased significantly, from 0.69 to 0.93 after two years on therapy (P<0.001) (Figure 6; Table 3). Muscle area remained unchanged.

These observations showed that ectopic fat (i.e. visceral fat or skeletal muscle fat infiltration) significantly increased over time on dialysis, while subcutaneous fat area and muscle area remained unchanged.

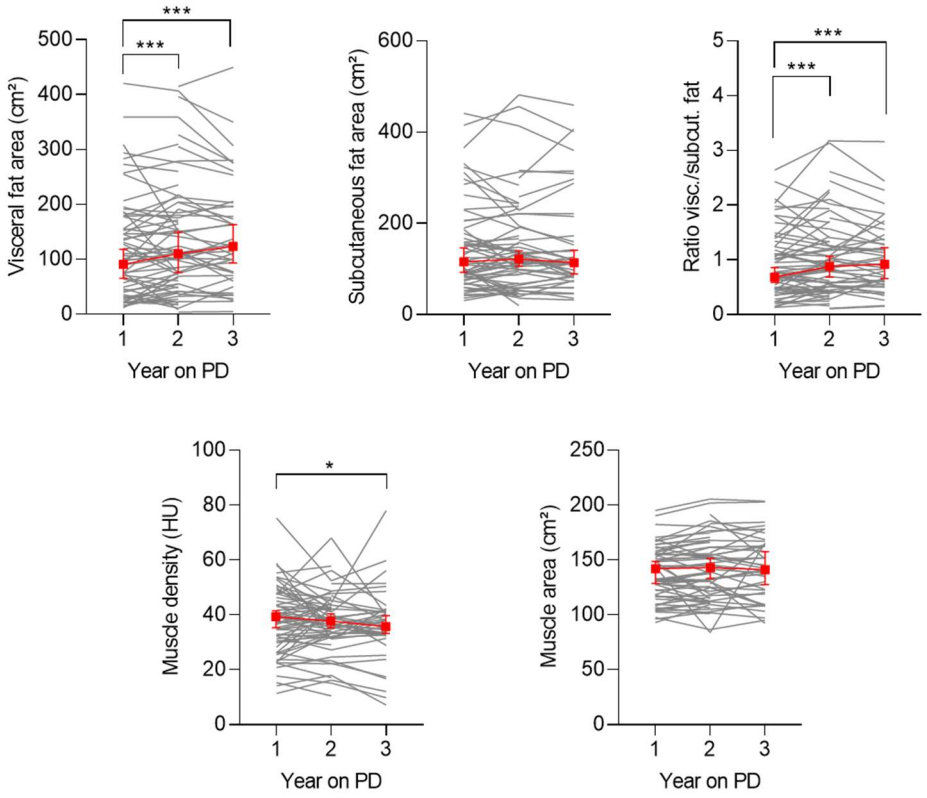


Figure 6. Changes in body mass composition over time on dialysis in the whole cohort. Data are individual values, red lines medians and 95% confidence interval. Comparison using a mixed-effects model considering repeated measures in individual patients.

Table 3. Changes in body composition over time on dialysis.

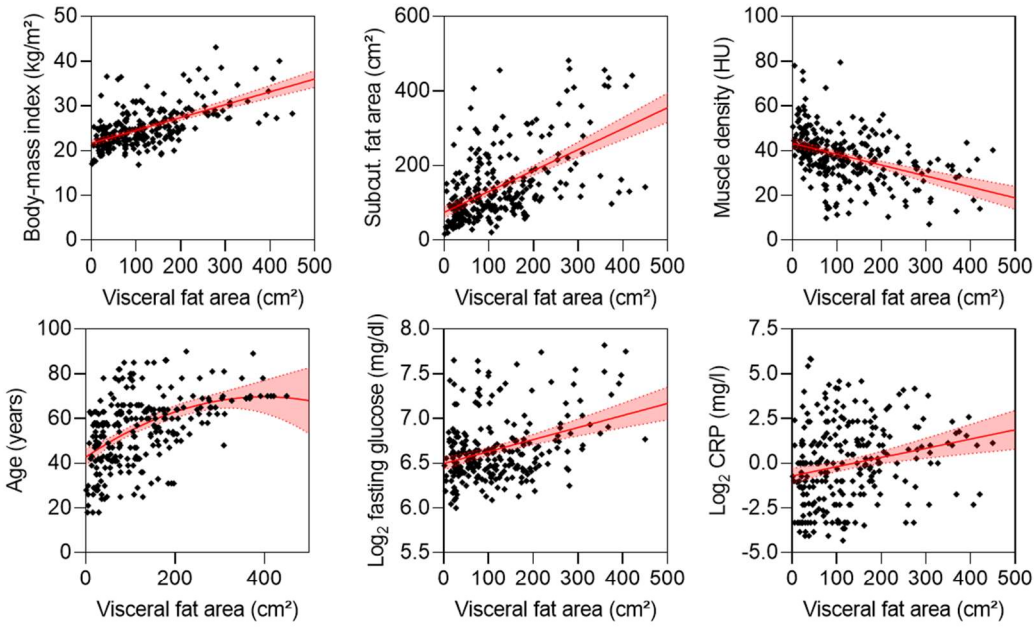
	Year on PD		
	1 **n=86**	**2** **n=78**	**3** **n=43**
Visc. Fat area – cm²	92 (67-119)	112 (77-151)[a]	125 (95-165)[a]
Subcut. Fat area – cm²	116 (93-146)	122 (106-140)	114 (89-141)
Ratio V/S – cm²	0.69 (0.60-0.87)	0.89 (0.69-1.07)[a]	0.93 (0.67-1.23)[a]
Muscle density – UH	39 (35-42)	38 (35-40)	36 (33-40)[b]
Muscle area – cm²	142 (129-149)	143 (134-152)	141 (128-158)

[a]P<0.001 vs. year 1 (baseline). [b]P<0.05 vs. year 1. Visc., visceral; subcut., subcutaneous; V/S, visceral-over-subcutaneous fat area. All values are medians (IQR).

Correlation between visceral fat area and demographic, biological and clinical covariates

To assess the determinants and associations of visceral fat area among patients on PD, we assessed the correlations between visceral fat area and a series of pre-specified covariates (Figure 7). We found significant correlations ($r < -0.20$ or $r > 0.20$, and $P<0.05$) between visceral fat area and body-mass index (0.59), subcutaneous fat area (0.57), muscle density (-0.47), age (0.49), fasting plasma glucose concentration (0.25), and systemic inflammation, assessed using plasma levels of C-reactive protein (0.27) (Figure 7).

Although visceral fat area did not, statistically significantly, differ between men and women at the start of dialysis, the increase during time on PD was steeper in male than in female patients, as shown on Figure 8.



	Spearman r	95% CI	P	n
Body-mass index (kg/m²)	0.59	0.50 to 0.66	<0.0001	253
Subcutaneous fat area (cm²)	0.57	0.48 to 0.64	<0.0001	270
Muscle density (UH)	-0.47	-0.56 to -0.36	<0.0001	270
Age (years)	0.49	0.40 to 0.58	<0.0001	270
Fasting glucose (mg/dl)	0.25	0.13 to 0.37	<0.0001	244
C-reactive protein (mg/l)	0.27	0.15 to 0.38	<0.0001	260

Figure 7. Association between visceral fat area and demographic, biological and clinical covariates. Data are individual values at baseline, red lines medians and 95% confidence interval.

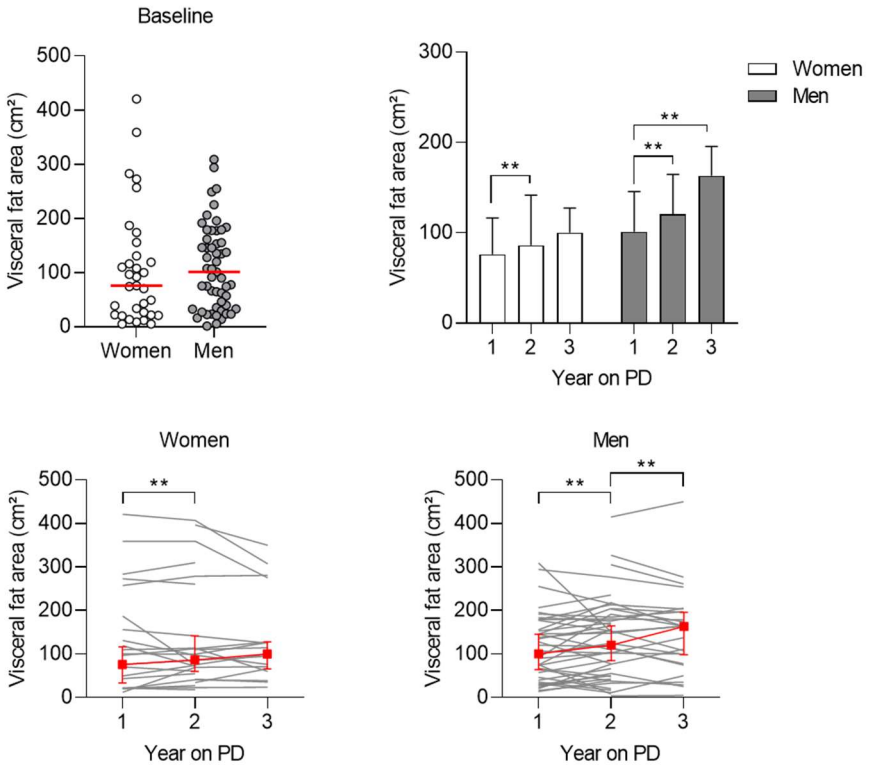


Figure 8. Visceral fat area at baseline and during PD according to gender. Data are individual values, red lines medians and 95% confidence interval. Comparison using a mixed-effects model considering repeated measures in individual patients. Correction for multiple comparisons using the Dunn's test.

Association between a common variant in the *AQP7* gene and fat accumulation during PD

The AQP7 is an aquaglyceroporin abundantly expressed in the plasma membrane of adipocytes where it operates as a glycerol efflux channel **(34)**. It plays an important role in glucose and lipid homeostasis. Its expression is increased during fasting and insulin deficiency, and downregulated after feeding and exposure to insulin. The deletion of *Aqp7* in mice results in accumulation of glycerol in adipocytes, which become hypertrophic, contributing to the development of obesity and associated metabolic complications **(35).** A common polymorphism in the promoter of the human AQP7 gene (rs2989924, A-953G) has been associated with reduced expression of AQP7 in adipocytes, obesity, and type 2 diabetes **(36).** As PD is responsible of a high caloric intake through glucose absorption **(21, 24)** and is associated with increase of abdominal fat **(26)**, we examined the potential association between genetic variation at rs2989924 and changes in body mass composition in patients treated with PD.

Minor allele frequency (MAF) at rs2989924 in our cohort (0.4953) was similar to that in European (0.4920) and to the whole population (0.4716) (data from gnomAD, accessed on march 04, 2022) (Figure 9). Twenty-six (24%), 57 (53%) and 25 (23%) of the patients in our cohort had an AA, GA and GG genotype at rs2989924, the G allele being considered as potential risk for fat accumulation based on previous reports **(36)**.

Over time on PD, however, we observed a striking and significant increase in visceral fat area among patients carrying one or two risk alleles (i.e. GA or GG genotype). The relative increase in visceral fat area from baseline, after one and two year on PD, was +34% and + 59% in patients with one risk allele, and +20% and +66% in those carrying two risk variants. In contrast, visceral fat area remained unchanged in patients with no risk variant (i.e. AA genotype), who even showed a non-significant trend toward decrease (-15% and -21% after one and two year on PD, respectively) (Figure 9 and Table 4). Of note, the association was independent from age, gender, glucose exposure, or peritoneal solute transfer rate (Table 2).

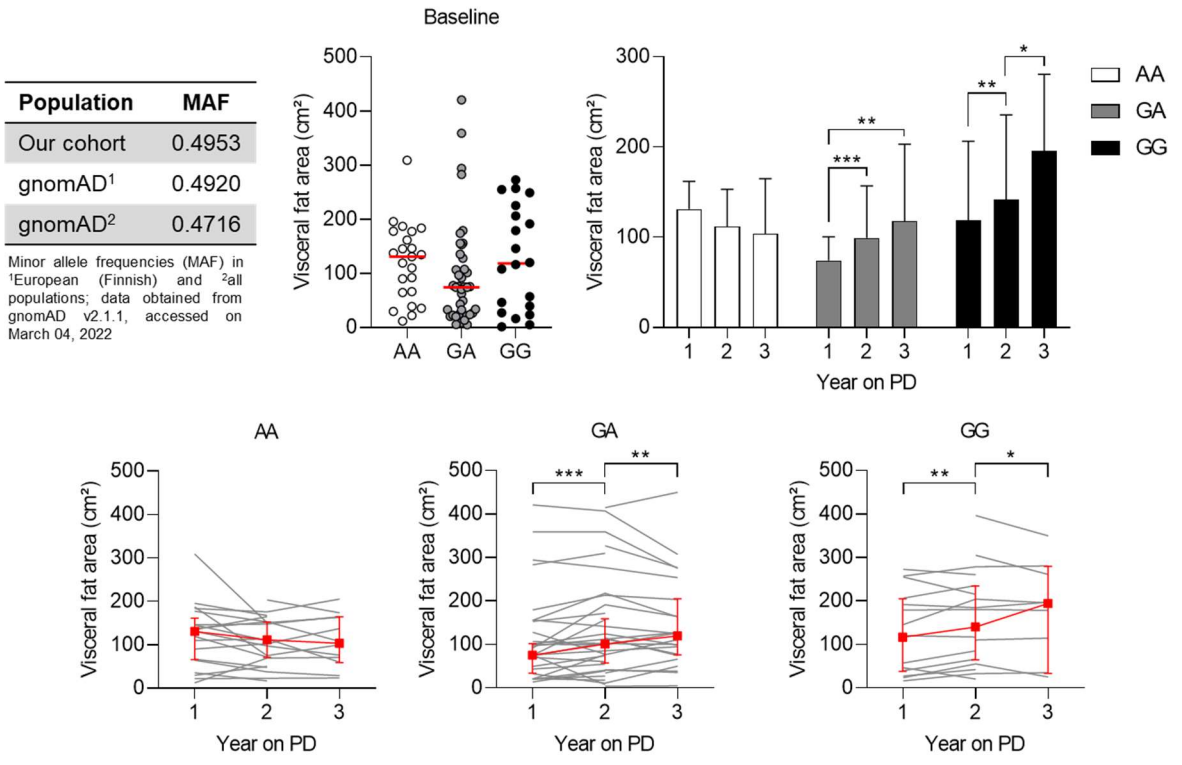


Figure 9. Visceral fat area at baseline and during the course of dialysis according to *AQP7* genotype at rs2989924 (-953,A-G). Data are individual values, medians and 95% confidence intervals. Comparison using a mixed-effects model considering repeated measures in individual patients.

Table 4. Changes in visceral fat area over time on PD stratified for *AQP7* genotype.

	Year on PD		
	1	**2**	**3**
***AQP7* rs2989924**	**n=86**	**n=78**	**n=43**
AA	131 (65-178)	112 (70-162)	104 (63-164)
GA	74 (24-128)	99 (42-213)[a]	118 (72-216)[b]
GG	118 (31-221)	142 (66-236)[c]	196 (75-271)[d]

[a]$P<0.0001$, [b]$P = 0.004$, [c]$P=0.002$, and [d]$P = 0.02$ vs. year 1 (baseline). All values are medians (IQR).

DISCUSSION

Our combined clinical, imaging and genetic data showed that kidney failure patients treated with PD, a population at high risk for cardiovascular disease events, have a significant increase in visceral fat area over time on dialysis. The increase in visceral fat area during PD was observed only among patients carrying a common risk variant in the promoter of the *AQP7* gene, which has previously been associated with fat distribution and cardiometabolic risk profile. In our cohort, visceral fat area was correlated with fasting glucose, systemic inflammation, age, gender, and ectopic fat accumulation in skeletal muscle.

In our cohort, we observed a significant increase in visceral fat area after one (+22%) and two (+36%) years on PD. These observations are in line with scarce previous studies, which suggested gain in visceral fat over time on dialysis **(26, 52)**. Changes in visceral fat area were paralleled by ectopic accumulation of fat in skeletal muscle and both parameters are linked with cardiovascular disease events in this high risk population **(51)**.

In our cohort, we demonstrated a strong association between a common variant in the *AQP7* promoter, A-953G, and fat accumulation. The significant increase in visceral fat area was observed only patients carrying one or two alleles at risk (GA : +34% and +59%, GG : +20% and +66% after one and two years on PD). In contrast, visceral fat area remained unchanged in patients without any allele at risk (AA : -15% and -21%). A previous study **(36)** associated this SNP in human's *AQP7* gene with obesity and related metabolic abnormalities. The hypothetic mechanism by which this variant promotes visceral fat accumulation revolves around a reduced transcriptional activity of the *AQP7* promoter in patients carrying an allele at risk, which reduces AQP7 expression in the plasma membrane of adipocytes, leading to reduced glycerol efflux from adipocytes, thus increased intracellular glycerol content, enhanced glycerol kinase enzymatic activity, leading to accumulation of triglycerides in adipocytes, adipocyte hypertrophy, and gain in visceral fat **(34, 36)**.

In our cohort, visceral fat area was correlated with fasting glucose, systemic inflammation, age, gender, and ectopic fat accumulation in skeletal muscle.

The strengths of this study include a well-phenotyped and representative cohort of patients treated with PD; the availability of systematic longitudinal follow-up; and accurate and non-invasive quantification of fat distribution using the gold standard method (abdominal CT analyzed using the Slice-O-Matic software).

However, we acknowledge some limitations, including the monocenter, retrospective design; the limited number of participants included; the single gene candidate approach; the lack of replication cohort; the lack of control group to assess the effect of kidney replacement therapy per se (e.g., hemodialysis controls); and the absence of mechanistic insight. It is very important to emphasize that the potential association between *AQP7* variant and changes in fat distribution (i) will need to be validated in an independent cohort, using for instance anthropometric measures such as the waist-to-hip ratio and/or body-mass index; (ii) does not mean causation. Mechanistic studies in transgenic mouse and cellular models are ongoing, to delineate the exact role of AQP7 in fat metabolism during PD and exogenous glucose exposure (Costa, Devuyst, Morelle, unpublished data).

In summary, kidney failure patients treated with PD have a significant increase in visceral fat area over time on dialysis. The increase in visceral fat area was only observed among patients carrying the common risk variant A-953G of the *AQP7* gene.

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