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*Original Article*

# The fat-mass and obesity-associated gene (FTO) predicts mortality in chronic kidney disease of various severity

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## Abstract

**Background.** Polymorphisms in the FTO (fat-mass and obesity-associated) gene have been associated with the body mass index, cancer, type 2 diabetes and hypertension.

**Methods.** We investigated the relationship between 17 tag single-nucleotide polymorphisms (SNPs) and all-cause mortality in three cohorts of dialysis patients (CREED-1, North Apulian and CREED-2 cohorts; $n = 783$) and in one cohort of stage 2–5 CKD patients ($n = 757$).

**Results.** We first explored the association between the 17 tag SNPs and all-cause mortality in the CREED-1 cohort and found that patients with the A allele of the FTO rs708259 polymorphism had an elevated risk of mortality (hazard ratio, HR: 1.52, 95% confidence interval (CI) 1.11–2.08; P = 0.008). Similarly, the A allele was associated with an increased risk of death also in the other two dialysis cohorts (North Apulian cohort, risk: +23%; CREED-2 cohort, risk: +21%). The elevated risk portended by this allele was even higher in the stage 2–5 CKD cohort (+97%). However, the risk of mortality associated with the A allele in the three confirmatory cohorts failed to achieve formal statistical significance. In a meta-analysis including the four cohorts ($n = 1540$; total deaths, $n = 381$), individuals with the A allele had a 42% excess risk of death (HR: 1.42, 95% CI 1.14–1.76, P = 0.002).

**Conclusion.** The A allele of the FTO rs708259 polymorphism is an independent predictor of all-cause mortality in patients with CKD of various severity. These data support our hypothesis that the FTO gene may be a relevant genetic risk factor for mortality in this population.

**Keywords:** chronic kidney disease; dialysis; FTO gene; meta-analysis; mortality

## Introduction

Obesity is an expanding epidemic and a public health priority worldwide. The causes of this epidemic are mainly environmental because obesity clusters with social contacts [1]. The genetic background contributes substantially to the risk of obesity and it is estimated that the genetic factors explain about half of the variation in adipose tissue mass [2]. Over the last two decades, a large series of genes associated with human obesity have been identified and for most of these genes the association with obesity has been replicated [3]. Among these genes, the FTO (fat-mass and obesity-associated gene) appears to be of particular interest because, beyond obesity, polymorphisms in this gene have been associated with mortality [4], cancer [5, 6, 7], diabetes [8, 9, 10] and hypertension [11].

Chronic kidney disease (CKD) is an emerging public health priority in economically developed and developing countries [12]. The relationship between the high body mass index (BMI) and survival in pre-dialysis [13] and dialysis [14] CKD patients seems to be complex. At variance with the general population, where excess adiposity is directly and linearly associated with the risk of death, the same association in CKD and in dialysis patients is either U shaped or inverse, suggesting that a high body mass may be protective in this population. Whether genetic variability in the FTO gene associates with elevated mortality in patients with CKD has not been investigated. The issue is of relevance because diabetes and hypertension, two risk factors which have been associated with the FTO gene, rank as major risk factors for CKD, while dialysis patients have an exceedingly high risk of incident cancers and diabetic patients on dialysis have a short survival. With this background in mind, we have, therefore, designed a genetic association study testing

whether the variability of the FTO gene may contribute to explaining mortality in CKD patients of varying severity.

## Materials and methods

### *Patients*

In the present study, we included three independent cohorts of dialysis patients (the CREED-1 cohort, the North Apulian cohort and the CREED-2 cohort) with a total number of 783 patients and one cohort of stage 2–5 CKD patients (see Table 1). CREED-1 [15] included an incident-prevalent cohort of 265 dialysis patients (age 61 ± 15 years; 56% males) treated in the urban areas of Reggio Calabria (Calabria region) and Catania (Sicily region). These patients (all Caucasian) have been on regular dialysis treatment for at least 6 months, with left ventricular ejection fraction ≥35% and without circulatory congestion, major infections (fever, infected vascular access or peritonitis or exit-site infection) or inter-current illnesses requiring hospitalization. Two hundred and fourteen haemodialysis patients were being treated thrice weekly with standard bicarbonate dialysis (Na 138 mmol/L, $HCO_3$ 5 mmol/L, K 1.5 mmol/L, Ca 1.25 mmol/L, Mg 0.75 mmol/L) either with cuprophan or with semi-synthetic membranes. The remaining 51 patients were on chronic ambulatory peritoneal dialysis (CAPD). One hundred and five patients were habitual smokers and 109 patients were treated with anti-hypertensive drugs. The incident/prevalent North Apulian cohort included 220 dialysis patients (age 58 ± 16 years; 53% males) [16] enrolled in the health district of Foggia (Puglia region). Two hundred and ten patients were treated thrice weekly with standard bicarbonate dialysis (Na 138 mmol/L, $HCO_3$ 5 mmol/L, K 1.5 mmol/L, Ca 1.25 mmol/L, Mg 0.75 mmol/L) with cuprophan or semi-synthetic membranes and 10 patients were on CAPD. Fifty-three patients were habitual smokers and 175 were on anti-hypertensive treatment. The CREED-2 cohort included 298 haemodialysis patients non-overlapping with the CREED-1 cohort. All patients in CREED-2 were of Caucasian descent and were being treated in 11 dialysis units in the two regions of southern Italy (Calabria and Sicily) and showed the same characteristics of CREED-1 and North Apulian cohort patients (see Table 1). All patients had been on regular haemodialysis with standard bicarbonate dialysis for a median time period of 42 months (inter-quartile range 20–81 months) and were being treated with non-cellulosic membrane filters of various types. One hundred and fifty-nine patients were habitual smokers and 174 patients were treated with anti-hypertensive drugs. The CKD cohort included 757 consecutive patients with stage 2–5 CKD of various aetiology. These patients were recruited from 22 nephrology units in southern Italy. All patients were in stable clinical condition and none had inter-current infections or acute inflammatory processes. The large majority of patients (97%) were being treated with anti-hypertensive drugs (51% were on mono/double therapy with ACE inhibitors, calcium antagonists, angiotensin II receptor antagonists, diuretics, alfa and beta blockers, and clonidine and the remaining 49% were consuming three or more various combinations of these drugs). The inclusion criteria were as follows: non-acute or rapidly evolving renal diseases; age ranging from 18 to 75 years; non-transplanted; not pregnant, not affected by cancer or diseases in the terminal phase. The main characteristics of these four cohorts are given in Table 1.

**Table 1.** Main characteristics of dialysis patients (CREED, North Apulian and CREED-2 cohorts) and of CKD patients

| | CREED-1 cohort | North Apulian cohort | CREED-2 cohort | CKD cohort |
|---|---|---|---|---|
| *N* | 265 | 220 | 298 | 757 |
| Age (years) | 61 ± 15 | 58 ± 16 | 61 ± 15 | 62 ± 11 |
| Male gender (%) | 56% | 53% | 63% | 60% |
| Diabetes (%) | 12% | 12% | 24% | 35% |
| BMI (kg/m$^2$) | 25 ± 4 | 23 ± 4 | 26 ± 5 | 28 ± 5 |
| Systolic BP (mmHg) | 140 ± 24 | 142 ± 19 | 136 ± 22 | 134 ± 18 |
| Diastolic BP (mmHg) | 77 ± 13 | 82 ± 8 | 74 ± 12 | 78 ± 11 |
| Enrolment period | Jan 1997–Feb 1998 | Nov 1999–May 1999 | May 2009–Oct 2010 | Oct 2005–Nov 2007 |

Data are expressed as mean ± SD or as percentage, as appropriate.

### *Follow-up study*

After the initial assessment, dialysis patients were followed up for a median time of 44 months (range: 0.20–154 months) in the CREED-1 study, for 66 months (range 1–90 months) in the North Apulian cohort and for 26 months (range 0.5–33 months) in the CREED-2 cohort. In the CKD cohort, patients were followed up for a median time period of 33 months (range 1–49 months). In all cohorts, during the observation period, all-cause mortality was accurately recorded and classified by trained outcome assessors.

### *Haplotype structure and SNP selection*

The haploblock structure of the FTO gene for the Central European population was defined using Haploview (http://www.broadinstitute.org/haploview/haploview) (version 3.0 release R2, accessed June 2009; Whitehead Institute for Biomedical Research, USA). Using a minor allele frequency of ≥5%, a pairwise approach and setting an $r^2 \geq 0.80$, 16 single-nucleotide polymorphisms (SNPs) (rs10163276; rs10521304; rs11075999; rs1125338; rs13334214; rs1078013; rs12935710; rs4784353; rs708259; rs7204916; rs8047395; rs860713; rs9924877; rs8044769; rs9926180; rs8050136), which were not in linkage disequilibrium, were sufficient to tag the haploblocks considered to be capturing most of the variability in the region. In addition, we determined the rs9939609 SNP which was in linkage disequilibium with the rs8050136. This polymorphism, which maps in intron 1, has been repeatedly associated with fat mass in overweight and obese patients in previous large studies [8, 17, 18, 19].

### *Genotyping of the selected SNPs*

Allelic discrimination of the selected 17 SNPs was performed using TaqMan SNP Genotyping assays provided by Applied Biosystems on a 7900HT Fast Real-Time PCR platform and its accompanying Sequence Detection System (SDS) Software version 2.4 (Applied Biosystems, Foster City, CA). Genomic DNA was extracted from peripheral blood leukocytes by a salting-out technique [20]. The reaction system contained 20 ng of genomic DNA, 12.5 μL of 2× TaqMan Universal PCR Master Mix No AmpErase UNG, 1.25 μL of 40× Assay mix (including unlabeled PCR primers, FAM and VIC dye-labelled TaqMan MGB probes) and $H_2O$ for a total volume of 25 μL. A random 10% of samples were independently repeated to confirm genotyping results. The genotype results for these samples were completely consistent.

### *Laboratory measurements*

In the whole study population, blood sampling was performed after an overnight fast always during a mid-week non-dialysis day for haemodialysis patients and at empty stomach for CAPD patients. Blood was drawn and put into tubes containing EDTA, and plasma supernatants were stored at −80°C until batch analyses. All analyses were done blinded to clinical information. Serum cholesterol, albumin and haemoglobin measurements were made using standard methods in the routine clinical laboratory.

### *Statistical analysis*

Data were expressed as mean ± SD, median and inter-quartile range (IQR) or as percent frequency and comparisons between groups were made by independent *t*-Test, Mann-Whitney *U* test or chi-square test, as appropriate.

The relationship between FTO rs708259 polymorphism and all-cause mortality was investigated by Cox regression analysis in which the centre effect was accounted by a stratified analysis. As potential confounders we considered Framingham risk factors (age, gender, smoking, diabetes, cholesterol and arterial pressure), anti-hypertensive treatment and factors peculiar to kidney failure (dialysis vintage, haemoglobin and albumin). A variable was considered as a confounder when it was related to both the exposure under investigation (the FTO rs708259 polymorphism) and the study outcome (all-cause mortality), was not an effect of the exposure and was not in the causal pathway between the exposure and outcome [21]. To account for multiple testing, a Monte Carlo permutation analysis (10 000 permutations) was done [22]. This analysis

provides an empirical P-value (permuted P-value) for the link between the FTO rs708259 polymorphism and all-cause mortality. The effect of the FTO rs708259 polymorphism on the risk of mortality was investigated separately in the four study cohorts as well as by a meta-analysis of these cohorts. The heterogeneity of the hazard ratios (HRs) for death associated with the FTO rs708259 polymorphism among the four cohorts was analysed by $I^2$ and $Q$-value [23]. Data were expressed as HR and 95% confidence interval (CI) and P value. All calculations were made by using two standard statistical packages (SPSS for Windows–9.01, Chicago, IL and Comprehensive Meta-Analysis – Version 2.2.064, BioStat, Englewood, NJ).).

## Results

We first investigated in the CREED-1 cohort study 17 SNPs (rs10163276; rs10521304; rs11075999; rs1125338; rs13334214; rs1078013; rs12935710; rs4784353; rs708259; rs7204916; rs8047395; rs860713; rs9924877; rs8044769; rs9926180; rs8050136; rs9939609) capturing most of the variability of the FTO gene. All these SNPs were in the Hardy–Weinberg equilibrium (P ranging from 0.09 to 0.98). Among these tag SNPs, the FTO rs708259 polymorphism was the only one to show an association with all-cause mortality (P = 0.008) (see below) in this cohort of dialysis patients. Then, we extended the analysis to other two dialysis cohorts (North Apulian and CREED-2 cohorts) and tested the relationship of the same polymorphism with mortality in a fourth cohort of stage 2–5 CKD patients.

### *FTO Rs708259 polymorphism*

The FTO rs708259 polymorphism, either in the CREED-1 study [GG, $n = 89$ (33.0%); AG, $n = 129$ (49.0%); AA, $n = 47$ (18.0%), $\chi 2 = 0.001$, P = 0.98] or in North Apulian [GG, $n = 43$ (19.0%); AG, $n = 96$ (44.0%); AA, $n = 81$ (37.0%), $\chi 2 = 2.22$, P = 0.14] and CREED-2 cohort [GG, $n = 94$ (31%); AG, $n = 148$ (50%); AA, $n = 56$ (19%), $\chi 2 = 0.03$, P = 0.87] did not deviate from the Hardy–Weinberg equilibrium. Similarly, in the CKD cohort ($n = 759$), the genotypic distribution of the FTO rs708259 polymorphism was in the Hardy–Weinberg equilibrium [GG, $n = 257$ (34.0%); AG, $n = 379$ (50.0%); AA, $n = 121$ (16.0%), $\chi 2 = 0.91$, P = 0.34].

In Table 2 the main demographic and clinical characteristics of patients of the three combined dialysis cohorts ($n = 783$) are described according to their genotypes. Patients with AA or AG genotypes did not differ from those homozygotes for the G allele. The same analysis carried out separately in the three dialysis cohorts provided similar results (data not shown).

### *FTO Rs708259 polymorphism and survival in dialysis patients*

During the follow-up period (median: 44 months, range: 0.2–154 months) of the whole study population of dialysis patients ($n = 783$), 339 patients died, 172 of them (51%) of cardiovascular causes. On univariate Cox regression analysis, the excess risk of death was 41% higher (HR: 1.41, 95% CI 1.10–1.81, P = 0.008; permuted P value = 0.006) in patients with AA or AG genotypes than in those with GG genotype (Figure 1), and the excess risk in the three combined cohorts did not differ from that observed in the three cohorts considered separately (CREED-1 cohort, excess risk: +52%; North Apulian cohort, excess risk: +23%; CREED-2 cohort, excess risk: +21%) (Figure 2). Adjustment for albumin (which was the only variable that tended to be different among genotypes) (see Table 2) had no material effect on the strength of the association between the FTO rs708259 polymorphism and the risk of mortality (HR: 1.41, 95% CI 1.10–1.82, P = 0.007, permuted P-value = 0.005).

**Table 2.** Main demographic, somatometric and clinical characteristics of the dialysis population (CREED-1, North Apulian and CREED-2 cohorts)

| | GG (rs708259) ($n = 226$) | AG/AA (rs708259) ($n = 557$) | P |
|---|---|---|---|
| Age (years) | 60 ± 15 | 60 ± 16 | 0.49 |
| Male sex *n*. (%) | 128 (57) | 325 (58) | 0.66 |
| Smokers *n*. (%) | 93 (41) | 224 (40) | 0.82 |
| Diabetics *n*. (%) | 45 (20) | 96 (17) | 0.35 |
| Dialysis vintage (months) | 43 (18–99) | 44 (20–99) | 0.91 |
| *BMI (kg/m$^2$) | 24.9 ± 4.5 | 25.2 ± 5.0 | 0.42 |
| Systolic pressure (mmHg) | 140 ± 22 | 139 ± 22 | 0.34 |
| Diastolic pressure (mmHg) | 77 ± 12 | 77 ± 12 | 0.96 |
| On anti-hypertensive treatment *n*. (%) | 127 (56) | 331 (59) | 0.42 |
| Cholesterol (mg/dL) | 181 ± 53 | 180 ± 52 | 0.75 |
| Haemoglobin (g/L) | 11.3 ± 1.7 | 11 ± 1.6 | 0.11 |
| Albumin (g/L) | 3.95 ± 0.48 | 3.87 ± 0.51 | 0.06 |

Data are expressed as mean ± SD, median and inter-quartile range or as percent frequency, as appropriate. Comparisons between groups were made by independent *T*-test, Mann–Witney *U* test or chi-square test, as appropriate.
*Available for 676 patients.

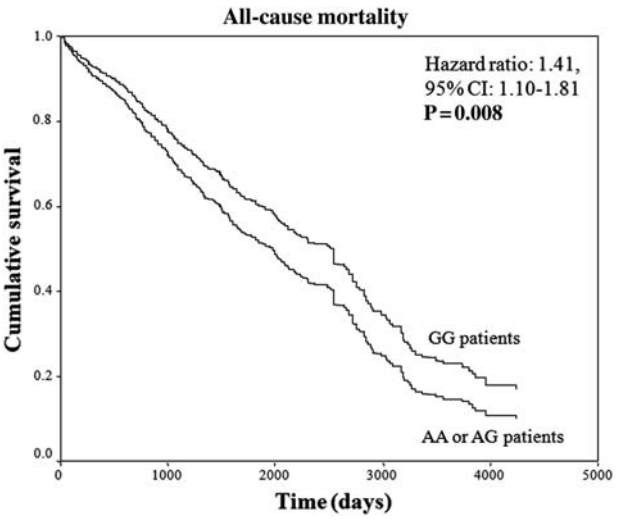


**Fig. 1.** Kaplan–Meier survival curves of all-cause mortality in the whole dialysis population (CREED-1, North Apulian and CREED-2 cohorts; $n = 783$) according to the genotypes of the FTO rs708259 polymorphism. Data are expressed as HR, 95% CIs and P value.

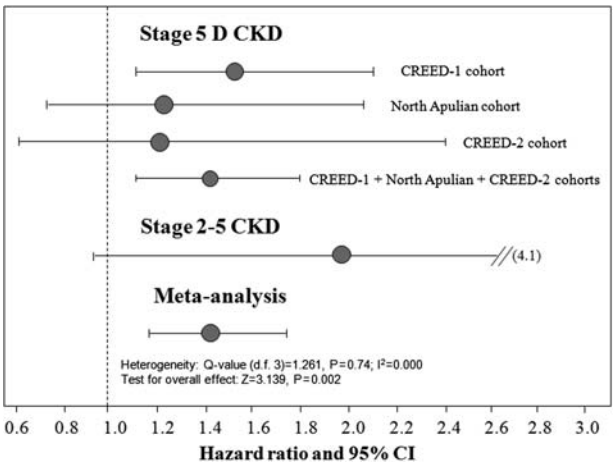


**Fig. 2.** HRs (and 95% CIs) of the FTO rs708259 polymorphism for all-cause mortality in the study cohorts. At the bottom of the figure, a pooled analysis of all cohorts based on a meta-analytic approach is reported.

### *FTO Rs708259 polymorphism and survival in stage 2–5 CKD patients*

During the follow-up period (33 months, range 1–49 months), 42 CKD patients died, 31 (74%) of them of CV causes. On univariate Cox regression analysis, CKD patients having AA or AG genotypes of the FTO rs708259 polymorphism had an excess risk of death almost doubled (+97%) as compared to patients with GG genotype and this excess risk did not differ (P = 0.32) from that observed in dialysis patients. However, probably due to the small number of death cases in this cohort of pre-dialysis patients, such a risk excess failed to achieve formal statistical significance (HR: 1.97, 95% CI 0.94–4.11; P = 0.07) in this cohort (Figure 2).

### *Meta-analysis of the four study cohorts*

There was no effect heterogeneity (P = 0.74) of FTO rs708259 polymorphism on the risk of mortality in patients with kidney failure and in those with stages 2–5 CKD and for this reason fixed- and random-effects models provided identical results. In a meta-analysis of the four cohorts (total number of patients, $n = 1540$; total deaths, $n = 381$), AA or AG individuals had a 42% excess risk of mortality when compared with those of homozygotes for the G allele (HR: 1.42, 95% CI 1.14–1.76, P = 0.002) (Figure 2), further confirming that the A allele of the FTO rs708259 polymorphism associates with reduced survival in CKD patients.

## Discussion

This study shows an association between the A allele of the FTO rs708259 polymorphism and all-cause mortality in pre-dialysis and dialysis patients.

### *FTO gene and obesity*

The fat-mass and obesity-associated (FTO) gene has been repeatedly associated with various obesity traits [8, 17, 18, 19, 24], insulin resistance and type 2 diabetes [8, 9, 10]. This gene codes for an enzyme that oxidatively demethylates single-stranded DNA [25] and, by this mechanism, it may modulate relevant epigenetic modifications of other fundamental genes regulating various biological processes. A common sequence variant in the first intron of this gene, the rs9939609, predisposes to type 2 diabetes through an effect on the BMI [8] and an association between this polymorphism and the BMI has been replicated in 13 external cohorts including 38 759 European subjects [8]. Furthermore, other FTO polymorphisms have been associated with severe obesity in individuals of French descent [18].

### *FTO Gene and renal disease*

Although studies performed so far have primarily focussed on the association between the FTO gene and obesity, variants in this gene associate also with other major clinical conditions, including cancer [5, 6, 7], hypertension [11], Alzheimer's disease [26] and kidney failure [27]. The FTO is one of the largest genes (>4 Mb) which have been implicated in human health. To study the association between genetic variants in the FTO gene, we selected 17 tag SNPs that reflected the haploblock structure of the gene. Of these SNPs, none of those localized in intron 1 was associated with mortality. However, in the first cohort enrolled in this study (CREED-1 cohort), we identified a new polymorphism, the rs708259 on intron 8 of the FTO gene, which was strongly associated with death in dialysis patients. When we extended the analysis to two replication cohorts, the North Apulian and the CREED-2 cohort, which included dialysis patients comparable for the main demographic and clinical characteristics with the first cohort, we observed again an excess risk of death (+23 and +21%, respectively) in A-allele carriers. Importantly, such an association was also confirmed in a third independent cohort of stage 2–5 CKD patients. Although the associations between the rs708259 polymorphism and mortality did not attain any formal statistical significance in the three confirmatory cohorts, a meta-analysis of the four cohorts showed that individuals harbouring the risk allele A of the FTO rs708259 polymorphism have a highly significant (P = 0.002) 42% excess risk of death when compared with individuals without such an allele.

To the best our knowledge, this is the first study investigating the role of the FTO gene in mortality in pre-dialysis and dialysis patients. The FTO rs708259 polymorphism in intron 8 is not in linkage disequilibrium with any of the SNPs in intron 1 of the gene. While the association between the FTO rs708259 polymorphism and mortality in pre-dialysis and dialysis patients in our data is statistically robust, the functional significance of this SNP is unknown. Because the FTO rs708259 polymorphism is unrelated to the BMI and diabetes, it seems unlikely that the FTO gene affects survival through its impact on the mechanisms regulating energy balance or glucose metabolism in this population. We speculate that this polymorphism may exert functional effects by modifying the bioavailability of the transcript and/or the protein product of FTO. Alternatively, this polymorphism may

enhance the risk of mortality via DNA methylation. Functional studies in appropriate models are needed to mechanistically interpret the association between the FTO rs708259 polymorphism and mortality in the CKD population.

In conclusion, our findings generate the hypothesis that CKD patients of various severity with the A allele of the FTO SNP rs708259 polymorphism may have a higher risk of death than those without such an allele. Functional studies will define the mechanism(s) whereby this polymorphism impacts upon survival in this population.

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