CANDIDATE GENE ANALYSIS OF MORTALITY IN DIALYSIS PATIENTS

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ABSTRACT

Background

Dialysis patients have high cardiovascular mortality risk. This study aimed to investigate the association between SNPs of genes involved in vascular processes and mortality in dialysis patients.

Methods

Forty two SNPs in 25 genes involved in endothelial function, vascular remodeling, cell proliferation, inflammation, coagulation and calcium/phosphate metabolism were genotyped in 1330 incident dialysis patients. The effect of SNPs on 5-years cardiovascular and non-cardiovascular mortality was investigated.

Results

The mortality rate was 114/1000 person-years and 49.4% of total mortality was cardiovascular. After correction for multiple testing, *VEGF rs699947* was associated with all-cause mortality (HR1.48, 95% CI 1.14-1.92). The other SNPs were not associated with mortality.

Conclusions

This study provides further evidence that a SNP in the *VEGF* gene may contribute to the comorbid conditions of dialysis patients. Future studies should unravel the underlying mechanisms responsible for the increase in mortality in these patients.

INTRODUCTION

Patients with end stage renal disease (ESRD) have a very high mortality risk as compared with the general population. Cardiovascular disease is a major cause of death in these patients, accounting for 40-50% of total mortality[1,2]. Recently, a large study showed that patients on chronic dialysis had an 8.8-times increased cardiovascular mortality risk as compared with the general population[3]. In addition to cardiovascular disease, declined kidney function and chronic kidney disease (CKD) are associated with increased hospitalization[4], infection[3,5,6], malignancies[7–9] and frailty[10] resulting in an 8.1-fold increased risk of non-cardiovascular mortality[3]. The latter illustrates the very high risk of both cardiovascular and non-cardiovascular death in these patients[3,3,6,11].

These increased mortality rates in ESRD patients are only in part explained by traditional risk factors, suggesting a role for CKD-related factors. CKD specific risk factors include chronic inflammatory state[12] and altered levels of circulating growth factors[13], the presence of uremic toxins[14], disturbed calcium/phosphate metabolism and coagulation[15] as well as endothelial dysfunction[16]. Alterations in the genetic profile of these processes in ESRD patients may further increase this dysbalance and enhance morbidity and mortality.

Interestingly, single nucleotide polymorphisms (SNPs) that influence the above mentioned processes have already been related to coronary restenosis[17–24] and vascular aneurysm formation[25,26] in the general population and to hemodialysis arteriovenous access failure[27–29] by changing vascular function through processes related to endothelial function and vascular remodeling, growth factors, inflammation, coagulation, and calcium/phosphate metabolism[20,24–31]. Thus far, the association between these SNPs and cardiovascular mortality has not been investigated in dialysis patients. Despite their strong cardiovascular link, these SNPs may not be exclusively related to cardiovascular morbidity and mortality. Indeed, the genes affected by these SNPs mediate a plethora of processes, and thus may also affect non-cardiovascular morbidity and mortality. Therefore, we hypothesized that SNPs involved in processes related to endothelial function, vascular remodeling, cell proliferation, inflammation, coagulation, and calcium/phosphate metabolism could influence cardiovascular and non-cardiovascular mortality in patients on dialysis. This study was performed in a large population of incident dialysis patients.

SUBJECTS AND METHODS

Patients

The Netherlands Cooperative Study on the Adequacy of Dialysis (NECOSAD) is a prospective multicenter cohort study in which incident adult ESRD patients from 38 dialysis centers in the Netherlands were included[32]. The study was performed in accordance with the Declaration of Helsinki. The Medical Review Ethics Committee of the Leiden University Medical Center approved the study. All patients gave written informed consent. Adult patients that did not receive any prior renal replacement therapy were eligible. Patients were followed from January 1997 until death or censoring, i.e. transfer to a nonparticipating dialysis center, withdrawal from the study, transplantation or

end of the follow-up period (June 2009). Data on dialysis modality, age, sex, and primary kidney disease were collected at the start of dialysis treatment. Primary kidney disease was classified according to the codes of the European Renal Association-European Dialysis and Transplant Association (ERA-EDTA)[33]. Patients were grouped into four classes of primary kidney disease: glomerulonephritis, diabetes mellitus, renal vascular disease and other kidney diseases. Other kidney diseases consisted of patients with interstitial nephritis, polycystic kidney diseases and kidney failure due to multisystem diseases. All-cause mortality was further subdivided in cardiovascular and non-cardiovascular mortality. Cardiovascular death was defined as death due to heart failure, myocardial infarction, ischemic or hemorrhagic stroke, sudden death without obvious non-cardiovascular cause, and death due to other cardiovascular causes. Non-cardiovascular death included all other causes of death.

SNP selection and genotyping

SNPs of interest were selected that could influence mortality risk by changing vascular processes in dialysis patients. Therefore, SNPs previously associated with vascular disease such as coronary restenosis, arteriovenous (AV) access failure and vascular aneurysm formation were selected after a systematic search of literature. Searching MEDLINE using keywords including 'hemodialysis', 'single nucleotide polymorphism', 'arteriovenous access failure', 'coronary restenosis', 'percutaneous coronary intervention' and 'aortic aneurysm' 42 SNPs in 25 candidate genes were identified[17–29,31,34–55]. Only SNPs with a minor allele frequency higher than 1% were included. The complete list of these candidate genes with associated outcomes is described elsewhere[32]. Two multiplex assays were designed using Assay designer software. When a SNP did not fit the multiplex, a proxy of that SNP was selected with the highest R^2 value. The final set included 42 SNPs in 25 genes related to growth factors[18,24,26,34–36,38–42] (Supplementary Table 1), inflammation[20,21,23,28,43–46] (Supplementary Table 2), endothelial function and vascular remodeling[17,27,55] (Supplementary Table 3), calcium/phosphate metabolism[22,24,29,47,48] (Supplementary Table 4) and coagulation[24,31,50,51,53,54] (Supplementary Table 5). All SNPs were genotyped by MALDI-TOF mass spectrometry, using the MassARRAY^{tm} methodology (Sequenom Inc., San Diego, CA, USA), following manufacturer's instructions. As quality control, 5% of the samples were genotyped in duplicate. No inconsistencies were observed. All the negative controls (2%) were negative.

Statistical analysis

Continuous variables are presented as median and interquartile range (IQR). Categorical variables are presented as number with percentages. Minor allele frequencies were calculated and a chi-squared test with 1 degree freedom was used to determine if observed and expected genotypes were in Hardy Weinberg equilibrium (HWE), using a p-value cut-off of <0.01, to reduce the likelihood of false positivity[32]. The hazard ratios (HRs) with 95% confidence intervals (95% CIs) were calculated using Cox regression analysis for heterozygote genotypes and homozygous mutant genotypes as compared with wild-type

genotypes for five-year mortality for the 42 SNPs. All analyses were performed using SPSS statistical software version 20.0 (SPSS, Chicago, Ill, USA). To adjust for multiple testing, the false discovery rate (FDR) was calculated using the method of Benjamini and Hochberg[56]. Although no universal FDR significance threshold has been defined, a cut-point of 0.20 has been suggested for candidate gene association studies, meaning that one should expect at most 20% of declared discoveries to be false[57]. Therefore a cutoff-point of 0.20 was chosen which resulted in a corrected level of significance of p=0.0048 instead of p=0.05.

RESULTS

A total of 1330 dialysis patients were genotyped for the 42 SNPs. Baseline characteristics of the patients are shown in Table 1. The median age was 62.2 years, 39.0% was female, and 14.3% had diabetes mellitus as their primary kidney disease. In addition, approximately 8% of the patients had diabetes as co-morbidity. Of the 1330 patients, 474 (35.6%) died within five years of dialysis treatment. The overall mortality rate was 114 per 1000 person-years. Cardiovascular mortality accounted for 234 of these deaths (49.4%), whereas 50.6% of total mortality was due to non-cardiovascular causes (Table 2).

Minor allele frequencies and HWE p-values are summarized in Supplementary Table 6. Three SNPs were not in equilibrium: *vitamin D receptor (VDR) rs4516035*, *interleukin-10 rs1800896* and *TGF-β receptor1 rs1626340*. Notably, none of these SNPs were significantly associated with mortality after correction for multiple testing.

SNPs and mortality

In total, 42 SNPs in 25 genes involved in vascular processes (endothelial function and vascular remodeling, growth factors, inflammation, coagulation, and calcium/phosphate metabolism) were genotyped. Without correction for multiple testing, three SNPs were associated with cardiovascular mortality. *Vascular endothelial growth factor (VEGF) rs2010963* (HR0.62; 95% CI 0.38-1.00) and *tumor necrosis factor rs1799964* (HR0.27; 95% CI 0.10-0.73) were associated with a decreased cardiovascular mortality, while *VEGF rs699947* (HR1.52; 95% CI 1.07-2.17) resulted in an increased risk. In addition,

Table 1. Baseline characteristics

		N=1330
Age (years, IQR)	62.2	(50.0-71.8)
Sex, female (n, %)	515	(39.0%)
Race, white (n, %)	1130	(91.4%)
Dialysis modality, hemodialysis (n, %)	812	(64.2%)
Primary kidney disease (n, %)		
Diabetes mellitus	189	(14.3%)
Glomerulonephritis	149	(11.3%)
Renal vascular disease	206	(15.6%)
Others	776	(58.8%)

Table 2. Cardiovascular and non-cardiovascular mortality

	N	%	
Cardiovascular	Myocardial infarction	41	8.7
	Heart failure	29	6.1
	Cerebrovascular accident	28	3.8
	Sudden death	41	8.7
	Other	105	22,1
	Total cardiovascular	234	49.4
Non-Cardiovascular	Infection	56	11.8
	Withdrawal	33	7.0
	Suicide/refusal treatment	59	12.4
	Malignancy	31	6.5
	Other non-cardiovascular	61	12.9
	Total non-cardiovascular	240	50.6

without correction for multiple testing, *matrix metalloproteinase*-1 *rs11292517* (HR0.67; 95% CI0.46-0.99) and *VDR rs2238135* (HR0.33; 95% CI0.13-0.80) were associated with decreased risk of non-cardiovascular mortality, while *rs9804922* (HR3.14; 95% CI1.17-8.46) in an intergenic region on *12q23.2*, *CD180 rs5744478* (HR3.25; 95% CI1.34-7.91) and *interleukin-6 rs1800795* (HR1.52; 95% CI1.02-2.25) were associated with an increased non-cardiovascular mortality risk.

However, after correction for multiple testing, *VEGF rs699947* only remained significantly associated with all-cause mortality (HR1.48, 95% CI 1.14-1.92, p=0.003*)*. Kaplan Meier curves for *VEGF rs699947* are depicted in Fig 1. The results of all other SNPs are summarized in Supplementary Tables 1-5.

DISCUSSION

Although it is widely recognized that patients on dialysis have substantially higher cardiovascular and non-cardiovascular mortality rates compared with the general population, little is known about the genetic predisposition to mortality of these vulnerable patients. In the present study, we investigated the association between mortality of chronic dialysis patients and 42 SNPs in 25 genes that have previously been linked to cardiovascular disease. We showed that, after correction for multiple testing, *VEGF rs699947* was associated with an increased all-cause mortality risk. This emphasizes that this SNP is not exclusively associated with cardiovascular mortality, but also influences non-cardiovascular mortality. In concordance with previous studies[3,6,58], we observed that the burden of cardiovascular mortality was comparable with non-cardiovascular mortality in our cohort.

The *VEGFA* gene is located on chromosome 6 and is composed of a 14kb coding region with 8 exons and 7 introns[59]. *VEGF rs699947* is situated in the promoter region and can thereby influence VEGF expression levels. Although we did not measure VEGF levels in our study, the effect of the *rs699947* SNP in the *VEGF*

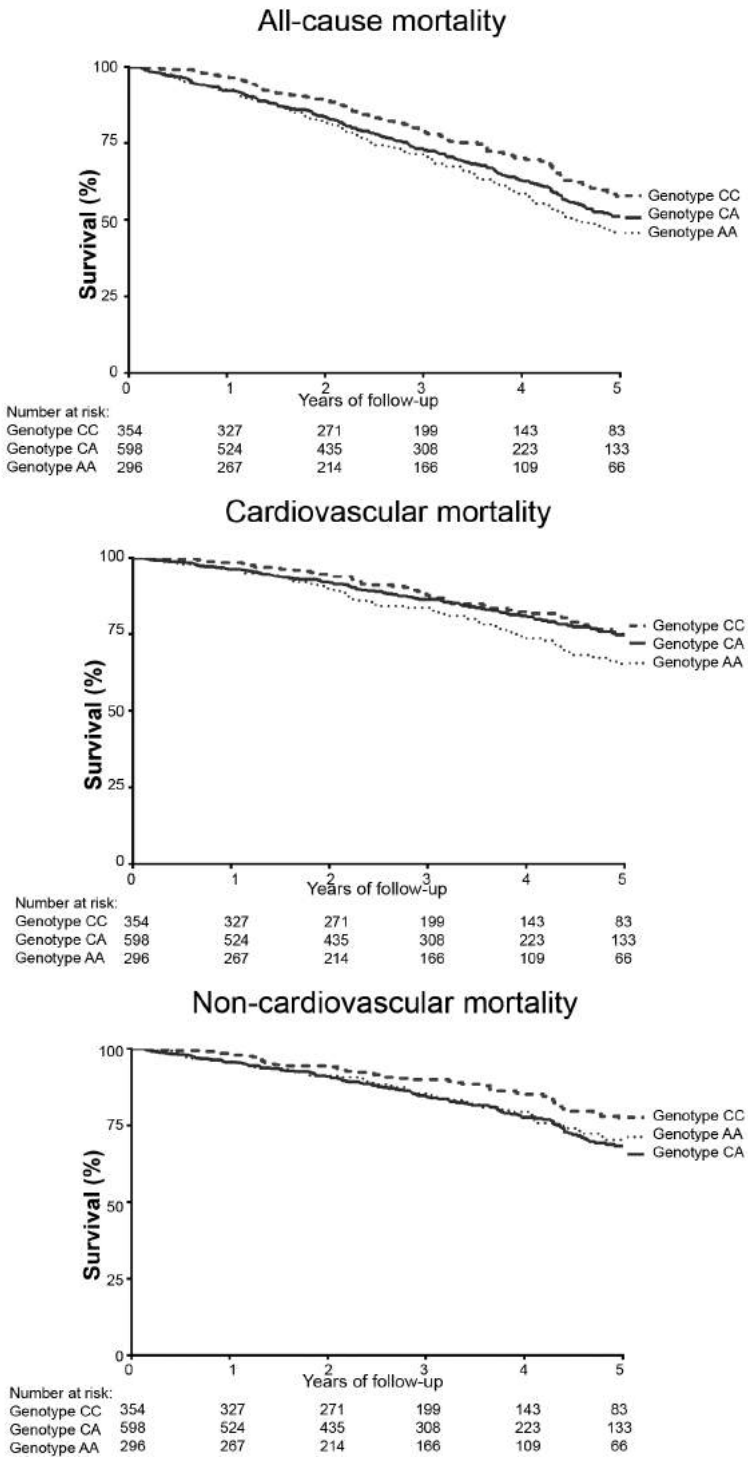

Fig. 1. Kaplan Meier survival curve for all-cause, cardiovascular and non-cardiovascular mortality for *VEGF rs699947*.

gene on VEGF protein levels is reported in other studies. Indeed, carriers of the mutant *A*-allele of *rs699947* on peritoneal dialysis have reduced serum VEGF levels[41]. Additional support for a detrimental effect of *rs699947* SNP of the *VEGF* gene comes from *in vitro* studies which revealed that peripheral blood mononuclear cells isolated from healthy controls produced significantly more VEGF when compared to mononuclear cells from subjects with the *AA* genotype[60]. In our study the mutant *AA* genotype was associated with all-cause mortality, suggesting this can be attributed to both cardiovascular and non-cardiovascular causes. VEGF is involved in angiogenesis, arteriogenesis, vascular permeability, and endothelial cell migration and proliferation[61]. As such, VEGF plays a pivotal role in cardiovascular homeostasis and dysregulation can result in cardiovascular disease. VEGF mediated angiogenesis is important in hypoxic situations such as myocardial infarction since adequate vascular collaterals can preserve the myocardium during ischemia[62] and decrease cardiovascular events[63]. Indeed, carriers of the *AA* genotype of *rs699947*, associated with low levels of VEGF, were shown to have an increased risk of developing coronary artery atherosclerosis[64,65]. In addition to cardiovascular disease, the *rs699947* SNP has also been associated to non-cardiovascular disease and mortality. *AA* carriers were shown to have an increased risk for thyroid cancer[66] and prostate cancer[67]. Moreover, patients with non-small cell lung cancer and *AA* genotype had poorer survival[68]. In addition to malignancies, *VEGF rs699947* is also associated to other non-cardiovascular pathophysiology. Despite low systemic levels of VEGF, patients on peritoneal dialysis with the *AA* genotype expressed high mRNA VEGF levels in their peritoneal dialysis effluent as compared to the *CC* genotype, which was associated with progressive increase in peritoneal transport and even increased mortality[41].

Additional support for the detrimental effects of low VEGF levels comes from non-genetic studies, which revealed that reduced VEGF levels are associated with renal podocyte loss in diabetic nephropathy and progression of renal disease[69]. In addition, selective inhibition of VEGF with bevacuzimab, a monoclonal antibody against VEGF used in oncology, can induce hypertension and proteinuria[70]. Furthermore, females with were shown to have elevated levels of soluble VEGF receptor-1, an endogenous VEGF antagonist [71].

Next to reduced levels of VEGF, very high VEGF levels have been reported to be detrimental as well. Indeed, previous studies demonstrated that highly elevated VEGF levels increase all-cause mortality risk in ESRD patients[13,72]. Besides its pro-angiogenic actions, VEGF can exert pro-inflammatory effects[72] by enhancing vascular permeability and inducing leukocyte adhesion molecules[73]. These data suggest that a dysbalance in VEGF levels, either decreased or largely increased, may potentially be pathogenic.

Our study has several potential limitations. The collective term cardiovascular disease comprises a plethora of disorders elicited by even more underlying processes. We investigated SNPs involved in endothelial function, vascular remodeling, cell proliferation, inflammation, coagulation and calcium/phosphate metabolism, as these processes play an important role in cardiovascular disease. Importantly, these processes are affected in CKD, and alterations in their genetic profile may further increase this dysbalance and enhance

morbidity and mortality. Nonetheless, more mechanisms are involved in the broad scope of cardiovascular disease and the selection of SNPs in this article is not exhaustive. For example, polymorphisms in iron metabolism and vascular calcification are missing, while these could be relevant in a dialysis population. Future studies could elaborate on the current selection. In addition, we primarily selected the SNPs on their cardiovascular interactions. Because the genes affected by these SNPs also exert important non-cardiovascular effects and dialysis patients also suffer from a large burden of non-cardiovascular mortality[3], we did not want to neglect this and also investigated their influence on non-cardiovascular mortality. However, other SNPs that may contribute to non-cardiovascular mortality were not appraised in this study. Considering the increasing attention to non-cardiovascular mortality in ESRD patients[74], future studies should investigate the effects of other SNPs primarily influencing non-cardiovascular disease.

Secondly, despite the large size of our cohort, in some SNPs there was a very small sample size in especially the variant genotypes. These groups are likely insufficiently powered to detect an association and this may lead to underestimation of the actual effect of the SNPs.

Furthermore, we did not measure plasma VEGF levels in this study. However, previous studies convincingly demonstrated decreased VEGF levels in the *rs699947 AA* genotype in both healthy individuals[60] and dialysis patients[41], thereby providing support for our assumption that the increased mortality as observed in dialysis patients carrying the *VEGF rs699947* SNP, might be explained by decreased serum VEGF levels.

In conclusion, this study provides evidence that *VEGF rs699947 AA* genotype is associated with all-cause mortality in a large cohort of dialysis patients, whereas there was no significant association with the other 41 SNPs. These results may help clarify the involved pathways in the increased mortality of these patients. Further studies should investigate the underlying mechanisms in order to develop new therapies aimed to reduce the dramatic mortality rates in dialysis patients. In addition, stratification of patients with genetic risk factors combined with clinical risk factors could be used to predict mortality for specific subgroups of dialysis patients and may facilitate tailored therapies.

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SUPPLEMENTARY TABLES

Suppl. Table 1. Polymorphisms in growth factor related genes and effect on five-years mortality

					All-Cause			Non-Cardiovascular			Cardiovascular		
Gene	Name	SNP	GT	N	HR (95% CI)		P	HR (95% CI)		P	HR (95% CI)		P
CDKN1B (p27kip1)	Cyclin-dependent kinase inhibitor 1B	rs36228499	CC	400	1	Ref		1	Ref		1	Ref	
			CA	623	1.06	0.86-1.30	0.61	1.20	0.89-1.62	0.24	0.93	0.69-1.25	0.63
			AA	242	1.02	0.78-1.34	0.88	1.09	0.74-1.60	0.68	0.97	0.66-1.41	0.86
CTGF	Connective Tissue Growth Factor	rs6918698	CC	377	1	Ref		1	Ref		1	Ref	
			CG	598	0.92	0.74-1.15	0.47	1.01	0.74-1.36	0.99	0.84	0.61-1.16	0.29
			GG	284	1.22	0.95-1.56	0.11	1.09	0.76-1.57	0.63	1.35	0.96-1.90	0.09
FGFR4	Fibroblast Growth Factor Receptor 4	rs351855	GG	573	1	Ref		1	Ref		1	Ref	
			GA	495	1.09	0.89-1.33	0.41	1.12	0.84-1.49	0.46	1.06	0.80-1.41	0.76
			AA	142	1.07	0.79-1.45	0.68	1.28	0.85-1.94	0.23	0.87	0.55-1.38	0.55
KLF5	Kruppel-like Factor 5	rs3812852	AA	1088	1	Ref		1	Ref		1	Ref	
			AG	179	1.00	0.77-1.30	0.99	1.02	0.71-1.48	0.91	1.00	0.67-1.43	0.90
			GG	5	NE			NE			NE		
PDGFD	Platelet Derived Growth Factor D	rs974819	CC	601	1	Ref		1	Ref		1	Ref	
			CT	534	0.77	0.64-1.94	0.009	0.84	0.64-1.10	0.20	0.71	0.53-0.94	0.02
			TT	132	0.74	0.53-1.03	0.070	0.65	0.40-1.07	0.09	0.82	0.52-1.28	0.37
PDGFD	Platelet Derived Growth Factor D	rs496339	AA	1019	1	Ref		1	Ref		1	Ref	
			AG	228	1.25	0.99-1.57	0.06	1.24	0.90-1.71	0.20	1.26	0.91-1.74	0.17
			GG	13	0.86	0.32-2.30	0.76	1.27	0.41-3.97	0.68	0.44	0.06-3.12	0.41
TGFBR1	Transforming Growth Factor β Receptor 1	rs1626340	GG	781	1	Ref		1	Ref		1	Ref	
			GA	368	0.97	0.79-1.19	0.76	0.95	0.71-1.27	0.73	0.99	0.74-1.32	0.93
			AA	67	0.64	0.40-1.02	0.006	0.81	0.45-1.46	0.48	0.47	0.22-1.01	0.054
TGFBR2	Transforming Growth Factor β Receptor 2	rs1036095	GG	716	1	Ref		1	Ref		1	Ref	
			GC	468	0.92	0.75-1.11	0.38	1.02	0.78-1.34	0.86	0.82	0.62-1.08	0.16

Suppl. Table 1. (continued)

					All-Cause			Non-Cardiovascular			Cardiovascular		
Gene	Name	SNP	GT	N	HR (95% CI)		P	HR (95% CI)		P	HR (95% CI)		P
			CC	77	0.85	0.56-1.29	0.44	1.11	0.65-1.89	0.71	0.61	0.31-1.20	0.15
TGFBR2	Transforming Growth Factor β Receptor 2	rs4522809	AA	381	1	Ref		1	Ref		1	Ref	
			AG	633	0.95	0.77-1.18	0.65	1.11	0.82-1.50	0.49	0.82	0.61-1.10	0.18
			GG	245	1.00	0.77-1.31	0.98	1.07	0.73-1.57	0.72	0.94	0.65-1.36	0.75
VEGF	Vascular Endothelial Growth Factor	rs2010963	GG	566	1	Ref		1	Ref		1	Ref	
			GC	538	0.87	0.72-1.06	0.17	0.83	0.63-1.09	0.17	0.92	0.70-1.21	0.56
			CC	157	0.75	0.55-1.02	0.06	0.87	0.58-1.31	0.50	0.62	0.38-1.00	0.05
VEGF	Vascular Endothelial Growth Factor	rs3025039	CC	958	1	Ref		1	Ref		1	Ref	
			CT	291	0.91	0.72-1.13	0.38	0.82	0.59-1.13	0.22	1.00	0.74-1.36	1.00
			TT	25	1.32	0.70-2.47	0.39	1.02	0.38-2.73	0.98	1.64	0.73-3.70	0.23
VEGF	Vascular Endothelial Growth Factor	rs699947	CC	354	1	Ref		1	Ref		1	Ref	
			CA	598	1.29	1.02-1.63	0.03	1.51	1.08-2.10	0.02	1.10	0.79-1.53	0.50
			AA	296	1.48	1.14-1.92	0.003	1.43	0.98-2.09	0.07	1.52	1.07-2.17	0.02

GT, genotype; SNP, single nucleotide polymorphism; N, number of subjects; HR, hazard ratio; CI confidence interval; NE, not estimable.

Suppl. Table 2. Polymorphisms related to inflammatory genes and effect on five-years mortality

					All-Cause			Non-Cardiovascular			Cardiovascular		
Gene	Name	SNP	GT	N	HR (95% CI)		P	HR (95% CI)		P	HR (95% CI)		P
CD180 (RP105)	CD180	rs5744478	TT	1084	1	Ref		1	Ref		1	Ref	
			TC	179	1.30	1.02-1.66	0.037	1.32	0.93-1.86	0.12	1.28	0.91-1.82	0.16
			CC	11	2.27	1.07-4.79	0.032	3.25	1.34-7.91	0.009	1.29	0.32-5.19	0.72
IL6	Interleukin 6	rs1800795	GG	504	1	Ref		1	Ref		1	Ref	
	CD180 (RP105)		GC	585	1.17	0.96-1.43	0.13	1.41	1.06-1.89	0.019	0.97	0.73-1.28	0.81
			CC	183	1.32	1.00-1.74	0.051	1.52	1.02-2.25	0.04	1.15	0.78-1.71	0.48
IL10	Interleukin 10	rs1800896	AA	367	1	Ref		1	Ref		1	Ref	
			AG	384	1.06	0.85-1.31	0.61	1.19	0.87-1.61	0.28	0.94	0.69-1.28	0.70
			GG	306	0.97	0.75-1.25	0.79	1.01	0.70-1.46	0.96	0.92	0.65-1.32	0.66
IL10	Interleukin 10	rs3024498	AA	682	1	Ref		1	Ref		1	Ref	
			AG	478	0.98	0.81-1.19	0.86	1.11	0.85-1.45	0.44	0.86	0.65-1.14	0.30
			GG	103	0.88	0.62-1.27	0.50	0.69	0.39-1.22	0.20	1.07	0.68-1.70	0.76
TNF	Tumor Necrosis Factor	rs1799964	TT	740	1	Ref		1	Ref		1	Ref	
			TC	449	1.09	0.90-1.32	0.36	1.19	0.91-1.56	0.21	1.00	0.78-1.32	0.96
			CC	73	0.60	0.37-0.98	0.04	0.96	0.55-1.71	0.90	0.27	0.10-0.73	0.01
TNF	Tumor Necrosis Factor	rs1800629	GG	855	1	Ref		1	Ref		1	Ref	
			GA	362	1.34	1.10-1.64	0.003	1.45	1.10-1.91	0.008	1.24	0.94-1.65	0.13
			AA	46	1.24	0.76-2.02	0.394	1.63	0.88-3.01	0.12	0.86	0.38-1.94	0.71
TNF	Tumor Necrosis Factor	rs361525	GG	1144	1	Ref		1	Ref		1	Ref	
			GA	113	0.90	0.65-1.26	0.55	0.93	0.58-1.48	0.75	0.88	0.54-1.42	0.60
			AA	6	1.70	0.64-4.55	0.29	2.55	0.82-7.99	0.11	0.85	0.12-6.06	0.87
TLR4	Toll-Like Receptor 4	rs4986790	AA	1101	1	Ref		1	Ref		1	Ref	
			AG	166	1.15	0.88-1.50	0.30	0.91	0.60-1.37	0.64	1.42	0.99-2.01	0.054
			GG	6	1.65	0.41-6.62	0.48	NE			3.50	0.87-14.10	0.07

GT, genotype; SNP, single nucleotide polymorphism; N, number of subjects; HR, hazard ratio; CI confidence interval; NE, not estimable.

Suppl. Table 3. Polymorphisms related to endothelial function and vascular remodeling and effect on five-years mortality

					All-Cause			Non-Cardiovascular			Cardiovascular		
Gene	Name	SNP	GT	N	HR (95% CI)		P	HR (95% CI)		P	HR (95% CI)		P
MMP1	Matrixmetalloproteinase 1	rs11292517	1G/1G	252	1	Ref		1	Ref		1	Ref	
			1G/2G	608	0.96	0.77-1.19	0.68	0.91	0.68-1.22	0.53	1	0.74-1.38	0.95
			2G/2G	276	0.86	0.66-1.12	0.26	0.67	0.46-0.99	0.045	1.07	0.75-1.55	0.70
NOS3	Nitric Oxide Synthase 3	rs1799983	GG	624	1	Ref		1	Ref		1	Ref	
			GA	525	0.97	0.80-1.18	0.77	0.89	0.68-1.17	0.40	1.06	0.81-1.40	0.66
			AA	114	0.95	0.67-1.34	0.76	0.91	0.56-1.47	0.69	1.00	0.61-1.62	0.99
ELN	Elastin	rs2071307	GG	486	1	Ref		1	Ref		1	Ref	
			GA	595	1.31	1.08-1.60	0.008	1.45	1.10-1.93	0.01	1.18	0.89-1.57	0.24
			AA	191	0.95	0.71-1.29	0.76	0.96	0.62-1.48	0.86	0.95	0.63-1.43	0.81
ANXA5	Annexin A5	rs4833229	CC	415	1	Ref		1	Ref		1	Ref	
			CT	608	0.89	0.72-1.09	0.25	0.87	0.65-1.17	0.37	0.90	0.67-1.20	0.46
			TT	238	0.95	0.74-1.23	0.71	1.10	0.78-1.57	0.59	0.81	0.55-1.18	0.27
ANXA5	Annexin A5	rs6830321	GG	377	1	Ref		1	Ref		1	Ref	
			GA	616	0.92	0.75-1.14	0.45	0.92	0.68-1.24	0.58	0.93	0.69-1.25	0.61
			AA	278	1.00	0.67-1.12	0.28	1.00	0.70-1.43	0.99	0.74	0.51-1.08	0.11
LRP1	Low Density Lipoprotein Receptor Related Protein 1	rs1466535	GG	560	1	Ref		1	Ref		1	Ref	
			GA	572	0.94	0.78-1.15	0.56	0.94	0.71-1.24	0.67	0.95	0.72-1.25	0.69
			AA	140	1.10	0.71-1.31	0.79	1.10	0.73-1.66	0.66	0.82	0.52-1.31	0.41
QKI	Quaking	rs3857504	CC	856	1	Ref		1	Ref		1	Ref	
			CT	355	0.98	0.80-1.22	0.91	0.98	0.73-1.32	0.90	1.00	0.74-1.34	0.98
			TT	30	0.90	0.51-1.60	0.72	0.44	0.14-1.38	0.16	1.37	0.70-2.69	0.36
QKI	Quaking	rs3763197	TT	885	1	Ref		1	Ref		1	Ref	
			TC	357	1.02	0.81-1.22	0.96	1.02	0.77-1.36	0.88	0.97	0.72-1.30	0.81
			CC	27	1.12	0.64-1.95	0.69	0.51	0.16-1.59	0.25	1.76	0.93-3.32	0.09
QKI	Quaking	rs2759393	CC	773	1	Ref		1	Ref		1	Ref	
			CA	416	1.03	0.78-1.16	0.62	1.03	0.78-1.37	0.83	0.87	0.65-1.17	0.36
			AA	68	1.03	0.58-1.35	0.57	1.03	0.58-1.81	0.93	0.75	0.40-1.42	0.38

Suppl. Table 3. (continued)

					All-Cause			Non-Cardiovascular			Cardiovascular		
Gene	Name	SNP	GT	N	HR (95% CI)		P	HR (95% CI)		P	HR (95% CI)		P
-	12q23.2	rs10861032	TT	858	1	Ref		1	Ref		1	Ref	
			TT	350	1.11	0.92-1.38	0.25	1.11	0.83-1.48	0.49	1.15	0.86-1.53	0.35
			CC	50	1.20	0.58-1.54	0.82	1.20	0.65-2.22	0.55	0.68	0.30-1.54	0.35
-	12q23.2	rs9804922	CC	1050	1	Ref		1	Ref		1	Ref	
			CT	201	1.11	1.00-1.61	0.05	1.11	0.78-1.58	0.55	1.43	1.03-1.98	0.03
			TT	10	3.14	0.61-4.38	0.33	3.14	1.17-8.46	0.02	NE		

GT, genotype; SNP, single nucleotide polymorphism; N, number of subjects; HR, hazard ratio; CI confidence interval; NE, not estimable.

Suppl. Table 4. Polymorphisms related to calcium/phosphate metabolism and effect on five-years mortality

					All-Cause			Non-Cardiovascular			Cardiovascular		
Gene	Name	SNP	GT	N	HR (95% CI)		P	HR (95% CI)		P	HR (95% CI)		P
Klotho	Klotho	rs9527025	GG	984	1	Ref		1	Ref		1	Ref	
			GC	269	0.82	0.64-1.04	0.10	1.17	0.86-1.58	0.32	0.51	0.34-0.76	0.001
			CC	17	0.41	0.13-1.27	0.12	0.29	0.04-2.08	0.22	0.51	0.13-2.06	0.35
Klotho	Klotho	rs564481	CC	464	1	Ref		1	Ref		1	Ref	
			CT	571	0.95	0.78-1.17	0.64	0.99	0.74-1.33	0.95	0.92	0.69-1.22	0.55
			TT	201	0.98	0.74-1.29	0.88	1.14	0.78-1.65	0.50	0.83	0.55-1.24	0.37
Klotho	Klotho	rs397703*	TT	868	1	Ref		1	Ref		1	Ref	
			TC	343	1.10	0.90-1.36	0.35	1.20	0.90-1.59	0.22	1.01	0.75-1.37	0.94
			CC	52	1.04	0.67-1.62	0.86	0.89	0.45-1.74	0.73	1.20	0.67-2.16	0.55
Klotho	Klotho	rs577912	GG	905	1	Ref		1	Ref		1	Ref	
			GT	310	1.12	0.91-1.38	0.30	0.99	0.73-1.34	0.94	1.26	0.94-1.69	0.12
			TT	40	1.31	0.81-2.10	0.27	1.11	0.54-2.45	0.78	1.52	0.80-2.89	0.20
VDR	Vitamin D Receptor	rs11574027	GG	1232	1	Ref		1	Ref		1	Ref	
			GT	40	0.80	0.44-1.45	0.46	0.57	0.21-1.54	0.27	1.03	0.48-2.18	0.95
			TT	1	NE			NE			NE		
VDR	Vitamin D Receptor	rs2238135	GG	745	1	Ref		1	Ref		1	Ref	
			GC	439	1.01	0.83-1.23	0.94	1.00	0.76-1.32	1.00	1.02	0.77-1.35	0.91
			CC	69	0.58	0.35-0.94	0.03	0.33	0.13-0.80	0.01	0.84	0.47-1.52	0.57
VDR	Vitamin D Receptor	rs4516035	AA	425	1	Ref		1	Ref		1	Ref	
			AG	567	1.01	0.82-1.25	0.91	1.21	0.90-1.64	0.22	0.84	0.62-1.14	0.27
			GG	260	1.09	0.84-1.40	0.52	1.15	0.80-1.67	0.46	1.03	0.73-1.47	0.86
AHSG	α2-HS Glycoprotein (Fetuin A)	rs4918	CC	586	1	Ref		1	Ref		1	Ref	
			CG	544	1.03	0.85-1.25	0.78	1.09	0.83-1.43	0.55	0.97	0.73-1.28	0.83
			GG	118	1.18	0.86-1.62	0.30	1.15	0.73-1.80	0.55	1.21	0.78-1.88	0.39

* rs397703 is a proxy for rs1207568 (R^2=0.70). GT, genotype; SNP, single nucleotide polymorphism; N, number of subjects; HR, hazard ratio; CI confidence interval; NE, not estimable.

Suppl. Table 5. Polymorphisms related to coagulation and effect on five-years mortality

					All-Cause			Non-Cardiovascular			Cardiovascular		
Gene	Name	SNP	GT	N	HR (95% CI)		P	HR (95% CI)		P	HR (95% CI)		P
FBG	Fibronogen β	rs1044291	CC	570	1	Ref		1	Ref		1	Ref	
			CT	542	0.94	0.77-1.14	0.52	1.03	0.78-1.36	0.82	0.85	0.64-1.12	0.24
			TT	147	1.19	0.89-1.58	0.25	1.18	0.78-1.79	0.43	1.19	0.80-1.77	0.40
FBG	Fibronogen β	rs1800787*	CC	789	1	Ref		1	Ref		1	Ref	
			CT	406	1.06	0.87-1.29	0.56	1.07	0.82-1.42	0.61	1.05	0.79-1.39	0.74
			TT	57	1.09	0.69-1.74	0.71	0.80	0.38-1.71	0.57	1.39	0.77-2.51	0.28
ITGB3	Integrin β3	rs17218711†	GG	924	1	Ref		1	Ref		1	Ref	
	(Platelet Glycoprotein IIIa)		GC	312	1.07	0.86-1.32	0.55	1.12	0.83-1.50	0.46	1.02	0.75-1.38	0.92
			CC	28	0.99	0.55-1.81	0.98	0.90	0.37-2.19	0.82	1.09	0.48-2.46	0.84

* rs1800787 was a proxy for rs1800790 (R^2=1.0). † rs1718711 was a proxy for rs5918 (R^2=0.93). GT, genotype; SNP, single nucleotide polymorphism; N, number of subjects; HR, hazard ratio; CI, confidence interval.

Suppl. Table 6. MAFs and HWE p-values

SNP	Variant allele frequency	HWE p-value
rs3763197	0.16	0.194
rs1799983	0.30	0.812
rs2071307	0.38	0.687
rs4833229	0.43	0.563
rs6830321	0.46	0.377
rs1466535	0.33	0.737
rs3857504	0.17	0.339
rs11292517	0.51	0.017
rs2759393	0.22	0.223
rs10861032	0.18	0.061
rs9804922	0.09	0.911
rs36228499	0.44	0.983
rs6918698	0.46	0.112
rs351855	0.32	0.029
rs3812852	0.07	0.408
rs974819	0.31	0.408
rs496339	0.10	0.951
rs1626340	0.21	0.008
rs1036095	0.25	0.964
rs4522809	0.45	0.536
rs2010963	0.34	0.099
rs3025039	0.13	0.598
rs699947	0.48	0.162
rs5744478	0.08	0.236
rs1800795	0.37	0.530
rs1800896	0.47	0.000
rs3024498	0.27	0.139
rs1799964	0.24	0.654
rs1800629	0.18	0.321
rs361525	0.05	0.082
rs4986790	0.07	0.924
rs9527025	0.12	0.775
rs564481	0.39	0.257
rs397703	0.18	0.016
rs577912	0.16	0.037
rs11574027	0.02	0.259
rs2238135	0.23	0.682
rs4516035	0.43	0.006
rs4918	0.31	0.610
rs1044291	0.33	0.296
rs1800787	0.21	0.606
rs17218711	0.15	0.783

MAF, minor allele frequency, HWE p-value, Hardy Weinberg equilibrium χ^2 test p-values. P-value <0.05 suggests a disequilibrium.