

Gender difference and genetic variance in lipoprotein receptor-related protein 1 is associated with mortality

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Abstract. Cardiovascular diseases are an important health resource problem and studies have shown a genetic association between single nucleotide polymorphisms (SNPs) and cardiovascular diseases. According to the literature, lipoprotein receptor-related protein 1 (LRP1) is associated with coronary artery disease. The aim of the present study was to evaluate a possible association between different genotypes of LRP1 and all-cause and cardiovascular mortality from a gender perspective. In the present study, 489 elderly community-living people were invited to participate. Clinical examination, echocardiography and blood sampling including SNP analyses of LRP1 (rs1466535) were performed, including the T/T, C/T and C/C genotypes, and the participants were followed for 6.7 years. During the follow-up period, 116 (24%) all-cause and 75 (15%) cardiovascular deaths were registered. In the female population, the LRP1 of the T/T or C/T genotype exhibited a 5.6-fold increased risk of cardiovascular mortality and a 2.8-fold increased risk of all-cause mortality compared with the C/C genotype. No such genotype differences could be seen in the male population. Gender differences could be seen regarding the risk of mortality in the different genotypes. Females with the LRP1 T/T or C/T genotypes exhibited a significantly increased risk of both all-cause and cardiovascular mortality compared with the C/C genotypes. Therefore, more individualized cardiovascular prevention and treatment should be prioritized. However, since this was a small study, the observations should only be regarded as hypothesis-generating.

Introduction

Cardiovascular diseases represent >30% of all deaths worldwide in 2008 (1). Heart failure alone, one of the consequences of ischemic heart disease, leads to ~2% of the health expenditure in the whole western world; therefore, the economic impact of cardiovascular diseases is huge. Therefore, it is important to identify at-risk patients early in order to optimize treatment and reduce costs. However, little is known regarding the association between genetic polymorphism and cardiovascular mortality risk.

Previous studies have shown a genetic association between single nucleotide polymorphisms (SNPs) and cardiovascular diseases. Special interest has been focused on the low-density lipoprotein receptor-related protein 1 (LRP1). In particular, the SNP rs1799986, of LRP1 is associated with increased rates of premature cardiovascular disease in familial hypercholesterolemia (2) and rs1466535 was identified in a genome-wide association study as a novel polymorphism associated with abdominal aortic aneurysm affecting the gene expression of LRP1 (3). According to Bown *et al* (3) functional analyses have demonstrated that rs1466535 might alter the sterol regulatory element-binding protein 1 binding site and therefore influence the activity at the locus.

Interestingly, the association between LRP1 and platelet-derived growth factor D (PDGF-D) was elucidated by Boucher *et al* (4). They demonstrated that LRP1 forms a complex of the PDGF receptor β and that inactivation of LRP1 causes abnormal activation of PDGF with increased risk of atherosclerosis as a result. Therefore, as PDGF has been shown to be associated with vascular diseases and stroke (5), LRP1 is even more interesting to evaluate. Therefore, the aim of this study was to investigate the possible influence of polymorphisms in LRP1, rs1466535, on all-cause and cardiovascular (CV) mortality in an elderly primary health care population, and to identify possible gender differences as the latter has not been studied before.

Materials and methods

Patient population. The study population consisted of 489 individuals (men: 248; females: 241) with a mean age of 77.0 years (range: 18 years) living in a rural municipality in the south-east of Sweden, who were all part of a longitudinal

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Abbreviations: CI, confidence interval; CV, cardiovascular; ECG, electrocardiogram; EF, ejection fraction; HR, hazard ratio; LRP1, low-density lipoprotein receptor-related protein 1; PDGF, platelet-derived growth factor; SD, standard deviation

Key words: low-density lipoprotein receptor-related protein 1, genotypes, elderly, genders, mortality

epidemiological study focusing on CV risk factors (6). All the participants in that study were invited to participate in the present sub-study conducted from 13th January 2003 through 18th June 2005. The blood samples were collected at the University Hospital of Linköping (Linköping, Sweden). All those living in the municipality within a specific age bracket were invited to participate in the longitudinal project in order to minimize bias in the selection process. The population that agreed to participate donated blood samples and submitted to echocardiographic examinations and an electrocardiogram (ECG). The New York Heart Association functional class was evaluated by the on-site physician based on the patient information.

All participants gave their written informed consent and the study was conducted in accordance with the Declaration of Helsinki principles. The study protocol was approved by the Regional Ethical Review Board of Linköping, Sweden (Dnr 95044). Mortality information was obtained from autopsy reports or from the National Board of Health and Welfare in Sweden, which registers all deaths.

Co-morbidity. In this study the following definitions have been used; hypertension was defined as a blood pressure of >140/90 mmHg measured in the right arm with the patient in a supine position after at least 30 min rest. Hypertension was also assumed if the participant had previously been diagnosed with hypertension and was receiving antihypertensive medication. Diabetes mellitus was defined as a previous diagnosis with on-going treatment, or a fasting blood glucose ≥ 7 mmol/l measured on a single occasion. Ischemic heart disease was defined as a history of angina pectoris/myocardial infarction or ECG-verified myocardial infarction. Heart failure was defined as a previous diagnosis with on-going treatment, or symptoms/signs of heart failure and objective demonstration of reduced cardiac function in terms of impaired cardiac function on echocardiography. CV death was defined as death caused by fatal arrhythmias, myocardial infarction, heart failure, or cerebrovascular insult.

Ultrasound examinations. Echocardiography examinations were performed using an Accuson XP-128c with the patient in a left supine position. Values for systolic function were expressed as left ventricular ejection fraction (EF), and were split into four classes with interclass limits of 30, 40 and 50%. Normal systolic function was defined as EF $\geq 50\%$ (7-9). Thus, only the systolic function was evaluated.

The abdominal aorta was examined through routine ultrasound examination, using an Accuson XP-128c ultrasound machine.

Determination of LRP1 levels in plasma. All blood samples (20 ml) were obtained while the patients were at rest in a supine position and all samples were collected in pre-chilled plastic Vacutainer tubes (Terumo EDTA K-3); however, 2 ml was used for subsequent analysis. Plasma was prepared by centrifugation at 3,000 \times g for 10 min at 4°C. All samples were stored at -70°C until used for analysis. None of the samples were thawed more than twice.

Plasma levels of LRP1 were measured using a commercially available ELISA kit (Cloud-Clone Corporation, Houston, TX,

USA) following the manufacturer's protocol. Plasma samples were diluted 1:2,000.

Genotype determination. Genomic DNA was isolated from peripheral blood using the QIAmp DNA Mini kit (Qiagen GmbH, Hilden, Germany), following the manufacturer's protocol. Genomic determination was performed as described in the literature (10). Briefly, DNA (10 ng) was mixed with TaqMan genotyping master mix coding rs1466535 in LRP1 gene (assay ID: C 7499211 10; Applied Biosystems; Thermo Fisher Scientific, Inc.) and with TaqMan Universal PCR Master mix II. The 7500 Fast Real-Time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.) was used with initial cycle at 50°C for 2 min, followed by one cycle at 95°C for 10 min and 40 cycles at 95°C for 15 sec and at 60°C for 1 min. The manual calling option in the allelic discrimination application ABI PRISM 7500 SDS software, version 1.3.1 (Applied Biosystems; Thermo Fisher Scientific, Inc.) was then used to assign the genotypes.

Statistical methods. Descriptive data are presented as percentages or mean and standard deviation (SD). Comparative analyses were performed using the Student's unpaired two-sided t-test, whereas the Chi-square test was used for discrete variables. Both univariate and multivariate Cox proportional hazard regression analyses were used to analyze and illustrate the risk of mortality during the follow-up period, where both all-cause mortality and CV mortality were analyzed. Kaplan-Meier graphs were used to illustrate CV mortality as a function of follow-up time. Censored patients were those who were still alive at the end of the study period or who had died of causes other than CV disease. Completed patients comprised those who had died due to CV disease. In the multivariate multivariable regression model, adjustments were made for the following co-variables: Age, hypertension, ischemic heart disease, diabetes, atrial fibrillation, Hb <120 g/l, EF <40%, ACE-inhibitors/Angiotensin receptor blockers, beta-blockers, diuretics, s-cholesterol, s-triglycerides, s-low density lipoprotein (LDL) and s-high density lipoproteins (HDL).

In the Cox regressions, the C/T and T/T genotypes were amalgamated because almost identical mortality figures were found during the follow-up with the two groups, and because the group size of the T/T group was small.

P<0.05 was considered to indicate a statistically significant difference. All data were analyzed using standard software packages (Statistica v. 13.1; Statsoft, Inc., Tulsa, OK, USA).

Results

The basic characteristics of the study population divided into gender and genotypes of LRP1 are presented in Table I.

Almost equal numbers of males and females were included (237 vs. 252). In the total study population, 383/489 (78.3%) of the participants had hypertension, 116/489 (23.7%) had diabetes, 115/489 (23.5%) had ischemic heart disease and 134/489 (27.4%) were receiving treatment with ACE-inhibitors or angiotensin receptor blockers. Of the population, 187/489 (38.2%) were receiving treatment with beta-blockers and 175/489 (35.8%) were receiving treatment with diuretics.

Table I. Basal characteristics of the study population divided into the three polymorphisms of LRP1.

Variable	LRP1			P-value
	TT	CT	CC	
n	65	222	202	
Males/females, n	25/40	107/115	105/97	
Age, mean (SD)	77.4 (4.0)	77.3 (3.6)	77.7 (3.9)	
History				
Diabetes, n (%)	13 (20.0)	56 (25.2)	47 (23.3)	0.66
Hypertension, n (%)	49 (75.4)	171 (77.0)	163 (80.7)	0.99
IHD, n (%)	12 (18.5)	52 (23.4)	51 (25.2)	0.53
NYHA I, n (%)	33 (50.8)	112 (50.5)	95 (47.0)	0.75
NYHA II, n (%)	17 (26.2)	67 (30.2)	72 (35.6)	0.27
NYHA III, n (%)	13 (20.0)	41 (18.5)	35 (17.3)	0.88
NYHA IV, n (%)	2 (3.1)	1 (0.5)	0	
Unclassified, n	0	1 (0.5)	0	
Medication				
Beta blockers, n (%)	24 (37)	70 (32)	93 (46)	0.08
ACEI/AII, n (%)	12 (19)	57 (26)	65 (32)	0.07
Diuretics, n (%)	21 (32)	81 (36)	73 (36)	0.81
Examinations				
BP, systolic mm Hg, mean (SD)	151 (20)	148 (24)	150 (19)	0.62
BP, diastolic mmHg, mean (SD)	75 (10)	75 (12)	75 (10)	0.97
IMT, thickness, mm (SD)	0.61 (0.22)	0.54 (0.16)	0.59 (0.18)	0.02
EF <40%, n (%)	5 (8)	14 (6)	20 (10)	0.31

ACEI, ACE inhibitor; AII, Angiotensin receptor blockers; BP, Blood pressure; EF, Ejection fraction; IHD, ischemic heart disease; IMT, intima media thickness; NYHA, New York Heart Association functional class; LRP1, low-density lipoprotein receptor-related protein 1; SD, standard deviation.

The abdominal aorta of in the study population was examined and a systolic dimension between 29.3 to 9.7 mm was found. Based on these measurements there were no signs of abdominal aortic aneurysms in the individuals examined.

When analyzing the two genders, it was found that significantly more females with the C/T genotype were receiving treatment with diuretics compared with the corresponding genotype among males (47.2% vs. 26.3%; $P=0.001$), which is in concurrence with other gender-divided reports (6). In the population with the C/C genotype, a higher systolic blood pressure was recorded among the females compared with the males with the same genotype. Also, it could be demonstrated that there was a larger proportion of participants with an impaired heart function out of the male population with the genotypes C/T and C/C, compared with the females with the corresponding genotypes.

Plasma levels of LRP1. In a small subsection of the study population ($n=221$) the level of LRP1 was evaluated using an ELISA. When evaluating the three genotypes, it could also be seen that the C/C genotype had a reduced level of LRP1 in comparison with the T/T genotype [mean 20,994 (SD 7131) pg/ml vs. 23,409 (SD 5651) pg/ml; $P=0.043$] and the C/T genotype with a level mean 21534 (SD 7,969) pg/ml]. No other differences in levels of LRP1 between clinical or gender groups could be found.

Mortality and genotypes. The median follow-up time of the population was 80 months (6.7 years) and during that time 116 (23.7%) all-cause and 75 (15.3%) CV deaths were registered. Analyzing the three genotypes and the total number of mortalities, significant differences could be detected in the distribution of both all-cause and CV mortality, with a higher mortality in the male population with the C/C genotype (Table II).

In a survival analysis, no significant difference in survival could be demonstrated between the three genotypes in the total study population, nor in the male population (Fig. 1).

However, evaluating the female population revealed significant differences in survival between the three genotypes, where increased all-cause mortality could be seen in the T/T and C/T genotypes ($P<0.05$; Fig. 2).

Analyzing mortality risk during the 6.7 years of follow-up in a multivariate model demonstrated a significantly increased risk both of all-cause mortality (HR 1.68; 95% CI 1.12-2.52; $P=0.01$) and CV mortality (HR 2.07; 95% CI 1.23-3.49; $P=0.006$) of the total study population for those with the T/T or C/T genotype (Table III).

Evaluating the female population with the same multivariate model showed increased risk for all-cause mortality (HR 2.75; 95% CI 1.28-5.90; $P=0.01$) and for CV mortality (HR 5.61; 95% CI 1.98-15.85; $P=0.001$) for those with the T/T

Table II. Cardiovascular and all-cause mortality in the study population distributed in the three genotypes of LRP1, and gender, during a follow-up period of 80 months.

LRP1	CV mortality			All-cause mortality		
	Females	Males	P-value	Females	Males	P-value
C/C, n (%)	14/97 (14.4)	32/105 (30.5)	$X^2=7.38$ $P=0.007$	22/97 (22.7)	47/105 (44.8)	$X^2=10.9$ $P=0.0009$
C/T, n (%)	20/115 (17.4)	21/107 (19.6)	$P=0.67$	35/115 (30.4)	37/107 (34.6)	0.51
T/T, n (%)	6/40 (15.0)	7/25 (28.0)	0.20	9/40 (23.0)	11/25 (44.0)	0.07

As the T/T genotype was small, the two genotype groups T/T and C/T were amalgamated into one group in the further mortality evaluations. LRP1, low-density lipoprotein receptor-related protein 1; CV, cardiovascular.

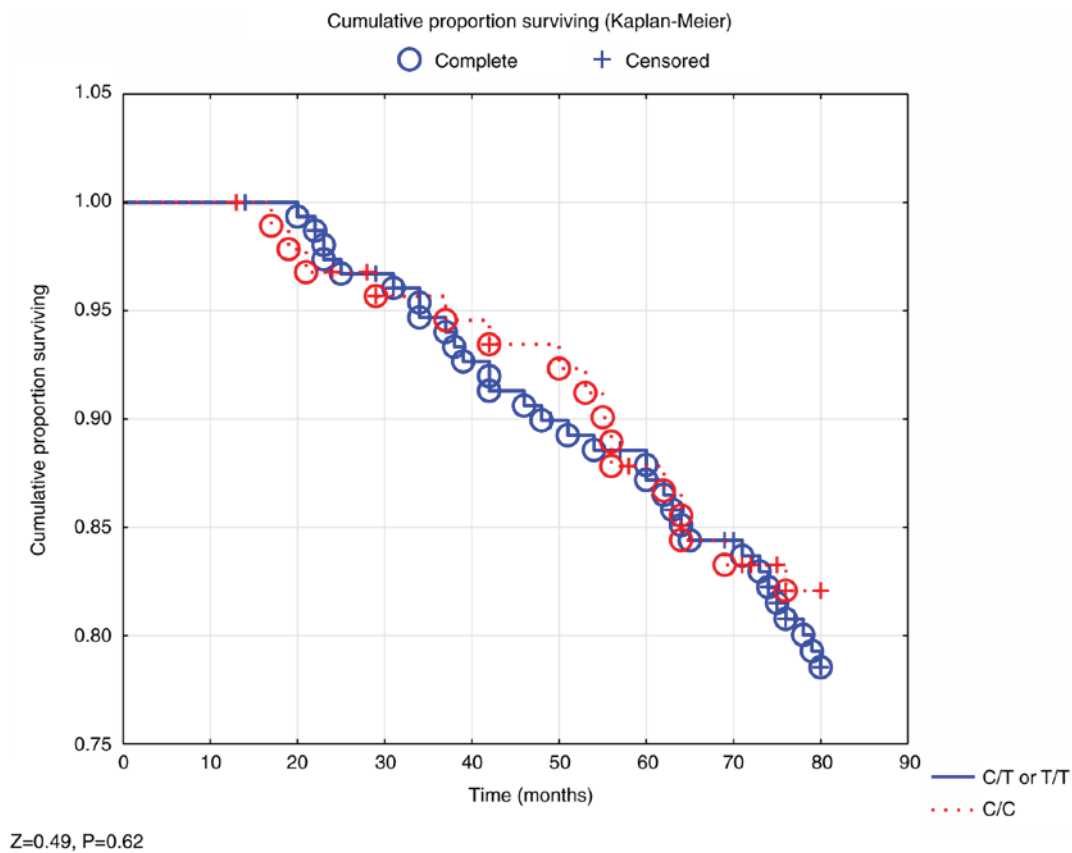


Figure 1. Kaplan-Meier analysis illustrating all-cause mortality in those with the low-density lipoprotein receptor-related protein 1 genotypes T/T or C/T vs. C/C in an elderly male population during a follow-up period of 80 months. Note: Censored participants were those still living at the end of the study period, or who had died for reasons other than cardiovascular disease. Completed participants were those who had died due to cardiovascular disease.

or C/T genotypes, as compared to those with the C/C genotype (Table IV).

No corresponding increased risk could be seen in the male population (for all-cause mortality HR 1.19; 95% CI 0.72-1.97; $P=0.49$; for CV mortality HR 1.39; 95% CI 0.74-2.60; $P=0.31$).

Discussion

In this study three genotypes of LRP1 were evaluated; C/C, C/T and T/T, and different risks of both all-cause, and CV mortality were demonstrated in different genotypes of the female population, but not in males. It should be noted

that the population evaluated in this study was an elderly community-living group and there, a representative one even though the sample size was not large. The results are interesting as they show gender-specific differences both regarding all-cause and to a greater extent CV mortality. The main project has been presented in several previous publications (11-22).

That genetic variance could result in effects on CV-associated mortality is well-known from the literature (23-25).

However, to the best of our knowledge, this is the first time that differences in gender-specific risk in mortality that are associated with a genetic variance have been demonstrated.

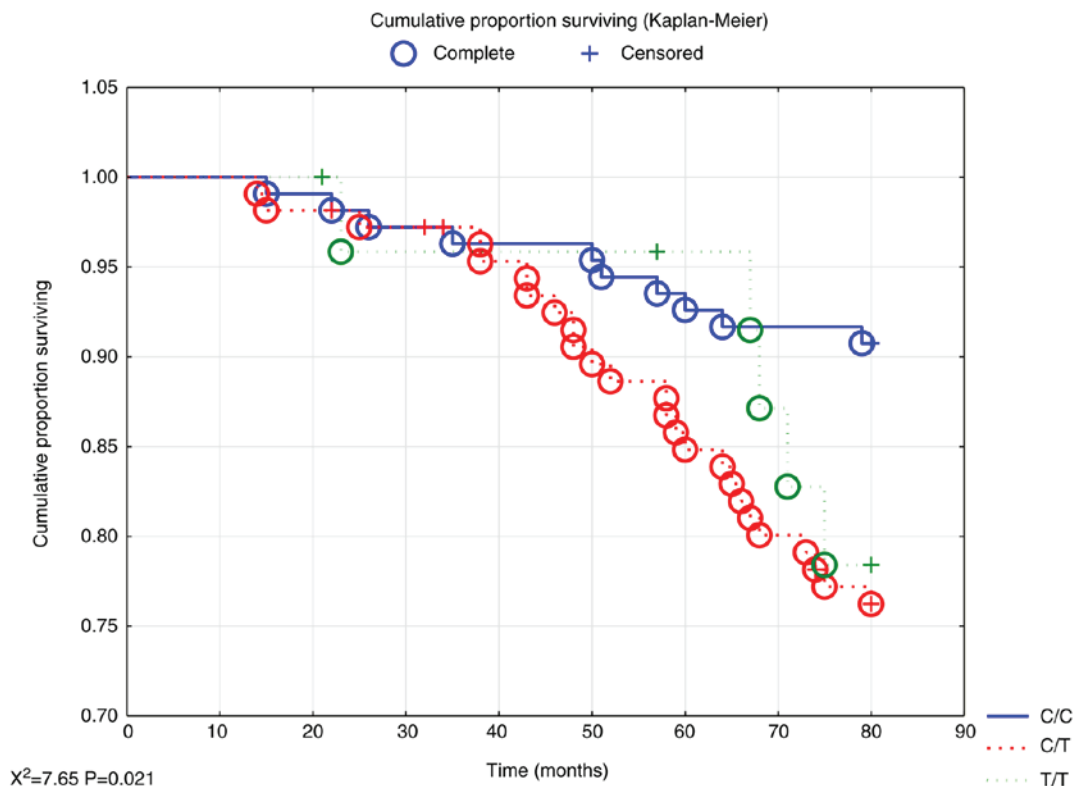


Figure 2. Kaplan-Meier analysis illustrating all-cause mortality in those with the low-density lipoprotein receptor-related protein 1 genotypes T/T or C/T or C/C in an elderly female population during a follow-up period of 80 months. Note: Censored participants were those still living at the end of the study period, or who had died for reasons other than cardiovascular disease. Completed participants were those who had died due to cardiovascular disease.

In recent years, special interest has been focused on LRP1 and its possible association with CV diseases. Gonias (26) demonstrated in an excellent review the important relationship between LRP1 and smooth muscle cell properties. In a report from Italy, Galora *et al* (27) demonstrated an interesting association between the T allele of rs1466535 and abdominal aortic aneurysms, but not with the other alleles investigated. The present study was able to confirm that the T allele also had an association with mortality, but only in one of the two genders. Ye *et al* (28) in a study of abdominal aneurysms, demonstrated significant sex differences, where a faster expansion of the aneurysms could be seen in the female group and therefore the present study intended to evaluate any sex differences in CV mortality in the different genotypes of rs1466535.

As Giusti *et al* (29) reported, the present study also found that the majority of participants had the C/C genotype and only a small number had the T/T genotype. After combining the T/T and the C/T genotypes, Giusti was able to report a greater proportion of patients suffering from carotid stenosis in this group compared with the C/C genotype. The same amalgamating process of the T/T and C/T genotypes in the evaluations was performed in the present study.

In the literature there are reports of a study with a larger number of participants compared to the present study, such as the huge genome-wide association study for coronary artery disease reported by Webb *et al* (30), which included more than 120,000 participants. However, the important difference compared with the present study is that mortality data for all participants is included; no one was lost during the follow-up period of 6.7 years. CV mortality is regarded as the definite

endpoint of the CV risks that the participants were exposed to, rather than getting diagnosed with coronary artery disease, as was done in the genome-wide association study mentioned above, which is why the small study sample reported in the present study is notable.

In the ELISA evaluation of the levels of LRP1, a significant difference was demonstrated in the levels between the T/T and the C/C genotypes, a fact that has not been reported before to the best of our knowledge. In the genome-wide study by Bown *et al* they reported a tendency toward an increased level of the C/C genotype of LRP1 in comparison with the T/T genotype (3). However, Bown *et al* reported on a population with abdominal aortic aneurysms, something that the population of the present study did not have and thus it is not surprising that the opposite results were reported regarding the level of the C/C genotype of LRP1.

The female population of individuals with the T/T or C/T genotypes of LRP1 demonstrated a 2.75-fold increased risk of all-cause mortality and a 5.61-fold increased risk of CV mortality when applying a multivariate model adjusting for certain well-known variables influencing the CV mortality risk. However, it should be pointed out that the confidence interval is wide in the evaluation, so it is important to interpret the point estimate of the HR with caution. The wide confidence interval is probably the result of the limited size of the different genotype groups analyzed. The important message is that an increased mortality risk exists in the C/T and T/T genotypes in comparison with the C/C genotype. In the male population no such prognostic differences between the different genotypes of LRP1 could be found. This could imply that irrespective of

Table III. Multivariate Cox proportional hazard regression illustrating risk of cardiovascular and all-cause mortality in the total population and with a follow-up period of 80 months.

Variables	CV-mortality			All cause mortality		
	Hazard ratio	95% confidence interval	P-value	Hazard ratio	95% confidence interval	P-value
Age	1.07	1.00-1.15	0.04	1.06	1.01-1.12	0.02
Hypertension	1.34	0.75-2.40	0.32	1.61	0.99-2.61	0.06
IHD	1.21	0.70-2.11	0.50	1.34	0.85-2.09	0.21
Atrial fibrillation	2.39	1.29-4.46	0.006	2.27	1.37-3.76	0.001
Diabetes	1.64	0.97-2.77	0.07	1.51	0.98-2.32	0.06
Hb <120 g/l	1.69	0.90-3.17	0.10	1.26	0.71-2.22	0.43
EF <40%	1.72	0.82-3.57	0.15	1.81	1.02-3.23	0.04
ACEI/ARB	0.79	0.46-1.38	0.41	0.75	0.48-1.18	0.21
Beta blockers	1.27	0.76-2.10	0.36	1.09	0.72-1.65	0.67
Diuretics	1.00	0.60-1.69	0.99	1.09	0.72-1.65	0.69
s-Chol	0.59	0.02-22.4	0.78	0.83	0.19-3.54	0.80
s-Triglyc	0.99	0.18-5.36	0.99	0.86	0.40-1.82	0.69
s-LDL	1.73	0.04-66.7	0.77	1.11	0.26-4.74	0.89
s-HDL	1.49	0.04-54.4	0.83	1.08	0.23-5.04	0.92
LRP1 C/T or T/T	2.07	1.23-3.49	0.006	1.68	1.12-2.52	0.01

ACEI/ARB, ACE-inhibitors, or Angiotensin receptor blockers; EF, Ejection fraction based on echocardiography; IHD, ischemic heart disease; s-Chol, s-Cholesterol, mmol/l; s-HDL, s-High Density Lipoproteins, mmol/l; s-LDL, s-Low Density Lipoproteins, mmol/l; s-Triglyc, s-Triglycerides, mmol/l; LRP1, low-density lipoprotein receptor-related protein 1; Hb, hemoglobin.

Table IV. Multivariate Cox proportional hazard regression illustrating risk of cardiovascular and all-cause mortality in the female population and with a follow-up period of 80 months.

Variables	CV-mortality			All cause mortality		
	Hazard ratio	95% confidence interval	P-value	Hazard ratio	95% confidence interval	P-value
Age	0.98	0.88-1.10	0.77	1.04	0.95-1.15	0.38
Hypertension	1.18	0.43-3.23	0.74	1.29	0.56-2.98	0.55
IHD	1.69	0.60-4.77	0.32	1.90	0.86-4.19	0.11
Atrial fibrillation	3.38	0.95-12.00	0.06	2.41	0.89-6.53	0.08
Diabetes	3.23	1.26-8.30	0.01	1.99	0.95-4.14	0.07
Hb <120 g/l	4.12	1.62-10.51	0.03	2.57	1.11-5.94	0.03
EF<40%	2.14	0.41-11.08	0.36	0.90	0.19-4.35	0.90
ACEI/ARB	0.60	0.22-1.65	0.32	1.08	0.50-2.32	0.85
Beta blockers	1.40	0.56-3.48	0.47	1.09	0.53-2.24	0.81
Diuretics	2.04	0.81-5.15	0.13	1.85	0.86-3.98	0.11
s-Chol	1.08	0.19-6.15	0.93	0.89	0.20-4.01	0.88
s-Triglyc	1.17	0.37-3.76	0.79	0.98	0.36-2.68	0.96
s-LDL	0.91	0.16-5.07	0.91	0.93	0.24-4.74	0.93
s-HDL	2.82	0.35-22.91	0.33	1.56	0.26-9.48	0.63
LRP1 C/T or T/T	5.61	1.98-15.85	0.001	2.75	1.28-5.90	0.01

ACEI/ARB, ACE-inhibitors, or Angiotensin receptor blockers; EF, Ejection fraction based on echocardiography; IHD, ischemic heart disease; s-Chol, s-Cholesterol, mmol/l; s-HDL, s-High Density Lipoproteins, mmol/l; s-LDL, s-Low Density Lipoproteins, mmol/l; s-Triglyc, s-Triglycerides, mmol/l; LRP1, low-density lipoprotein receptor-related protein 1; Hb, hemoglobin.

the LRP1 genotype, there are stronger factors that determine the mortality risk in the male group, for example mortality based on ischemic heart disease, a condition with a substantial preponderance in the male population.

In the literature there are a multitude of reports indicating that there are gender differences regarding CV mortality in general and a higher mortality is generally reported in the male population of corresponding age, compared to the female population (31,32). The major reason for this is the higher incidence of ischemic heart disease among males (33). It is possible that the SNP may in some (secondary) way be influenced by variation in genes situated on the sex chromosomes, or by hormonal differences between men and women, and this should be a focus of further study.

The presented gender differences in some of the genotypes of LRP1 are of importance in clinical routine since individuals with increased risk for CV mortality can be identified. Even if it is not possible to modify the genetic constitution, it is possible to provide more intense prevention procedures and an individualized follow-up program for patients, which also may reduce CV mortality and health care costs.

It should also be noted that the genetic analyses exhibited in the present study were conducted at a cost that was below the cost for analyses of the golden-standard biomarker used to identify patients with heart failure, the N-terminal fragment of the natriuretic peptide proBNP. Thus, it was concluded that the presented genetic analyses are also cost-effective in the clinic.

It could be argued that it would only be relevant to evaluate the at-risk genotypes in an at-risk population. However, this assumption is only correct in a population with known or assumed increased risk and this is usually not the case in the clinical routine. Therefore, elderly persons from a community-living population with mixed CV risks were included to better reflect the situation that clinicians are faced with every day. Despite this fact, it was possible to obtain significant and interesting data indicating that certain individuals are exposed to an increased risk that can be identified through genotype evaluation.

Therefore, out of the female group, those with the C/T or T/T genotypes of the LRP1 gene, with a more than 5-fold increased risk of CV mortality, constituted a high-risk population and thus it was important to identify them, even though the obtained point estimate should be interpreted with caution due to the limited sample size.

However, from a clinical and economical point of view it is also interesting to identify those with a low risk of mortality. As the present study demonstrated above, the females with the C/C genotype of LRP1 only exhibited a 9.3% all-cause and a 4.6% CV mortality during almost seven years of follow-up in spite of being an elderly patient group. From this it could be concluded that identifying both those at high as well as those at low risk of death is important from a clinical point of view and the use of genetic information could further aid in this identification process.

The presented research is a community-based study and thus includes most patients with discrete or no CV symptoms. Therefore, those with CV disease are in a minority and thus the size of the CV group is small, which results in wide CIs in risk evaluations, thus making their interpretation uncertain.

Also, the presented study population is an elderly one and therefore it is not possible to extrapolate the obtained results into other age groups without uncertainty.

Finally, as the subgroups of those with certain genotypes are small, the results of some of the evaluations should be interpreted with caution and the results should be regarded as hypothesis-generating. However, the results of the present study are in line with and strengthen previous findings and a hypothesis that the investigated genes and SNPs could play an important role as markers to identify individuals at risk.

In conclusion, information regarding the LRP1 gene was applied to an elderly primary health care population that was followed for almost seven years and where all mortality was registered. Specific gender differences were noted, and in the female group those with the LRP1 T/T or C/T genotype had a 5.61-fold increased risk for CV mortality in the multivariate analysis.

In the male group however, no such gender difference could be found.

Applying a combined analysis using, for the females, those with LRP1 C/T or T/T could substantially increase the possibility of identifying individuals with high CV risk and thereby help to optimize CV prevention and treatment.

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Availability of data and materials

Under Swedish Law, the datasets generated/analyzed are not publicly available but are available from the corresponding author upon reasonable request and with permission of the University of Linköping.

Authors' contributions

Conceived and designed the experiments: UA and DW; performed the experiments: UA and DW; analyzed the data: UA and DW; contributed reagents/material/analysis tools: DW; wrote the manuscript: UA and DW.

Ethics approval and consent to participate

All participants gave their written informed consent and the study was conducted in accordance with the Declaration of Helsinki principles. The study protocol was approved by the Regional Ethical Review Board of Linköping, Sweden (Dnr 95044).

Patient consent for publication

All participants gave their written informed consent.

Competing interests

The authors declare that they have no competing interests.

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