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Sara Mutikainen

Genetic and Environmental Effects on Resting Electrocardiography and the Association between Electrocardiography and Physical Activity, Walking Endurance and Mortality in Older People

JYVÄSKYLÄN YLIOPISTO

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ABSTRACT

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The purpose of this study was, first, to examine genetic and environmental effects on resting electrocardiographic (ECG) variables in older people, and, second, to examine the associations between resting ECG variables and long-term physical activity, walking endurance and all-cause mortality.

Two twin data sets were used, both drawn from the Finnish Twin Cohort. The subjects for the Finnish Twin Study on Aging were recruited on the basis of age and zygosity, and comprised 86 monozygotic and 91 dizygotic female twin pairs, aged 63-76 years. The subjects for the TWINACTIVE study were recruited on the basis of long-term (32 years) discordance for leisure time physical activity and comprised 5 monozygotic and 7 dizygotic female and male twin pairs, aged 50-67 years. Resting ECG recordings were obtained from each subject. Walking endurance was assessed using the six-minute walking test (6MWT) and mortality data were obtained from the official register. The statistical analyses included quantitative genetic modelling, within-pair analyses and individual-based analyses.

Most of the resting ECG variables were moderately to highly (32-72%) affected by genetic factors. The effect of genetic factors was especially strong for T wave amplitudes, left ventricular hypertrophy (LVH) indices and resting heart rate. The remaining variation (28-100%) was explained by environmental factors. The genetic correlation between ECG LVH and the repolarisation phase (T wave amplitudes) was very high (-0.93), suggesting that, to a large extent, the same genes operate in both cases, while the low environmental correlation (-0.05) suggested that mainly distinct environmental factors affect these two traits. Within-pair analyses showed that the principal adaptation to long-term physical activity is lowering of resting heart rate. The active co-twins had 8.8 (95% confidence interval (CI) 1.3-16.4) beats per minute (bpm) lower resting heart rate than their inactive co-twins. In the individual-based analyses, resting ECG variables explained 0-11% of the variation in walking endurance. The best predictors of better walking endurance (longer walking distance in the 6MWT) were high T wave amplitudes and low LVH indices. Resting heart rate had the strongest association with all-cause mortality. Subjects in the highest tertile (≥ 73 bpm) had over twofold risk for death (age-, physical activity- and beta-blocker–adjusted hazard ratio 2.48, 95% CI 0.95-6.44) compared with subjects in the lowest tertile (≤ 62 bpm). Within-pair analyses gave similar results both for walking endurance and all-cause mortality.

The results suggest that both genetic and environmental factors are important in explaining the variation in resting ECG variables in old age. Long-term physical activity may be one of the environmental factors explaining the variation. The main adaptation to long-term physical activity, when genetic liability was controlled for, was lowering of resting heart rate. This is an important adaptation from the public health perspective, since high resting heart rate is associated with increased risk for death. The role of resting heart rate and the other resting ECG variables as predictors of walking endurance, however, is relatively small.

Keywords: resting electrocardiography, genetic effects, environmental effects, leisure time physical activity, walking endurance, all-cause mortality, twin, older people

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LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following papers, which will be referred to by their Roman numerals.

I Mutikainen, S., Ortega-Alonso, A., Alén, M., Kaprio, J., Karjalainen, J., Rantanen, T., Kujala, U.M. 2009. Genetic influences on resting electrocardiographic variables in older women: A twin study. Annals of Noninvasive Electrocardiology 14, 57-64.

II Mutikainen, S., Ortega-Alonso, A., Alén, M., Kaprio, J., Karjalainen, J., Rantanen, T., Kujala, U.M. 2009. Electrocardiographic indices of left ventricular hypertrophy and repolarization phase share the same genetic influences: A twin study. Annals of Noninvasive Electrocardiology 14, 346-354.

III Mutikainen, S., Perhonen, M., Alén, M., Leskinen, T., Karjalainen, J., Rantanen, T., Kaprio, J., Kujala, U.M. 2009. Effects of long-term physical activity on cardiac structure and function: A twin study. Journal of Sports Science and Medicine 8, 533-542.

IV Mutikainen, S., Rantanen, T., Alén, M., Kauppinen, M., Karjalainen, J., Ortega-Alonso, A., Kaprio, J., Kujala, U.M. 2010. Electrocardiographic and other clinical correlates of walking ability in older women. Archives of Gerontology and Geriatrics 51, 216-221.

V Mutikainen, S., Rantanen, T., Alén, M., Karjalainen, J., Kauppinen, M., Kaprio, J., Kujala, U.M. Walking ability and all-cause mortality in older women. International Journal of Sports Medicine, in press.

ABBREVIATIONS

A	Additive genetic effects
AIC	Akaike's information criterion
BMI	Body mass index
bpm	Beats per minute
C	Common environmental effects
CI	Confidence interval
D	Dominance genetic effects
DZ	Dizygotic
E	Unique environmental effects
ECG	Electrocardiographic
FITSA	Finnish Twin Study on Aging
HR	Hazard ratio
ICC	Intra-class correlation coefficient
LTPA	Leisure time physical activity
LV	Left ventricular
LVH	Left ventricular hypertrophy
LVM	Left ventricular mass
MET	Metabolic equivalent
MZ	Monozygotic
QTc	Heart rate-corrected QT interval
RaVL	R wave amplitude in lead aVL
RP	Repolarisation phase
RV_5	R wave amplitude in lead V_5
6MWD	Six-minute walking distance
6MWT	Six-minute walking test
SV_1	S wave amplitude in lead V_1
SV_3	S wave amplitude in lead V_3
XZ	Unknown zygosity

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1 INTRODUCTION

Resting electrocardiography (ECG) is one of the most widely used tools in clinical practice, as there are many situations in which it may provide helpful information. Resting ECG records the heart's electrical activity and this can be used to detect, for example, potentially life-threatening rhythm disturbances, conduction abnormalities or signs of evolving myocardial infarction. Resting ECG findings can also be used to assess prognosis. Additionally, resting ECG is relatively easy and fast to perform and inexpensive compared with many other diagnostic tools such as echocardiography or magnetic resonance imaging.

Considerable variability between individuals may exist in different variables measured by resting ECG. A well-known example is resting heart rate, which usually is about 60 beats per minute (bpm), but may be over 100 bpm in people with chronic diseases while it can lower as low as 30 bpm in highly-trained endurance athletes. Even between individuals in relatively similar situations, such as equally highly-trained endurance athletes, variability may still exist. This variability in resting heart rate and other resting ECG variables may originate both from genetic and environmental factors. Previous twin studies have shown that genetic factors explain a moderate to high proportion of the variation in resting ECG variables (Havlik et al. 1980, Møller et al. 1982, Russell et al. 1998, Busjahn et al. 1999, Carter et al. 2000, Jedrusik et al. 2003, Snieder et al. 2003, Dalageorgou et al. 2008). These studies have mainly been performed in young and middle-aged persons and often in groups combining both men and women. When only men or women have been studied, the age range sampled has been relatively wide, such as from 20 to 80 years. This may be problematic, since estimates of genetic effects are often gender-specific and tend to vary with age (Plomin et al. 2001). To date, rather little is known about the relative contribution of genetic and environmental factors to resting ECG variables in older women, the target population of the present study. Study of this population is important for many reasons. For one, the prevalence of resting ECG abnormalities strongly increase with aging (Furberg et al. 1992, De Bacquer et al. 2000, Larsen et al. 2002, Oberman et al. 2006) and, for another, the proportion of older women will increase in the future along with increased life

expectancy. Accordingly, it is reasonable to assume that the proportion of older women with resting ECG abnormalities will also increase in the future. Understanding the role of genetic and environmental effects on resting ECG variables may therefore be helpful in the prevention of the development of resting ECG abnormalities.

Cardiovascular diseases remain one of the leading causes of death worldwide which sets challenges for health care systems and highlights the importance of preventive procedures. Physical activity or training is a well-documented way to reduce the risk of hypertension (Whelton et al. 2002) and occurrence of coronary heart disease (Batty 2002). The benefits of physical activity in reducing the risk of cardiovascular diseases may in part be mediated through its positive effects on cardiac structure and/or function. The results of previous studies (e.g., Bjørnstad et al. 1991, Stolt et al. 1997, Sharma et al. 1999) have indicated that physical activity or training has effects on resting ECG variables (such as lowering of the resting heart rate or heightening of T wave amplitudes), i.e., it may be one of the environmental factors which explain the variation between individuals in resting ECG variables. Some of the adaptations caused by physical activity may buffer against the resting ECG abnormalities which increase with aging or which have been reported to be associated, for example, with increased risk for death.

However, the majority of the previous studies have been conducted in athletes and sedentary controls, while the number of longitudinal studies is relatively low. The effects of athletic training on resting ECG variables are well-established, but less is known about the long-term effects of leisure time physical activity, especially in non-athletic populations. Research in this area is challenging as, on the one hand, it is difficult to carry out long-term randomized controlled exercise trials, while, on the other hand, observational population-based follow-ups may include genetic selection bias. The latter may also have caused problems in the interpretation of the results of case-control studies comparing athletes and sedentary controls, since resting ECG variables (e.g., Havlik et al. 1980, Busjahn et al. 1999, Carter et al. 2000, Dalageorgou et al. 2008) as well as participation in physical activity (Stubbe et al. 2006) are all moderately to highly affected by genetic factors. This may make it easier for some people to engage in athletic training or other vigorous physical activity, resulting in an apparent but non-causal association of physical activity with resting ECG variables. To minimize such issues, a co-twin control study design can be utilized by carrying out within-pair analyses of resting ECG variables in twin pairs who have been identified on the basis of their long-term discordance for physical activity. By studying both monozygotic (MZ, identical) and dizygotic (DZ, non-identical) twin pairs, genetic liability can be partially (MZ and DZ twin pairs) or fully (MZ twin pairs) controlled for, as MZ co-twins share all and DZ co-twins on average share half of their segregating genes. MZ and DZ co-twins usually share their childhood environment so that using this study design the effects of childhood environment can also be controlled for.

The ability to walk longer distances is a prerequisite for coping with activities of daily living and maintaining independence in old age. An important factor in the endurance performance required in long-distance walking is the functioning of the cardiovascular system. Therefore the disturbances in cardiac function, including the heart's electrical function, may reduce endurance performance and the ability to walk longer distances. One of the advantages of resting ECG recording is its ability to reveal negative asymptomatic changes in cardiac function, also in persons without overt, diagnosed cardiac disease. These asymptomatic changes may include, for example, repolarisation abnormalities, which often are manifested by ST segment depression and/or T wave inversions. T wave inversions may also be signs of myocardial ischemia (Achar et al. 2005). All these abnormalities may reduce walking endurance. Additionally, aging can cause some changes in some resting ECG variables (e.g., T wave changes, left axis deviation) (Yasumura & Shibata 1989) which partially resemble those caused by cardiac diseases, and therefore resting ECG variables may be associated with walking endurance. A previous study (Evers Larsson & Reynisdottir 2008) has shown that resting heart rate predicted walking endurance (measured by the six-minute walking test (6MWT)) in obese subjects. Otherwise knowledge in this area is relatively sparse.

The prognostic significance of the resting ECG variables is well-established. Many previous studies (e.g. Rose et al. 1978, Dyer et al. 1980, Vakili et al. 2001) have shown that resting ECG abnormalities, such as high resting heart rate, inverted T waves and electrocardiographically detected left ventricular hypertrophy (LVH), are associated with increased risk for both cardiovascular and all-cause mortality. These studies have included both men and women with wide age ranges. However, genetic factors and childhood environment may also play some role in this case, hence use of the co-twin control design can be justified in exploring whether these associations are independent of genetic factors and childhood environment.

The purpose of this study was to examine genetic and environmental effects on resting ECG variables in older people. The purpose was also to examine the associations between resting ECG variables and long-term physical activity, walking endurance and all-cause mortality.

2 REVIEW OF THE LITERATURE

2.1 Genetic and environmental effects on resting electrocardiographic variables

2.1.1 Genetic effects on single resting electrocardiographic variables

One of the earliest studies in this field dates from the beginning of the twentieth century, when Wise et al. (1939) compared resting ECG recordings of 32 MZ and 18 DZ twin pairs. They classified the recordings into three categories on the basis of similarity of QRS complex amplitudes: the categories were "close similarity", "some similarity" and "no similarity". After comparison of the categories between MZ and DZ twin pairs they concluded that *"a close degree of similarity is more often to be found in the electrocardiograms of identical twins than in the electrocardiograms of nonidentical twins"*. Since these observations, the methodology for studies of genetic effects on different traits has developed dramatically, and nowadays it is possible to present quantitative estimations for genetic effects. In the following sub-sections, the previous results concerning genetic effects on resting ECG variables are reported. However, comparison of the results between the studies in the different sub-sections has to be done with caution, since different study designs (twin vs. family study) and differences in the statistical methods used may lead to different estimates of genetic effects. The estimations are also population- and gender-specific, and likely to change with aging (Plomin et al. 2001).

Twin studies using quantitative genetic modelling
Some twin studies have used quantitative genetic modelling (see 2.4.1) in the analysis of genetic and environmental effects on resting ECG variables. In these studies, the most widely investigated variable is heart rate–corrected QT interval (QTc) (correction made using Bazett´s formula; QT interval/$\sqrt{}$RR interval). Genetic effects explained 52% of the variation in QTc in young and middle-aged twins (both male and female, mean age 34 years) (Busjahn et al.

1999) and 25% in 18- to 71-year-old female twins (Carter et al. 2000), whereas Dalageorgou et al. (2008) reported that the variation in Bazett-corrected QTc accounted only for common and unique environmental effects in 21- to 80-year-old female twins. However, when Dalageorgou et al. (2008) analyzed QTc corrected by formulas QT interval + [(1000 - RR interval)/7] and QT interval + [0.154 x (1000 – RR interval)], the estimates of genetic effects were 52% and 50%, respectively. Genetic effects explained 60% of the variation in the uncorrected QT interval.

Previous results are also available for resting heart rate. Relatively high estimates of genetic effects have been reported: 64% in 10- to 26-year-old male and female twins (Snieder et al. 2003), 59% in adult male and female twins (mean age 35±8 years) (Jedrusik et al. 2003) and 55% in 21- to 80-year-old female twins (Dalageorgou et al. 2008). For the other resting ECG variables, the following estimates of genetic effects have been reported: P duration 46%, RR interval, PR interval and P axis 0%, QRS duration 40%, QRS axis 59% and T axis 52% in young and middle-aged twins (Busjahn et al. 1999).

Twin studies using other statistical methods

Other twin studies have mainly used the Falconer's formula ($h^2 = 2[r_{MZ} – r_{DZ}]$) (Plomin et al. 2001, Rijsdijk & Sham 2002) for the estimation of genetic effects on resting ECG variables (h^2 refers to heritability, i.e., the proportion of genetic effects, and r refers to the intra-class correlation coefficient). Varying results have been reported for different variables. Genetic effects explained 54% of the variation in resting heart rate and 34% in PR interval in 42- to 56-year-old male twins while QRS duration and QT interval were only environmentally affected in this group of twins (Havlik et al. 1980). In 12- to 73-year-old male and female twins the proportion of the variation in PR interval explained by genetic effects was 78% (Møller et al. 1982). Russell et al. (1998) reported that genetic effects accounted for 77% of the variation in RR interval and 36% of the variation in QT interval among 52- to 66-year-old male twins.

Family studies

Some family studies (including both men and women, from adolescents to older people) have investigated genetic effects on resting ECG variables, and they have used varying statistical methods for this purpose. The most widely investigated variable in these family studies has also been QTc (most commonly corrected for heart rate using Bazett's formula). The estimates of genetic effects for QTc have ranged between 30 and 40% (Friedlander et al. 1999, Hong et al. 2001, Newton-Cheh et al. 2005). ECG LVH indices and estimates of left ventricular mass (LVM) have also been studied. The following estimates of genetic effects have been reported (see calculation of indices in 4.2.1): Sokolow Lyon voltage 39 – 41%, Cornell voltage 19 – 25%, Cornell product 28 – 32% and ECG LVM 12 – 18% (Mayosi et al. 2002).

2.1.2 Association between left ventricular hypertrophy and repolarisation phase, and its underlying factors

LVH is a structural adaptation of the heart to an abnormal hemodynamic load or chronic neurohumoral activation. Thickening of the left ventricular (LV) wall is due to the hypertrophy of myocytes, and it reduces wall stress and maintains cardiac output when the mechanical load is increased, such as in chronic hypertension. (Arnett 2000, Lorell & Carabello 2000.) Other factors which increase the risk for LVH includes for example age, race, obesity and valvular heart disease (Levy et al. 1988, Oberman et al. 2006). Initially the hypertrophied LV wall may improve LV function, for example by increasing stroke volume, but ultimately, the hypertrophied LV wall causes detrimental changes in cardiac function, such as abnormalities in myocardial relaxation and passive filling during diastole. These events may partly be explained by fibrotic and adaptational cellular changes, which occur in hypertrophy. (Arnett 2000, Lorell & Carabello 2000.) LVH-related abnormalities in cardiac function may also be seen in resting ECG, since LVH has been reported to be associated with repolarisation abnormalities (Carter & Estes 1964, Devereux & Reichek 1982, Moore et al. 1984, Porthan et al. 2007). These can be manifested by ST segment depression and/or inverted T waves.

LVH can be most reliably detected and diagnosed using echocardiography or magnetic resonance imaging techniques. However, these are expensive and less immediately available methods, hence sometimes, for example in research settings, resting ECG may be a more useful method for this purpose, although its accuracy in the diagnosis of LVH has been reported to be unsatisfactory. In their systematic review, Pewsner et al. (2007) reported the sensitivities and specificities of six commonly used resting ECG criteria for LVH (Sokolow Lyon voltage, Cornell voltage, Cornell product, Gubner index and Romhilt-Estes scores with two thresholds for positive finding). Specificities ranged between 87 and 96%, but sensitivities were only between 13 and 25%. After comparing the different criteria, they concluded that no one of them is clearly superior to the others. Mean sensitivities for more recent criteria Cornell voltage and Cornell product in different studies were 16% and 20%, and mean specificities 95% and 91%, respectively.

From the public health perspective, both ECG LVH and repolarisation abnormalities (either in isolation or in combination) are important changes in the heart. Firstly, the prevalence of these abnormalities increases with age (e.g., De Bacquer et al. 2000, Larsen et al. 2002, Oberman et al. 2006). For example, Larsen et al. (2002) reported that in men and women (25-74 years) the prevalence of both ECG LVH with negative T wave and LVH with ST segment depression and negative T wave increased with aging. Among the youngest women (25-34 years) the prevalence of these abnormalities was 0%, but it had increased to 1.8% and 1.6%, respectively, in the oldest women (65-74 years). Very similar results were observed in the study by De Bacquer et al. (2000). Secondly, LVH with or without repolarisation abnormalities is associated with increased risk for cardiovascular morbidity and mortality (Vakili et al. 2001).

Larsen et al. (2002) found that subjects who have ECG LVH with negative T waves have over twofold risk for cardiovascular mortality compared with subjects who do not have these abnormalities. Associations with cardiovascular morbidity and mortality were stronger if ST depression was also present.

The association between LVH and relaxation/repolarisation abnormality is rather well established, but less is known about the factors underlying this association (i.e., whether it originates from genetic and/or environmental factors and to what extent), since no previous studies exist in this area. According to the information available (see 2.1.1), genetic effects explain a moderate proportion of the variation (19 – 41%) in ECG LVH indices (Mayosi et al. 2002) and a low to high proportion of the variation (0 – 60%) in QT interval (which includes both the ventricular depolarisation and repolarisation phases) (Havlik et al. 1980, Russell et al. 1998, Busjahn et al. 1999, Friedlander et al. 1999, Carter et al. 2000, Hong et al. 2001, Newton-Cheh et al. 2005, Dalageorgou et al. 2008), whereas T waves (reflecting the ventricular repolarisation phase) are less studied variables and quantitative estimates for their heritability cannot be given.

2.2 Effects of physical activity on resting electrocardiographic variables

Previous studies, conducted mainly in athletes, have shown that long-term and intensive physical training have several effects on the heart which are of physiological origin and accordingly benign in nature, but which can mimic the changes observed in heart diseases. In addition to well-documented changes detected by echocardiography (e.g., enlarged cavity dimension, increased wall thickness) (Pluim et al. 2000), adaptations to physical activity/training can also be seen in the heart's electrical function.

2.2.1 The most common electrocardiographic adaptations caused by physical activity and their underlying mechanisms

The most common resting ECG alterations associated with physical activity/training include sinus bradycardia (resting heart rate < 60 bpm), first degree atrioventricular block (PR interval > 220 ms), incomplete right bundle branch block (QRS duration < 120 ms), early repolarisation (usually characterized in Caucasian populations by an elevated ST segment with an upward concavity which ends in positive [peaked and tall] T wave) and isolated QRS voltage criteria for LVH (e.g., S wave in lead V_1 + R wave in lead V_5 > 3.5 mV) (Holly et al. 1998, Fagard 2003, Maron & Pelliccia 2006, Corrado et al. 2009). Physiological LVH observed in trained persons can often be distinguished from pathological LVH, since physiological LVH usually exhibit

normal QRS axis, normal atrial and ventricular activation patterns and normal repolarisation (Holly et al. 1998, Corrado et al. 2009).

The alterations observed in resting ECG are usually associated both with adaptations of the cardiac autonomic nervous system (increased parasympathetic tone and/or decreased sympathetic tone) and increased cardiac mass and volume. The former mechanism may explain such adaptations as sinus bradycardia and first-degree atrioventricular block, since they usually disappear during exercise. The latter mechanism, on the other hand, may explain the increased QRS voltage or right ventricular conduction delay. Resting ECG alterations may also be explained by lower intrinsic heart rate and non-homogeneous repolarisation of the ventricles. (Holly et al. 1998, Fagard 2003, Corrado et al. 2009.)

The prevalence and significance of the resting ECG alterations associated with physical activity may be affected by factors such as gender, age, race, type of sporting discipline and intensity of physical activity. Physical activity–related alterations in resting ECG are usually more often observed in males, younger age-groups, Blacks and persons engaged in top-level endurance disciplines such as cross-country skiing and rowing. (Pelliccia et al. 2000, Pelliccia et al. 2007, Magalski et al. 2008.) The greater prevalence of resting ECG alterations observed in endurance athletes may partially be explained by large cardiac output which results in increases in LV cardiac dimension and wall thickness (Corrado et al. 2009). The male preponderance observed in resting ECG alterations may be explained by several factors, such as the ability of men to train at higher intensity levels than women, and their possibly higher participation rates in certain sporting disciplines (such as rowing) which have great impact on resting ECG findings. One explanation for the observation that physical activity–related resting ECG alterations are more prevalent in younger age-groups might be that the heart's electrical function is more vulnerable to physical activity–induced alterations during body growth and maturation.

Numerous case-control studies in athletes and sedentary controls have been performed to study the effects of physical activity/training on specific resting ECG variables. Longitudinal studies in this area are less numerous, but they have also been performed in non-athletic populations (deMaria et al. 1978, Adams et al. 1981, Schuit et al. 1998). One previous co-twin control study (see the principles of this study design in 2.4.2) also exists on this topic, in which young male MZ co-twins discordant for physical activity and fitness were studied (Hannukainen et al. 2005). Due to the large number of different resting ECG variables, the effects of physical activity/training are presented only for those variables which are relevant in the light of the results of the present study, namely resting heart rate, LVH indices and T wave amplitudes.

One of the most widely studied variables is resting heart rate and almost without exception, case-control studies have reported lower heart rates in endurance athletes compared with sedentary controls. This applies both to male and female athletes as well as younger and older athletes (e.g., Beckner & Winsor 1954, van Ganse et al. 1970, Parker et al. 1978, Northcote et al. 1989,

Bjørnstad et al. 1991, George et al. 1995, Stolt et al. 1997). Some selected case-control studies are presented in Table 1. Similar results have also been observed after training periods in longitudinal studies (deMaria et al. 1978, Adams et al. 1981, Schuit et al. 1998). However, when male MZ twins were studied, resting heart rate was similar between the more and less active co-twins (56±6 vs. 56±10 bpm, respectively, $p = 0.84$) (Hannukainen et al. 2005).

Physical activity–induced physiological LVH can also be detected in resting ECG. Sokolow Lyon voltage as an index for LVH (Sokolow & Lyon 1949) is widely used in different studies and many of them have reported significantly higher voltages in athletes compared with sedentary controls (van Ganse et al. 1970, Parker et al. 1978, Douglas et al. 1988, Northcote et al. 1989, Bjørnstad et al. 1991) or after an endurance training programs in non-athletic persons (deMaria et al. 1978). Indirect evidence of LVH can also be seen among highly trained junior athletes; the prevalence of the Sokolow Lyon voltage criterion (> 3.5 mV) was significantly higher among athletes (45%) than non-athletic controls (23%) (Sharma et al. 1999). Sokolow Lyon voltage also tended to be higher among more active male MZ twins than their less active co-twins (Hannukainen et al. 2005), whereas among female athletes, Sokolow Lyon voltage (voltage itself or prevalence of LVH determined using this method) was not different from that of sedentary controls (George et al. 1995, Stolt et al. 1997).

Cornell voltage is a less studied variable and the few existing studies have yielded varying results. Cornell voltage was significantly higher among more active male MZ twins (Hannukainen et al. 2005) and female endurance athletes (Stolt et al. 1997) than more sedentary controls, but it was not different between male endurance athletes and sedentary controls, despite the fact that the majority of the male athletes exhibited an echocardiographically detected LVH (Douglas et al. 1988). In the study by Stolt et al. (1997), Cornell product was also greater among female endurance athletes than less active controls.

One of the manifestations of repolarisation abnormalities is lowered T wave amplitudes or T wave inversions. In athletes, resting ECG is often characterized by tall T waves (Hanne-Paparo et al. 1976, Sharma et al. 1999) or higher T wave amplitudes when compared with non-athletic controls (George & Winsor 1954, van Ganse et al. 1970, Bjørnstad et al. 1991). For example, the prevalence of tall T waves was significantly greater in junior athletes (22%) compared with sedentary controls (6%) (Sharma et al. 1999). However, studies also exist, in which T wave amplitudes have been similar between physically trained and sedentary/less active persons. These studies have been performed among female athletes (Stolt et al. 1997) and non-athletic populations (Hannukainen et al. 2005).

TABLE 1 Resting heart rate in athletes and controls in selected case-control studies. Values are mean ± SD unless otherwise stated.

Authors (year)	Participants	Age (years)	Heart rate in athletes (bpm)	Heart rate in controls (bpm)	p-value
Beckner & Winsor (1954)	Athletes: 165 M marathon runners Controls: 40 M non-runners	27.9 (range 18-48) 25.5 (range 18-42)	57 (range 40-72)	65 (range 55-85)	-
van Ganse et al. (1970)	Athletes: 30 M cyclists Controls: 30 M non-athletes	21.9±3.0 21.7±3.1	53.2±7.4	70.8±10.6	< 0.001
Northcote et al. (1989)	Athletes: 20 M endurance runners Controls: 20 M with sedentary lifestyle	56±7 56±7	51±7	72±10	< 0.001
Bjørnstad et al. (1991)	Athletes: 757 M and 542 F athletic students Controls: 76 M and 75 F sedentary students and office workers	24.2±4.3 24.8±3.2	62.2±13.2	68.2±13.1	0.0001
George et al. (1995)	Athletes: 10 F endurance athletes 10 F power-trained athletes Controls: 10 F non-athletes	22±1 22±1 22±1	Endurance: 47±3 Power: 65±5	70±3	Endurance vs. control: < 0.05 Power vs. control: > 0.05
Stolt et al. (1997)	Athletes: 30 F endurance athletes Controls: 30 F non-athletes	25.6±5.6 27.5±4.1	53.2±7.2	67.0±10.8	< 0.0001

M, males; F, females

2.2.2 Effects of cessation of physical activity and clinical significance of resting electrocardiographic alterations

Both structural and functional adaptations in the heart caused by physical activity/training are mainly reversible (Ehsani et al. 1978, Fagard et al. 1983, Pelliccia et al. 2002, Luthi et al. 2008, Bjørnstad et al. 2009). With regard to resting ECG, Fagard et al. (1983) reported that athletes had a higher resting heart rate and lower R wave amplitudes in the non-competition season than in the competition season. In the study by Pelliccia et al. (2002), for example, resting heart rate was higher and the prevalence of LVH determined by Sokolow Lyon voltage lower in athletes after long-term detraining than during the period of peak training.

Although the cardiac-related adaptations to physical activity/training often mimic signs of structural heart disease, they usually are not associated with adverse clinical events (Pelliccia et al. 2002, Bjørnstad et al. 2009). Abnormal resting ECG patterns are often associated with factors such as increased cardiac dimensions, whereas structural cardiovascular diseases are rarely responsible for these alterations (Pelliccia et al. 2000). Extreme alterations in resting ECG, however, must be taken seriously and be carefully evaluated. For example, although the prevalence of a prolonged QTc interval (males > 440 ms, female > 460 ms) in elite athletes is low, a QTc interval > 500 ms is highly suggestive of a long QT syndrome (Basavarajaiah et al. 2007). Marked repolarisation abnormality (T wave inversion ≥ 2 mm in at least three leads) in athletes is also uncommon, but if present, it is associated with increased risk for structural cardiac disease, such as hypertrophic cardiomyopathy (Pelliccia et al. 2008). Sinus bradycardia and a prolonged PQ interval may be important risk factors for lone atrial fibrillation in highly-trained endurance athletes (Grimsmo et al. 2010).

2.3 Association of resting electrocardiographic variables with walking endurance and all-cause mortality

2.3.1 Walking ability and endurance in old age

The ability to walk is an important part of functional capacity, as it enables coping with activities of daily living and thus independence. Several factors have an effect on walking ability, including age, body mass index (BMI), chronic diseases, physical activity and lower-limb muscle strength (Ho et al. 1997, Rantanen et al. 1999, Newman et al. 2003, Patel et al. 2006, Feinglass et al. 2009, Stenholm et al. 2009, Vincent et al. 2010). If walking ability is decreased, it has several important consequences. Decreased walking ability has been reported to be associated with, for example, mobility limitation, mobility disability, incident cardiovascular disease and mortality (Newman et al. 2006).

The term walking ability can refer to many different aspects of walking, such as the movements of specific body segments, walking speed or walking endurance. The latter is needed to be able to walk longer distances (several hundred meters or several minutes continuously), which, in turn, is needed for the performance of many activities of daily living, such as getting from home to a bus stop or going to buy food from the corner shop. Adequate function of many body systems are needed in long distance walking, including function of the cardiovascular system. Accordingly, disturbances and disorders related to the cardiovascular system may often compromise walking endurance and the ability to walk longer distances. For example, the presence of major resting ECG abnormalities, peripheral arterial disease, stroke and hypertension were associated with significantly longer performance time in the 400-meter corridor walk test among older, well-functioning men and women (Newman et al. 2003).

Walking endurance can be assessed by both self-reports and objective tests, such as the 6MWT and 400-meter walking test. 6MWT measures the distance which a subject can walk during six minutes on a hard and even surface (American Thoracic Society 2002). It is a widely used test, which has been shown to be both valid and reliable in the assessment of submaximal functional exercise capacity in older people and in people with chronic diseases. It is usually well-tolerated and reflects the ability to perform the activities of daily living better than the other walking tests. (Solway et al. 2001.)

2.3.2 Predictors of walking performance in the six-minute walking test

Many factors can predict the result of the 6MWT. In apparently healthy older subjects, the most common factors reported to be associated with the six-minute walking distance (6MWD) are age, gender, height and weight (Enright & Sherrill 1998, Troosters et al. 1999, Gibbons et al. 2001, Lord & Menz 2002, Enright et al. 2003, Hulens et al. 2003, Bautmans et al. 2004, Camarri et al. 2006, Jenkins et al. 2009). The 6MWD usually shortens with increasing age and weight, while longer 6MWD is more often seen in men compared with women and in taller persons compared with shorter persons. The latter phenomenon is probably explained by greater length of the lower extremities and consequent longer stride length in taller persons. The proportion of the variation in 6MWD explained by these factors has varied widely in different studies. All these four factors together explained 36 – 66% of the variation in the studies by Enright & Sherrill (1998), Troosters et al. (1999) and Camarri et al. (2006), while in the study of Gibbons et al. (2001), age and gender explained 41% of the variation in 6MWD. The proportion of the variation explained by height alone has been approximately 30% (Troosters et al. 1999, Camarri et al. 2006) and by age alone 25% (Gibbons et al. 2001). In addition to weight, BMI has also been found to be a predictor of 6MWD, especially in obese subjects. BMI alone has explained 38 – 59% of the variation in 6MWD (Hulens et al. 2003, Evers Larsson & Reynisdottir 2008).

Numerous other factors have also been reported to predict 6MWD. These include peakVO_2 (Hulens et al. 2003), lower-limb muscle strength (Lord &

Menz 2002, Hulens et al. 2003), participation in physical activity (Hulens et al. 2003), health status (Lord & Menz 2002, Bautmans et al. 2004), medication (Lord & Menz 2002, Enright et al. 2003), cognitive status (Enright et al. 2003) and lung function (FEV_1) (Enright et al. 2003, Camarri et al. 2006). However, less information is available regarding heart function. Resting heart rate was associated with 6MWD in obese subjects (Evers Larsson & Reynisdottir 2008) and moderate-to-severe LV wall motion abnormality was significant predictor of 6MWD in older women (Enright et al. 2003).

2.3.3 Association of resting electrocardiographic variables with mortality

Associations between resting ECG abnormalities and cardiovascular and all-cause mortality have been widely studied both in patient populations and general populations. Both major and minor abnormalities in resting ECG have been associated with increased risk for death in both women and men, also in old age. However, the significance of resting ECG abnormalities may vary to some extent. On the one hand, some abnormalities may be relatively common at the population level, such as left axis deviation, but their prognostic significance is rather small (De Bacquer et al. 1998). On the other hand, some resting ECG abnormalities may be strongly predictive of cardiovascular morbidity and mortality, but they concern only a small number of people. An example is a markedly prolonged QTc interval in persons with inherited long QT syndrome (Goldenberg & Moss 2008). Due to the large number of resting ECG variables, only resting heart rate, LVH indices and T wave amplitudes as predictors of cardiovascular and all-cause mortality are reported here, with the emphasis on all-cause mortality, since these are important variables with regard to the results of the present study.

Many studies have reported that increased resting heart rate is a predictor of both cardiovascular and all-cause mortality in the general population (Dyer et al. 1980, Kannel et al. 1987, Gillum et al. 1991, Shaper et al. 1993, Mensink & Hoffmeister 1997, Reunanen et al. 2000). Mensink & Hoffmeister (1997) reported that each 20 bpm increment in resting heart rate was associated with 70% higher risk for all-cause mortality in middle-aged and older men during a 12-year follow-up, while for women the corresponding figure was 40%. Similar results were observed in relation to cardiovascular mortality. In the study by Gillum et al. (1991) among middle-aged and older men and women, resting heart rate > 84 bpm significantly predicted all-cause mortality over 6-13 years in men (multivariately adjusted relative risks ranged between 1.60 and 1.81 across age groups, compared with men whose resting heart rate was < 74 bpm). Increased resting heart rate (≥ 90 bpm) was associated with over threefold increased risk for ischemic heart disease mortality among middle-aged men in an 8-year follow-up compared with men whose resting heart rate was < 60 bpm (Shaper et al. 1993). In a Finnish long-term (23 years) follow-up study among middle-aged and older men and women, increased resting heart rate was a significant predictor of all-cause and cardiovascular mortality, but only in the analyses adjusted for age and smoking (Reunanen et al. 2000).

According to the review by Vakili et al. (2001), both echocardiographically and electrocardiographically detected LVH predicts cardiovascular morbidity as well as all-cause mortality. These findings apply to various populations and ethnic groups. Voltage–based LVH indices (Sokolow Lyon voltage, Cornell voltage and Cornell product) are rather widely used and two single examples of their associations with all-cause mortality are presented here. Cornell voltage-based LVH (Cornell voltage > 2.0 mV in women and > 2.8 mV in men) was associated with increased 10-year all-cause mortality in middle-aged and older women (hazard ratio 1.48, 95% confidence interval 1.15-1.91) but not in men in the study by Havranek et al. (2008). A similar result was observed for Cornell product-based LVH (LVH is suggested to be present if the product is greater than 2436 mm x ms). However, in men, both Cornell voltage and Cornell product-based LVH were associated with 5- and 10-year cardiovascular mortality whereas in women, these LVH indices were not associated with cardiovascular mortality. Cornell voltage and Cornell product also significantly predicted all-cause mortality in the study by Sundström et al. (2001) among older men. Those with LVH had a 2.5-4-fold increased risk for death compared with subjects without LVH. In this same study, subjects with Sokolow Lyon voltage-based LVH (Sokolow Lyon voltage ≥ 3.5 mV) had twofold increased risk for death from all causes compared with subjects without LVH.

Also flattened or inverted T waves, either isolated or in combination with ST segment depression, are strong predictors of cardiovascular and all-cause mortality (Rose et al. 1978, Cullen et al. 1982, Ristola 1983, Sutherland et al. 1993, De Bacquer et al. 1994, Tervahauta et al. 1996, De Bacquer et al. 1998, Daviglus et al. 1999, Larsen et al. 2002, Greenland et al. 2003, Yamazaki et al. 2005, Rautaharju et al. 2006). Isolated T wave abnormalities (flattened or inverted T waves) seem to be more strongly related to increased cardiovascular than all-cause mortality, but significant associations with all-cause mortality have been observed, especially in men (De Bacquer et al. 1994, De Bacquer et al. 1998, Greenland et al. 2003). For example, in the study by De Bacquer et al. (1998) among middle-aged and older men and women, 10-year cardiovascular and coronary heart disease mortality was 2-2.5-fold higher in subjects with flattened or inverted T waves compared with those with normal T waves, while all-cause mortality was about 40% higher among subjects with T wave abnormalities. When men and women were studied separately, the results were rather similar between the sexes for cardiovascular and coronary heart disease mortality, but not for all-cause mortality. T wave abnormalities were significantly associated with all-cause mortality only in men. The predictive value of T wave amplitudes in specific leads has also been studied. Among middle-aged and older men, negative or flattened T wave amplitudes in leads I and V_5 were the strongest predictors of cardiovascular mortality (Yamazaki et al. 2005), and among middle-aged Finnish men, negative T wave amplitudes in leads II and V_5 were most strongly associated with cardiovascular mortality. Rather similar results were also observed for women. (Ristola 1983.)

2.4 Quantitative genetic modelling using twin data and co-twin control study

2.4.1 Quantitative genetic modelling

The aim of quantitative genetics is to investigate the extent to which variation in a trait is accounted for by genetic and environmental effects. Quantitative genetics gives an estimation of the proportions of these effects, without specifying any single gene or environmental factor that affects the trait. This estimation can be done using twin, adoption and/or family data. A commonly used design is the classical twin method, in which two types of twin pairs, MZ and DZ, are used to disentangle genetic effects from environmental effects on a trait. (Plomin et al. 2001, Boomsma et al. 2002, Rijsdijk & Sham 2002, Posthuma et al. 2003.) MZ twins arise from one fertilized egg, which splits into two separate individuals, and therefore MZ co-twins are genetically identical, i.e., they share all of their genes. DZ twins, in turn, share on average 50% of their segregating genes, since they arise from two eggs, which have been fertilized by two sperm. (Hall 2003.) On account of their identical genetic make-up, MZ co-twins are more alike than DZ co-twins. The environment shared by both types of co-twins increase the similarities between the co-twins whereas environment which is specific to each co-twin contribute to differences between the co-twins. (Plomin et al. 2001, Posthuma et al. 2003.)

In quantitative genetics, variation in a trait can be decomposed into different variance components which originate from genetic or environmental effects. In this context, environmental effects include all those sources of variation which are not explained by genetic effects. Both genetic and environmental effects can be divided into two different components. Additive genetic effects (labelled A) refer to the additive effects of individual alleles at all those loci that affect the trait while non-additive or dominance genetic effects (D) represent interactions between alleles at the same or different loci. Common environmental effects (C) include factors that are shared by both co-twins, such as those related to their childhood environment, whereas unique environmental effects (E) consist of exposures that are not shared by the co-twins, such as diseases that have affected only one sibling within a pair. E also includes measurement error as this is a random effect not correlated between the co-twins. (Neale & Cardon 1992, Plomin et al. 2001, Posthuma et al. 2003.) The term heritability refers to the proportion of variation which is accounted for by genetic effects. Broad-sense heritability includes both additive and dominance genetic effects while narrow-sense heritability includes only additive genetic effects. (Neale & Cardon 1992, Plomin et al. 2001, Boomsma et al. 2002.) Heritability is a statistic which is age, gender and population-specific (Neale & Cardon 1992, Plomin et al. 2001).

In twin data, the estimation of the variance components is based on both the different degrees of correlation for additive and dominance genetic effects

and the same degrees of correlation for the common and unique environmental effects in MZ and DZ twins (Figure 1). The correlation is 1 for both additive and dominance genetic effects in MZ pairs, whereas the respective values for DZ pairs are 0.5 and 0.25. The correlation is 1 for common environmental effects and 0 for unique environmental effects in both types of twin pairs. The limitation of classical twin study, in which MZ and DZ twins reared together are studied, is that dominance genetic effects and common environmental effects cannot be estimated simultaneously since they are confounded in this study design. (Neale & Cardon 1992, Plomin et al. 2001, Rijsdijk & Sham 2002, Posthuma et al. 2003.) However, twin correlations can be used to assess which of these two variance components is more likely to be present and this can then be included in the genetic models which are to be modelled. On the basis of the correlations given above, the following guidelines can be presented. If the DZ correlation is less than half the MZ correlation, dominance genetic effects are indicated, while common environmental effects are indicated if the DZ correlations are greater than half the MZ correlations. Additive genetic effects are indicated if the MZ correlation is about twice the DZ correlation, but this does not exclude the presence of dominance genetic or common environmental effects. (Plomin et al. 2001, Rijsdijk & Sham 2002.) In addition to the confounding effects of dominance genetic and common environmental effects in the same model, it should be borne in mind that a model that includes only dominance genetic and unique environmental effects is biologically implausible since dominance genetic effects are highly unlikely if additive genetic effects are not also present (Posthuma et al. 2003).

A basic univariate twin model is presented in Figure 1. Univariate analysis decomposes the variation in a single trait into the variance components presented earlier. The observed (measured) variables are shown in rectangles whereas the components of genetic and environmental variance to be estimated are presented in circles. Single-headed arrows are causal paths which point from the variance components to the observed variables. As noted above, dominance genetic and common environmental effects cannot be modelled simultaneously, but in this theoretical model they are all presented at the same time to show the correlations between the different variance components. (Plomin et al. 2001, Rijsdijk & Sham 2002.)

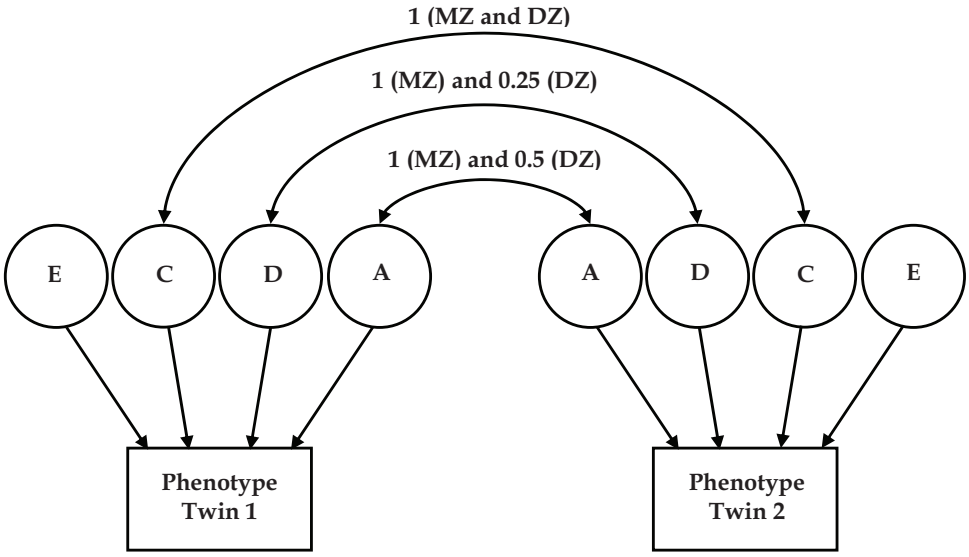


FIGURE 1 Path diagram for a univariate twin model. Correlations for additive genetic effects (A), dominance genetic effects (D) and common environmental effects (C) in monozygotic (MZ) and dizygotic (DZ) twin pairs are presented above the double-headed arrows. E refers to unique environmental effects which are uncorrelated between the co-twins of MZ and DZ pairs. (Modified from Rijsdijk & Sham 2002)

If there more than one observed trait is available per subject, then a multivariate genetic analysis can be performed. In multivariate genetic analysis, it is the cross-covariance between relatives (i.e., whether trait X in one family member is associated with trait Y in another family member) which is analyzed. When the phenotypic correlation between traits is observed, it can be decomposed into genetic and environmental components, and their relative contribution can be estimated. In addition, by calculating genetic and environmental correlations, the overlap of genetic or environmental effects between the traits can also be estimated. This reveals whether the same or distinct genetic or environmental factors affect the traits and to what extent. If there are two traits, for example X and Y, the genetic correlation between them can be calculated by dividing the genetic covariance between traits X and Y by the square root of the product of the genetic variances of traits X and Y. The environmental correlation is calculated similarly. Exactly the same genetic factors affect both traits if the genetic correlation equals 1, and if it equals 0, then distinct genetic factors affect the traits. If the correlation is between 0 and 1, then some factors are shared between the traits and some are specific to each trait. If the genetic correlation is high, it does not necessarily mean that the genetic factors affect both variables to the same magnitude or in a similar way (additive vs. dominance). The interpretation of the environmental correlation is similar. (Neale & Cardon 1992, Plomin et al. 2001, Posthuma et al. 2003.)

To study whether two or more traits share the same genetic and/or environmental effects, different methods can be used. The selection of the best-fitting model (see later) can then be made by comparing the results of different modelling methods. One option is a Cholesky decomposition model. In a bivariate Cholesky decomposition model (including additive genetic, common environmental and unique environmental effects), there are both additive genetic effects, which are shared by both observed traits, and additive genetic effects, which are specific to only one trait. Common and unique environmental effects behave in a similar way. Similar principles also apply to the models which include three or more observed traits. (Neale & Cardon 1992.) The second option is an independent pathway model. Using this method it can be studied whether there are genetic and/or environmental effects which are common to all the observed traits included in the model and whether there are genetic and/or environmental effects which are specific to each trait. Their relative contributions can also be quantified. The third option, a common pathway model (Figure 2) is based on the assumption that there are one or more latent variables (factors) onto which the common genetic and environmental effects load. The term latent factor means that the factor is not directly observable but can be statistically estimated through the information available from the observed traits. In the model, the latent factor loads onto the observed traits. (Neale & Cardon 1992, Plomin et al. 2001, Rijsdijk & Sham 2002.) The degree of relationship between a latent factor and an observed trait can be determined by calculating factor loadings (Neale & Cardon 1992). Trait-specific genetic and environmental effects load onto each observed trait. These describe the proportion of the variation not explained by the genetic or environmental effects of a latent factor. (Plomin et al. 2001, Rijsdijk & Sham 2002.) To estimate the overlap of the common genetic and environmental effects between latent factors, genetic and environmental correlations can be calculated as described earlier.

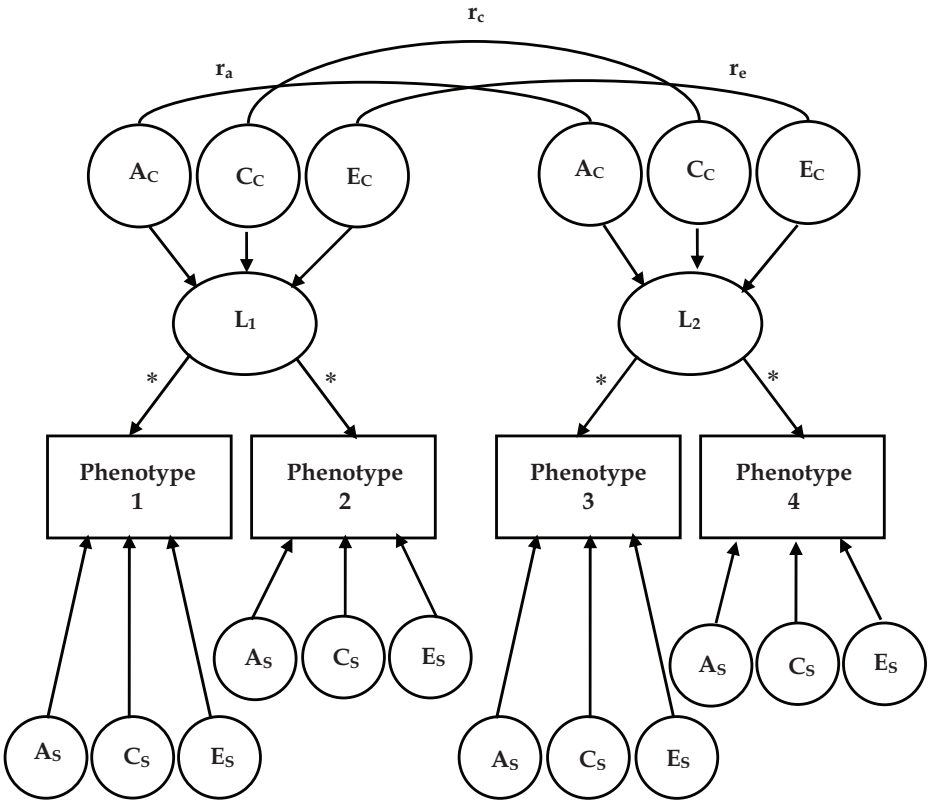


FIGURE 2 A common pathway model with two latent factors for one twin, including additive genetic effects (A), common environmental effects (C) and unique environmental effects (E). Lower-case c refers to genetic and environmental effects which are common to a latent factor (L_1 or L_2). Lower-case s refers to phenotype or trait-specific genetic and environmental effects. r_a, r_c and r_e = correlations for additive genetic, common environmental and unique environmental effects (respectively) between the two latent factors. The factor loadings of latent factors on the observed phenotypes are shown with *.

A method currently used to analyze both univariate and multivariate data is called model fitting. Model fitting is based on constructing both the expected and observed variance-covariance matrices for family members (e.g., MZ and DZ twins), including various parameters (variance components). The expected variance-covariance matrices are formed from different combinations of model parameter values and the observed variance-covariance matrices are calculated from the data. These two types of matrices are then fitted against each other to see how well the expected variance-covariance matrix describes the observed data. The aims of model fitting are to create expected variance-covariance matrices with parameter values which match the observed data as closely as possible and to find a well fitting model with smallest number of parameters. Using model fitting, it is also possible to compare the fit of different models.

After these procedures, the best-fitting model is selected to provide the estimates of genetic and environmental effects. (Plomin et al. 2001.)

Model fitting can be performed using the Mx program, for example. In Mx, data can be entered either as raw data or summary statistics (e.g., covariance matrices), but entering raw data is usually preferred since it allows greater flexibility in the handling of data (e.g., missing cases or variables). (Neale & Cardon 1992, Neale et al. 2003.) Model parameters are estimated by minimizing a goodness-of-fit statistic between the expected and observed variance-covariance matrices. There are many criteria which can be used to test this. (Plomin et al. 2001, Rijsdijk & Sham 2002.) One of these criteria is the Akaike's Information Criterion (AIC = -2 x log-likelihood – 2 x degrees of freedom) (Akaike 1987) which compares models on the basis of parsimony, taking into account the likelihood of every model and its degrees of freedom: the smaller the AIC, the better the fit of the model.

There are many assumptions in the classical twin study. The most important assumption is that MZ and DZ pairs share their environment to the same extent. It is also assumed that the traits studied in twins do not differ from those in the general population and that mating occurs at random. In addition, it is assumed that there are no gene-environment correlations or gene-environment interactions for the studied traits, or if so, then are at least minimal. (Plomin et al. 2001, Rijsdijk & Sham 2002, Posthuma et al. 2003.) One important way to check these assumptions is to test the equality of the means and variances of the studied traits between MZ and DZ twin pairs, since their unequality may be an indication of violations of the above-mentioned assumptions (Neale & Cardon 1992). Violations of the assumptions may lead to biased estimates of variance components. (Plomin et al. 2001, Rijsdijk & Sham 2002, Posthuma et al. 2003.)

2.4.2 Co-twin control study

The study of twin pairs, in which the co-twins are discordant for some property, offer an ideal case-control study design (co-twin control study), since the co-twins are closely matched for age, gender and familial background. In this study design, the co-twin with the property of interest is the case and his/her own co-twin without this property serves as a control. The special advantage of this design is the study of discordant MZ twin pairs, since they are identical at the chromosomal sequence level. In this case, the effects of genetic factors can be controlled for. This control is partial when DZ twin pairs are studied, since they share on average half of their segregating genes. (Plomin et al. 2001, Boomsma et al. 2002.)

3 AIMS OF THE STUDY

The purpose of this study was to examine genetic and environmental effects on resting ECG variables in older people. The purpose was also to examine the associations between resting ECG variables and physical activity, walking endurance and mortality.

The specific aims of the study were:

1. To examine the relative contribution of genetic and environmental effects on resting ECG variables (Study I) and whether genetic and environmental effects are shared between electrocardiographically determined LVH and repolarisation phase (Study II).

2. To examine the effects of long-term leisure time physical activity (LTPA) vs. inactivity on resting ECG variables when partially or fully controlled for genetic liability and childhood environment (Study III).

3. To examine how resting ECG variables are associated with walking endurance (Study IV) and all-cause mortality (Study V).

4 PARTICIPANTS AND METHODS

4.1 Participants

4.1.1 Recruitment

The present study is part of both the Finnish Twin Study on Aging (FITSA) (Pajala et al. 2004) and the TWINACTIVE study (Leskinen et al. 2009). FITSA is a study of genetic and environmental effects on the disablement process in older women, and the TWINACTIVE study investigates the metabolic and cardiovascular consequences of physical activity vs. inactivity using a study design of twin pairs persistently discordant for LTPA. The participants of both study projects were recruited from the nationwide Finnish Twin Cohort (Kaprio et al. 1978, Kaprio & Koskenvuo 2002) which is a longitudinal study of the genetic and environmental factors of chronic diseases and risk factors. The cohort includes all same-sex twin pairs born before 1958 and with both co-twins alive in 1975.

The Finnish Twin Cohort contained 1260 female twin pairs born in 1924-1937 who responded to the baseline questionnaire in 1975. Of this group, an invitation to participate in the FITSA study was sent in 2000 to every female MZ twin pair (n=178), every third female DZ twin pair (n=212) and to 24 female twin pairs whose zygosity was uncertain (XZ). Altogether the number of invited twin pairs was 414, and they were selected solely on the basis of their age and zygosity. To be included in the study, both co-twins had to agree to participate (Figure 3). Participating twin pairs arrived for the laboratory measurements the previous evening, staying overnight in a hotel. Both co-twins came to the laboratory at the same time, and they received their individual test schedules on arrival (Figure 4).

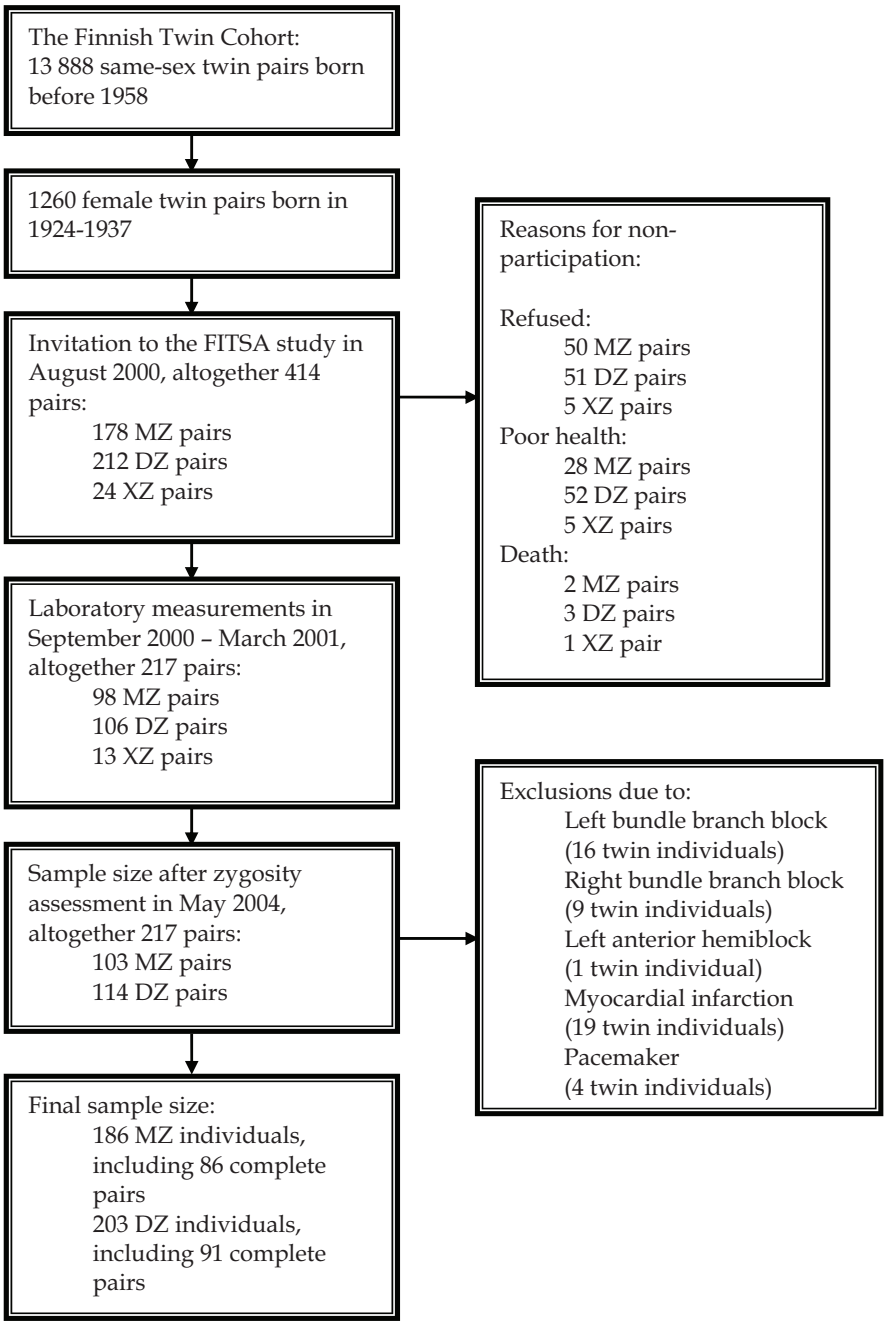


FIGURE 3 Recruitment of the participants for the Finnish Twin Study on Aging (FITSA). MZ, monozygotic; DZ, dizygotic; XZ, unknown zygosity

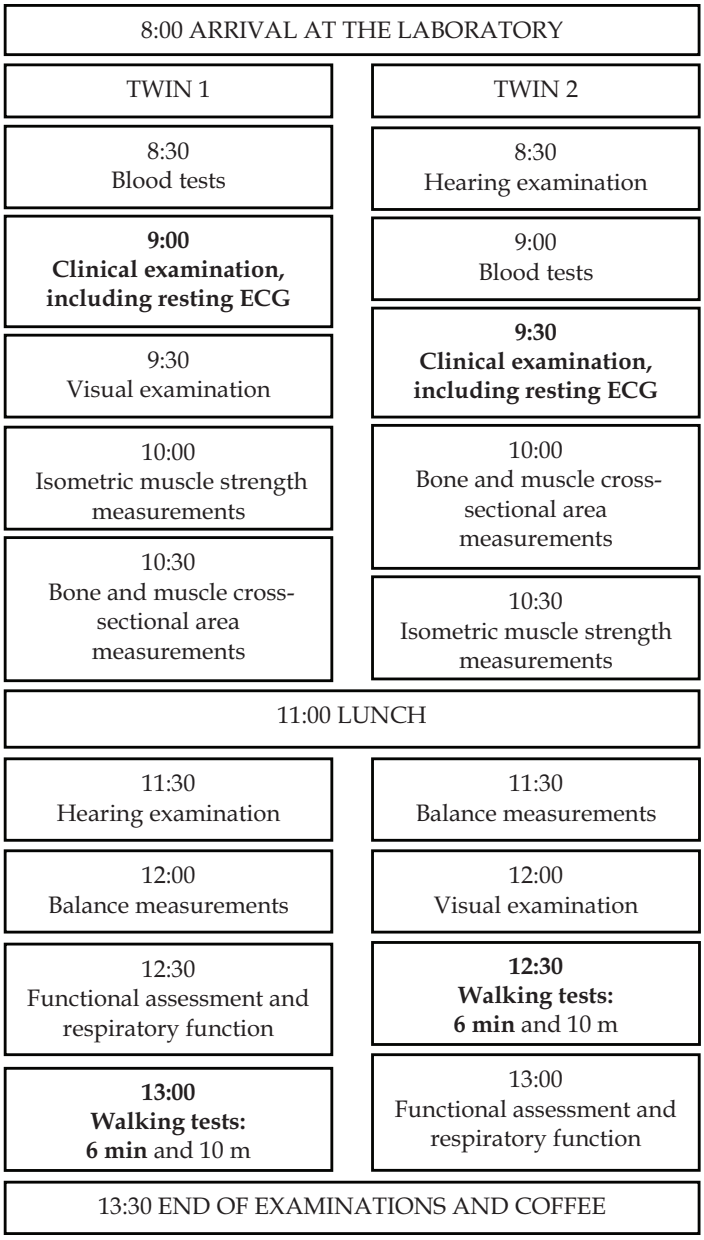
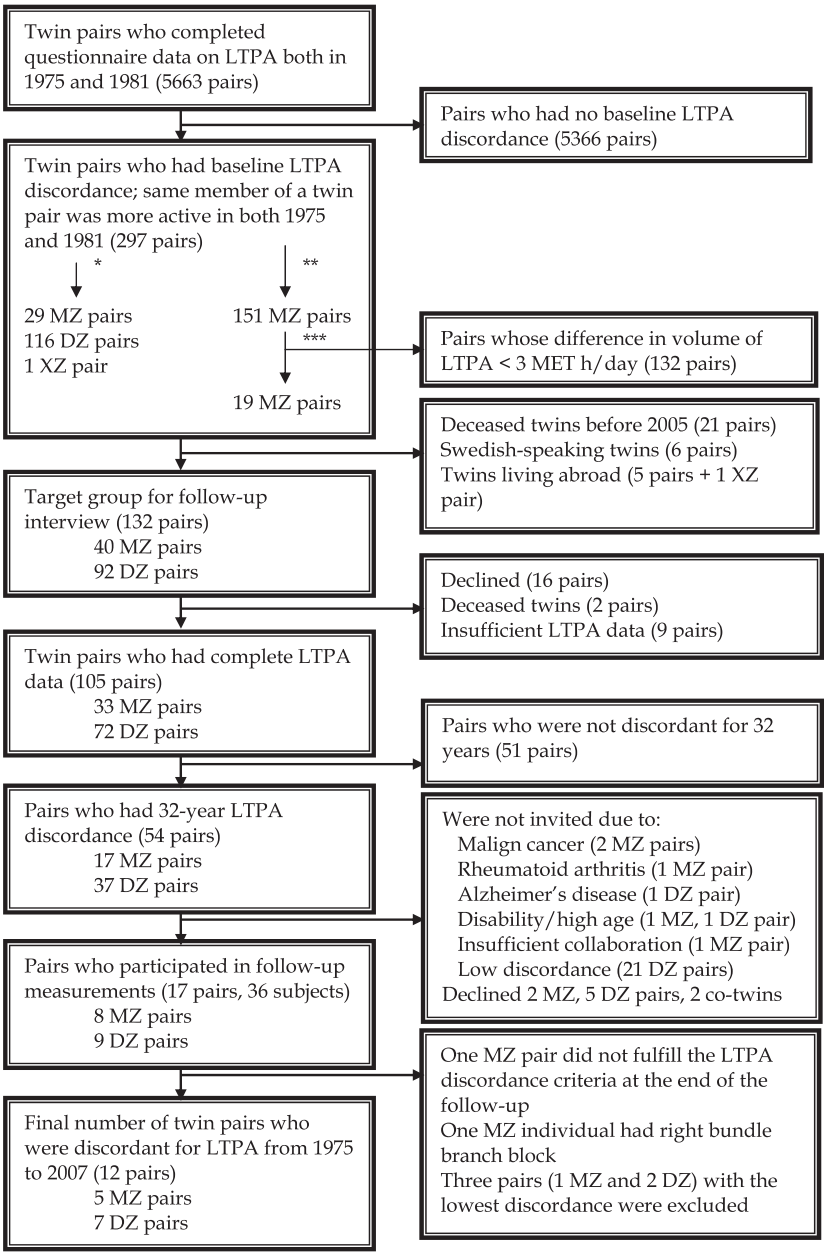


FIGURE 4 Test schedule for twin 1 and twin 2 in the Finnish Twin Study on Aging. ECG, electrocardiography

The participants of the TWINACTIVE study were recruited from the Finnish Twin Cohort on the basis of both leisure time and commuting physical activity data collected at several time points during the 32-year follow-up period from 1975 to 2007. Identification of eligible twin pairs began with the pairs in the Finnish Twin Cohort who were 24-60 years old, employed and healthy in 1981

(altogether 5663 pairs). They had all completed the LTPA questionnaire in both 1975 and 1981 (Kujala et al. 2002, Leskinen et al. 2009). On the basis of the questionnaire data, leisure time metabolic equivalent (MET) indices (MET h/day, see 4.2.2) were calculated for the assessment of LTPA volume (Leskinen et al. 2009). LTPA discordance was considered to be present, if the difference between the co-twins was at least 2 MET h/day both in the volume and intensity of LTPA or at least 3 MET h/day in the volume of LTPA. One hundred and sixty-five out of 5663 twin pairs fulfilled these criteria for LTPA discordance in both 1975 and 1981. Of these 165 twin pairs, 132 twin pairs formed the target group for a retrospective follow-up interview on LTPA from 1980 to 2005 (with 5-year intervals). Fifty-four twin pairs fulfilled the LTPA discordance criteria as described in the retrospective follow-up (discordance between the co-twins was found in at least four out of the six assessed time points). After exclusions and refusals, the final study sample consisted of 5 MZ pairs and 7 DZ pairs (50-67 years, 9 male pairs, 3 female pairs) (Figure 5). Their LTPA discordance was also determined in laboratory measurements conducted in 2007. The participants attended the laboratory measurements (visit 1) (Table 2) either alone or together with his/her co-twin, staying overnight in a hotel.



* Baseline discordance in volume (2 MET criterion) and intensity of LTPA
** Baseline discordance only in volume of LTPA (2 MET criterion)
*** Baseline discordance in volume of LTPA (3 MET criterion)

FIGURE 5 Selection of twin pairs discordant for leisure time physical activity (LTPA) in the TWINACTIVE study. MZ, monozygotic; DZ, dizygotic; XZ, unknown zygosity

TABLE 2 Test schedule in the TWINACTIVE study.

VISIT 1	
Before	Assessments of 5-d diet and 7-d physical activity using diaries
Day 1	
11:00	Blood tests
12:00	Standardized interview to assess smoking habits, use of alcohol, dietary habits and exercise attitudes
13:00	Resting echocardiography
13:45	Clinical examination
14:15	**Resting electrocardiography**
14:30	Symptom-limited maximal clinical exercise test
15:15	Muscle strength measurements
16:00	Measurement of bone properties
22:00	Fast begins
Day 2	
7:30	Anthropometric measurements and assessment of body composition
8:00	Fasting blood and DNA samples
8:15	Oral glucose tolerance test
8:00-10:00	Standardized leisure time physical activity interview
12:00	MR imaging from abdomen and thigh, MR angiography of macroscopic arteries
VISIT 2	
Before	Structured instructions of exercise before/after biopsy. Overnight fast.
Day 3	
8:00-10:00	Muscle and subcutaneous adipose tissue biopsies

DNA, deoxyribonucleic acid; MR, magnetic resonance

4.1.2 Zygosity

The zygosity of the participating twin pairs in both the FITSA and TWINACTIVE studies was initially determined by a validated questionnaire (Sarna et al 1978) at the baseline study in 1975. It was later verified in both studies using DNA extracted from a venous blood sample by a battery of ten highly polymorphic gene markers. These analyses were carried out at the Paternity Testing Laboratory of the National Public Health Institute in Helsinki, Finland.

4.1.3 Ethical aspects

The present study was conducted according to the guidelines for good clinical and scientific practice laid down by the Declaration of Helsinki. All the participants in both the FITSA and TWINACTIVE studies were informed about the study and they signed a written informed consent prior to any measurements. The study plans of both studies were approved by the Ethics Committee of the Central Finland Health Care District.

4.1.4 Total number of participants in substudies

The total number of studied individuals and twin pairs varied in the different studies (Table 3). In each study, participants with left bundle branch block, right bundle branch block, left anterior hemiblock, myocardial infarction and/or pacemaker were excluded, if applicable, since they make it impossible to interpret resting ECG recordings in a meaningful way. In Study III, the selection of twin pairs discordant for physical activity was made solely on the basis of LTPA (ignoring the discordance for commuting physical activity) and three pairs with the lowest discordance were excluded. In Study IV, the exclusions also concerned participants who had incomplete resting ECG data and who were unable to perform the 6MWT.

TABLE 3 Total number of studied monozygotic (MZ) and dizygotic (DZ) twin pairs and individuals in studies I-V.

Studies	Project	MZ pairs	DZ pairs	MZ individuals	DZ individuals
I	FITSA	86	91	186	203
II	FITSA	86	91	186	203
III	TWINACTIVE	5	7	-	-
IV	FITSA	16*	-	154†	166†
V	FITSA	8*	10*	186†	203†

All twin pairs in the Finnish Twin Study on Aging (FITSA) are female twin pairs, aged 63-76 years. The TWINACTIVE study comprised 3 female and 9 male twin pairs, aged 50-67 years after exclusions.

* Number of twin pairs used in within-pair analyses

† Number of twin individuals used in individual-based analyses

4.2 Measurements

4.2.1 Electrocardiographic measurements

Standard 12-lead resting ECGs were recorded at 25 mm/s and 1 mV/cm standardization using a Nihon Kohden Cardiofax V Ecaps 12 (FITSA) and at 50 mm/s and 1 mV/cm standardization using a GE Medical Systems IT CardioSoft V5.02 (TWINACTIVE). Recordings were performed after a five-minute rest interval. In both studies, T wave amplitudes in leads V_1, V_5 and II as well as R wave amplitude in lead aVL (RaVL) and S wave amplitude in lead V_3 (SV_3) were measured manually while the other variables were collected from the automatic listings of the ECG recorder. In the TWINACTIVE study, S wave amplitude in lead V_1 (SV_1) and R wave amplitude in lead V_5 (RV_5) were also measured manually. QTc interval was calculated by the equation: QT interval + [(1000 - RR interval)/7] (Katsuoka et al. 1998). LVH indices were calculated as follows: Sokolow Lyon voltage (SV_1 + RV_5) (Sokolow & Lyon 1949), Cornell

voltage (RaVL + SV_3) (Casale et al. 1985) and Cornell product (Cornell voltage x QRS duration) (Molloy et al. 1992). ECG LVM was calculated using the following formulas: [0.02 x (RaVL + SV_3)] + (1.12 x body weight) + 36.2 (women) and [0.026 x (RaVL + SV_3)] + (1.25 x body weight) + 34.4 (men) (Rautaharju et al. 2000). All ECG measurements were carried out blinded to other data.

4.2.2 Assessment of physical activity

In the FITSA study, the present status of physical activity was assessed using the self-report scale designed by Grimby (Grimby 1986), with slight modifications. The 7-point scale ranged from hardly any activity to participation in competitive sports. Participants were considered sedentary if they reported no other activity than light walking once or twice a week. In all other cases participants were considered physically active.

In the TWINACTIVE study, the assessment of LTPA volume (leisure time MET index) was based on a series of structured questions on LTPA and physical activity during journeys to and from work (Kujala et al. 1998, Leskinen et al. 2009). A similar assessment was performed at the baseline in 1975 and 1981, in the retrospective follow-up interview from 1980 to 2005, and in the laboratory measurements in 2007 (Leskinen et al. 2009). The leisure time MET index was calculated by assigning a multiple of the resting metabolic rate to each form of physical activity (intensity of activity x duration of one session x monthly frequency) which was then expressed as a sum score of leisure time MET h/day. The mean MET index was calculated for both the active and inactive co-twins at 5-year intervals from 1975 to 2005, and for the year 2007. The co-twins with higher leisure time MET index were considered physically active while their co-twins with lower leisure time MET index were considered physically inactive.

4.2.3 Assessment of walking endurance

In the FITSA study, walking endurance was assessed using a validated 6MWT (Solway et al. 2001, American Thoracic Society 2002). The participants were requested to walk up and down a 50-m indoor straight track for six minutes and to complete as many laps as possible. Except for the length of the indoor track, the protocol and security conditions followed the instructions subsequently published in the American Thoracic Society Statement (American Thoracic Society 2002). The distance covered by the end of the six minutes was recorded as the outcome.

On the basis of walking test results, within-pair analyses for worse and better walking MZ co-twins were performed. In 63 MZ pairs both co-twins completed the 6MWT. The pairs (n=16) with the greatest and clinically most significant discordance for walking endurance were selected for the within-pair analyses; the within-pair difference in the 6MWT result had to be at least 75 m (Wise & Brown 2005). The co-twins with the longer walking distances were

considered the better walking co-twins and their twin-sisters with shorter walking distances were considered the worse walking co-twins.

4.2.4 Mortality data

The mortality follow-up (FITSA study) began on September 18, 2000 and continued until May 31, 2009. Death dates were received from the Population Register Centre of Finland. Deaths from all causes were taken into account. In addition to individual-based analyses, within-pair analyses on the risk for death were also carried out for all the twin pairs who were discordant both for survival status and resting ECG findings (n=18).

4.2.5 Health and medication

To ensure the safety of the measurements, participants' present health status, chronic diseases and exercise eligibility as well as use of medication were evaluated by a physician in a clinical examination. The examination also included basic anthropometric and blood pressure measurements.

4.3 Statistical methods

4.3.1 General statistical methods

General statistical analyses were performed using SPSS 14.0 (SPSS 2005), SAS 8.2 (SAS 2001) and Stata 8.0 (Stata Corp. 2003) software. The normality of the data and other assumptions for statistical analyses, including equality of means and variances (quantitative genetic modelling), were checked. Individual-based analyses (e.g., linear regression analysis, Cox regression analysis) were performed by taking into account the clustering of possibly correlated observations from the twin pairs (Williams 2000). Within-pair analyses were also used (paired-samples Student's t-test, Wilcoxon signed ranks test, Cox regression analysis). In the Cox regression analyses, the tested variables were classified into tertiles and the lowest tertile was used as a reference group. The level of statistical significance was set at $p < 0.05$ (two-sided). All analyses (including quantitative genetic modelling) were performed adjusted for age and use of beta-blockers.

4.3.2 Quantitative genetic modelling

To obtain preliminary information on the importance of the genetic contribution to each studied resting ECG variable, intra-class correlation coefficients (ICC) were computed separately for the MZ and DZ pairs. Thereafter, both univariate and multivariate genetic analyses were carried out.

Univariate genetic analysis (Study I)
Univariate genetic analyses were carried out to estimate the relative contribution of genetic and environmental effects to each resting ECG variable. The combinations of variance components tested were ACE, ADE, AE, DE, CE and E. Since D and C are confounded in twin studies in which MZ and DZ pairs reared together are investigated, they could not be modelled simultaneously (Neale & Cardon 1992, Rijsdijk & Sham 2002).

Multivariate genetic analysis (Study II)
Multivariate genetic modelling was used to study whether the ECG LVH indices and T wave amplitudes (reflecting the repolarisation phase) share the same genetic and/or environmental effects. The data were analyzed using all three methods available for this kind of multivariate modelling (Cholesky decomposition model, independent pathway model and common pathway model). The results of each analysis were then compared and the common pathway model with two latent factors gave the most parsimonius explanation for the data.

The two latent factors involved in the common pathway model were named LVH and the repolarisation phase (RP). They condensed information obtained from the measured LVH indices (Cornell voltage and Cornell product) and T wave amplitudes (leads V_5 and II), respectively.

The combinations of variance components tested were ACE, AE, CE and E. D was excluded since the univariate analyses showed no dominance genetic effects on any single LVH index or T wave amplitude. Both factor-specific (LVH or RP) and trait-specific (Cornell voltage, Cornell product, T wave amplitudes in leads V_5 and II) variance components were estimated. Factor loadings from the latent factors to the observed data were also calculated. To estimate the overlap between the sets of genes influencing the LVH and RP factors, the genetic correlation between the two sets was calculated. Correlations for common and unique environmental effects were calculated similarly.

Model fitting
The tested genetic models were fitted to the raw data with Mx software (Neale & Cardon 1992, Neale et al. 2003) using maximum likelihood algorithms and treating unobserved data as missing at random. The analyses were started with the hypothetical full ACE or ADE model which included all the plausible parameters. The significance of the estimates for the variance components was tested by removing them sequentially in different subsequent models. The fit of the different submodels was compared against the fit of the hypothetical full model. This procedure eventually led to a model in which the pattern of variances and covariance was explained by as few effects as possible, providing an acceptable fit to the data. In Study I, all the models were not nested and the usual likelihood-ratio test could not be applied to compare the models. Instead, the AIC (Akaike 1987) was calculated to obtain the resulting best-fitting model. The AIC was also used in Study II, since its use is recommended in large

models which have a high number of observations (Christensen et al. 2003). Final estimates of genetic and environmental effects were obtained from the most parsimonious and theoretically acceptable univariate (Study I) or multivariate (Study II) model.

5 RESULTS

5.1 Characteristics of participants (Studies I-V)

The baseline characteristics of the participants in the FITSA study are shown in Table 4. The proportion of MZ twins who smoked was significantly higher than that of DZ twins, while Sokolow Lyon voltage was higher in the DZ twins than MZ twins. Otherwise there were no significant differences in baseline characteristics by zygosity. The variances of the resting ECG variables were similar between MZ and DZ twins.

TABLE 4 Baseline characteristics, including resting electrocardiographic (ECG) variables, by zygosity in the Finnish Twin Study on Aging. Continuous variables are mean ± SD.

	MZ individuals (n=186)	DZ individuals (n=203)	Total (n=389)	p-value*
Age (years)	68.1±5.2	68.8±4.3	68.5±4.7	0.13
Height (cm)	158.0±8.7	159.1±6.8	158.6±7.9	0.19
Weight (kg)	69.6±15.3	70.6±14.7	70.1±15.0	0.52
Body mass index (kg/m^2)	28.0±6.3	27.9±5.7	28.0±5.9	0.87
Systolic blood pressure (mmHg)	149.4±24.7	149.4±25.4	149.4±25.1	0.99
Diastolic blood pressure (mmHg)	86.2±12.4	86.1±12.1	86.1±12.2	0.92
Total cholesterol (mmol/l)	5.5±1.0	5.7±1.1	5.6±1.0	0.13
Chronic diseases, yes (n)	125 (67%)	146 (72%)	271 (70%)	0.31
Use of beta-blockers, yes (n)	47 (25%)	63 (31%)	110 (28%)	0.21
Physical activity status, active (n)	136 (73%)	146 (72%)	282 (73%)	0.79
Smoking, current smoker (n)	32 (17%)	19 (9%)	51 (13%)	0.02
Heart rate (bpm)	69.2±14.3	68.2±11.3	68.7±12.8	0.42
PR interval (ms)	164.3±31.6	166.1±28.5	165.2±30.2	0.55
QRS duration (ms)	86.2±9.4	87.1±9.4	86.6±9.5	0.37
QT interval (ms)	395.3±35.2	398.5±32.1	397.0±33.5	0.35
QTc (ms)	411.3±26.3	413.6±23.4	412.5±24.9	0.38
P axis (degree)	46.2±21.3	48.2±22.4	47.2±21.9	0.35
QRS axis (degree)	27.7±34.4	30.5±29.4	29.2±32.0	0.40
T axis (degree)	43.1±36.8	45.5±31.8	44.4±34.3	0.49
Sokolow Lyon voltage† (mV)	2.2±0.8	2.4±0.7	2.3±0.8	0.02
Cornell voltage‡ (mV)	1.1±0.6	1.2±0.6	1.1±0.6	0.64
Cornell product§ (mV x ms)	98.9±56.6	102.5±57.7	100.8±57.2	0.54
ECG left ventricular mass** (g)	114.2±17.2	115.3±16.5	114.8±16.8	0.53
T wave amplitude, lead V_1 (mV)	0.01±0.14	-0.01±0.14	0.00±0.20	0.28
T wave amplitude, lead V_5 (mV)	0.17±0.14	0.20±0.14	0.18±0.20	0.07
T wave amplitude, lead II (mV)	0.15±0.14	0.17±0.14	0.16±0.20	0.06

* For the difference between monozygotic (MZ) and dizygotic (DZ) twin individuals.
† $SV_1 + RV_5$
‡ RaVL + SV_3
§ Cornell voltage (RaVL + SV_3) x QRS duration
** Calculated by the formula: [0.02 x (RaVL + SV_3)] + (1.12 x body weight) + 36.2 (women)

Table 5 presents both the baseline and follow-up characteristics of the TWINACTIVE participants. There were no significant differences in these characteristics between the active and inactive co-twins, except for the leisure time MET indices and VO_{2peak} which were significantly higher in the active co-twins. During the follow-up period, the active co-twins were on average 8.2±4.0 MET h/day more active than their inactive co-twins which is the equivalent of, for example, a 2-hour walk daily. Resting ECG variables are presented in Table 8.

TABLE 5 Baseline and follow-up characteristics of the TWINACTIVE study participants. Continuous variables are mean ± SD.

	Inactive co-twins (n=12)	Active co-twins (n=12)	p-value*
Baseline in 1975			
Age (years)	27 (range 18-35)		
Height† (cm)	176.9±8.9	175.1±10.6	0.33
Weight (kg)	71.0±17.8	66.8±9.8	0.36
Body mass index† (kg/m^2)	22.8±4.9	22.1±2.1	0.57
Leisure time MET index‡ (h/day)	0.1±0.2	3.1±2.4	0.002
Work-related physical activity (n)			0.51
Sedentary	3	5	
Standing or walking at work	2	3	
Light manual work	7	4	
Heavy manual work	0	0	
Follow-up in 2007			
Age (years)	59 (range 50-67)		
Height (cm)	174.8±9.9	173.2±10.4	0.32
Weight (kg)	82.3±20.1	73.0±12.8	0.09
Body mass index (kg/m^2)	26.7±3.9	24.2±2.3	0.08
Leisure time MET index (h/day)	1.2±1.1	9.4±3.8	< 0.001
VO_{2peak} (ml/kg/min)	28.1±3.7	34.4±4.9	0.003
Systolic blood pressure (mmHg)	145.0±27.2	141.6±19.0	0.66
Diastolic blood pressure (mmHg)	90.3±14.4	86.3±9.7	0.39
Use of beta-blockers (yes; n)	2	1	0.32
Work-related physical activity (n)			0.26
Sedentary	3	5	
Standing or walking at work	1	2	
Light manual work	4	0	
Heavy manual work	0	1	

* For the difference between the inactive and active co-twins.
† n=11
‡ Includes both leisure time physical activity and physical activity to and from work.

5.2 Genetic and environmental effects on resting electrocardiographic variables (Studies I and II)

Study I. Table 6 shows the ICCs for the MZ and DZ twin pairs. Except for QTc, the ICCs were higher in the MZ pairs than DZ pairs, suggesting the presence of additive genetic effects. There were also some variables the ICCs of which in the MZ pairs were more than twice that in the DZ pairs, suggesting the presence of dominance genetic effects in addition to additive genetic effects.

TABLE 6 Within-pair intra-class correlation coefficients (ICC) for resting electrocardiographic (ECG) variables in monozygotic (MZ) and dizygotic (DZ) twin pairs.

	MZ twin pairs (n=86)		DZ twin pairs (n=91)	
	ICC	**95% CI**	**ICC**	**95% CI**
Heart rate (bpm)	0.51	0.34-0.65	0.19	-0.01-0.38
PR interval (ms)	0.43	0.24-0.59	0.16	-0.05-0.36
QRS duration (ms)	0.35	0.15-0.53	0.11	-0.10-0.31
QT interval (ms)	0.21	0.00-0.40	0.15	-0.05-0.35
QTc (ms)	0.17	-0.04-0.37	0.19	-0.02-0.38
P axis (degree)	0.07	-0.15-0.28	0.05	-0.16-0.25
QRS axis (degree)	0.51	0.34-0.65	0.22	0.01-0.40
T axis (degree)	0.32	0.12-0.50	0.16	-0.05-0.35
Sokolow Lyon voltage* (mV)	0.59	0.44-0.72	0.18	-0.03-0.37
Cornell voltage† (mV)	0.53	0.36-0.67	0.36	0.17-0.53
Cornell product‡ (mV x ms)	0.53	0.36-0.67	0.36	0.17-0.53
ECG left ventricular mass§ (g)	0.61	0.46-0.73	0.42	0.24-0.58
T wave amplitude, lead V_1 (mV)	0.54	0.37-0.67	0.08	-0.13-0.28
T wave amplitude, lead V_5 (mV)	0.70	0.57-0.80	0.29	0.09-0.47
T wave amplitude, lead II (mV)	0.56	0.40-0.69	0.27	0.07-0.45

* $SV_1 + RV_5$

† RaVL + SV_3

‡ Cornell voltage (RaVL + SV_3) x QRS duration

§ Calculated by the formula: [0.02 x (RaVL + SV_3)] + (1.12 x body weight) + 36.2 (women)

The results of the univariate genetic modelling are presented in Table 7. The model of additive genetic effects and unique environmental effects (AE) was the best-fitting model for the majority of the resting ECG variables. The estimates of additive genetic effects ranged from 32% for the T axis to 72% for T wave amplitude in lead V_5. Dominance genetic effects were found for QRS duration, Sokolow Lyon voltage and T wave amplitude in lead V_1, and the estimates ranged between 36% and 57%. Common environmental effects contributed only to the variations of QT interval and QTc, whereas unique environmental effects contributed to the variation of each studied resting ECG variable.

TABLE 7 Standardized estimates (and 95% confidence intervals) of genetic and environmental effects on resting electrocardiographic (ECG) variables based on best-fitting quantitative genetic models.

	A	D	C	E
Heart rate (bpm)	0.48 (0.32-0.60)	-	-	0.52 (0.40-0.68)
PR interval (ms)	0.40 (0.23-0.55)	-	-	0.60 (0.45-0.77)
QRS duration (ms)	-	0.36 (0.17-0.51)	-	0.64 (0.49-0.83)
QT interval (ms)	-	-	0.19 (0.04-0.33)	0.81 (0.67-0.96)
QTc (ms)	-	-	0.19 (0.03-0.33)	0.81 (0.67-0.97)
P axis (degree)	-	-	-	1.00 (1.00-1.00)
QRS axis (degree)	0.43 (0.27-0.56)	-	-	0.57 (0.44-0.73)
T axis (degree)	0.32 (0.14-0.48)	-	-	0.68 (0.52-0.86)
Sokolow Lyon voltage* (mV)	-	0.57 (0.43-0.68)	-	0.43 (0.32-0.57)
Cornell voltage† (mV)	0.55 (0.40-0.67)	-	-	0.45 (0.33-0.60)
Cornell product‡ (mV x ms)	0.56 (0.41-0.67)	-	-	0.44 (0.33-0.59)
ECG left ventricular mass§ (g)	0.65 (0.52-0.74)	-	-	0.35 (0.26-0.48)
T wave amplitude, lead V_1 (mV)	-	0.53 (0.37-0.65)	-	0.47 (0.35-0.63)
T wave amplitude, lead V_5 (mV)	0.72 (0.61-0.80)	-	-	0.28 (0.20-0.39)
T wave amplitude, lead II (mV)	0.61 (0.46-0.71)	-	-	0.39 (0.29-0.54)

* $SV_1 + RV_5$

† RaVL + SV_3

‡ Cornell voltage (RaVL + SV_3) x QRS duration

§ Calculated by the formula: [0.02 x (RaVL + SV_3)] + (1.12 x body weight) + 36.2 (women)

A, additive genetic effect; D, dominance genetic effect; C, common environmental effect; E, unique environmental effect

Study II. Significant phenotypic correlations between the LVH indices (Cornell voltage, Cornell product) and T wave amplitudes (leads V_5, II) were observed, ranging from -0.28 between Cornell product and T wave amplitude in lead II to -0.31 between Cornell voltage and T wave amplitude in lead V_5. The ICCs of these four resting ECG variables (Table 6) suggested the presence of genetic effects, and subsequent univariate genetic modelling (Table 7) revealed that each variable was best explained by the model of additive genetic and unique environmental effects.

Multivariate genetic modelling showed that the common pathway model with two latent factors gave the most parsimonious explanation for the data (Figure 6). Both the LVH and RP factors were explained by additive genetic and unique environmental effects. The estimate of additive genetic effects was larger for RP (74%) than for LVH (16%). Common environmental effects also accounted for the variation of LVH (35%). The genetic correlation for additive genetic effects between the LVH and RP factors was -0.93, while the respective correlation for unique environmental effects was -0.05.

Trait-specific additive genetic effects and/or unique environmental effects were found for each measured resting ECG variable involved in the model, but their proportions of the overall variations were small (1 - 8%), except for trait-specific unique environmental effects on T wave amplitude in lead II (21%).

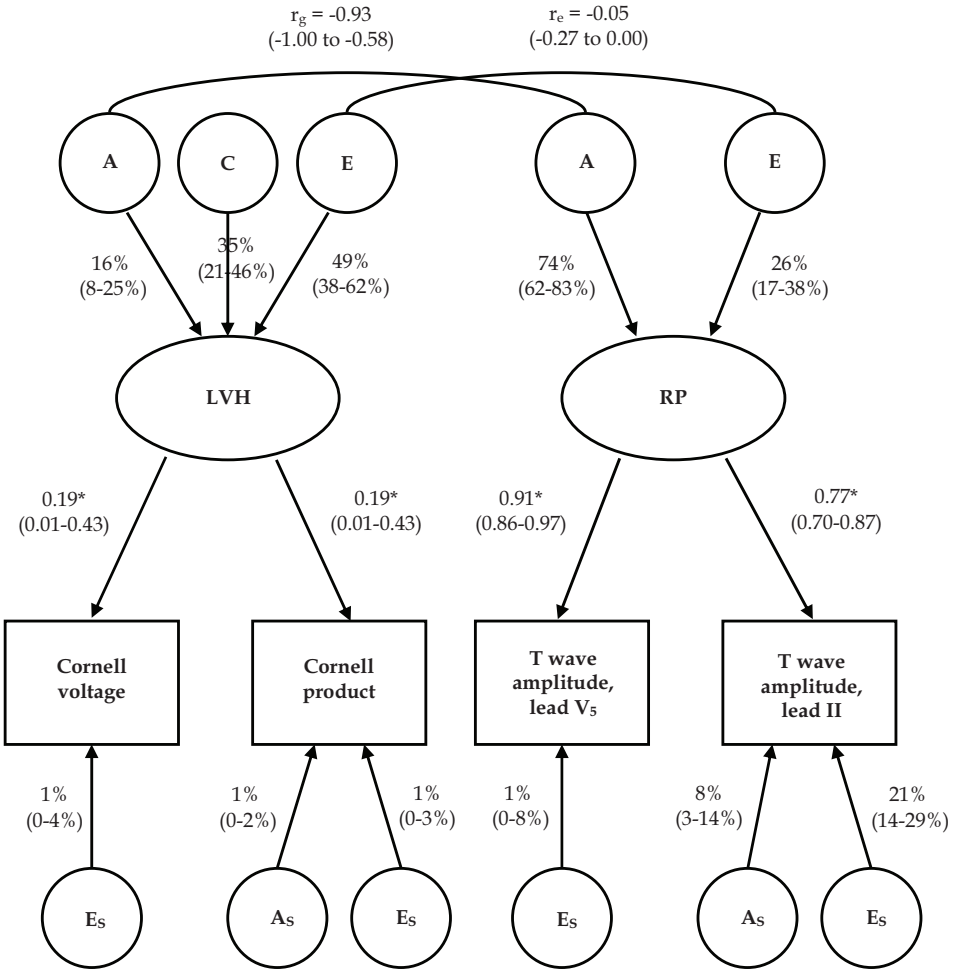


FIGURE 6 The best-fitting common pathway model for the electrocardiographic (ECG) left ventricular hypertrophy (LVH) and repolarisation phase (RP) factors which combines the variation from the measured LVH indices and T wave amplitudes. The percentages (95% confidence intervals) in the upper part of the figure are the proportions of the total variation of each latent factor explained by additive genetic (A), common environmental (C) or unique environmental (E) effects. The percentages (95% confidence intervals) in the lower part of the figure are the proportions of each measured resting ECG variable explained by trait-specific additive genetic (A_S) and unique environmental (E_S) effects. r_g and r_e refer to the correlation of additive genetic and unique environmental effects between the LVH and RP factors, respectively. The factor loadings of the LVH and RP factors on the measured resting ECG variables are shown with *. Cornell voltage = RaVL + SV_3; Cornell product = Cornell voltage x QRS duration.

5.3 Effects of physical activity on resting electrocardiographic variables (Study III)

Among all the twin pairs, the main adaptation to long-term LTPA was lowered resting heart rate (Table 8). At the end of the follow-up, the resting heart rate was 8.8 (95% CI 1.3 to 16.4) bpm lower in the active co-twins than in their inactive co-twins (p=0.03). QT interval was 5% longer in the active co-twins (p=0.02), but QTc was similar between the co-twins. There was a tendency for T wave amplitude in lead II to be higher (15%) in the active co-twins (p=0.08). No significant differences between the co-twins were observed in the other resting ECG variables.

Similar trends were observed when MZ and DZ pairs were studied separately (Table 8). Among the MZ pairs, the intra-pair differences were even larger than among all the pairs in some of the resting ECG variables, although they did not attain statistical significance (except for QT interval). Resting heart rate was 9.6 (95% CI -4.2 to 23.4) bpm lower in the active co-twins than inactive co-twins. The results for all the pairs as well as for the MZ pairs separately remained similar when an MZ pair discordant for use of beta-blockers was excluded.

TABLE 8 Follow-up results for resting electrocardiographic (ECG) variables in 12 twin pairs, whose within-pair difference in leisure time physical activity was 8.2±4.0 MET h/day. Values are mean ± SD.

	Inactive co-twins	Active co-twins	Mean difference (95% CI)	p-value*
All 12 pairs				
Heart rate (bpm)	68.0±10.2	59.2±5.1	8.8 (1.3 to 16.4)	0.03
RR interval (ms)	902.3±134.1	1011.7±80.1	-109.4 (-216.4 to -2.3)	0.05
P duration (ms)	91.8±15.8	103.8±12.1	-12.0 (-27.8 to 3.8)	0.12
PR interval (ms)	157.5±21.9	166.7±26.7	-9.2 (-21.2 to 2.8)	0.12
QRS duration (ms)	88.8±10.5	89.2±14.0	-0.4 (-9.5 to 8.8)	0.94
QT interval (ms)	400.7±25.6	422.0±18.2	-21.3 (-39.2 to -3.5)	0.02
QTc (ms)	414.6±21.1	420.3±14.9	-5.7 (-17.1 to 5.7)	0.29
P axis (degree)	49.3±18.3	45.9±16.4	3.4 (-7.0 to 13.8)	0.49
QRS axis (degree)	32.0±20.2	40.3±22.9	-8.3 (-31.7 to 15.0)	0.45
T axis (degree)	37.8±13.6	39.8±14.3	-2.0 (-15.1 to 11.1)	0.74
Sokolow Lyon voltage† (mV)	2.7±0.7	2.9±0.9	-0.2 (-1.0 to 0.7)	0.88
Cornell voltage‡ (mV)	1.2±0.5	1.5±0.6	-0.3 (-0.7 to 0.2)	0.22
Cornell product§ (mV x ms)	110.5±42.0	136.7±67.7	-26.2 (-64.8 to 12.6)	0.17
ECG left ventricular mass** (g)	135.7±26.8	124.2±17.8	11.5 (-2.4 to 25.3)	0.10
T wave amplitude, lead V_1 (mV)	0.08±0.20	0.11±0.12	-0.03 (-0.20 to 0.15)	0.75
T wave amplitude, lead V_5 (mV)	0.42±0.13	0.46±0.20	-0.04 (-0.13 to 0.06)	0.42
T wave amplitude, lead II (mV)	0.26±0.06	0.30±0.09	-0.04 (-0.09 to 0.01)	0.08
5 MZ pairs				
Heart rate (bpm)	69.6±5.1	60.0±6.9	9.6 (-4.2 to 23.4)	0.13
RR interval (ms)	858.8±83.4	997.6±102.5	-138.8 (-363.0 to 85.4)	0.16
P duration (ms)	86.4±19.7	110.8±13.0	-24.4 (-60.4 to 11.6)	0.13
PR interval (ms)	158.4±26.2	167.6±27.8	-9.2 (-31.5 to 13.1)	0.32
QRS duration (ms)	90.8±16.0	88.4±13.7	2.4 (-13.6 to 18.4)	0.70
QT interval (ms)	394.8±25.3	424.4±24.6	-29.6 (-44.6 to -14.6)	0.01
QTc (ms)	415.0±30.4	424.7±18.2	-9.7 (-34.5 to 15.0)	0.34
P axis (degree)	49.6±5.5	39.6±14.1	10.0 (-2.3 to 22.3)	0.09
QRS axis (degree)	34.8±6.6	36.2±25.5	-1.4 (-28.3 to 25.5)	0.89
T axis (degree)	42.8±15.6	39.2±3.7	3.6 (-15.1 to 22.3)	0.62
Sokolow Lyon voltage† (mV)	2.8±0.7	2.9±0.6	-0.1 (-1.3 to 1.1)	0.89
Cornell voltage‡ (mV)	1.1±0.5	1.5±0.7	-0.4 (-1.4 to 0.6)	0.32
Cornell product§ (mV x ms)	102.0±44.9	141.0±75.2	-39.0 (-126.5 to 48.6)	0.29
ECG left ventricular mass** (g)	132.1±15.6	133.0±18.4	-0.9 (-12.8 to 11.2)	0.86
T wave amplitude, lead V_1 (mV)	0.10±0.27	0.12±0.14	-0.02 (-0.42 to 0.39)	0.93
T wave amplitude, lead V_5 (mV)	0.37±0.16	0.37±0.15	0.00 (-0.17 to 0.17)	1.00
T wave amplitude, lead II (mV)	0.23±0.07	0.27±0.07	-0.04 (-0.12 to 0.04)	0.21

(continues)

TABLE 8 (continues)

	Inactive co-twins	Active co-twins	Mean difference (95% CI)	p-value*
7 DZ pairs				
Heart rate (bpm)	66.9±13.0	58.6±4.0	8.3 (-4.0 to 20.6)	0.15
RR interval (ms)	933.4±160.1	1021.7±67.0	-88.3 (-245.9 to 69.3)	0.22
P duration (ms)	95.7±12.5	98.9±9.3	-3.1 (-20.7 to 14.4)	0.68
PR interval (ms)	156.9±20.5	166.0±28.1	-9.1 (-28.6 to 10.3)	0.29
QRS duration (ms)	87.4±4.9	89.7±15.3	-2.3 (-17.1 to 12.6)	0.72
QT interval (ms)	404.9±26.9	420.3±14.0	-15.4 (-48.1 to 17.3)	0.29
QTc (ms)	414.4±14.2	417.2±12.5	-2.8 (-18.9 to 13.2)	0.68
P axis (degree)	49.1±24.4	50.4±17.4	-1.3 (-18.9 to 16.4)	0.86
QRS axis (degree)	30.0±26.6	43.3±22.5	-13.3 (-55.7 to 29.1)	0.47
T axis (degree)	34.1±11.9	40.1±19.1	-6.0 (-28.5 to 16.5)	0.54
Sokolow Lyon voltage† (mV)	2.7±0.7	2.9±1.1	-0.2 (-1.6 to 1.3)	0.87
Cornell voltage‡ (mV)	1.3±0.5	1.5±0.6	-0.2 (-0.6 to 0.4)	0.56
Cornell product§ (mV x ms)	116.6±42.2	133.6±67.9	-17.0 (-69.7 to 35.7)	0.46
ECG left ventricular mass** (g)	138.2±33.7	118.0±15.8	20.2 (-2.4 to 42.7)	0.07
T wave amplitude, lead V_1 (mV)	0.07±0.16	0.10±0.12	-0.03 (-0.27 to 0.20)	0.74
T wave amplitude, lead V_5 (mV)	0.45±0.10	0.52±0.22	-0.07 (-0.21 to 0.09)	0.35
T wave amplitude, lead II (mV)	0.27±0.05	0.32±0.10	-0.05 (-0.13 to 0.04)	0.25

* For the difference between the inactive and active co-twins.
† $SV_1 + RV_5$
‡ RaVL + SV_3
§ Cornell voltage (RaVL + SV_3) x QRS duration
** Calculated by the formulas: [0.02 x (RaVL + SV_3)] + (1.12 x body weight) + 36.2 (women) and [0.026 x (RaVL + SV_3)] + (1.25 x body weight) + 34.4 (men)
MZ, monozygotic; DZ, dizygotic

5.4 Association of resting electrocardiographic variables with walking endurance and all-cause mortality (Studies IV and V)

Study IV. Participants walked on average 533±75 m during 6MWT. Resting ECG variables as cross-sectional predictors of 6MWD (individual-based analyses) are presented in Table 9. As a whole, LVH indices, QRS axis and T wave amplitudes significantly explained the variation in 6MWD. Specifically, the best predictors of long walking distance were low ECG LVM and Cornell voltage, and high T wave amplitudes in leads V_5 and II. The increase in the explanation rates of these variables in addition to age and use of beta-blockers ranged from 0.030 to 0.106. The role of low ECG LVM and high T wave amplitudes (leads V_5 and II) as predictors of long walking distance was enhanced when only participants with high systolic blood pressure (≥ 160 mmHg, n=102) were studied: the explanation rates were 0.150, 0.093 and 0.058, respectively.

TABLE 9 Resting electrocardiographic (ECG) variables as predictors of six-minute walking distance.

	Regression coefficient (95% CI)	p-value	R^{2*}	$R^{2\dagger}$
Heart rate (bpm)	-0.4 (-1.3 to 0.4)	0.32	0.068	0.004
PR interval (ms)	-0.3 (-0.6 to 0.1)	0.10	0.072	0.008
QRS duration (ms)	0.2 (-0.9 to 1.3)	0.73	0.065	0.001
QT interval (ms)	0.0 (-0.3 to 0.3)	1.00	0.064	0.000
QTc (ms)	-0.2 (-0.5 to 0.2)	0.29	0.067	0.003
P axis (degree)	0.4 (0.0 to 0.8)	0.06	0.076	0.012
QRS axis (degree)	0.4 (0.0 to 0.7)	0.04	0.079	0.015
T axis (degree)	0.1 (-0.2 to 0.3)	0.62	0.065	0.001
Sokolow Lyon voltage‡ (mV)	16.9 (5.0 to 28.9)	0.01	0.088	0.024
Cornell voltage§ (mV)	-26.0 (-43.4 to -8.6)	0.004	0.094	0.030
Cornell product** (mV x ms)	-0.2 (-0.4 to -0.1)	0.01	0.087	0.023
ECG left ventricular mass†† (g)	-1.8 (-2.4 to -1.2)	< 0.001	0.170	0.106
T wave amplitude, lead V_1 (mV)	46.6 (-44.2 to 137.4)	0.31	0.068	0.004
T wave amplitude, lead V_5 (mV)	105.1 (49.3 to 160.9)	< 0.001	0.109	0.045
T wave amplitude, lead II (mV)	129.6 (57.6 to 201.7)	< 0.001	0.101	0.037

* Cumulative explanation rate for resting ECG variable, age and use of beta-blockers.
† Increase in explanation rate when explanation rate of age and use of beta-blockers has been subtracted from explanation rate of resting ECG variable, age and use of beta-blockers; explanation rate of age and use of beta-blockers = 0.064.
‡ $SV_1 + RV_5$
§ RaVL + SV_3
** Cornell voltage (RaVL + SV_3) x QRS duration
†† Calculated by the formula: [0.02 x (RaVL + SV_3)] + (1.12 x body weight) + 36.2 (women)

The 6MWD of the worse walking MZ co-twins was 451.6±97.0 m and that of the better walking co-twins 577.2±65.4 m (mean difference -125.6 m, $p < 0.001$, range 75 - 240 m). The resting ECG variables that differed significantly between the co-twins (within-pair analyses) were ECG LVM and T wave amplitude in lead V_5 (Table 10). ECG LVM was lower and T wave amplitude in lead V_5 higher in the better walking co-twins. There was also a tendency for Sokolow Lyon voltage and Cornell voltage to be lower and P axis to be more vertical in the better walking co-twins. The results remained similar when 5 MZ pairs discordant for the use of beta-blockers were excluded.

TABLE 10 Resting electrocardiographic (ECG) variables in worse and better walking monozygotic co-twins whose within-pair difference in six-minute walking distance is ≥ 75 m. Values are mean ± SD.

	Worse walking co-twins (n=16)	Better walking co-twins (n=16)	Mean difference (95% CI)	p-value*
Heart rate (bpm)	73.4±9.9	71.3±12.1	2.1 (-4.0 to 8.3)	0.46
PR interval (ms)	152.0±17.8	153.0±25.7	-1.0 (-15.3 to 13.3)	0.88
QRS duration (ms)	85.3±9.5	84.0±10.3	1.3 (-4.2 to 6.7)	0.63
QT interval (ms)	390.3±22.2	383.0±37.9	7.3 (-14.8 to 29.3)	0.89
QTc (ms)	414.6±18.1	403.0±25.5	11.6 (-8.6 to 31.9)	0.28
P axis (degree)	43.1±25.5	56.2±16.8	-13.1 (-28.3 to 2.0)	0.09
QRS axis (degree)	26.6±27.2	34.4±31.6	-7.8 (-20.4 to 4.7)	0.20
T axis (degree)	46.7±18.4	48.3±38.2	-1.6 (-18.0 to 14.9)	0.84
Sokolow Lyon voltage† (mV)	2.3±0.7	2.0±0.6	0.3 (0.0 to 0.6)	0.07
Cornell voltage‡ (mV)	1.2±0.5	1.0±0.5	0.2 (0.0 to 0.4)	0.09
Cornell product§ (mV x ms)	102.7±44.7	88.4±52.9	14.3 (-5.9 to 34.3)	0.15
ECG left ventricular mass** (g)	117.7±10.4	111.4±9.6	6.3 (2.1 to 10.5)	0.01
T wave amplitude, lead V_1 (mV)	0.02±0.09	0.01±0.07	0.01 (-0.04 to 0.06)	0.69
T wave amplitude, lead V_5 (mV)	0.16±0.12	0.23±0.16	-0.07 (-0.13 to -0.01)	0.03
T wave amplitude, lead II (mV)	0.15±0.10	0.19±0.13	-0.04 (-0.10 to 0.01)	0.10

* For the difference between the worse and better walking co-twins.
† $SV_1 + RV_5$
‡ RaVL + SV_3
§ Cornell voltage (RaVL + SV_3) x QRS duration
** Calculated by the formula: [0.02 x (RaVL + SV_3)] + (1.12 x body weight) + 36.2 (women)

Study V. The mean length of the follow-up until death or the end of the follow-up was 8.4±1.2 years. During that time, 33 of the participants (8.5%) died. Crude mortality rates per 1000 person-years in each resting ECG variable tertile are presented in Table 11. Mortality was significantly higher in the highest T axis tertile compared with the lowest tertile and the same was true for the highest Cornell voltage tertile when compared with the middle tertile. Among the other variables there were no significant differences in mortality.

In the individual-based Cox regression models, the best predictors of all-cause mortality were heart rate and T axis (Table 11). In the analyses adjusted for age, physical activity and use of beta-blockers, the middle heart rate tertile had only a mildly increased risk for all-cause mortality [hazard ratio (HR) 1.24, 95% confidence interval (CI) 0.50-3.06], whereas the highest tertile had an almost 2.5-fold risk (HR 2.48, 95% CI 0.95-6.44), compared with the lowest tertile. The risk for all-cause mortality was very similar between the two lowest T axis tertiles, while those in the highest tertile had a twofold risk for mortality (HR 2.01, 95% CI 0.82-4.89) compared with those in the lowest tertile. The trends remained similar after adjustments for other potential confounders (BMI, systolic and diastolic blood pressure, total cholesterol, chronic diseases, smoking).

The pairwise Cox regression models did not reveal congruent trends in the results compared with the individual-based results. The associations between the resting ECG variable tertiles and all-cause mortality both strengthened, weakened and remained unchanged. Among all the twin pairs, when adjusted for physical activity and use of beta-blockers, the association between the highest heart rate tertile and all-cause mortality strengthened (HR 7.89, 95% CI 0.60-103.09), while the converse was true for the highest T axis tertile (HR 1.41, 95% CI 0.33-6.09).

TABLE 11 Associations between resting electrocardiographic (ECG) variables and all-cause mortality. Individual-based Cox regression models for risk of death in each tertile.

	Person years	Deaths	Rate (‰)	Hazard ratio (95% confidence interval)			
				Model 1	Model 2	Model 3	Model 4
Heart rate							
≤ 62 bpm	1055	7	6.6	1.00	1.00	1.00	1.00
63-72 bpm	1163	10	8.6	1.30 (0.53-3.20)	1.24 (0.50-3.07)	1.17 (0.48-2.89)	1.24 (0.50-3.06)
≥ 73 bpm	1050	16	15.2	2.32 (0.93-5.79)	2.22 (0.89-5.54)	2.24 (0.91-5.54)	2.48 (0.95-6.44)
PR interval							
≤ 152 ms	1131	8	7.1	1.00	1.00	1.00	1.00
153-172 ms	1048	11	10.5	1.49 (0.59-3.77)	1.59 (0.62-4.10)	1.54 (0.61-3.91)	1.54 (0.61-3.89)
≥ 173 ms	1058	13	12.3	1.74 (0.71-4.25)	1.43 (0.60-3.42)	1.39 (0.59-3.26)	1.39 (0.59-3.28)
QRS duration							
≤ 80 ms	936	12	12.8	1.00	1.00	1.00	1.00
81-88 ms	1323	13	9.8	0.76 (0.36-1.62)	0.74 (0.36-1.53)	0.78 (0.38-1.61)	0.78 (0.38-1.62)
≥ 89 ms	1009	8	7.9	0.62 (0.25-1.54)	0.60 (0.24-1.50)	0.66 (0.26-1.66)	0.66 (0.26-1.66)
QT interval							
≤ 380 ms	1004	13	12.9	1.00	1.00	1.00	1.00
381-404 ms	1098	11	10.0	0.77 (0.34-1.73)	0.72 (0.33-1.60)	0.69 (0.31-1.51)	0.68 (0.30-1.51)
≥ 405 ms	1166	9	7.7	0.59 (0.24-1.48)	0.55 (0.22-1.34)	0.52 (0.21-1.29)	0.51 (0.21-1.28)
QTc							
≤ 403 ms	1084	13	12.0	1.00	1.00	1.00	1.00
404-419 ms	1105	9	8.1	0.68 (0.29-1.59)	0.69 (0.30-1.57)	0.66 (0.29-1.50)	0.67 (0.29-1.51)
≥ 420 ms	1080	11	10.2	0.85 (0.36-1.96)	0.83 (0.36-1.93)	0.75 (0.31-1.79)	0.75 (0.31-1.82)
P axis							
≤ 42 degree	1103	15	13.6	1.00	1.00	1.00	1.00
43-58 degree	1056	9	8.5	0.61 (0.28-1.35)	0.70 (0.31-1.58)	0.72 (0.32-1.62)	0.72 (0.32-1.63)
≥ 59 degree	1079	8	7.4	0.52 (0.23-1.17)	0.58 (0.26-1.32)	0.59 (0.26-1.34)	0.59 (0.26-1.37)

(continues)

TABLE 11 (continues)

	Person years	Deaths	Rate (‰)	Hazard ratio (95% confidence interval)			
				Model 1	Model 2	Model 3	Model 4
QRS axis							
≤ 16 degree	1090	10	9.2	1.00	1.00	1.00	1.00
17-44 degree	1126	8	7.1	0.77 (0.31-1.91)	0.90 (0.36-2.26)	0.81 (0.32-2.05)	0.80 (0.32-2.02)
≥ 45 degree	1053	15	14.2	1.56 (0.67-3.64)	1.96 (0.85-4.53)	1.81 (0.78-4.22)	1.85 (0.78-4.39)
T axis							
≤ 39 degree	1217	9	7.4	1.00	1.00	1.00	1.00
40-57 degree	1082	8	7.4	1.00 (0.37-2.71)	1.06 (0.39-2.86)	1.03 (0.39-2.73)	1.02 (0.38-2.74)
≥ 58 degree	969	16	16.5	2.25 (0.94-5.36)	2.02 (0.85-4.82)	1.95 (0.83-4.62)	2.01 (0.82-4.89)
Sokolow Lyon voltage*							
≤ 1.97 mV	1057	11	10.4	1.00	1.00	1.00	1.00
1.98-2.50 mV	1102	13	11.8	1.13 (0.50-2.55)	1.16 (0.51-2.63)	1.19 (0.52-2.72)	1.19 (0.52-2.71)
≥ 2.51 mV	1083	9	8.3	0.80 (0.32-1.97)	0.83 (0.34-2.02)	0.84 (0.35-2.00)	0.84 (0.35-2.00)
Cornell voltage†							
≤ 0.95 mV	1192	13	10.9	1.00	1.00	1.00	1.00
0.96-1.40 mV	1136	7	6.2	0.56 (0.21-1.49)	0.57 (0.21-1.52)	0.63 (0.23-1.74)	0.63 (0.23-1.75)
≥ 1.41 mV	854	13	15.2	1.39 (0.61-3.21)	1.11 (0.49-2.53)	1.23 (0.53-2.85)	1.23 (0.53-2.85)
Cornell product‡							
≤ 73.60 mV x ms	962	11	11.4	1.00	1.00	1.00	1.00
73.61-120.00 mV x ms	1292	8	6.2	0.54 (0.20-1.44)	0.56 (0.20-1.56)	0.62 (0.22-1.75)	0.62 (0.22-1.77)
≥ 120.01 mV x ms	980	14	14.3	1.25 (0.52-2.98)	1.01 (0.43-2.38)	1.11 (0.47-2.67)	1.12 (0.47-2.67)
ECG LVM§							
≤ 107.78 g	1061	13	12.3	1.00	1.00	1.00	1.00
107.79-120.12 g	1078	11	10.2	0.83 (0.35-1.99)	0.87 (0.36-2.12)	0.85 (0.36-2.02)	0.83 (0.34-2.03)
≥ 120.13 g	1103	9	8.2	0.66 (0.27-1.64)	0.73 (0.29-1.82)	0.66 (0.26-1.68)	0.64 (0.24-1.70)

(continues)

TABLE 11 (continues)

	Person years	Deaths	Rate (‰)	Hazard ratio (95% confidence interval)			
				Model 1	Model 2	Model 3	Model 4
T wave amplitude, lead V_1							
≤ -0.05 mV	1460	14	9.6	1.00	1.00	1.00	1.00
-0.04 to 0.05 mV	1122	10	8.9	0.93 (0.39-2.18)	0.98 (0.43-2.27)	1.02 (0.45-2.31)	1.05 (0.44-2.47)
≥ 0.06 mV	687	9	13.1	1.34 (0.60-3.11)	1.39 (0.64-3.02)	1.45 (0.69-3.05)	1.48 (0.69-3.18)
T wave amplitude, lead V_5							
≤ 0.10 mV	1248	14	11.2	1.00	1.00	1.00	1.00
0.11-0.25 mV	1224	9	7.4	0.65 (0.25-1.68)	0.69 (0.27-1.75)	0.65 (0.25-1.68)	0.66 (0.25-1.70)
≥ 0.26 mV	788	10	12.7	1.13 (0.53-2.43)	1.22 (0.57-2.58)	1.33 (0.64-2.77)	1.33 (0.64-2.77)
T wave amplitude, lead II							
≤ 0.10 mV	1319	14	10.6	1.00	1.00	1.00	1.00
0.11-0.20 mV	1061	11	10.4	0.98 (0.42-2.26)	0.97 (0.41-2.25)	0.94 (0.40-2.19)	0.95 (0.40-2.23)
≥ 0.21 mV	889	8	9.0	0.85 (0.36-1.99)	0.91 (0.39-2.09)	1.03 (0.45-2.36)	1.03 (0.45-2.36)

Model 1: No adjustments
Model 2: Adjusted for age
Model 3: Adjusted for age and physical activity status
Model 4: Adjusted for age, physical activity status and use of beta-blockers
* $RV_5 + SV_1$
† $RaVL + SV_3$
‡ $(RaVL + SV_3) \times$ QRS duration
§ $(0.02 \times [RaVL + SV_3]) + (1.12 \times$ body weight) + 36.2 (women)
LVM, left ventricular mass

6 DISCUSSION

The present study investigated the effects of genetic and environmental factors and long-term LTPA on resting ECG variables in older people using quantitative genetic modelling and a co-twin control design. In addition, the associations of the resting ECG variables with walking endurance and all-cause mortality were studied using both individual-based analyses and the co-twin control design.

The majority of the resting ECG variables were moderately to highly (32-72%) affected by genetic factors, the remainder of the variation being explained by environmental factors. Genetic effects were especially strong for the LVH indices, T wave amplitudes and resting heart rate. The majority of the genetic factors were shared between the LVH indices and T wave amplitudes (genetic correlation -0.93), while environmental factors were mainly distinct for these two traits (environmental correlation -0.05), suggesting that the association established between LVH and repolarisation abnormalities mainly originates from genetic factors. The main ECG adaptation to long-term LTPA after controlling for genetic liability and childhood environment was lowering of the resting heart rate. The resting heart rate was also the strongest predictor of increased all-cause mortality. The best ECG predictors of walking endurance were the LVH indices and T wave amplitudes.

When comparing the present results with the findings of the previous studies (discussed below), it should be noted that in the vast majority of the previous studies, the authors have not reported the length of the rest period prior to conducting the ECG recording. The duration of the rest period may have effects on the ECG results.

6.1 Genetic and environmental effects on resting electrocardiographic variables

In the present study, genetic effects were especially strong for T wave amplitudes (53-72%), ECG LVM (65%) and the LVH indices (55-57%). Also, resting heart rate was rather strongly affected by genetic factors (48%). This study is one of the first studies to quantify the genetic and environmental effects on T wave amplitudes. The strong genetic components of T wave amplitudes were somewhat unexpected in the light of the previous results reported for QTc interval (about 0-50%) (Havlik et al. 1980, Russell et al. 1998, Busjahn et al. 1999, Friedlander et al. 1999, Carter et al. 2000, Hong et al. 2001, Newton-Cheh et al. 2005, Dalageorgou et al. 2008) which also includes the ventricular repolarisation phase. On the other hand, QTc interval measures the time spent on ventricular depolarisation and repolarisation, and not the amplitudes of the QRS complex or T waves which may reflect different aspects of depolarisation and repolarisation.

The genetic effects on ECG LVM and LVH indices in the present study (55-65%) were considerably greater than those reported for the same indices (12-41%) in the family study by Mayosi et al. (2002) among middle-aged (mean age 52 years) men and women. The discrepancies between the results may be due to differences in the study design (twin vs. family study), statistical methods and/or populations studied (older women vs. middle-aged men and women). However, the results of the present study are in accordance with the results of a previous twin study (female and male twins, aged 25-79 years) which reported that genetic effects amounted to 59% for echocardiographic LVM. For women only, the respective estimate was 47%. (Sharma et al. 2006.) The results of the present study are also in line with those of the previous twin studies in relation to resting heart rate, where the estimates of genetic effects on resting heart rate were only slightly higher (54-64%) (Havlik et al. 1980, Jedrusik et al. 2003, Snieder et al. 2003, Dalageorgou et al. 2008). A recently published family study found a smaller estimate (21%) for genetic effects (Li et al. 2009).

In the multivariate analysis, genetic effects accounted for 16% for ECG LVH (formed from Cornell voltage and Cornell product) and 74% for RP (T wave amplitudes in leads V_5 and II). The latter finding confirmed the results of the univariate analysis, i.e., T wave amplitudes, and therefore the RP, are under strong genetic control. On the other hand, differences in the results were found for ECG LVH. Genetic effects on single LVH indices were greater than observed for the LVH factor in the multivariate analysis. However, in the multivariate analysis, common environmental effects were found for LVH, whereas they were not detected in the univariate analysis for single LVH indices. One possible reason for this may be that multivariate analysis, because it takes into account all the available information, has higher statistical power to discriminate common environmental effects from additive genetic effects. The genetic correlation between ECG LVH and RP was very high (-0.93) while the

unique environmental correlation was low (-0.05). These results suggest that, to a large extent, the same genes operate in both cases, while the environmental effects are mainly distinct for these two traits. The negative genetic correlation indicates that while some genes may increase the degree of LVH, they may lower T wave amplitudes, i.e., lead to repolarisation abnormalities. These genes may include genes that cause heritable hypertrophic disorders (e.g., hypertrophic cardiomyopathy) and genes that otherwise contribute to the development of LVH. However, it should be remembered that LVH is not always associated with repolarisation abnormalities. LVH is usually associated with repolarisation abnormalities when it is pathological, such as when it is the result of hypertrophic cardiomyopathy, hypertension or aortic stenosis, but not in the case of physiologically induced LVH, such as observed in highly trained athletes (Moore et al. 1984).

Despite the rather strong genetic effects found for some resting ECG variables, environmental effects were also important, explaining 28-100% of the variation in the univariate analyses. Rather similar results were observed for ECG LVH and RP in the multivariate analysis. These are in large part in accordance with the results of the previous studies. Using quantitative genetic modelling, the environmental factors which affect certain resting ECG variables cannot be specified, but some suggestions can be made on the basis of existing knowledge. These factors include, for example, cardiovascular diseases and medications used to treat these diseases, obesity (Fraley et al. 2005) and physical activity (Fagard 2003).

6.2 Effects of physical activity on resting electrocardiographic variables

The effect of physical activity or training on cardiac structure and function is a widely studied area. Many case-control studies have been conducted in athletes and sedentary controls, longitudinal studies being less numerous. The present study, along with the other previously published studies, showed that the proportions of the variation in the studied resting ECG variables that were explained by genetic factors are moderate to high. These results suggest that some of the differences observed in resting ECG variables (and other characteristics of the heart) between athletes and sedentary controls may be due to genetic factors. Due to their inherited traits, for example larger hearts, some people may be more prone to participate in athletic activities and other vigorous physical activity, since it may be easier for them than for persons with smaller hearts. In case-control studies this may cause bias in the interpretation of the results between athletes and controls. Similar problems may also arise in longitudinal studies conducted in unrelated individuals, since genetic factors may modify the myocardial response to physical training. For example, the angiotensinogen gene M235T (Karjalainen et al. 1999) and angiotensin-

converting enzyme gene I/D polymorphisms (Montgomery et al. 1997, Tanriverdi et al. 2005) have been reported to be associated with the variability in LVM induced by physical training. Therefore, the co-twin control study offers an ideal opportunity to take into account the effects of genetic factors, since MZ co-twins are genetically identical at the chromosomal sequence level with the rare exception of somatic mutations. Epigenetic differences between MZ co-twins, however, may exist (Fraga et al. 2005).

In the present study, the main ECG adaptation to long-term LTPA was lowering of the resting heart rate. The present and previous studies (Havlik et al. 1980, Jedrusik et al. 2003, Snieder et al. 2003, Dalageorgou et al. 2008, Li et al. 2009) have reported moderate to high estimates of genetic effects (about 20-65%) on resting heart rate, the estimates of environmental effects ranging from 35 to 80%. These findings, on the one hand, highlight the importance of the use of a co-twin control design to control for genetic factors, but on the other hand, demonstrate that the environmental factors also are important in explaining the variation in resting heart rate. The present study confirmed the previous findings that physical activity is an important environmental factor affecting resting heart rate. The heart rate of the active co-twins was almost 10 bpm lower than the heart rate of their inactive co-twins. Similar or even larger differences have been observed between athletes and sedentary controls (e.g., Beckner & Winsor 1954, van Ganse et al. 1970, George et al. 1995, Stolt et al. 1997). However, a twin study on young male MZ twins discordant for physical activity found no difference between more and less active co-twins (Hannukainen et al. 2005). In addition to age and difference in the duration of physical activity discordance, one possible explanation for this discrepancy between the present and previous twin study may be the differences in the sports disciplines performed. In the present study, the twins mainly engaged in endurance type sport disciplines, such as walking and jogging, while the twins in the previous study participated widely also in strength training and ball games.

Other resting ECG variables, including LVH indices and T wave amplitudes, did not differ significantly between the active and inactive co-twins. This is contrary to the results of the previous studies. Higher Sokolow Lyon voltages (van Ganse et al. 1970, Parker et al. 1978, Douglas et al. 1988, Northcote et al. 1989, Bjørnstad et al. 1991) and Cornell voltages (Stolt et al. 1997) as well as T wave amplitudes (van Ganse et al. 1970, Bjørnstad et al. 1991) have been reported for athletes compared with sedentary or less active controls, while other adaptations include for instance prolonged PR interval (Sharma et al. 1999) and a more vertical QRS axis (Parker et al. 1978, Bjørnstad et al. 1991). There are a number of possible reasons for the discrepancy between the present and previous studies. Firstly, moderate intensity physical activity may not be enough to induce adaptations of these kinds in the heart. Secondly, these "adaptations" may not be revealed when genetic factors and childhood environment are taken into account. However, this does not mean that physical training has not contributed to the differences observed in the previous athlete

studies since the intensity of training in athletes is significantly greater than in the twins in the present study and often also includes differences in training during childhood and adolescence. Thirdly, the small sample size may have led to lack of statistical power to detect differences between the co-twins. The results of the present study also differ from those of the twin study by Hannukainen et al. (2005) which reported significant differences between the more and less active male MZ co-twins in, for example, Cornell voltage.

Although significant differences in the other resting ECG variables were not detected, it is nevertheless very important that the effects of long-term LTPA can be seen at least in resting heart rate after adjustment for genetic factors. The lower resting heart rate observed in the active co-twins may have many health-related advantages. For example, previous studies have shown that a higher resting heart rate is associated with increased risk for cardiovascular and all-cause mortality (Dyer et al. 1980, Kannel et al. 1987, Gillum et al. 1991, Shaper et al. 1993, Mensink & Hoffmeister 1997, Reunanen et al. 2000), and similar results were also observed in the present study with regard to all-cause mortality (see later). Accordingly, lower resting heart rate caused by physical activity may decrease the risk for death, and therefore a physically active lifestyle can be recommended. Low physical activity by itself is also a predictor for cardiovascular and all-cause mortality (Barengo et al. 2004). The amount and/or intensity of physical activity at which the lowering of resting heart rate was observed in the present study is realistically achievable for normal, healthy people.

6.3 Association of resting electrocardiographic variables with walking endurance and all-cause mortality

The best ECG predictor of walking endurance was ECG LVM, followed by T wave amplitudes (leads V_5 and II) and LVH indices. The explanation rate was relatively high (11%) for ECG LVM, but rather small (2-5%) for T wave amplitudes and LVH indices. Body weight is needed for the calculation of ECG LVM, and thus body weight may at least partly explain the greater explanation rate for ECG LVM compared with LVH indices (and also with T wave amplitudes). Body weight alone explained 9% of the variation in 6MWD.

The observed results are logical. Higher LVH indices (except for Sokolow Lyon voltage) were associated with shorter 6MWD, whereas higher T wave amplitudes were associated with longer 6MWD. These individual-based results were confirmed in the within-pair analyses which suggested that the observed associations are independent of genetic factors. ECG LVM was significantly higher in the worse walking MZ co-twins and T wave amplitude in lead V_5 was significantly higher in the better walking MZ co-twins. Similar trends, although non-significant, were observed for the other LVH indices and T wave amplitudes.

If pathological, LVH is usually associated with systolic and diastolic dysfunction which in turn may lead to decreased exercise capacity (Olsen et al. 2001, Pierson et al. 2004) and subsequently to shorter 6MWD. Echocardiographic measurements were not performed in the FITSA study to determine whether systolic and/or diastolic abnormalities were present. The above-mentioned mechanism may still be probable, since on the one hand there were no highly-training athletes in the FITSA study (who might have had physiological LVH) and on the other hand 33% of these participants had hypertension.

Positive (high) T wave is usually a sign of normal ventricular repolarisation while a low or negative (inverted) T wave may be a sign of disturbed repolarisation and reduced cardiac function, and accordingly, can be associated with reduced exercise capacity. This may partly explain why lower T wave amplitudes were associated with shorter 6MWD or higher amplitudes with longer 6MWD. The higher T wave amplitudes seen in the better walking subjects may also be explained by their higher level of physical activity or physical fitness which contributed to their better 6MWT performance. Previous studies have reported that the T wave amplitudes are higher in athletes compared with sedentary controls (e.g., Bjørnstad et al. 1991) and in persons with a higher fitness level compared with persons whose fitness level is lower (Bjørnstad et al. 1993). A trend toward higher T wave amplitude (lead II) in the physically active subjects was also observed in the present study.

Somewhat unexpectedly, resting heart rate was not significantly associated with 6MWD in the present study. Persons with good physical fitness usually have lower resting heart rates compared with persons whose fitness level is lower; accordingly, it could have been expected that lower heart rate would be more strongly associated with longer 6MWD. The present result is in contrary to the findings of a previous study which reported that in obese subjects, resting heart rate significantly predicted 6MWD (Evers Larsson & Reynisdottir 2008).

However, resting heart rate was the strongest predictor of all-cause mortality in the present study. Higher heart rate was associated with increased risk for death which is in accordance with the results of the previous studies (Dyer et al. 1980, Kannel et al .1987, Gillum et al. 1991, Mensink & Hoffmeister 1997). High resting heart rates have also been reported to be associated with increased risk for cardiovascular death (Kannel et al. 1987, Gillum et al. 1991, Shaper et al. 1993, Mensink & Hoffmeister 1997, Hsia et al. 2009). Unfortunately, it was not meaningful to perform cause-specific mortality analyses in the present study due to the relatively small sample size and low number of deaths. However, the observed association between resting heart rate and all-cause mortality was clinically significant, since the subjects in the highest resting heart rate tertile (≥ 73 bpm) had 2.5-fold risk for death compared with those in the lowest tertile (≤ 62 bpm). In the within-pair analyses among all the MZ and DZ twin pairs, above-mentioned risk was almost eight-fold. This suggests that the

observed association is at least partially independent of genetic factors and childhood environment.

Many mechanisms may explain the association between high resting heart rate and increased risk for mortality. One explanation could be that the elevated heart rate, resulting from increased sympathetic tone, may be a marker of subclinical cardiovascular disease. On the other hand, Mensink & Hoffmeister (1997) excluded early mortality cases (i.e., deaths occurring during the first two years of follow-up) from their analyses and observed that their original results (resting heart rate significantly predicted mortality) remained almost unchanged. This might indicate that the association between resting heart rate and mortality may not be due to the heart rate as an indicator of subclinical or pre-existing disease. Another explanation may be that increased heart rate is associated with hypertension and cardiovascular diseases (Tjugen et al. 2010). However, the majority of the studies reporting associations between resting heart rate and increased mortality (including the present study) have adjusted the analyses for many risk factors, such as blood pressure and cholesterol levels, so that these should not explain the observed association.

Previous studies have reported that Cornell voltage and Cornell product-based LVH significantly predicted all-cause mortality in middle-aged and older women (Havranek et al. 2008) as well as in older men (Sundström et al. 2001). Among older men, Sokolow Lyon voltage-based LVH also predicted all-cause mortality (Sundström et al. 2001). Significant predictors of all-cause mortality also include T wave flattening or inversions, especially among men (De Bacquer et al. 1994, De Bacquer et al. 1998, Greenland et al. 2003). However, similar results were not observed in the present study. The discrepancy between the results of the present and the previous studies with regard to ECG LVH indices may result from the differences in the use of LVH indices, i.e., previous studies have used dichotomized LVH indices (LVH either present or absent according to the criteria established for each LVH index) whereas in the present study, LVH indices were classified into tertiles. The proportion of the subjects with Cornell voltage-, Cornell product- or Sokolow Lyon-based LVH was rather small (5.1%, 0.8% and 5.1%, respectively) which may have contributed to the absence of a significant association between ECG LVH and all-cause mortality in the present study. Methodological differences may also explain the discrepancies between the present and previous studies with regard to the prognostic value of T wave amplitudes. T wave amplitudes were classified into tertiles in the present study whereas in the previous studies (De Bacquer et al. 1994, De Bacquer et al. 1998, Greenland et al. 2003) the Minnesota classification system was used. Also, gender may explain the discrepancy. For example, the Finnish study by Ristola (1983) reported that negative T wave amplitudes in leads II and V_5 were strongly associated with cardiovascular mortality in middle-aged men but the association was not as clear in women.

6.4 Methodological considerations

One of the most important strengths of the present study is the utilization of twin pairs. On the one hand, this enabled the use of a more sophisticated method (quantitative genetic modelling) for the estimation of genetic and environmental effects on the variation in the studied resting ECG variables. On the other hand, within-pair analyses (co-twin control design) enabled genetic liability and childhood environment to be partially or fully controlled for when the associations between the resting ECG variables and long-term LTPA, walking endurance and mortality were studied. The co-twin control study probably represents one of the best controlled long-term study designs available in humans that allow adjustment for genetic and familial factors owing to the complete or close match for genetic factors, age, gender and the intrauterine and childhood environment. Another strength is that the study samples were population-based. The main limitation of the study is that the sample sizes were relatively low and therefore the statistical power may have been limited. Additionally, the participants were mainly older women which partly limits the generalizability of the results, as also does the exclusion of the twin pairs with the poorest health.

Shared environmental effects were detected only for two resting ECG variables in the univariate heritability analyses. Relatively low sample size may have led to insufficient statistical power to discriminate common environmental effects from additive genetic effects. Insufficient statistical power may also explain the discrepancies in the estimations of genetic and environmental effects observed between single LVH indices and LVH factor in the univariate and multivariate analyses, respectively. Multivariate analysis takes into account all the available information, leading to increased statistical power and subsequent better ability to discriminate common environmental effects from additive genetic effects. Therefore the reliability of the results of the multivariate analyses may be improved. Additionally, the inclusion criteria of the FITSA study (e.g., both members of the twin sister pair had to be willing to participate and capable of travelling to the study centre) may have led to the exclusion of pairs with at least one sister in poor health. This may have reduced the variation in resting ECG variables, increased the similarity within the pairs, and thus affected the estimates of genetic effects.

One of the advantages of the TWINACTIVE study is that the follow-up period was long, over 30 years, thus covering most of the participants' adult lives. A limitation, however, is that the LTPA habits of the participating twin pairs were collected retrospectively. This may have caused problems in recalling the intensity and amount of physical activity, possibly leading to incorrect MET index values. However, the intra-class correlation between the LTPA MET index from the questionnaire performed in 1981 and the retrospective MET index for the year 1980 obtained from the telephone interview conducted in 2005 was relatively high (0.56) (Waller et al. 2008),

showing that the participants were able to recall their physical activity habits rather well even for the longest retrospective time point. In the FITSA study, only the present status of physical activity (at the baseline measurements) was collected.

When studying the associations of the resting ECG variables with walking endurance and mortality, one advantage was the reliability of the outcome variables. Firstly, the 6MWT (6MWD) has been reported to be both a valid and reliable method for the assessment of walking endurance (Solway et al. 2001) and, secondly, taking into account deaths from all causes does not cause problems in the classification of the causes of death. However, the small sample size and relatively low number of deaths did not allow cause-specific mortality analyses. A further limitation was that the study design was cross-sectional for the resting ECG variables and walking endurance; however, a cross-sectional design was considered to be better choice than a longitudinal design (in the FITSA study, the follow-up study was conducted three years after the baseline measurements), since in the follow-up measurements, the number of participants capable or willing to perform the 6MWT was too low to allow for meaningful analyses.

6.5 Future directions

Several possibilities exist for future studies. The heritability of resting ECG variables could be studied in many different population groups across a relatively narrow age range to obtain more reliable estimates of genetic effects, since these estimates are population-specific and tend to vary with age (Plomin et al. 2001). An especially interesting possibility would be to study same subjects longitudinally from childhood to old age to see whether there are changes in the relative proportion of genetic and environmental effects with aging. Since in the present study among older women the heritability of different ECG variables was moderate to high, there would also be reason to conduct studies with the aim of identifying specific genes/gene variations for resting ECG characteristics.

In the future, using a similar co-twin control study design as in the present study, it would be important to study the effects of long-term LTPA on resting ECG variables separately for men and women, since the adaptation of the heart to physical activity is gender-specific (Spirito et al. 1994). In these separate analyses, it would also be interesting to study variables measured by echocardiography or magnetic resonance imaging to obtain information on whether long-term LTPA has effects on these variables after controlling for genetic factors and childhood environment. A similar study could also be conducted among younger persons. One advantage of doing this is that younger persons tend to be free of the kinds of cardiac or other diseases (or medications) which cause changes in cardiac function. Another interesting

possibility would be to study subjects who have been physically active or inactive throughout their lives, not only during adulthood, and subjects who have been active during childhood and adolescence, but not in adulthood, and vice versa.

Although the resting ECG variables studied were not strongly associated with walking endurance in the present study, cardiac function as a predictor of walking ability among relatively healthy older subjects may still be worth investigating. Firstly, in larger and more heterogeneous populations (reflecting "normal" populations with different degrees of functional capacity and differing health status) resting ECG may have some importance in this regard, while the inclusion of older subjects (> 76 years) might also be relevant since age-related or asymptomatic ECG changes may be more prevalent in older age groups. Secondly, the inclusion of echocardiographic measurements in such studies would also be worthwhile since they could yield additional information on the role of cardiac function (and structure) as a predictor of walking endurance. Since the association between resting ECG variables and mortality is well-established and that physical activity has effects on resting ECG variables, it would be interesting, and from the preventive point of view important, to study whether physical activity modifies the association between cardiac function and mortality.

7 MAIN FINDINGS AND CONCLUSIONS

The main findings of the present study can be summarized as follows:

1. Genetic effects explained a moderate to high proportion (32-72%) of the variation in the majority of the resting ECG variables. Genetic effects were especially strong for T wave amplitudes, ECG LVM and LVH indices, and heart rate, whereas the other variables (intervals and frontal plane axes) were more affected by environmental effects.

2. The majority of genetic effects were shared between LVH and repolarisation phase (genetic correlation -0.93), suggesting that the previously established association between LVH and repolarisation abnormalities mainly originates from genetic factors.

3. The main ECG adaptation to long-term LTPA is lowered resting heart rate after partially or fully controlling for genetic liability and childhood environment.

4. The role of the resting ECG variables as (cross-sectional) predictors of walking endurance was rather small. The best predictors were T wave amplitudes, ECG LVM and LVH indices which explained less than 11% of the variation in walking endurance.

5. Resting heart rate was the ECG variable most strongly associated with increased risk for all-cause mortality. Those in the highest resting heart rate tertile had over twofold risk for death compared with subjects in the lowest tertile.

In conclusion, among older people, of the resting ECG characteristics studied T wave amplitudes, LVH indices and resting heart rate are strongly affected by genetic factors, may be modifiable by long-term LTPA or have prognostic value with regard to walking endurance and all-cause mortality. The results of the present study suggest that while some people may be more prone to resting ECG abnormalities in old age due to their genetic predisposition, the role of environmental effects is also important. The implication of this finding is that by modifying lifestyle factors it may be possible to prevent the development of resting ECG abnormalities. After controlling for genetic liability and childhood environment, long-term LTPA may be one of the environmental factors which explain the variation in resting ECG variables, especially in resting heart rate.

Lowering of the resting heart rate is an important adaptation to physical activity, since a high resting heart rate is associated with increased risk for death, also after controlling for genetic liability and childhood environment.

YHTEENVETO

Lepo-EKG –muuttujien periytyvyys sekä yhteydet fyysiseen aktiivisuuteen, kävelykestävyyteen ja kuolleisuuteen iäkkäillä henkilöillä

Lepoelektrokardiografia (lepo-EKG) on yksi käytetyimmistä menetelmistä terveystarkastuksissa ja sydänsairauksien diagnostiikassa. Sitä käytetään muun muassa rytmihäiriöiden rekisteröintiin sekä sydämen hapenpuutteen arviointiin. Lepo-EKG:ssa havaitut poikkeavuudet lisääntyvät iän myötä ja niillä on usein diagnostista ja ennusteellista arvoa. Lepo-EKG –poikkeavuuksien kliinisen merkityksen kannalta on tärkeää ymmärtää, mitkä tekijät selittävät yksilöiden välisiä eroja lepo-EKG –löydöksissä ikääntyneillä henkilöillä.

Yksilöiden väliset erot lepo-EKG:sta mitatuissa muuttujissa voivat selittyä sekä geneettisillä tekijöillä että ympäristötekijöillä. Aikaisemmat tutkimukset ovat osoittaneet, että ainakin nuoremmilla henkilöillä geneettiset tekijät vaikuttavat kohtalaisen tai hyvin voimakkaasti yksilöiden välisiin eroihin. Iäkkäämmillä henkilöillä, etenkin naisilla, tätä on tutkittu vähemmän. Geneettisten tekijöiden ja ympäristötekijöiden selitysosuuksia yksilöiden välisistä eroista voidaan selvittää tutkimalla identtisiä ja ei-identtisiä kaksosia geneettisen mallinnuksen avulla. Identtiset kaksoset ovat perimältään samanlaisia kun taas ei-identtiset kaksoset ovat perimältään noin 50-prosenttisesti samanlaisia. Jos identtiset kaksoset ovat tutkittavan ominaisuuden suhteen samankaltaisempia verrattuna ei-identtisiin kaksosiin, on se osoitus siitä, että perimä selittää yksilöiden välisiä eroja ko. ominaisuudessa.

Lukuisten aiempien, pääasiassa urheilijoilla ja fyysisesti inaktiivisilla kontrollihenkilöillä tehtyjen tutkimusten mukaan fyysinen harjoittelu vaikuttaa lepo-EKG –muuttujiin (esimerkiksi leposyke madaltuu, T aallot ovat korkeampia) ja voi näin ollen olla yksi niistä ympäristötekijöistä, jotka selittävät yksilöiden välisiä eroja lepo-EKG –muuttujissa. Muissa aiemmissa tutkimuksissa on kuitenkin havaittu, että geneettiset tekijät vaikuttavat sekä lepo-EKG –muuttujiin että fyysiseen harjoitteluun tai liikuntaan osallistumiseen. Tämä saattaa aiheuttaa vääristymiä urheilijoilla ja ei-urheilevilla henkilöillä tehtyihin tapaus-verrokkitutkimuksiin, sillä henkilöiden, joilla esimerkiksi on luonnostaan suuri sydän, voi olla helpompi osallistua rasittavaan liikuntaan kuin henkilöiden, joilla on pienempi sydän. Geneettisten tekijöiden vaikutus liikunnan ja lepo-EKG –muuttujien väliseen yhteyteen voidaan huomioida vertailemalla sellaisia identtisiä ja ei-identtisiä kaksospareja, jotka eroavat liikuntatottumustensa suhteen. Tällaisissa kaksospareissa toinen kaksossisarus harrastaa selkeästi runsaammin liikuntaa kuin hänen oma kaksossisaruksensa.

Lepo-EKG –poikkeavuuksien yhteyttä sairastuvuuteen ja kuolleisuuteen on aiemmin tutkittu runsaasti. Suurentuneeseen kuolemanriskiin ovat yhteydessä muun muassa kohonnut leposyke, T aaltojen madaltumat tai inversiot sekä lepo-EKG:n avulla määritelty vasemman kammion hypertrofia. Geneettiset tekijät voivat sekoittaa myös näitä yhteyksiä, joten myös lepo-EKG

–muuttujien yhteyttä kuolleisuuteen on perusteltua tutkia geneettisesti kontrolloidussa tutkimusasetelmassa kaksosilla. Lepo-EKG –muuttujien yhteyttä kävelykestävyyteen sen sijaan ei ole paljon tutkittu. On kuitenkin syytä olettaa, että lepo-EKG –muuttujat voisivat olla yhteydessä kävelykestävyyteen, sillä vaikka ilmeistä sydänsairautta ei olisikaan, lepo-EKG:n avulla voidaan havaita vakaviakin, mutta oireettomia häiriöitä sydämen toiminnassa. Lisäksi ikääntyminen aiheuttaa joitakin sellaisia muutoksia lepo-EKG –muuttujiin, jotka ovat samankaltaisia kuin sydänsairauksien aiheuttamat muutokset ja voivat näin ollen ennustaa heikentynyttä kävelykestävyyttä.

Tämän väitöskirjatutkimuksen tarkoituksena oli selvittää miten geneettiset tekijät ja ympäristötekijät selittävät yksilöiden välisiä eroja lepo-EKG –muuttujissa sekä miten pitkäaikainen vapaa-ajan liikunta vaikuttaa lepo-EKG –muuttujiin geneettisesti kontrolloidussa tutkimusasetelmassa ikääntyneillä kaksosilla. Tutkimuksen tavoitteena oli myös selvittää miten lepo-EKG –muuttujat ovat yhteydessä kävelykestävyyteen ja kokonaiskuolleisuuteen.

Tutkimuksessa käytettiin kahta kaksosaineistoa, jonka osallistujat valittiin Suomen kaksoskohorttitutkimuksesta (The Finnish Twin Cohort Study). Finnish Twin Study on Aging (FITSA) –tutkimuksen osallistujat valittiin iän ja tsygoottisuuden perusteella 63-76-vuotiaista naiskaksosista. FITSA–tutkimukseen osallistui 103 identtistä ja 114 ei-identtistä kaksosparia, joista 86 identtistä ja 91 ei-identtistä paria oli käytettävissä tähän väitöskirjatutkimukseen. TWINACTIVE–tutkimuksen osallistujat (5 identtistä ja 7 ei-identtistä nais- ja mieskaksosparia, iältään 50-67 vuotta) puolestaan valittiin pitkäaikaisen (32 v) vapaa-ajan liikunnan suhteen tapahtuneen eroavaisuuden perusteella, toisin sanoen kaksosparin toinen jäsen harrasti ko. ajanjakson ajan selkeästi enemmän vapaa-ajan liikuntaa kuin hänen inaktiivisempi kaksosensa. Lepo-EKG –mittaus suoritettiin kaikille tutkimuksiin osallistuneille kaksosille. Kävelykestävyys arvioitiin kuuden minuutin kävelytestin perusteella ja kuolintiedot saatiin Suomen Väestörekisterikeskuksesta. Tilastollisina analyysimenetelminä käytettiin geneettistä mallinnusta, parittaisia analyysejä sekä yksilöperusteisia analyysejä.

Tutkimuksen tulokset osoittivat, että geneettiset tekijät selittävät kohtalaisen tai hyvin suuren osan (32-72%) yksilöiden välisistä eroista suurimmassa osassa lepo-EKG –muuttujia. Erityisen voimakkaasti geneettiset tekijät selittivät yksilöiden välisiä eroja T aaltojen amplitudeissa, vasemman kammion hypertrofia –indekseissä sekä leposykkeessä. Myös ympäristötekijät selittivät yksilöiden välisiä eroja tutkituissa lepo-EKG –muuttujissa (28-100%). Geneettinen korrelaatio EKG:lla määritetyn vasemman kammion hypertrofian ja repolarisaatiovaiheen (T aaltojen amplitudit) välillä oli hyvin korkea (-0.93) antaen viitteitä siitä, että suurimmaksi osaksi samat geenit olisivat yhteydessä sekä hypertrofiaan että repolarisaatiovaiheeseen. Pitkäaikainen vapaa-ajan liikunta vaikutti selvimmin leposykkeeseen. Vapaa-ajan liikuntaa harrastaneilla kaksosilla oli 8.8 (95%:n luottamusväli 1.3-16.4) lyöntiä/minuutti alhaisempi leposyke kuin heidän vapaa-ajan liikuntaa huomattavasti vähemmän harrastaneilla kaksossisaruksillaan. Lepo-EKG –muuttujat selittivät 0-11% kävelykestävyyden eroista. Kävelykestävyyden parhaat elektrokardiografiset ennustajat olivat T aaltojen

amplitudit sekä vasemman kammion hypertrofia–indeksit. Kokonaiskuolleisuuteen puolestaan oli voimakkaimmin yhteydessä leposyke. Henkilöillä, joiden leposyke oli ≥ 73 lyöntiä/minuutti, oli yli kaksinkertainen riski kuolla (iällä, fyysisellä aktiivisuudella ja beetasalpaajien käytöllä vakioitu riskisuhde 2.48, 95%:n luottamusväli 0.95-6.44) verrattuna henkilöihin, joiden leposyke oli ≤ 62 lyöntiä/minuutti.

Tutkimuksen tulokset viittaavat siihen, että sekä geneettiset tekijät että ympäristötekijät selittävät yksilöiden välisiä eroja lepo-EKG –muuttujissa iäkkäillä. Pitkäaikainen vapaa-ajan liikunnan harrastaminen saattaa olla yksi näistä eroja selittävistä ympäristötekijöistä, etenkin leposykkeen kohdalla, sillä leposyke oli liikuntaa harrastavilla selkeästi matalampi kuin vähemmän liikuntaa harrastaneilla, kun geneettiset tekijät otettiin huomioon. Madaltunut leposyke on tärkeä liikunnan aiheuttama suojaava adaptaatio, sillä korkea leposyke oli tässäkin tutkimuksessa yhteydessä suurentuneeseen kuolemanriskiin. Lepo-EKG –muuttujien rooli kävelykestävyyden ennustajana sen sijaan oli vähäinen.

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ORIGINAL PAPERS

I

GENETIC INFLUENCES ON RESTING ELECTROCARDIOGRAPHIC VARIABLES IN OLDER WOMEN: A TWIN STUDY

by

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Genetic Influences on Resting Electrocardiographic Variables in Older Women: A Twin Study

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Background: Previous studies in young and middle-aged men and women have shown that resting electrocardiographic (ECG) variables are influenced by genetic factors. However, the extent to which resting ECG variables are influenced by genetic factors in older women is unknown. Thus, the aim of this study was to estimate the relative contribution of genetic and environmental influences to individual differences in resting ECG variables among older female twins without overt cardiac diseases.

Methods: Resting ECG recordings were obtained from 186 monozygotic and 203 dizygotic twin individuals, aged 63–76 years. Quantitative genetic modeling was used to decompose the phenotypic variance in each resting ECG variable into additive genetic, dominance genetic, shared environmental, and unique environmental influences.

Results: The results showed that individual differences in the majority of the resting ECG variables were moderately to highly explained by additive genetic influences, ranging from 32% for T axis to 72% for TV_5. The results also suggested dominance genetic influences on QRS duration, TV_1, and Sokolow–Lyon voltage (36%, 53%, and 57%, respectively). Unique environmental influences were important for each resting ECG variable, whereas shared environmental influences were detected only for QT interval and QTc.

Conclusion: In older women without overt cardiac diseases, genetic influences explain a moderate to high proportion of individual differences in the majority of the resting ECG variables. Genetic influences are especially strong for T-wave amplitudes, left ventricular mass, and hypertrophy indices, whereas other variables, including heart rate, intervals, and axes, are more affected by environmental influences.

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electrocardiography; heritability; quantitative genetic modeling; aging

Cardiovascular diseases are one of the leading causes of death worldwide.[1] Alterations in resting electrocardiogram (ECG) have prognostic significance for future health since some resting ECG abnormalities, such as left ventricular hypertrophy (LVH) and T-wave inversions, are associated with increased risk for cardiovascular disease morbidity and mortality.[2–7]

Few twin and family studies have provided information about genetic influences on resting ECG

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variables.[8–14] To our knowledge, at least three previous studies have used more sophisticated biometric methods to estimate genetic contribution to resting ECG variables. The results of Busjahn et al.[11] in young adult men and women (100 monozygotic (MZ) twin pairs and 66 dizygotic (DZ) twin pairs, mean age 34 years) showed that additive genetic influences explained 59% of individual differences in QRS axis, 52% in T axis and QTc, 46% in P duration, and 40% in QRS duration, while RR interval, PR interval, and P axis were influenced by environmental factors. Carter et al.[12] reported in women aged 18–71 years (103 MZ and 198 DZ twin pairs) that additive genetic influences explained 25% of the individual differences in QT interval. The study by Dalageorgou et al.[14] showed that the proportions of additive genetic influences were 55% for resting heart rate, 60% for QT interval, and about 50% for QTc intervals in 21- to 80-year-old women (105 MZ and 256 DZ pairs).

Because both gender[15] and age[16] have effects on heart function characteristics, it is important to study genetic and environmental influences on resting ECG variables separately for men and women within a narrow age range. Until now, genetic influences on resting ECG variables in older women are unknown. Thus, the aim of this study was to estimate the relative contribution of genetic and environmental influences to individual differences in resting ECG variables among 63- to 76-year-old female twins without overt cardiac diseases.

METHODS

Study Participants

The present study is part of the Finnish Twin Study on Aging (FITSA), a study of genetic and environmental influences on the disablement process in older women. The study sample was recruited from the nationwide Finnish Twin Cohort,[17] which comprises all same-sex twins born in Finland before 1958 with both co-twins alive in 1975. On the basis of age and zygosity, an invitation to participate in FITSA was sent to 414 twin sister pairs aged 63–76 years surviving in 2000. Details on recruitment have been described earlier[18] and are briefly summarized here. To be included, both twin pair members had to agree to participate. The reasons for nonparticipation were that one or both sisters were unwilling to take part (106 pairs), had poor health status (85 pairs), or had died after the last update of vital status for all cohort members (6 pairs). As a result of the procedures, the study group consisted of 217 twin sister pairs, comprising 103 MZ and 114 DZ pairs. The zygosity of the participating pairs was initially determined through a validated questionnaire[19] and was later confirmed using a battery of 10 highly polymorphic gene markers in DNA extracted from a venous blood sample.

Because specific conduction abnormalities make it impossible to interpret ECG features in a meaningful way, participants with left bundle branch block (n = 16), right bundle branch block (n = 9), and left anterior hemiblock (n = 1) were excluded from the statistical analyses. Conduction abnormalities were confirmed by a specialist in internal medicine (J. Karjalainen). Myocardial infarction has an effect on resting ECG variables and thus participants with myocardial infarction (n = 19) were also excluded from the analyses. In addition, participants with pacemakers (n = 4) were excluded. After these exclusions, the number of participants included in the statistical analyses was 186 MZ and 203 DZ individuals (number of complete pairs was 86 MZ and 91 DZ pairs). Twin individuals without their co-twins can be included in the analyses since the statistical methodology used in the analyses can also utilize data from pairless twin individuals, giving more accurate estimates of genetic and environmental influences than if using only data from complete twin pairs.

The study was conducted according to the ethical rules stated in the Declaration of Helsinki. All the participants were informed about the study and they signed a written informed consent. The study was approved by the ethics committee of the Central Hospital of Central Finland.

Electrocardiographic Measurements

Standard 12-lead resting ECG was recorded at 25 mm/s and 1 mV/cm standardization using a Nihon Kohden Cardiofax V Ecaps 12. The ECG variables measured are presented in Table 1. T-wave amplitudes in leads V_1, V_5, and II as well as R-wave amplitude in lead aVL and S-wave amplitude in lead V_3 for the calculation of Cornell voltage were measured manually, while the other variables were collected from the automatic listings of the ECG recorder. Heart rate-corrected QT interval was calculated by the equation: QT interval + (1000 – RR interval)/7. LVH indices

Table 1. Participant Characteristics and Resting Electrocardiographic Variables by Zygosity

Characteristic	Mean ± SD		
	MZ Individuals (n = 186)	DZ Individuals (n = 203)	Total (n = 389)
Age (years)	68.1 ± 3.7*	68.8 ± 3.1*	68.5 ± 3.4
Height (cm)	158.0 ± 6.4	159.1 ± 5.7	158.6 ± 6.0
Weight (kg)	69.6 ± 12.0	70.6 ± 12.4	70.2 ± 12.2
Body mass index (kg/m^2)	28.1 ± 4.9	28.0 ± 4.8	28.0 ± 4.8
Heart rate (beats/min)	69 ± 12	68 ± 10	69 ± 11
PR interval (ms)	164 ± 26	166 ± 26	165 ± 26
QRS duration (ms)	86 ± 8	87 ± 9	87 ± 9
QT interval (ms)	395 ± 31	399 ± 30	397 ± 30
QTc (ms)	411 ± 24	414 ± 22	413 ± 23
P axis (degree)	46 ± 20	48 ± 22	47 ± 21
QRS axis (degree)	28 ± 28	31 ± 26	29 ± 27
T axis (degree)	43 ± 35	46 ± 30	44 ± 33
Sokolow–Lyon voltage† (mV)	2.2 ± 0.7‡	2.4 ± 0.7‡	2.3 ± 0.7
Cornell voltage§ (mV)	1.1 ± 0.5	1.2 ± 0.5	1.2 ± 0.5
Cornell product** (mV × ms)	98.9 ± 45.3	102.5 ± 48.8	100.8 ± 47.1
ECG left ventricular mass†† (g)	114.2 ± 13.5	115.3 ± 14.0	114.8 ± 13.7
TV_1 (mV)	0.0 ± 0.1	0.0 ± 0.1	0.0 ± 0.1
TV_5 (mV)	0.2 ± 0.1	0.2 ± 0.2	0.2 ± 0.2
TII (mV)	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1

*Statistically significant difference in variances between the MZ and DZ individuals ($P < 0.05$), based on variance ratio test. The equality of the variances was analyzed using variables, which were standardized by age and use of beta-blockers.
†$SV_1 + RV_5$.
‡Statistically significant difference in means between the MZ and DZ individuals ($P < 0.05$), based on adjusted Wald test. The equality of the means was analyzed using variables, which were standardized by age and use of beta-blockers.
§RaVL + SV_3.
**Cornell voltage (RaVL + SV_3) × QRS duration.
††Calculated by the formula: 0.02 × (RaVL + SV_3) + 1.12 × body weight + 36.2.
DZ = dizygotic; ECG = electrocardiographic; MZ = monozygotic.

were calculated as follows: Sokolow–Lyon voltage ($SV_1 + RV_5$),[20] Cornell voltage (RaVL + SV_3),[21] and Cornell product (Cornell voltage × QRS duration).[22] Electrocardiographic left ventricular mass (LVM) was calculated using the following formula: 0.02 × Cornell voltage + 1.12 × body weight + 36.2.[23] All ECG measurements were carried out blinded to zygosity and twin sister's data.

Clinical Examination

All participants underwent a 30-minute clinical examination by a physician. Self-reports of acute and chronic diseases and medication had been obtained earlier and were confirmed in the clinical examination. These included the history of myocardial infarction, the presence of a pacemaker (exclusion criteria), and the use of beta-blockers, which were taken into account in the statistical analyses as described below. Beta-blocker users numbered 47 MZ and 63 DZ individuals, and nonusers, 139 MZ and 140 DZ individuals.

Statistical Analysis

The sample description and tests for data normality (Kolmogorov–Smirnov) were computed using SPSS 14.0 (SPSS Inc., Chicago, IL, USA). The resting ECG variables were normalized and then standardized by age and use of beta-blockers, using the PROC RANK and PROC STANDARD procedures in SAS 8.2 (SAS Institute Inc., Cary, NC, USA). This transformation both reduces nonnormality and standardizes the resting ECG variables so that the means and variances are independent of age variability and use of beta-blockers in the sample. Thus, age and use of beta-blockers will not bias the parameter estimates in subsequent genetic analyses. The equality of the means of the participants' characteristics and the resting ECG variables between the MZ and DZ twin individuals was analyzed with the adjusted Wald test, and the equality of variances was tested with the variance ratio test, taking into account the dependence of observations between the

co-twins (Stata 8.0, Stata Corp., College Station, TX, USA).

The within-pair resemblance in each resting ECG variable was estimated separately for the MZ and DZ groups using intraclass correlation coefficients (ICCs) (SPSS 14.0). Afterward, using biometric methods, univariate genetic analyses were carried out to estimate the relative contribution of genetic and environmental influences to each resting ECG variable. Quantitative genetic modeling is based on the fact that MZ co-twins share 100% of their genes, whereas DZ co-twins share on average 50% of their segregating genes. Both types of twins are assumed to share their environment to the same extent. Genetic influences make MZ co-twins more similar than DZ co-twins, while the shared environment leads both MZ and DZ co-twins to be more similar. The influence of the environment not shared by the co-twins contributes to differences between them. Consequently, the total variance in a phenotype can be decomposed into additive genetic influences (A), dominance genetic influences (D), shared environmental influences (C), and unique environmental influences (E). A refers to the sum of the effects of individual alleles over the loci, whereas D refers to interactions between alleles at the same or different loci. Hence, A + D is considered (broad sense) heritability or overall genetic influence on the trait under study. C includes factors that are shared by both co-twins, such as those related to their childhood environment, and E consists of exposures that are not shared by the co-twins, such as diseases that have affected only one sibling within a pair. E also includes measurement error as this is a random effect not correlated between twins.[24]

Genetic models were fitted to the raw data, using Mx software[25] with full information maximum likelihood methods. The possible hypothetical combinations of the different influences (ACE, AE, CE, ADE, DE, and E) were built and tested. As only data from two different groups of relatives are available (MZ and DZ twin sisters), all four possible influences (A, D, C, and E) cannot be modeled simultaneously. Consequently, not all the models are nested and the usual likelihood ratio test cannot be applied to compare all six biometric models. Instead, the Akaike's Information Criterion (AIC = $-2 \times$ log-likelihood $- 2 \times$ degrees of freedom)[26] was calculated and used to obtain the resulting best-fitting model. The AIC compares models on the basis of parsimony, taking into account the likelihood of every model and its degrees of freedom. The AIC is smaller for better fitting models.

RESULTS

The general characteristics and resting ECG variables of the MZ and DZ individuals are presented in Table 1. There were no statistically significant differences between the MZ and DZ individuals in means and variances except for the mean of Sokolow–Lyon voltage and variance of age.

Except for QTc, the ICCs were higher in MZ pairs than in DZ pairs (Table 2), suggesting the presence of genetic influences. In many resting ECG variables (e.g., QRS duration and TV_1) the ICCs of MZ twins were more than twice that of DZ pairs, suggesting the presence of dominance genetic influences in addition to additive genetic influences. Thus, the subsequent genetic modeling analyses also tested for dominance genetic influences.

The resulting best-fitting models (and their 95% confidence intervals) for the resting ECG variables are shown in Table 3. Details on the genetic modeling, including all the possible models, are available from the first author. Most of the resting ECG variables were best explained by the models with additive genetic and unique environmental influences (AE). Additive genetic influences were moderate to high, ranging from 32% for T axis to 72% for TV_5. For QRS duration, TV_1, and Sokolow–Lyon voltage, the best-fitting model included dominance genetic and unique environmental influences (DE). In these cases, dominance genetic influences were also moderate to high, being 36%, 53% and 57%, respectively. However, from the biological point of view and taking into account the results of genetic modeling, the most reasonable model for QRS duration and Sokolow–Lyon voltage is the model which includes both additive and dominance genetic influences, and unique environmental influences (ADE), since pure dominance genetic influences (interactions between certain alleles) are highly unlikely if additive genetic influences are not present. For QRS duration, the proportion of additive genetic influences was 12% and that of dominance genetic influences 23%. For Sokolow–Lyon voltage these estimates were 24% and 33%, respectively. In relatively small samples such as ours, the power to distinguish additive genetic influences from dominance genetic influences is limited, and hence for such models the

Table 2. Within-Pair Intraclass Correlation Coefficients for Resting Electrocardiographic Variables in Monozygotic and Dizygotic Twin Pairs

	MZ Twin Pairs (n = 86)		DZ Twin Pairs (n = 91)	
Resting ECG Variable	**ICC**	**95% CI**	**ICC**	**95% CI**
Heart rate (beats/min)	0.51	0.34–0.65	0.19	−0.01–0.38
PR interval (ms)	0.43	0.24–0.59	0.16	−0.05–0.36
QRS duration (ms)	0.35	0.15–0.53	0.11	−0.10–0.31
QT interval (ms)	0.21	0.00–0.40	0.15	−0.05–0.35
QTc (ms)	0.17	−0.04–0.37	0.19	−0.02–0.38
P axis (degree)	0.07	−0.15–0.28	0.05	−0.16–0.25
QRS axis (degree)	0.51	0.34–0.65	0.22	0.01–0.40
T axis (degree)	0.32	0.12–0.50	0.16	−0.05–0.35
Sokolow–Lyon voltage* (mV)	0.59	0.44–0.72	0.18	−0.03–0.37
Cornell voltage† (mV)	0.53	0.36–0.67	0.36	0.17–0.53
Cornell product‡ (mV × ms)	0.53	0.36–0.67	0.36	0.17–0.53
ECG left ventricular mass§ (g)	0.61	0.46–0.73	0.42	0.24–0.58
TV_1 (mV)	0.54	0.37–0.67	0.08	−0.13–0.28
TV_5 (mV)	0.70	0.57–0.80	0.29	0.09–0.47
TII (mV)	0.56	0.40–0.69	0.27	0.07–0.45

*$SV_1 + RV_5$.
†RaVL + SV_3.
‡Cornell voltage (RaVL + SV_3) × QRS duration.
§Calculated by the formula: 0.02 × (RaVL + SV_3) + 1.12 × body weight + 36.2.
DZ = dizygotic; ECG = electrocardiographic; ICC = intraclass correlation coefficient; MZ = monozygotic.

overall genetic influence (A + D) is the best estimate of genetic versus environmental influences. Thus, for TV_1, the overall genetic influence was 53%, but the true proportions of additive versus dominance genetic influences could not be clearly established.

QT interval, QTc, and P axis were the only variables unaffected by either additive or dominance

Table 3. Standardized Estimates (and 95% Confidence Intervals) of Genetic and Environmental Influences on Resting Electrocardiographic Variables in Older Women, Based on Best-Fitting Quantitative Genetic Models

Resting ECG Variable	**A**	**D**	**C**	**E**
Heart rate (beats/min)	0.48 (0.32–0.60)	–	–	0.52 (0.40–0.68)
PR interval (ms)	0.40 (0.23–0.55)	–	–	0.60 (0.45–0.77)
QRS duration (ms)	–	0.36 (0.17–0.51)	–	0.64 (0.49–0.83)
QT interval (ms)	–	–	0.19 (0.04–0.33)	0.81 (0.67–0.96)
QTc (ms)	–	–	0.19 (0.03–0.33)	0.81 (0.67–0.97)
P axis (degree)	–	–	–	1.00 (1.00–1.00)
QRS axis (degree)	0.43 (0.27–0.56)	–	–	0.57 (0.44–0.73)
T axis (degree)	0.32 (0.14–0.48)	–	–	0.68 (0.52–0.86)
Sokolow–Lyon voltage* (mV)	–	0.57 (0.43–0.68)	–	0.43 (0.32–0.57)
Cornell voltage† (mV)	0.55 (0.40–0.67)	–	–	0.45 (0.33–0.60)
Cornell product‡ (mV × ms)	0.56 (0.41–0.67)	–	–	0.44 (0.33–0.59)
ECG left ventricular mass§ (g)	0.65 (0.52–0.74)	–	–	0.35 (0.26–0.48)
TV_1 (mV)	–	0.53 (0.37–0.65)	–	0.47 (0.35–0.63)
TV_5 (mV)	0.72 (0.61–0.80)	–	–	0.28 (0.20–0.39)
TII (mV)	0.61 (0.46–0.71)	–	–	0.39 (0.29–0.54)

*$SV_1 + RV_5$.
†RaVL + SV_3.
‡Cornell voltage (RaVL + SV_3) × QRS duration.
§Calculated by the formula: 0.02 × (RaVL + SV_3) + 1.12 × body weight + 36.2.
A = additive genetic influence; C = shared environmental influence; D = dominance genetic influence; E = unique environmental influence; ECG = electrocardiographic.

genetic influences. QT interval and QTc were best explained by the models with shared and unique environmental influences (CE), whereas P axis was best explained by the model with only unique environmental influences (E).

DISCUSSION

The results of our study provide new and valuable information about the genetic influences on resting ECG variables in older women. Resting ECG abnormalities strongly increase with age[27] so that understanding of the role of genetic influences on resting ECG variables in older age is important. Our study showed that a moderate to high proportion of the individual differences in resting ECG variables were explained by genetic influences. The proportions of additive genetic influences were 32–72% in the majority of the resting ECG variables and those of dominance genetic influences 36–57% in QRS duration, TV_1, and Sokolow–Lyon voltage.

Our study provides new information, especially about genetic influences on T wave amplitudes since, to the best of our knowledge, no previous studies have been conducted to quantify the genetic influences on these ECG variables. Understanding of the role of genetic influences on T waves is important because T-wave alterations (flattenings and inversions) are known to be associated with increased risk for cardiovascular morbidity and mortality.[2,6,7] We found that the proportion of the individual differences in T-wave amplitudes explained by both additive and dominance genetic influences was high, ranging from 53% to 72%. These findings suggest that in healthy older women variation exists in ventricular repolarization, which is largely due to genetic factors. This is important to take into account when interpreting T waves and their possible abnormalities.

Our study also showed rather strong genetic influences for LVH indices (55–57%). A previous family study (395 men and 473 women, mean age 52 years), which used different statistical methods, reported lower estimates for these same indices (19–41%).[13] The estimate of genetic influences for electrocardiographic LVM was also high in our study (65%). This is in accordance with the results of a previous twin study (182 MZ and 194 DZ male and female twin pairs, aged 25–79 years) which reported that genetic influences amounted to 59% for echocardiographic LVM.[28] Our findings of strong genetic influences on LVH indices in older women are important since LVH is one of the resting ECG abnormalities that increase with age[6,27] and is of significant prognostic value for cardiovascular morbidity and mortality.[5]

We found similar results with the previous studies, especially with respect to frontal plane axes. In our study, additive genetic influences explained 43% and 32% of the individual differences in QRS axis and T axis, respectively, while Busjahn et al.[11] reported slightly larger estimates for young adult men and women (59% and 52%, respectively). Relatively high estimates of genetic influences on QRS axis were found in both studies. Our study showed strong genetic influences on LVH indices (about 60%), which may contribute to the estimate of genetic influences on QRS axis since the direction of QRS axis is partly influenced by the degree of LVH. P axis, in turn, seems to be unaffected by genetic factors. We found only unique environmental influences while Busjahn et al.[11] found both shared and unique environmental influences.

Interestingly, some of our results differ markedly from those obtained by Busjahn et al.,[11] Carter et al.,[12] and Dalageorgou et al.,[14] who used the same biometric methods as we did. We found moderate additive genetic influences on PR interval (40%), whereas Busjahn et al.[11] found only environmental influences in young adult men and women. However, another twin study in middle-aged men, using different statistical methods, reported quite similar results to those of our study (34%).[8] On the other hand, we found that QT interval and QTc were unaffected by genetic factors, whereas Carter et al.[12] and Dalageorgou et al.[14] reported that the proportion of additive genetic influences in QT interval was 25% in 18- to 71-year-old women and 60% in 21- to 80-year-old women, respectively. A twin study using different statistical methods reported an estimate of genetic influences to be 36% for QT interval in middle-aged and older men.[10] Genetic influences have also been reported for QTc in young adult men and women (52%)[11] and in 21- to 80-year-old women (50–52%, depending on the correction method used).[14]

Somewhat unexpectedly, we did not detect genetic influences on QT interval and QTc. On the basis of previous results, it seems that in younger women (and men) there are genetic influences on QT interval and QTc.[10–12,14] However, because our selection criterion for the best-fitting model was purely statistical (AIC), it does not totally exclude

the possibility of the presence of genetic influences on QT interval and QTc also in older women. The model with additive genetic (23% for QT interval and 22% for QTc) and unique environmental influences fitted almost equally well as the model with shared and unique environmental influences, which was selected to the best-fitting model according to AIC. This almost equally well-fitting model (AE) would be in line with the previous studies. In addition, there can be other reasons why we did not detect genetic influences on these two variables. One reason may be the lack of statistical power to discriminate additive genetic influences from shared environmental influences. Also, heritable QT syndromes are different from normal variation and in our sample nobody had long QT syndrome. Normal variation in QT intervals can be more environmentally affected, for example by physical activity.

Comparing the present results with those of the previous studies, however, must be done cautiously. Different study designs (twin vs family study) and differences in the statistical methods used in some previous studies may lead to different estimates of genetic and environmental influences. These estimates are also population-specific and likely to change with aging.[29] In addition, the estimates from different studies have relatively wide confidence intervals, and hence some of the differences between studies may be due to sampling variation rather than true differences between populations due to gender, age, health status, or genetic makeup.

It should be remembered that finding genetic influences on resting ECG variables does not mean that environmental influences are unimportant. In our study, unique environmental influences explained varying proportion of the individual differences in each resting ECG variable (28–100%), which in large part is in agreement with other studies.[11,12,14] We are not able to specify what the unique environmental factors (or shared environmental factors, as discussed below) are that influence the resting ECG variables. The literature suggests that among such factors are, for example, physical activity[30] and medications. Shared environmental influences were detected for only two variables and their role was rather small. One reason why shared environmental influences were not detected in other variables may be that moderately sized twin studies may not have the statistical power to do this. Shared environmental factors affecting resting ECG variables may include, for example, obesity,[31] resulting from similar eating habits in a family. However, twin and family studies have generally not found any or at most a minor role for shared environmental factors in obesity.[32,33]

Use of beta-blockers has influences on resting ECG variables, most commonly influencing heart rate and intervals. Beta-blocker medication is used in the treatment of cardiovascular abnormalities, which can originate from both genetic and environmental factors. The use of beta-blockers may not only increase the environmental component in the statistical analyses as could be expected, but it can also decrease it. We did the analyses also without beta-blocker adjustment. The results remained mainly unchanged, but there were five variables—PR interval, QT interval, QTc, Sokolow–Lyon voltage, and TV_5—that had different results without and with beta-blocker adjustment. In these cases (except for TV_5), unadjusted results showed smaller environmental components.

One of the strengths of our study is that it was population-based. However, since estimates of genetic influences are always population-specific and likely to change with aging,[29] caution must be exercised when applying the results to other populations than to Caucasian older women. In addition, participants with significant cardiac diseases were excluded from the statistical analyses, which means that the results apply only to older women without overt cardiac diseases. Although our study sample was population-based, the inclusion criteria may have lead to the exclusion of pairs with at least one sister in poor health. This may have reduced the variance in the resting ECG phenotypes, increased the similarity within the pairs, and thus affected the estimates of genetic influences.

In conclusion, in older women without overt cardiac diseases, genetic influences explain a moderate to high proportion of the individual differences in the majority of the resting ECG variables. Genetic influences are especially strong for T-wave amplitudes, LVM, and hypertrophy indices, whereas other variables, including heart rate, intervals and frontal plane axes, are more affected by environmental influences.

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II

ELECTROCARDIOGRAPHIC INDICES OF LEFT VENTRICULAR HYPERTROPHY AND REPOLARIZATION PHASE SHARE THE SAME GENETIC INFLUENCES: A TWIN STUDY

by

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Electrocardiographic Indices of Left Ventricular Hypertrophy and Repolarization Phase Share the Same Genetic Influences: A Twin Study

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Background: Both left ventricular hypertrophy (LVH) and repolarization phase (RP) are known to be attributable to genetic influences, but less is known whether they share same genetic influences. The aim of this study was to investigate to what extent individual differences in electrocardiographic (ECG) LVH and RP are explained by genetic and environmental influences and whether these influences are shared between these two traits.

Methods: Resting ECG recordings were obtained from 186 monozygotic and 203 dizygotic female twin individuals, aged 63 to 76 years. Latent factors, called LVH and RP, were formed to condense the information obtained from LVH indices (Cornell voltage and Cornell product) and T-wave amplitudes (leads V_5 and II), respectively. Multivariate quantitative genetic modeling was used both to decompose the phenotypic variances into additive genetic, common environmental, and unique environmental influences, and for the calculation of genetic and environmental correlations between LVH and RP.

Results: Additive genetic influences explained 16% of individual differences in LVH and 74% in RP. The remaining individual differences were explained by both common and unique environmental influences. The genetic correlation and unique environmental correlation between LVH and RP were −0.93 and −0.05, respectively.

Conclusions: In older women without overt cardiac diseases, RP is under stronger genetic control than LVH. The majority of genetic influences are shared between LVH and RP whereas environmental influences are mainly specific to each.

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heritability; shared genetic influences; left ventricular hypertrophy; repolarization phase; aging

Left ventricular hypertrophy (LVH), most commonly resulting from chronic hypertension, obesity and/or increasing age,[1,2] is a significant predictor of cardiovascular morbidity and mortality.[3] The hypertrophied left ventricular wall finally causes detrimental changes in cardiac function, such as repolarization abnormalities,[4–6] manifested by ST segment depression and inverted T waves. T wave changes (both isolated and those related to LVH) are also associated with increased cardiovascular morbidity and mortality.[7–10] The prevalence of both LVH and T-wave abnormalities strongly increase with age[9,11] so that study of the underlying factors (either genetic or environmental) is

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important, especially in women due to their longer life expectancy.[12]

Previous studies have shown that left ventricular mass (LVM) and hypertrophy are affected by genetic factors in both men and women. Echocardiographic studies have reported varying estimates of the relative contribution of genetic influences to LVM, ranging from approximately 20–30%[13–15] to 60–70%.[16,17] The estimates for electrocardiographic (ECG) LVH indices are similar. For example, in a large family study Mayosi et al.[14] reported that about 20–40% of interindividual differences in LVH indices (Sokolow-Lyon voltage, Cornell voltage, and Cornell product) were attributable to genetic influences whereas a previous twin study in older women showed slightly higher estimates for these indices (55–57%).[18] T-wave characteristics also are strongly heritable traits. In older women, estimates of genetic influences have been reported to be about 50–70%.[18] However, less is known whether these genetic influences are the same or distinct for LVM/LVH and T waves, that is, whether the same genetic (and/or environmental) factors explain the association between increased ventricular wall thickness/mass and subsequent repolarization abnormalities.

The aim of this study was to determine to what extent individual differences in ECG LVH and repolarization phase (RP) are explained by genetic and environmental influences and whether these influences are shared between these two traits in older female twins without overt cardiac diseases.

METHODS

Participants

The present study is part of the Finnish Twin Study on Aging (FITSA), a study of genetic and environmental influences on the disablement process in older women. The study sample was recruited from the nationwide Finnish Twin Cohort,[19] which comprises all same-sex twins born in Finland before 1958 with both cotwins alive in 1975. On the basis of age and zygosity, an invitation to participate in FITSA was sent to 414 twin-sister pairs aged 63 to 76 years surviving in 2000. Details on recruitment have been described earlier[20] and are briefly summarized here. To be included, both twin pair members had to agree to participate. The reasons for nonparticipation were that one or both sisters were unwilling to take part (106 pairs), had poor health status (85 pairs), or had died after vital status was last updated for all cohort members (6 pairs). As a result of the procedures, the study group consisted of 217 twin sister pairs, comprising 103 monozygotic (MZ) and 114 dizygotic (DZ) pairs. The zygosity of the participating pairs was determined using a battery of 10 highly polymorphic gene markers in DNA extracted from a venous blood sample.

Because specific conduction abnormalities make it impossible to interpret resting ECG features in a meaningful way, participants with left bundle branch block (n = 16), right bundle branch block (n = 9), and left anterior hemiblock (n = 1) were excluded from the statistical analyses. Myocardial infarction has an effect on resting ECG variables and thus participants with myocardial infarction (n = 19) were also excluded from the analyses. In addition, participants with pacemakers (n = 4) were excluded. After these exclusions, the number of participants included in the statistical analyses was 186 MZ and 203 DZ individuals (number of complete pairs was 86 MZ and 91 DZ pairs). Twin individuals without their cotwins can be included in the analyses since the statistical methodology used in the present analyses can also utilize data from unpaired twin individuals, enabling more accurate estimates of genetic and environmental influences than would be obtained if data from complete twin pairs alone are used.

The study was conducted according to the ethical standards stated in the Declaration of Helsinki. All the participants were informed about the study and they signed a written informed consent. The study was approved by the ethics committee of the Central Hospital of Central Finland.

ECG Measurements

Standard 12-lead resting ECG was recorded at 25 mm/s and 1 mV/cm standardization using a Nihon Kohden Cardiofax V Ecaps 12 (Nihon Kohden Corporation, Tokyo, Japan). The ECG variables used in the statistical analysis were predetermined and they were selected as follows. Cornell voltage ($RaVL + SV_3$)[21,22] and Cornell product (Cornell voltage × QRS duration)[23] were used as indices of LVH, since these indices are among the most commonly used in clinical practice and have somewhat greater sensitivities and specificities in diagnosing of LVH compared to some other

widely used LVH indices.[24] T-wave amplitudes were measured from leads V_5 and II, which are among the T-wave variables most significantly associated with cardiovascular morbidity and mortality.[7–9] T-wave amplitudes and the components of Cornell voltage were measured manually, and QRS duration was collected from the automatic listing of the ECG recorder. All ECG measurements were carried out blinded to zygosity and cotwin's data.

Clinical Examination

All participants were clinically examined by a physician. Self-reports of acute and chronic diseases and medication had been obtained earlier and were confirmed in the clinical examination. These included the history of myocardial infarction and the presence of a pacemaker (exclusion criteria), and the use of beta-blockers, which was taken into account in the statistical analyses as described below. Beta-blocker users numbered 47 MZ and 63 DZ individuals and nonusers 139 MZ and 140 DZ individuals.

Statistical Analysis

The sample description and data normality tests (Kolmogorov-Smirnov) were computed using SPSS 14.0 (SPSS Inc., Chicago, Illinois, USA). LVH indices and T-wave variables were normalized and then standardized by age and use of beta-blockers using the PROC RANK and PROC STANDARD procedures in SAS 8.2 (SAS Institute Inc., Cary, NC, USA). This transformation both reduces nonnormality and standardizes the variables so that the means and variances are independent of age variability and use of beta-blockers in the sample. Thus, age and use of beta-blockers will not bias the parameter estimates in subsequent genetic analyses. The equality of the means of the participants' characteristics and studied ECG variables between the MZ and DZ twin individuals was analyzed with the adjusted Wald test and the equality of variances was tested with the variance ratio test, taking into account the dependence of observations between the cotwins (Stata 8.0, Stata Corp., College Station, TX, USA). Phenotypic correlations between the LVH indices and T-wave amplitudes in the whole sample were calculated with Mx software,[25] which also takes into account the dependence of observations between cotwins.

The relative contribution of genetic and environmental influences to each latent factor (see later) or measured ECG variable was estimated using quantitative genetic modeling. The method is based on the fact that MZ cotwins share 100% of their genes whereas DZ cotwins share on average 50% of their segregating genes. Both types of twins are assumed to share to the same extent their environment relevant to the traits under study. Genetic influences cause greater similarity between MZ cotwins than DZ cotwins while shared environment leads to greater similarity between both MZ and DZ cotwins. Environmental influences not shared by the cotwins contribute to differences between them. Consequently, the total variance in a phenotype can be decomposed into additive genetic influences (A), dominance genetic influences (D), common environmental influences (C) and unique environmental influences (E). A refers to the sum of the effects of the individual alleles over the loci whereas D refers to interactions between alleles at the same or different loci. Hence, A + D is considered (broad sense) heritability or overall genetic influence on the trait under study. C includes factors that are shared by both cotwins, such as those related to their childhood environment, and E consists of exposures that are not shared by the cotwins, such as diseases that have affected only one sibling within a pair. E also includes measurement error as this is a random effect not correlated between twins.[26]

To obtain preliminary information on the importance of the genetic contribution to LVH indices and T-wave amplitudes, intraclass correlation coefficients (ICC) were computed separately for the MZ and DZ pairs (SPSS 14.0, SPSS Inc.). The involvement of genetic influences is supported if the pair-wise correlation for a phenotype in MZ pairs is significantly higher than that in DZ pairs. Afterward, univariate genetic models were computed to estimate the relative contribution of genetic and environmental influences to each of the four phenotypes.

Multivariate quantitative genetic modeling was used for final analyses. As significant phenotypic correlations between the two LVH indices and between the two T-wave variables were observed, a common pathway model with two latent factors, called LVH and RP, were built and tested. The term "latent factor" means that the factor is not directly observable but it can be statistically estimated through the information available from the observed (measured) variables. In the present study, latent factors LVH and RP condensed the

information obtained from LVH indices and T-wave amplitudes, respectively.

The tested models (including all the possible combinations of A, C, and E; D was excluded since univariate analyses showed no dominance genetic influences on single ECG variables) were fitted to the raw data by Mx software[25] using maximum likelihood algorithms and treating unobserved data as missing-at-random. This enabled the estimation of both factor-specific (LVH or RP) and trait-specific (Cornell voltage, Cornell product, TV_5, TII) genetic and environmental influences. The latter describe the proportion of individual differences not explained by the genetic or environmental influences of a latent factor. Factor loadings from the latent factors to the observed data were also calculated to determine the degree of relationship between a latent factor and an observed variable.[26]

The significance of the estimates for A, C, and E was tested by removing them sequentially in different submodels. The fit of the different models to the raw data were tested by analyzing standard likelihood-ratio tests and the Akaike's Information Criterion (AIC = $-2 \times$ log-likelihood $- 2 \times$ degrees of freedom).[27] The AIC compares models on the basis of parsimony, taking into account the likelihood of every model and its degrees of freedom. Smaller AIC values indicate a better fit of the model. This sequential procedure eventually led to a model in which the pattern of variances and covariance was explained by as few influences as possible, providing an acceptable fit to the data. Final estimates of factor- and trait-specific genetic and environmental influences with 95% confidence intervals were obtained from the most parsimonious and theoretically acceptable multivariate model. To estimate the overlap between the sets of genes influencing the LVH and RP factors, the genetic correlation between the two sets was calculated. Correlations for common and unique environmental influences were calculated similarly.

RESULTS

The general characteristics, LVH indices and T-wave amplitudes of the MZ and DZ individuals are presented in Table 1. There were no statistically significant differences between the MZ and DZ individuals in means and variances, except for variance of age. Phenotypic correlations (for the whole sample) between the LVH indices and T- wave amplitudes ranged from −0.28 between Cornell product and TII to −0.31 between Cornell voltage and TV_5. The phenotypic correlation between TV_5 and TII was 0.78 and between Cornell voltage and Cornell product it was 0.98.

The ICCs of the LVH indices and T-wave amplitudes were higher in the MZ than DZ pairs, suggesting the presence of genetic influences. The results of univariate genetic modeling were previously published as a part of another article,[18] and are only briefly summarized here. All four

Table 1. Participant Characteristics, Electrocardiographic Left Ventricular Hypertrophy Indices and T-Wave Amplitudes by Zygosity

Characteristic	MZ Individuals (n = 186) Mean ± SD	DZ Individuals (n = 203) Mean ± SD	Total (n = 389) Mean ± SD	P*	P†
Age, years	68.1 ± 3.7	68.8 ± 3.1	68.5 ± 3.4	0.135	0.007
Height, cm	158.0 ± 6.4	159.1 ± 5.7	158.6 ± 6.0	0.191	0.097
Weight, kg	69.6 ± 12.0	70.6 ± 12.4	70.2 ± 12.2	0.517	0.628
Body mass index, kg/m^2	28.1 ± 4.9	28.0 ± 4.8	28.0 ± 4.8	0.866	0.767
Cornell voltage,‡ mV	1.1 ± 0.5	1.2 ± 0.5	1.2 ± 0.5	0.724	0.119
Cornell product,§ mV × ms	98.9 ± 45.3	102.5 ± 48.8	100.8 ± 47.1	0.640	0.179
TV_5, mV	0.2 ± 0.1	0.2 ± 0.2	0.2 ± 0.2	0.138	0.661
TII, mV	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.053	0.879

DZ = dizygotic; MZ = monozygotic; SD = standard deviation.
*For the equality of the means between the MZ and DZ individuals, based on adjusted Wald test. In four electrocardiographic variables, the equality of the means was analysed using variables standardized by age and use of beta-blockers;
†For the equality of the variances between the MZ and DZ individuals, based on the variance ratio test. In four electrocardiographic variables, the equality of the variances was analyzed using variables standardized by age and use of beta-blockers;
‡RaVL + SV_3.
§Cornell voltage (RaVL + SV_3) × QRS duration.

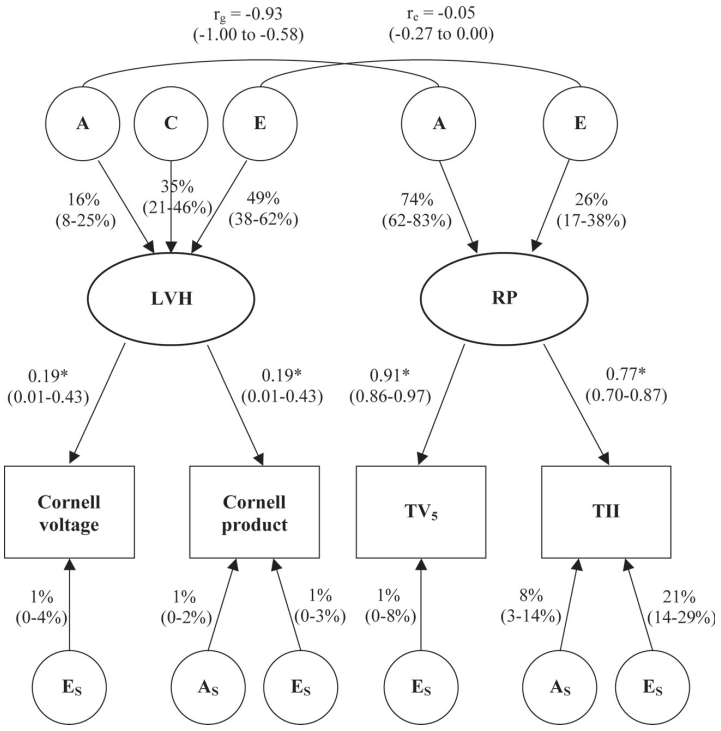

Figure 1. The best fitting common pathway model for the electrocardiographic (ECG) left ventricular hypertrophy (LVH) and repolarization phase (RP) factors, which combine the variance from the measured LVH indices and T-wave amplitudes. The percentages (95% confidence intervals) in the upper part of the figure are the proportions of the total variance of each latent factor explained by additive genetic (A), common environmental (C), or unique environmental (E) influences. The percentages (95% confidence intervals) in the lower part of the figure are the proportions of each measured ECG variable explained by trait-specific additive genetic (A_S) and unique environmental (E_S) influences. r_g and r_e refer to the correlation of additive genetic and unique environmental influences between the LVH and RP factors, respectively. The factor loadings of the LVH and RP factors on the measured ECG variables are shown with *. Cornell voltage = RaVL + SV_3; Cornell product = Cornell voltage × QRS duration.

variables were best explained by the models with additive genetic and unique environmental influences (AE). The estimates of additive genetic influences ranged from 55% for Cornell voltage to 72% for TV_5.

The best fitting common pathway model with two latent factors is shown in Figure 1 and the model-fitting statistics for all the submodels in Table 2. Additive genetic (16% for LVH and 74% for RP) and unique environmental influences (49% for LVH and 26% for RP) explained individual differences in both the LVH and RP factors whereas common environmental influences (35%) were found only for LVH. Between the LVH and RP factors, the genetic correlation for additive genetic influences was −0.93, while the correlation for unique environmental influences was −0.05. The factor loadings of the LVH and RP factors on

Table 2. Multivariate Model-Fitting Statistics for all the Submodels

Influences on Latent Factors*			Trait-Specific Influences*				Fitting Statistics†				
LVH	**RP**	**r**	**Cornell Voltage**	**Cornell Product**	**TV_5**	**TII**	**−2LL**	**df**	**χ^2 (Δdf)**	***P***	**AIC**
ACE	ACE	r_g, r_c, r_e	A_s, C_s, E_s	A_s, C_s, E_s	A_s, C_s, E_s	A_s, C_s, E_s	2040.334	1528	–	–	−651.666
ACE	ACE	r_g, r_c, r_e	A_s, E_s	A_s, E_s	A_s, E_s	A_s, E_s	2405.370	1532	1.036 (4)	0.904	−658.630
ACE	ACE	r_g, r_c, r_e	C_s, E_s	C_s, E_s	C_s, E_s	C_s, E_s	2407.399	1532	3.065 (4)	0.547	−656.601
ACE	ACE	r_g, r_c, r_e	E_s	A_s, E_s	E_s	A_s, E_s	2045.370	1534	1.036 (6)	0.984	−662.630
ACE	ACE	r_g, r_c, r_e	E_s	E_s	E_s	E_s	2418.644	1536	14.310 (8)	0.074	−653.356
ACE	**AE**	**r_g, r_e**	**E_s**	**A_s, E_s**	**E_s**	**A_s, E_s**	**2407.662**	**1536**	**3.328 (8)**	**0.853**	**−664.662**
ACE	AE	r_e	E_s	A_s, E_s	E_s	A_s, E_s	2428.154	1536	24.623 (8)	0.002	−643.043
AE	AE	r_g, r_e	A_s, E_s	A_s, E_s	A_s, E_s	A_s, E_s	47059.141	1536	44654.807 (7)	<0.001	43989.141

AIC = Akaike's information criterion (−2 × log-likelihood −2 × degrees of freedom); Cornell product = Cornell voltage × QRS duration; Cornell voltage = RaVL + SV_3; df = degrees of freedom; χ^2 (Δdf) = difference chi-square (and increment in degrees of freedom) with respect to the saturated (full) model (the submodel in the first row); LL = log-likelihood; LVH = electrocardiographic left ventricular hypertrophy; RP = repolarization phase.

*Each row represents one submodel, that is, a specific combination of genetic/environmental influences (on latent factors or measured electrocardiographic variables) and genetic/environmental correlations between latent factors. A, C, and E refer to additive genetic, common environmental, and unique environmental influences, respectively, which affect each latent factor, whereas A_s, C_s, and E_s are trait-specific additive genetic, common environmental, and unique environmental influences, respectively. r_g, r_c, and r_e represent genetic, common environmental, and unique environmental correlations, respectively;

†Fitting statistics describe how well a hypothetical submodel fits to the present data. The smaller the value of AIC, the better the fit of the model. For more details, see the Statistical Analysis section and reference no. 26.

The best fitting common pathway model is shown in bold.

the measured ECG variables ranged from 0.19 to 0.91.

Trait-specific additive genetic and unique environmental influences were also found for the measured LVH indices and T-wave amplitudes. Trait-specific additive genetic influences were found for Cornell product and TII, but they accounted for a small proportion of the overall variance (1% and 8%, respectively). Trait-specific unique environmental influences explained individual differences in each variable; these were 21% for TII and 1% for the other variables.

The data were also analysed using the Cholesky decomposition model and independent pathway model, but based on AIC, a common pathway model with two latent factors gave the most parsimonious explanation for the present data. The results of the Cholesky decomposition and independent pathway models are available from the first author.

DISCUSSION

Our study showed that ECG LVH and RP are affected by both genetic and environmental influences in older women. The contribution of additive genetic influences to individual differences in LVH was minor (16%), but for RP it was strong (74%). The remaining individual differences were explained by common and unique environmental influences in LVH and unique environmental influences in RP. The high correlation between additive genetic influences (−0.93) underlying LVH and RP suggest that to a large extent the same genes operate in both cases. On the other hand, the negligible correlation between unique environmental influences (−0.05) suggest that LVH and RP are mainly affected by distinct environmental factors.

The proportion of additive genetic influences in LVH (16%) was much less than might have been expected from our previous univariate results for single LVH indices (55% for Cornell voltage and 56% for Cornell product).[18] However, in the present multivariate analysis we found common environmental influences (35%) for the LVH factor whereas in the univariate analyses they were not detected for single LVH indices. The discrepancies between the results of the multivariate and univariate analyses (concerning both the proportion of additive genetic influences and presence/absence of common environmental influences) might be explained by the higher statistical power of the multivariate analysis, which, because it takes into account all the available information, enables common environmental influences to be discriminated from additive genetic influences. For the RP factor, the estimate of additive genetic influences was nearly the same (74%) as for the single T-wave variables reported previously (72% for TV_5 and 61% for TII).[18]

The strong genetic correlation (−0.93) between additive genetic influences underlying the LVH and RP factors suggests that, to a large extent, the same genes that regulate LVH may also regulate RP. The negative correlation indicates that while these genes may increase the degree of LVH, they can lower T-wave amplitudes (i.e., lead to repolarization abnormalities). The negligible environmental correlation in turn indicates that the majority of the environmental factors affecting LVH and RP are distinct. Thus, these results suggest that the association between LVH and repolarization abnormalities mainly originates from genetic factors. These may include both genes that cause heritable hypertrophic disorders (e.g., hypertrophic cardiomyopathy) and genes that otherwise contribute to the development of LVH.

Searching for specific genes for electrocardiographic or echocardiographic LVH has been under intensive study during recent years. For example, variations in the angiotensin-converting-enzyme gene,[28] angiotensinogen gene,[29] insulin-like growth factor-I gene,[30] estrogen receptor β gene,[31] and ghrelin receptor gene[32] have been proposed to be associated with LVH. Based on our present results, it can be suggested that all these specific gene variations found for LVH might also be associated, at least to some extent, with T-wave characteristics and RP. Since minor trait-specific additive genetic influences were also found (1% for Cornell product and 8% for TII), it is possible that there might also exist some genes, which are specific only for LVH or RP.

An important consideration is that LVH does not necessarily always lead to repolarization changes. For example, Moore et al.[6] reported that LVH associated with repolarization abnormalities in pathological LVH (resulting from hypertrophic cardiomyopathy, hypertension, aortic stenosis, or coarctation), but not in physiological LVH in high-trained athletes. Our data did not include many athletes, but is still in agreement that environmental factors may be more important in the etiology of LVH than in the etiology of repolarization

abnormalities. However, the common genetic component of LVH and abnormal repolarization is large, indicating that the same genes play a role in the etiology of pathologic LVH and repolarization abnormalities. In our data, among those who had a repolarization abnormality (negative T wave in lead V_5 and/or II), 20% had LVH, according to the Cornell voltage criterion (>2.0 mV),[21,22] whereas among those who did not have a repolarization abnormality, only 4% had LVH.

Environmental influences accounted for individual differences in both LVH and RP. Given the absence of a significant unique environmental correlation, for these two traits the unique environmental influences seem mainly to be distinct. Also, trait-specific unique environmental influences were found for each LVH index and T-wave amplitude, but their role was small (1%), except for TII (21%). We are not able to specify what these unique environmental factors (or common environmental factors for LVH) are, but based on previous studies some suggestions can be made. Common environmental factors affecting LVH can include, for example, similar physical activity habits adopted in childhood and continued throughout adulthood. Alternatively, this can also be considered as a unique environmental factor since physical activity habits may not always be similar across the life span in twin pairs; studies in adults have not found evidence for shared environmental effects on physical activity.[33] Other unique environmental factors affecting LVH can be pressure overload[1,2] or valvular disease. The respective factors for RP and T wave changes may be coronary artery disease-related ischemia[34] and medications. However, although the correlation for unique environmental influences between LVH and RP was negligible, it is nevertheless possible that unique environmental factors exist that are common to both LVH and RP.

One of the strengths of our study is that it was population based. However, since estimates of genetic influences are always population specific and likely to vary with age,[35] caution must be exercised when applying the results to populations other than Caucasian older women. Also, participants with significant cardiac diseases were excluded from the statistical analyses so that the results apply only to older women without overt cardiac diseases. However, our study sample is particularly important since older women are at increased risk for the development of LVH and T-wave abnormalities.[9,11] Although our study sample was population based, the inclusion criteria may have led to the exclusion of pairs with at least one sister in poor health. This may have reduced the variance in the LVH indices and T-wave amplitudes, increased the similarity within the pairs and thus affected the estimates of genetic influences. In addition, we determined LVH using ECG, which is also a limitation in our study, since ECG is not as sensitive in detecting LVH as echocardiography.

In conclusion, ECG LVH and RP are affected by both genetic and environmental influences in older women without overt cardiac diseases. RP seems to be a more heritable trait than LVH. Genetic influences underlying LVH and RP overlap to a large extent suggesting that the association between LVH and repolarization abnormalities mainly originates from genetic factors.

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III

EFFECTS OF LONG-TERM PHYSICAL ACTIVITY ON CARDIAC STRUCTURE AND FUNCTION: A TWIN STUDY

by

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IV

ELECTROCARDIOGRAPHIC AND OTHER CLINICAL CORRELATES OF WALKING ABILITY IN OLDER WOMEN

by

Mutikainen, S., Rantanen, T., Alén, M., Kauppinen, M., Karjalainen, J., Ortega-Alonso, A., Kaprio, J., Kujala, U.M. 2010

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Electrocardiographic and other clinical correlates of walking ability in older women

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ABSTRACT

The purpose of this study was to examine how resting electrocardiographic (ECG) and other clinical variables, which can be included in a routine clinical examination, predict walking ability in older women. Three hundred and twenty women (63–75 years) without overt cardiac diseases and apparent mobility limitations were studied. Measurements performed were clinical examination (standard 12-lead resting ECG, assessment of physical activity level, presence of chronic diseases, use of beta-blockers, body mass index (BMI), ability to squat, resting blood pressure) and six-minute walking test. Participants walked 533 ± 75 m in the six-minute walking test. The best electrocardiographic predictors of long walking distance were high TV_5 and TII, but their explanation rates were small (4.5% and 3.8%, respectively). In hypertensive participants (systolic blood pressure = SBP ≥ 160 mmHg), the respective values were 9.3% and 5.8%. The best predictors of long walking distance were ability to squat without limitations and low BMI (15.5% and 13.6%, respectively). Altogether the studied variables explained 36% of the variation in walking distance. The data gathered in clinical examination give useful information for the assessment of walking ability in relatively healthy older women. Resting ECG does not give clinically significant additional information for the assessment in subjects without overt cardiac disease.

1. Introduction

Ability to walk several hundreds of meters in a reasonable time is a significant factor for maintaining independence in old age. The inability to walk 400 m is associated with increased risk for persistent mobility limitation and/or mobility disability, thereby increasing the risk of losing independence. Decreased walking ability is also associated with increased risk for mortality (Newman et al., 2006).

The six-minute walking test is a reliable and valid method to estimate functional exercise capacity and walking ability in older persons (Solway et al., 2001). Many factors are known to be associated with the results of this test. In our population of older women genetic factors explained about 20% of the variability in six-minute walking distance (Ortega-Alonso et al., 2006). According to population-based studies, reduced six-minute walking distance is associated with increasing age (Lord and Menz, 2002; Enright et al., 2003) and BMI (Hulens et al., 2003), female gender (Camarri et al., 2006), shorter height (Camarri et al., 2006) and presence of several chronic diseases, such as heart failure (Bittner et al., 1993) and stroke (Eng et al., 2002). Six-minute walking distance can be improved by increasing physical activity (Hulens et al., 2003) and lower limb muscle strength (Lord and Menz, 2002; Hulens et al., 2003).

Until now, the association between electrocardiographic (ECG) variables and walking ability in a non-clinical, older population has been unknown. Although a person does not have an overt, diagnosed cardiac disease, ECG can reveal negative asymptomatic changes in cardiac function, which may be associated with decreased exercise capacity and walking ability. In addition, aging can cause changes in ECG variables (e.g., T wave changes, left axis deviation) (Yasumura and Shibata, 1989), which are partly similar as those caused by cardiac diseases and thus the ECG variables in older people may be associated with decreased exercise capacity

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and walking ability. The ECG variables are also affected, for example, by genetic factors (Mutikainen et al., 2009), obesity (Alpert et al., 2000) and physical activity (Schuit et al., 1998), which are in large part the same factors as those predicting walking performance in the six-minute walking test.

Numerous ECG criteria have been proposed for the diagnosis of left ventricular hypertrophy (LVH). Most of these are dependent on fixed voltage thresholds, for example the Cornell voltage (R wave amplitude in lead aVL + S wave amplitude in lead $V_3 > 28$ mm for men and >20 mm for women) (Casale et al., 1985, 1987). LVH has been shown to be associated with decreased exercise capacity (Olsen et al., 2001; Pierson et al., 2004) and this can negatively affect walking ability. T wave inversions may be signs of myocardial ischemia (Achar et al., 2005), which can also reduce walking ability.

In a clinical examination, in addition to interview and other measurements, there are not always possibilities (time or room) to perform six-minute walking test for the assessment of walking ability although it is an important part of the assessment of functional capacity in older people. Therefore it is beneficial to know how the information usually collected by a physician predicts walking performance in the six-minute walking test. Many factors known to have associations with walking ability (e.g., BMI, physical activity level) (Hulens et al., 2003) can easily be evaluated in a routine clinical examination.

The aim of this study was to determine (1) how resting ECG variables and clinical variables (such as BMI, chronic diseases, physical activity level), which can be included in a routine clinical examination, predict distance covered in the six-minute walking test in a group of relatively healthy older women without overt cardiac diseases and/or apparent mobility limitations and (2) how the resting ECG variables predict walking distance when adjusted for the other clinical variables included in this study. In addition, since our study sample consisted of twins, we also utilized that and aimed at investigate (3) whether the studied ECG variables and other clinical variables differ between better and worse walking monozygotic (MZ) co-twins. Because MZ co-twins share all of their genes, by studying them we were able to control for genetic factors.

2. Subjects and methods

2.1. Participants

Four hundred and thirty four women (217 twin-sister pairs including 103 MZ pairs and 114 dizygotic (DZ) pairs) aged 63–76 years participated in this study including resting ECG recording and six-minute walking test. The study is part of the Finnish Twin Study on Aging (FITSA). In the statistical analyses concerning the first and second research questions, the study group was treated as a set of individuals taking into account the clustering of possibly correlated observations from twin-sisters (see later). For the third research question, within-pair analyses were carried out for the selected MZ pairs, as explained in detail below.

The study sample was recruited from the Finnish Twin Cohort Study (Kaprio and Koskenvuo, 2002), which comprises all same-sex twins born in Finland before 1958 with both co-twins alive in 1975. An invitation to participate in FITSA was sent to 414 twin-sister pairs aged 63–76 years surviving in 2000. To be included, both twin pair members had to agree to participate. The reasons for non-participation were that one or both sisters were unwilling to take part (106 pairs), had poor health status (85 pairs) or had died after vital status was last updated for all cohort members (6 pairs). As a result of the procedures, the study group consisted of 217 twin-sister pairs (434 individuals). The zygosity of the participating pairs was initially determined through a validated questionnaire (Sarna et al., 1978) and was later confirmed using a battery of 10 highly polymorphic gene markers in DNA extracted from a venous blood sample.

The six-minute walking test was performed by 359 individuals. Reasons for not completing the test were refusal by the examining physician to grant permission to perform the test ($n = 54$), lack of time ($n = 12$) and participant's inability or unwillingness to initiate or finalize the test ($n = 9$). Of these 359 participants, 30 had incomplete ECG data. In addition, nine participants had a history of myocardial infarction, which can affect resting ECG. These participants were also excluded from the statistical analyses. Thus, the number of participants included in the final analyses (concerning the first and second research questions) was 320 (age range 63–75).

Among these 320 included participants, there were 63 complete MZ pairs. Of these, those pairs ($n = 16$) with the greatest and clinically significant discordance in walking ability were selected into the within-pair analyses (the third research question). The within-pair difference in the six-minute walking test result had to be at least 75 m. The co-twins with the longer walking distances were considered as better walking co-twins and their twin-sisters with shorter walking distances were considered as worse walking co-twins.

The study was conducted according to the ethical standards stated in the Declaration of Helsinki. All the participants were informed about the study and they signed a written informed consent prior to their inclusion in the study. The study was approved by the ethics committee of the Central Hospital of Central Finland.

2.2. Six-minute walking test

Maximal walking distance was assessed using a six-minute walking test. The participants were requested to walk up and down a 50 m indoor straight track for six minutes and to complete as many laps as possible. Except for the length of the indoor track, the protocol and security conditions followed the instructions subsequently published in the American Thoracic Society Statement (American Thoracic Society, 2002). The distance covered by the end of the six minutes was recorded as the outcome.

2.3. Electrocardiographic measurements

Standard 12-lead resting ECG was recorded at 25 mm/s and 1 mV/cm standardization using a Nihon Kohden Cardiofax V Ecaps 12. Recording was performed before the six-minute walking test. The ECG variables measured are presented in Table 1. Components of Cornell voltage (R wave amplitude in lead aVL and S wave amplitude in lead V_3) and T wave amplitudes in leads V_1, V_5 and II were measured manually and the other variables were collected from the automatic listings of the ECG recorder. All ECG measurements were carried out blinded to other data.

2.4. Health ascertainment and assessment of physical activity

All participants underwent a 30-minute clinical examination by a physician. Self-reports of acute and chronic diseases and medication had been obtained earlier and were confirmed in a clinical examination. Chronic diseases taken into account were arthrosis of hip ($n = 22$), knee ($n = 85$) and ankle ($n = 8$), rheumatoid arthritis ($n = 11$), asthma ($n = 19$), emphysema ($n = 2$), cerebral hemorrhage and stroke ($n = 18$) and Parkinson's disease ($n = 1$), all of which may affect the results of the six-minute walking test. Diseases were dichotomized as present ($n = 124$) or absent ($n = 196$), as was also the use of beta-blockers (yes, $n = 81$; no, $n = 239$). As a functional test, every participant was asked to squat so that the thighs reached the horizontal level and 189 participants

Table 1
Age, anthropometry, six-minute walking distance, resting blood pressure and resting electrocardiography in older women.

	Mean ± S.D.	p^a	n
Age (years)	68.2 ± 3.3		320
Height (cm)	158.8 ± 6.0		320
Weight (kg)	69.7 ± 12.0		320
Body mass index (kg/m^2)	27.7 ± 4.8		320
Six-minute walking dist. (m), all participants	532.5 ± 75.3		320
Beta-blocker users	510.6 ± 68.1	0.002	81
Non-beta-blocker users	539.9 ± 90.0		239
SBP (mmHg), all participants	149.8 ± 20.9		320
Beta-blocker users	152.4 ± 23.4	0.254	81
Non-beta-blocker users	148.9 ± 20.0		239
DBP (mmHg), all participants	86.4 ± 10.3		319
Beta-blocker users	86.8 ± 10.9	0.712	81
Non-beta-blocker users	86.3 ± 10.1		238
Resting heart rate (bpm), all participants	68.4 ± 11.1		320
Beta-blocker users	65.4 ± 11.2	0.010	81
Non-beta-blocker users	69.4 ± 10.8		239
PR interval (ms)	165.1 ± 25.3		320
QRS duration (ms)	86.3 ± 8.4		320
P axis (degree)	48.2 ± 21.1		320
QRS axis (degree)	30.6 ± 26.5		320
T axis (degree)	42.8 ± 31.4		320
Cornell voltage (RaVL + SV_3) (mm)	11.3 ± 5.0		320
T wave amplitude in lead V_1 (mm)	0.0 ± 1.0		320
T wave amplitude in lead V_5 (mm)	1.9 ± 1.5		320
T wave amplitude in lead II (mm)	1.7 ± 1.1		320

[a] For the mean difference between beta-blocker users and non-users, based on the adjusted Wald test.

were able to do so. If the participant was unable to attain the required position due to pain or some other limitation, squatting was regarded as painful or limited (n = 131). BMI was calculated from measured body weight and height by dividing weight in kilograms by height squared in meters. Blood pressure was measured once in the supine position.

In the assessment of the present status of physical activity, the self-report scale designed by Grimby (1986) was used, with slight modifications. The 7-point scale ranged from hardly any activity to participation in competitive sports. Participants were considered sedentary if they reported no other activity than light walking once or twice a week (n = 82). In all other cases participants were considered physically active (n = 238).

2.5. Statistical analysis

Data were analyzed using Stata Version 8 (Stata Corp., College Station, TX, USA) and SPSS 14.0 (SPSS Inc., Chicago, IL, USA). Descriptive data were calculated as mean ± standard deviation. Mean differences between two groups (e.g., included and excluded participants, beta-blocker users and non-users) were analyzed using an adjusted Wald test to take into account the sampling scheme of twins clustered in twin pairs. The predictive value of the ECG and other clinical variables for walking distance was analyzed using linear regression analysis. Age-adjusted models were used both for the ECG variables and for the other clinical variables. In addition, using the forward–enter method a multivariate linear regression analysis was performed separately for each ECG variable together with the other clinical variables included in this study. The assumptions of linear regression analysis (linearity, normal distribution and scattering of residuals, multicollinearity) were tested and they were found to be reasonably valid. In the abovementioned analyses, clustering of possibly correlated observations from twin pairs was taken into account by computing standard errors of coefficients using robust estimators of variance (Williams, 2000). The within-pair analyses were carried out as follows: paired samples Student's t-test were used for normally distributed continuous variables (normality was tested using Shapiro–Wilk test), Wilcoxon signed ranks test for non-normally distributed continuous variables and the symmetry test for categorical variables for matched pairs. Significance was set at $p < 0.05$ (two-sided) for all tests.

3. Results

Mean six-minute walking distance of the included participants (n = 320) was 533 ± 75 m. The walking distance of all the participants excluded from final statistical analyses because of a history of myocardial infarction (n = 9) or incomplete ECG data (n = 30) differed statistically significantly from the distance covered by those included (507 ± 70 m vs. 533 ± 75 m, p = 0.032). The difference between included participants and those with a history of myocardial infarction was not statistically significant (533 ± 75 m vs. 479 ± 82 m, p = 0.055). Those participants who were unable to perform the six-minute walking test (n = 75) were older (p = 0.002), shorter (p = 0.005) and more obese (p = 0.004) than participants who were able to perform this test. In addition, participants who were unable to walk six minutes had longer QRS duration (p = 0.044), smaller P axis (p = 0.025) and QRS axis (p = 0.020), and lower TV_5 (p = 0.004) and TII (p = 0.006) compared with those capable of walking six minutes. Physical activity level and squatting ability were higher and the prevalence of chronic diseases and use of beta-blockers were lower in those who performed the six-minute walking test compared with those who did not perform it ($p < 0.001$ for all these variables).

Age, anthropometry, blood pressure and results of the six-minute walking test and ECG measurements in the final study

Table 2
Age-adjusted results of clinical variables as predictors of six-minute walking distance (linear regression analysis) in 63- to 75-year-old women (n = 320).

	Regression coefficient (95% CI)	p	R^{2a}	R^{2b}
Age (years)	−4.52 (−7.43 to 1.61)	0.002	0.040	
Physical activity (active/inactive)	42.06 (24.12 to 59.99)	<0.001	0.099	0.059
Chronic diseases (present/absent)	−46.81 (−62.87 to −30.74)	<0.001	0.131	0.091
Beta-blockers (yes/no)	−27.23 (−45.04 to −9.43)	0.003	0.064	0.024
Body mass index (kg/m^2)	−5.86 (−7.53 to −4.18)	<0.001	0.176	0.136
Squatting (normal/painful or limited)	−60.20 (−76.72 to −43.69)	<0.001	0.195	0.155
SBP (mmHg)[c]	−0.39 (−0.83 to 0.06)	0.086	0.076	0.012
DBP (mmHg)[c]	−1.29 (−2.12 to −0.47)	0.002	0.096	0.032
Resting heart rate (bpm)[c]	−0.43 (−1.28 to 0.42)	0.316	0.068	0.004

[a] Cumulative explanation rate for clinical variable and age.
[b] Increase in explanation rate when explanation rate of age (in cases of heart rate and systolic and diastolic blood pressure explanation rate of age and beta-blockers) has been subtracted from explanation rate of clinical variable and age (in cases of heart rate and systolic and diastolic blood pressure explanation rate of heart rate/blood pressure, age and beta-blockers); explanation rate of age = 0.040, explanation rate of age and beta-blockers = 0.064.
[c] Adjusted for age and use of beta-blockers.

Table 3
Age-adjusted results of electrocardiographic measurements as predictors of six-minute walking distance (linear regression analysis) in 63- to 75-year-old women ($n = 320$).

	Regression coefficient (95% CI)	p	R^{2a}	R^{2b}
P axis (degree)	0.40 (0.00 to 0.80)	0.048	0.052	0.012
QRS axis (degree)	0.39 (0.06 to 0.73)	0.023	0.058	0.018
Cornell voltage (RaVL + SV_3) (mm)	−2.63 (−4.39 to −0.88)	0.004	0.070	0.030
T wave amplitude in lead V_5 (mm)	10.59 (4.95 to 16.23)	<0.001	0.085	0.045
T wave amplitude in lead II (mm)	13.42 (6.18 to 20.66)	<0.001	0.078	0.038

[a] Cumulative explanation rate for ECG variable and age.
[b] Increase in explanation rate when explanation rate of age has been subtracted from explanation rate of ECG variable and age; explanation rate of age = 0.040.

group ($n = 320$) are shown in Table 1. Of the clinical variables, ability to squat without pain or other limitations, low BMI, absence of chronic diseases and participation in physical activity were the best age-adjusted predictors of distance walked during six minutes (Table 2). Those participants who were able to squat normally walked 557 ± 83 m during six minutes and those who were unable to squat or whose squatting was painful walked 497 ± 70 m ($p < 0.001$). Resting heart rate and systolic blood pressure (adjusted for both age and use of beta-blockers) were the only variables that did not attain statistical significance. Altogether the clinical variables explained 35% of the variation in walking distance.

Of the nine ECG measurements performed those presented in Table 3 reached statistical significance in explaining the variation in walking distance, with high TV_5, high TII and low Cornell voltage being the best age-adjusted predictors of long walking distance. In those participants ($n = 102$), whose systolic blood pressure was ≥160 mmHg, TV_5 and TII explained the variation in walking distance better, the increases in explanation rates in addition to age being 0.093 and 0.058, respectively. The cumulative explanation rate for all the statistically significant ECG variables could not be calculated due to multicollinearity between certain ECG variables (e.g., TV_5 and TII). Cornell voltage and TV_5 had the greatest independent explanation rate of the variables reflecting the QRS complex and T waves, respectively, and they explained 6% of the variation in walking distance. When ECG variables were adjusted for the variables presented in Table 2, every ECG variable lost its statistical significance. Together TV_5, Cornell voltage and other clinical variables, including age, explained 36% of the variation in walking distance.

The results of the within-pair analyses are presented in Table 4. The mean difference between better and worse walking MZ co-twins was 125.6 m, ranging from 75 m to 240 m. Of the clinical variables, BMI and squatting ability differed statistically significantly between better and worse walking co-twins whereas TV_5 was the only resting ECG variable differing between these two groups. TV_5 was statistically significantly higher in better walking co-twins compared with their worse walking co-twins.

4. Discussion

We found that among a non-clinical group of women aged 63–75 years, the predictive value of resting ECG variables for six-minute walking distance was small. However, when analyses were restricted to participants with hypertension, correlations between T wave amplitudes and six-minute walking distance became stronger. In our comparisons of twins discordant for walking endurance, we observed that TV_5 was significantly higher in better walking co-twins compared with their worse walking co-twins, showing that at least part of this association is independent of genetic factors.

These analyses provide information about correlations between resting ECG variables and walking endurance among people at very early phase of mobility decline typically observed with age. Among them, the role of each single ECG variable in explaining variation in walking distance was rather small, probably because participants with significant cardiac disease (myocardial infarction) were excluded from the analyses. In older persons without overt cardiac diseases, cardiac function determined by resting ECG does not limit exercise capacity and thus walking ability in the same way as in persons with known cardiac disease (e.g., myocardial infarction, heart failure). In this study, participants with a history of myocardial infarction walked shorter distances during six minutes than the other participants. However, the shortest distance walked by any participant of our study was 270 m showing that none of the participants had severe mobility limitation. The truncated distribution of the walking test results caused by the fact that the participants had no apparent mobility limitations may also underlie the lack of association between resting ECG variables and walking endurance.

Table 4
Six-minute walking distance, clinical variables and resting electrocardiographic variables in worse and better walking MZ co-twins, whose within-pair difference in six-minute walking distance is ≥75 m (range 75–240 m), mean ± S.D.

n	Worse walking 16	Better walking 16	Mean difference (95% CI)	p^a
Six-minute walking distance (m)	451.6 ± 97.0	577.2 ± 65.4	−125.6 (−153.3 to −97.9)	<0.001
Physical activity (active/inactive)				0.180
Chronic diseases (present/absent)				0.257
Beta-blockers (yes/no)				0.655
BMI (kg/m^2)	28.4 ± 4.5	26.3 ± 4.2	2.1 (0.6 to 3.8)	0.011
Squatting (normal/painful or limited)				0.046
SBP (mmHg)[b]	148.5 ± 22.4	142.3 ± 20.7	6.2 (−3.5 to 15.9)	0.185
DBP (mmHg)[b]	87.3 ± 9.8	84.4 ± 11.7	2.9 (−4.1 to 9.9)	0.377
Resting heart rate (bpm)[b]	74.1 ± 10.5	70.8 ± 12.0	3.3 (−3.2 to 9.8)	0.083
P axis (degree)	43.1 ± 25.5	56.2 ± 16.8	−13.1 (−28.3 to 2.0)	0.112
QRS axis (degree)	26.6 ± 27.2	34.4 ± 31.6	−7.8 (−20.4 to 4.7)	0.244
Cornell voltage (RaVL + SV_3) (mm)	12.0 ± 5.0	10.0 ± 5.0	2.0 (0.0 to 4.0)	0.214
T wave amplitude in lead V_5 (mm)	1.6 ± 1.2	2.3 ± 1.6	−0.7 (−1.0 to 0.0)	0.034
T wave amplitude in lead II (mm)	1.5 ± 1.0	1.9 ± 1.3	−0.4 (−1.0 to 0.0)	0.110

[a] For the difference between worse and better walking co-twins, based on paired samples Student's t-test for normally distributed continuous variables, Wilcoxon signed ranks test for non-normally distributed continuous variables and the symmetry test for categorical variables.
[b] Five pairs discordant for use of beta-blockers excluded.

TV_5, TII and Cornell voltage showed the clearest correlations with six-minute walking distance in the age-adjusted analyses. For both T wave amplitudes, the regression coefficients were positive, indicating that the higher the T wave amplitude, the longer the six-minute walking distance, or vice versa. A positive (high) T wave is usually a sign of normal ventricular repolarization (Yan et al., 2003) while a low or negative (inverted) T wave may be a sign of reduced cardiac function and thus limit exercise capacity and walking ability. Among the participants whose systolic blood pressure was $\geq$160 mmHg, correlations of TV_5 and TII with six-minute walking distance were stronger than for the entire study group. This association may be explained by at least two different mechanisms. Firstly, high blood pressure can cause myocardial ischemia, even independent of the presence of LVH or coronary artery disease (Asmar et al., 1996; Stramba-Badiale et al., 1998). Presence of myocardial ischemia can be manifested not only by ST segment depression, but also by lowered or inverted T waves. Secondly, if high blood pressure has been longstanding, it may have caused the development of LVH. This, in turn, can be associated with repolarization abnormalities (Moore et al., 1984) and lowered or inverted T waves.

The result of within-pair analysis further strengthened the association between TV_5 and walking ability since by studying MZ twin pairs we were able to control for genetic factors. This controlling was important because about 20% of the variation in six-minute walking distance (Ortega-Alonso et al., 2006) and 72% of the variation in TV_5 (Mutikainen et al., 2009) in this study population is explained by genetic factors. Even after controlling for genetic factors, TV_5 was significantly higher in better walking MZ co-twins compared with their worse walking co-twins. Previous studies in non-related individuals have shown that the T wave amplitudes are higher in athletes compared with sedentary controls (Bjørnstad et al., 1991) and in persons with higher fitness level compared with persons whose fitness level is lower (Bjørnstad et al., 1993). Higher fitness level in better walking co-twins compared with their worse walking co-twins can be one explanation for our results since the six-minute walking test evaluates especially walking endurance. Achievement of good walking endurance requires normal cardiac function whereas decreased walking endurance can be caused by, for example, disturbances in cardiac function, such as repolarization abnormalities or myocardial ischemia.

The regression coefficient of Cornell voltage was negative, indicating that low Cornell voltage is associated with long walking distance, and vice versa. Generally, LVH is a pathological state resulting from, for example, chronic hypertension. In pathological LVH, systolic and diastolic function is disturbed and weakened, and it is associated with decreased exercise capacity (Olsen et al., 2001; Pierson et al., 2004).

After adjustment for the other clinical variables included in this study, the ECG variables which were statistically significant in the age-adjusted analyses lost their statistical significance. One possible reason for this is that among the other clinical variables were factors which are known independently to cause changes in ECG findings (e.g., obesity, age) (Yasumura and Shibata, 1989; Alpert et al., 2000).

In our study, normal ability to squat, low BMI, absence of chronic diseases and participation in physical activity were the best predictors of good result in the six-minute walking test. BMI, chronic diseases and physical activity have been shown to be good predictors of six-minute walking distance also in other studies (Eng et al., 2002; Hulens et al., 2003). Squatting is a simple functional test which can easily be performed during a clinical examination and it reveals a great deal about the patient's functional ability. High BMI limits walking ability by increasing workload (Mattsson et al., 1997) and/or causing mechanical complications such as osteoarthritis or pain in the lower limbs (Messier et al., 1994; Oliveria et al., 1999). In addition to causing pain or other limitations in walking, chronic diseases are often one of the most important reasons for fear of exertion in older people (Murphy et al., 2002), restricting their walking ability.

Also many other factors can be associated with walking ability. Possible predictors of walking performance in the six-minute walking test not investigated in this study are, for example, height, lower limb muscle strength and peak VO_2, which have been shown to be predictors of six-minute walking distance in other studies (Lord and Menz, 2002; Hulens et al., 2003; Camarri et al., 2006). Other possible predictors are such factors as decreased mental status (Enright et al., 2003) or motivation.

There are some limitations in this study. Our study population was truncated as those who had overt difficulties in walking did not take part in this study. In addition, participants with significant cardiac diseases were excluded from the analyses. Thus, the results of this study are applicable only to relatively healthy older women without overt cardiac diseases and apparent mobility limitations. Such women, however, make up a substantial proportion of the women in these age-groups, and will increase in size as populations age and become healthier.

5. Conclusions

The ability to squat normally, low BMI, absence of chronic diseases and participation in physical activity are the most significant predictors of good walking ability in relatively healthy older women. The predictive value of the ECG variables is smaller, except for TV_5 and TII in those persons with high systolic blood pressure. From the clinical point of view, the factors studied here give valuable information for the assessment of walking ability and functional status in older women. These results may also be used as a basis for future prospective analyses on changes in health.

Conflict of interest statement

None.

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WALKING ABILITY AND ALL-CAUSE MORTALITY IN OLDER WOMEN

by

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Walking Ability and All-Cause Mortality in Older Women

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Abstract

In this study self-reported ability to walk 2 km and six-minute walking test (6MWT) performance were examined as predictors of all-cause mortality in 434 women, aged 63-76 years. The primary outcome measure was all-cause mortality (follow-up: eight years). Predictors were self-reported difficulties in walking 2 km and six-minute walking distance tertiles of ≤495, 496-560 and ≥561 m, and no test result due to refusal by the physician to grant permission to perform the test or participant's inability or unwillingness to perform the test. During the follow-up, 39 participants died. Participants reporting minor (age- and body mass index-adjusted hazard ratio 2.53, 95% confidence interval 1.12-5.69) or major (7.93, 3.49-18.05) difficulties in walking 2 km had increased risk of death compared with those reporting no difficulties. Participants with no 6MWT result (6.99, 2.46-19.86) were at an increased risk of death when compared with participants who walked ≥561 m. A similar trend (2.47, 0.81-7.56) was found for participants with walking distance of ≤495 m during the 6MWT. The trends remained similar after adjustments for other confounders. In conclusion, self-reported difficulties in walking 2 km are associated with an increased risk of death in older community-dwelling women. Objectively measured walking ability gives similar results.

INTRODUCTION

The ability to walk briskly for a moderate distance (several hundred meters) requires good exercise capacity and is a significant factor for maintaining independence in old age. In addition to risk for loss of independence, reduced exercise capacity and walking ability also have other important implications. Both factors have been reported to be associated with increased risk of morbidity [14,19,21] and mortality [3,4,9,10,18,19,24]. For example, a previous study [19] in a community-based cohort of well-functioning older adults reported that the inability to walk 400 m was a significant predictor of both mobility limitation and mobility disability, as also was each additional minute of longer performance time among those who were able to walk 400 m. Longer performance time was also associated with increased risk of incident cardiovascular disease and all-cause mortality.

Walking ability can be assessed both by self-reports and objective tests. In clinical practice, however, there is not always the time or opportunity to perform walking tests, and on such occasions the self-report may be the only useful way to estimate a patient's walking ability. If self-reported walking ability can be "validated" using an objective measurement of walking ability, then self-report can be regarded as both an easy and useful way to estimate walking ability and, accordingly, also a prognosis. Previous studies on self-reported walking ability have reported that, for example, difficulties in walking 500 m [24] and ½ mile (about 800 m) [10] are associated with increased risk of death in older people.

There are several tests for the objective assessment of walking ability, such as the six-minute walking test (6MWT) and 400-meter walk test. The 6MWT is widely used and has been shown to be a reliable and valid method for the assessment of functional exercise capacity and walking ability both in the elderly and in people with chronic diseases [23]. The reduced six-minute walking distance (6MWD) has been reported to be a predictor of death, for example in patients with chronic obstructive pulmonary disease [6], left ventricular dysfunction [2], and peripheral artery disease [15]. However, less is known about the association between 6MWD and mortality in older community-dwelling populations. The aim of our study was to investigate self-reported ability to walk 2 km and 6MWT performance as predictors of all-cause mortality in older community-dwelling women. Since our study group consisted of twin pairs, we also utilized that and studied abovementioned associations among twin pairs who were discordant both for walking ability and survival status. By studying twin pairs, we were able to control for genetic liability and childhood environment.

METHODS

Participants

Four hundred and thirty four women (217 twin sister pairs) aged 63-76 years participated in this study, which forms part of the Finnish Twin Study on Aging (FITSA). The aim of the FITSA study is to investigate genetic and environmental influences on the disablement process in older women. Women were chosen for the target group since they usually live longer than men and consequently the disablement process may be more clearly seen among them than among men. The study group was mainly treated as a set of individuals taking into account the clustering of possibly correlated observations from twin pairs. Within-pair analyses were also performed for twin pairs discordant for both walking ability and survival status (see below).

The study sample was recruited from the Finnish Twin Cohort Study [12], which comprises all same-sex twins born in Finland before 1958 with both co-twins alive in 1975. Details on recruitment have been described earlier [20] and are briefly summarized here. An invitation to participate in FITSA was sent to 414 twin sister pairs aged 63-76 years surviving in 2000. To be included, both twin pair members had to agree to participate. The reasons for non-participation were that one or both sisters were unwilling to take part (106 pairs), had poor health status (85 pairs), or had died after vital status was last updated for all cohort members (6 pairs). As a result of the procedures, the study group consisted of 217 twin sister pairs (434 individuals). In the statistical analyses some exclusions were made. Twelve participants did not have time to perform the 6MWT (see also below) and accordingly they were excluded, leaving 422 participants for the 6MWT-related analyses. The zygosity of the participating pairs was initially determined through a validated questionnaire [22] and later confirmed using a battery of 10 highly polymorphic gene markers in DNA extracted from a venous blood sample.

The study was conducted according to the ethical standards of the International Journal of Sports Medicine [11]. All the participants were informed about the study and they signed a written informed consent. The study was approved by the ethics committee of the Central Hospital of Central Finland.

Self-reported ability to walk 2 km

Self-reported ability to walk 2 km was assessed by a questionnaire before the laboratory measurements. Participants were asked if they had no difficulties (n=296), minor difficulties (n=101), or major difficulties (n=37) in walking 2 km. The latter group also included participants who needed help or were unable to walk 2 km. The group with no difficulties served as a reference group. The ability to walk 2 km (instead of some shorter distance such as 500 m) was chosen for self-report because the study group consisted of high functioning older women. In this target group, difficulties in walking such a long distance can be used as an early sign of mobility decline.

Six-minute walking test

Maximal walking distance was assessed using the 6MWT. The participants were requested to walk up and down a 50-m indoor straight track for six minutes and to complete as many laps as possible. Except for the length of the indoor track, the protocol and security conditions followed the instructions subsequently published in the American Thoracic Society Statement [1]. The distance covered by the end of the six minutes was recorded as the outcome.

Seventy-five participants were unable to perform the 6MWT. Reasons for not completing the test were refusal by the examining physician to grant permission to perform the test [n=54; specifically, these were severe coronary artery disease and/or chronic heart failure (n=19), severe arthrosis of hip, knee or ankle (n=19), COPD (n=2), and general health status (n=14)], lack of time (n=12), and participant's inability or unwillingness to initiate or finalize the test (n=9). The participants who had no time to perform the 6MWT (n=12) were excluded from the analyses. The remaining 63 participants were considered as a single group, labelled "no result". The other participants (n=359) performed the 6MWT and were divided into tertiles according to their 6MWD. The highest tertile (n=118) walked ≥ 561 m, the middle tertile (n=118) 496 – 560 m, and the lowest tertile (n=123) ≤ 495 m. The highest tertile was considered as a reference group.

Assessment of mortality data

The mortality follow-up began on September 18, 2000 and continued until May 31, 2009. Death dates were received from the Population Register Centre of Finland. Deaths from all causes were included in the analyses.

Assessment of confounders

All participants were clinically examined by a physician. Self-reports of acute and chronic diseases and medication had been obtained earlier and were confirmed in a clinical examination. Chronic diseases taken into account were arthrosis of hip (n=35), knee (n=124), and ankle (n=15), rheumatoid arthritis (n=18), coronary artery disease (n=53), myocardial infarction (n=19), hypertension (n=161), chronic heart failure (n=21), claudication (n=4), asthma (n=35), COPD (n=4), cerebral haemorrhage and stroke (n=30), Parkinson's disease (n=1), and cancer (n=55), all of which may affect walking ability and/or mortality. Diseases were dichotomized as present (n=310) or absent (n=124). Other dichotomized variables were the use of beta-blockers (yes, n=137; no, n=297), smoking status (never smoked, n=374; current smokers, n=59), and physical activity status. The current status of physical activity was assessed using the self-report scale designed by Grimby [8], with slight modifications. The 7-point scale ranged from hardly any activity to participation in competitive sports. Participants were considered sedentary if they reported no other activity than light walking once or twice a week (n=123). In all other cases participants were considered physically active (n=311). Age, body mass index (BMI), systolic and diastolic blood pressure, total cholesterol, and maximal isometric knee extension strength were treated as continuous covariates as also were resting electro-

cardiographic (ECG) variables, which were recorded and measured as previously described [17].

Statistical analysis

Data were analyzed using Stata Version 8 (Stata Corp., College Station, TX, USA) and SPSS 14.0 (SPSS Inc., Chicago, IL, USA). Descriptive data for continuous variables were calculated as mean ± SD. Differences in baseline characteristics between surviving and deceased participants were analyzed with the adjusted Wald test for continuous variables. The respective test for the walking ability groups was the analysis of variance or its non-parametric version (Kruskall-Wallis test or Welch test). Categorized variables were analyzed using cross-tabulation with chi-squared test.

Mortality rates were expressed as number of deaths per 1000 person-years. Follow-up time was calculated from the beginning of the baseline measurements to the date of death or to the end of the follow-up. The Kaplan-Meier procedure with log-rank test was used to estimate mortality functions for the walking ability groups. Cox regression models were used to assess hazard ratios (HR) with 95% confidence intervals for all-cause mortality. The proportional hazard assumption was found to be valid. The analyses were first performed without adjustments and thereafter each covariate was added to the model one at a time. Finally the covariates, which were significantly associated with mortality, were added to the model at the same time. Clustering of possibly correlated observations from twin pairs was taken into account in each individual-based analysis by computing standard errors of coefficients using robust estimators of variance [26]. Within-pair analyses (co-twin control design) were carried out for the twin pairs discordant for both walking ability and survival status. In these analyses, the data were stratified by pair and therefore the risk estimates were within-pair estimates. Significance was set at p less than 0.05 (two-sided) for all tests.

RESULTS

During the follow-up period, 39 participants (9%) died. When participants who did not have time to perform the 6MWT were excluded (6MWT-related analyses), the number of deaths was 38. The mean ± SD length of the follow-up until death or the end of the follow-up was 8.4 ± 1.2 years. Participants who died were older than those who survived and the proportion of participants with a sedentary lifestyle was greater among the deceased than surviving participants ($p < 0.05$) (Table 1). The proportion of participants who reported minor or major difficulties in walking 2 km was significantly greater among those who died, as was also the proportion of participants who did not have 6MWT result.

Baseline characteristics by self-reported ability to walk 2 km are also shown in Table 1. When baseline characteristics were studied according to the 6MWD tertiles, the results resembled those for self-reported walking ability, except for diastolic blood pressure, presence of bundle branch blocks, myocardial

infarction and/or pacemaker, Sokolow Lyon voltage, and Cornell voltage, which differed only between the 6MWD tertiles (results not shown).

Table 2 presents the 6MWT results according to self-reported ability to walk 2 km. The majority of the participants who reported being able to walk 2 km with no difficulties were also able to perform the 6MWT, and 68% of these participants walked ≥ 496 m. Sixty-five percent of the participants, who reported major difficulties in walking 2 km, had no result in the 6MWT.

Crude mortality rates per 1000 person-years are presented in Table 3 and survival functions according to self-reported ability to walk 2 km and 6MWT results in Figs. 1 and 2. Survival differed significantly between the walking ability groups.

Self-reported ability to walk 2 km predicted all-cause mortality (Table 3). In the age- and BMI-adjusted analyses, participants with minor (HR 2.53, 95% CI 1.12-5.69) and major (7.93, 3.49-18.05) difficulties in walking 2 km had significantly increased risk of all-cause mortality compared with those who had no difficulties. The results remained similar after adjustments for other potential confounders, including chronic diseases. Significantly increased risk was also observed when participants with minor and major difficulties in walking 2 km were considered as a single group. Participants with difficulties of any kind in walking 2 km had over threefold age-adjusted risk (3.16, 1.65-6.06) of all-cause mortality compared with participants who reported no difficulties.

All-cause mortality was also predicted by the 6MWT performance (Table 3). The age- and BMI-adjusted risk of mortality in the middle tertile was similar (1.01, 0.29-3.51) to that in the highest tertile, but the risk was over twofold (2.47, 0.81-7.56) in the lowest tertile and almost sevenfold (6.99, 2.46-19.86) in participants with no test result compared with the highest tertile. These results remained similar when adjusted for other potential confounders. When the subjects were dichotomized into those whose 6MWD was ≤ 495 m (lowest tertile and no test result) and into those who walked ≥ 496 m (middle and highest tertile), after adjusting for age and BMI those who walked ≤ 495 m had almost fourfold risk of mortality compared with the better walkers (3.73, 1.78-7.83).

When self-reported walking ability and the 6MWT result (dichotomized as result available/no result) were studied in the same model, self-reported major difficulties in walking 2 km remained a significant predictor of all-cause mortality (age- and BMI –adjusted HR 4.62, 95% CI 1.78-12.02). Those with minor difficulties in walking 2 km had twofold risk of death (2.15, 0.91-5.05) compared with participants who reported no difficulties. The results remained unchanged after adjustments for potential confounders, including physical activity. In this same model, HR for the dichotomized 6MWT result was 2.52 (95% CI 1.12-5.65). The 6MWT result as a continuous variable (meters walked during six minutes) in the abovementioned model was not significant among the participants who were capable of performing the 6MWT.

Of all the twin pairs, 81 pairs were discordant for self-reported ability to walk 2 km and 34 pairs were discordant for survival status. Of these pairs, 22 pairs (6 monozygotic and 16 dizygotic) were discordant for both self-reported

walking ability and survival status. Of these 22 pairs there were 20 pairs in which the co-twin reporting more difficulties in walking 2 km had died during the follow-up, while her co-twin reporting fewer difficulties in walking 2 km survived. The converse was true for only two pairs. The respective numbers for monozygotic pairs were 6 and 0. Thus, the within-pair analyses among all pairs showed that, compared with the twin sisters who reported no difficulties in walking 2 km, their co-twins, who reported minor (10.7, 1.4-82.8) or major (68.6, 4.0-1176) difficulties, had increased risk of death. The results persisted after adjustments for potential confounders.

DISCUSSION

Our study on elderly community-dwelling women showed that self-reported walking ability is a significant predictor of all-cause mortality. The participants with minor self-reported difficulties in walking 2 km had over twofold age- and BMI-adjusted risk of mortality compared with those who had no difficulties, while the respective risk was almost eightfold in the participants who reported major difficulties, needed help, or were unable to walk 2 km. Similar results were observed when walking ability was assessed using the 6MWT. Both self-reported and objectively measured walking ability remained significant predictors of all-cause mortality after adjustments for chronic diseases and other potential confounders, including unmeasured within-pair factors.

Previous studies have also shown that self-reported difficulties in walking longer distances are associated with increased risk of mortality in older people [10,24]. For example, the study by Tilvis et al. [24] in men and women aged 65 years and older showed that self-reported difficulties in walking more than 500 m are associated with approximately two- to fourfold increased age-adjusted risk of mortality compared with those who do not have any difficulties. When we reanalysed the data after classifying the participants into those with no difficulties and those with difficulties of any kind in walking 2 km, the results were rather similar to those obtained by Tilvis et al. [24] (age-adjusted risk of all-cause mortality was over threefold in those with difficulties in walking 2 km compared with those who had no difficulties). However, it seems that by using three categories (no difficulties, minor difficulties, major difficulties), the risk can be estimated more precisely. In addition, self-reported major difficulties in walking 2 km remained a significant predictor of all-cause mortality when the 6MWT result (i.e., whether participant has test result or not) was taken into account. The results of our study were further confirmed in the within-pair analyses in the twin pairs discordant for both self-reported walking ability and survival status. These analyses gave results similar to those of the individual-based analyses and suggested that the association between reduced self-reported walking ability and increased all-cause mortality is independent of genetic factors.

Since the ability to walk 2 km was self-reported, it is not possible to propose any specific criteria for determining whether the subject had major or minor difficulties in walking 2 km. However, some speculations can be presented.

When we analyzed some of the background characteristics in relation to the difficulties in walking 2 km, we observed that a greater proportion of the participants reporting minor or major difficulties had chronic diseases (e.g., cardiovascular diseases and musculoskeletal disorders), were obese or were physically inactive compared with those reporting no difficulties.

The usefulness of the 6MWT in the assessment of exercise capacity and prognosis in people with chronic disease is well-established [2,5,6,15,16]. Our results support these findings by showing similar trends in a population of older community-dwelling women. No result in the 6MWT was a strong predictor of all-cause mortality, even after adjustments for potential confounders, including chronic diseases affecting mortality and/or walking ability. Similar results have been obtained in studies which have used the 400-meter walk test. Vestergaard et al. [25] reported that the inability to walk 400 m and 400-meter walking time were associated with increased risk of death among men and women aged ≥ 65 years. 400-meter walking time was also associated with all-cause mortality in the study by Newman et al. [19] among 70- to 79-year-old men and women. Dumurgier et al. [7] reported that low walking speed was associated with increased risk of cardiovascular mortality in older people. We also analyzed whether 10 m walking speed predicts all-cause mortality, but in our study cohort its predictive value was much lower (results not shown) than that reported by Dumurgier et al. [7]. In our study, both the simple question on the ability to walk 2 km and walking endurance assessed by the 6MWT were associated with increased risk of death, and can accordingly be used as early indicators of health deterioration among high functioning older women.

There are some possible mechanisms that may explain our results. Firstly, the ability to walk 2 km and completion of the 6MWT is an indicator of sufficient exercise capacity to undertake daily tasks, while reduced exercise capacity, in turn, is associated with increased risk of mortality [13]. Secondly, reduced walking ability may be an indicator of poor health, in which case poor health status could partly explain the association between reduced walking ability and increased mortality. However, the twin pairs with the poorest health did not participate in our study, and we adjusted the analyses for chronic diseases and also for resting ECG variables, which can reveal serious but asymptomatic problems in cardiac function. Reduced walking ability remained an important predictor of increased all-cause mortality.

One of the strengths of our study is that we compared self-reported walking ability against objectively measured data. The participants were quite reliably able to estimate their walking ability by themselves. Although the number of study participants was rather small, they were community-dwelling, relatively healthy older women. Such women make up a substantial proportion of the women in these age-groups and their proportion will increase in the future as populations age and become healthier. In addition, the use of the twin design (within-pair analyses) confirmed the results of the individual-based analyses, suggesting that the observed association between reduced walking ability and increased all-cause mortality is independent of genetic factors and

childhood environment. Among limitations of our study are the relatively low outcome rate and the dominance of judgments by a physician to exclude participants from the 6MWT. More studies on this topic are needed among more disabled women, and also among men and among different age-groups to see whether similar trends in the association between self-reported difficulties in walking longer distances and all-cause mortality are also observed in these populations.

In conclusion, self-reported difficulties in walking 2 km, no result in the 6MWT, and walking a distance of ≤ 495 m in the 6MWT are associated with increased risk of all-cause mortality in older community-dwelling women. The results suggest that self-reported walking ability is one useful way to identify subjects who may be at increased risk of death in this target group.

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Table 1 Baseline characteristics by survival status and self-reported ability to walk 2 km in 63- to 76-year-old women. Results are mean ± SD for continuous variables.

		Survival status			Self-reported ability to walk 2 km			
	All (n=434)	Survived (n=395)	Died (n=39)	p	No difficulties (n=296)	Minor difficulties (n=101)	Major difficulties* (n=37)	p
Age (yrs)	68.6±3.4	68.4±4.6	70.6±4.3	0.002	68.3±3.3	69.0±3.3	70.2±3.8	0.002
Body mass index (kg/m²)	28.0±4.7	28.0±5.8	27.4±4.5	0.39	27.3±4.2	29.1±5.6	30.5±5.1	< 0.001
Systolic blood pressure (mmHg)	149.7±21.9	149.5±25.8	151.5±23.8	0.60	150.1±21.7	148.6±23.1	148.9±20.2	0.68
Diastolic blood pressure (mmHg)	85.9±10.3	86.0±12.3	84.5±9.6	0.37	86.2±10.2	85.5±11.0	84.8±9.8	0.73
Total cholesterol (mmol/l)	5.5±0.9	5.5±1.0	5.6±1.2	0.85	5.5±0.9	5.7±1.0	5.5±0.9	0.47
Maximal isometric knee extension strength (N)	334.8±186.0	334.5±241.7	337.1±282.7	0.95	336.6±148.6	316.0±230.1	371.0±292.7	0.53
Six-minute walking test result†; yes, n	359 (85%)	337 (85%)	22 (56%)	< 0.001	268 (93%)	79 (80%)	12 (35%)	< 0.001
Physical activity status; active, n	311 (72%)	291 (74%)	20 (51%)	0.003	234 (79%)	65 (64%)	12 (32%)	< 0.001
Chronic diseases‡; yes, n	310 (71%)	280 (71%)	30 (77%)	0.42	186 (63%)	89 (88%)	35 (95%)	< 0.001
Chronic diseases§; yes, n	228 (53%)	202 (51%)	26 (67%)	0.07	140 (47%)	60 (59%)	28 (76%)	0.002
Bundle branch block, pacemaker and/or MI; yes, n	45 (10%)	39 (10%)	6 (15%)	0.27	26 (9%)	14 (14%)	5 (14%)	0.30
Beta-blockers; yes, n	137 (32%)	121 (31%)	16 (41%)	0.18	81 (27%)	40 (40%)	16 (43%)	0.02
Smoking status; current smoker, n	59 (14%)	51 (13%)	8 (21%)	0.24	38 (13%)	16 (16%)	5 (14%)	0.77
Heart rate (bpm)	68.5±13.2	68.3±13.1	70.6±12.1	0.25	68.7±11.7	67.7±10.6	68.8±10.6	0.80
P axis (degree)	47.2±21.4	47.8±21.7	40.7±25.2	0.12	48.6±20.9	43.9±22.4	44.5±22.3	0.12
QRS axis (degree)	29.2±26.9	28.5±31.7	36.2±31.9	0.19	30.6±26.6	29.7±26.0	15.8±29.5	0.03
Sokolow Lyon voltage¶ (mV)	2.3±0.7	2.3±0.8	2.3±0.7	0.69	2.3±0.6	2.2±0.8	2.2±0.6	0.33
Cornell voltage** (mV)	1.1±0.5	1.1±0.6	1.2±0.6	0.36	1.1±0.5	1.2±0.5	1.3±0.5	0.08
Cornell product†† (mV x ms)	100.8±47.1	100.2±56.2	106.8±53.7	0.49	97.5±45.7	104.8±51.2	117.3±44.3	0.04
Electrocardiographic LVM‡‡ (g)	114.8±13.7	114.9±16.6	113.2±13.6	0.48	113.1±12.2	117.9±16.2	120.6±15.8	0.004
T wave amplitude, lead V_5 (mV)	0.18±0.15	0.19±0.19	0.16±0.17	0.33	0.20±0.15	0.18±0.15	0.11±0.17	0.003
T wave amplitude, lead II (mV)	0.16±0.11	0.17±0.19	0.14±0.11	0.34	0.17±0.11	0.15±0.11	0.11±0.10	0.009

Abbreviations: MI, myocardial infarction; LVM, left ventricular mass.

* Includes participants who need help or are unable to walk 2 km;

† Participants with no time to perform the six-minute walking test excluded (n=12);

‡ Includes cardiovascular, pulmonary, neurological and musculoskeletal diseases, and cancer;

§ Includes cardiovascular diseases, COPD, and cancer;

¶ $SV_1 + RV_5$;

** $RaVL + SV_3$;

†† $(RaVL + SV_3) \times QRS$ duration;

‡‡ $(0.02 \times [RaVL + SV_3]) + (1.12 \times \text{body weight}) + 36.2$

Table 2 Six-minute walking test result according to self-reported ability to walk 2 km in 63- to 76-year-old women.

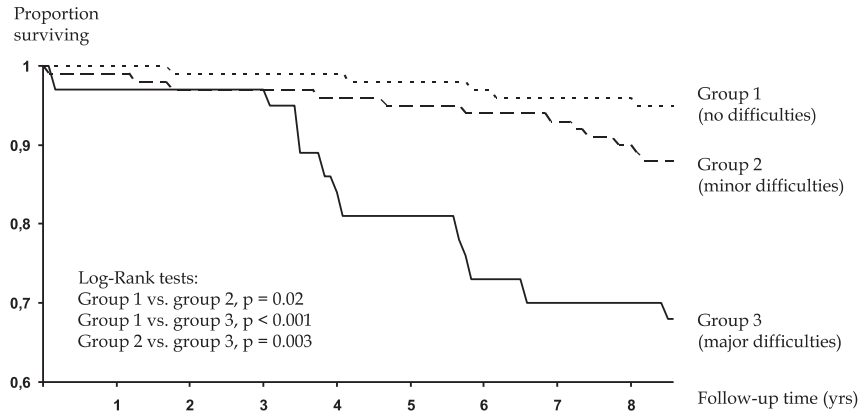
	Six-minute walking test result*			
Self-reported ability to walk 2 km	**≥ 561 m**	**496-560 m**	**≤ 495 m**	**No result†**
No difficulties, n	105 (36%)	92 (32%)	71 (25%)	21 (7%)
Minor difficulties, n	12 (12%)	21 (21%)	46 (47%)	20 (20%)
Major difficulties‡, n	1 (3%)	5 (15%)	6 (18%)	22 (65%)

* Participants with no time to perform the six-minute walking test excluded (n=12);
† Examining physician did not grant permission to perform the test or participant was unwilling or unable to initiate or finalize the test;
‡ Includes participants who need help or are unable to walk 2 km

Table 3 Associations between self-reported ability to walk 2 km, six-minute walking test (6MWT) result and all-cause mortality among 63-to 76-year-old women. Cox regression models for risk of death in each self-reported walking ability group and 6MWT result group.

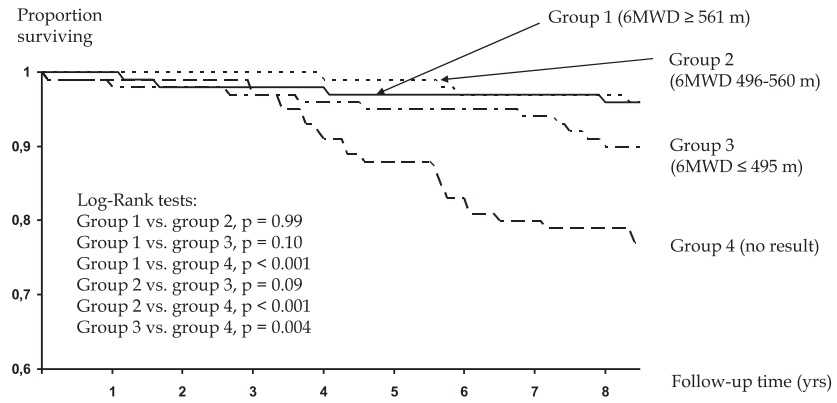
	Person-years	Deaths	Rate (‰)	Hazard ratio (95% confidence interval)	
				Model 1	Model 2
Self-reported ability to walk 2km					
No difficulties*	2526	15	5.9	1.00	1.00
Minor difficulties	839	12	14.3	2.18 (0.98-4.85)	2.53 (1.12-5.69)†
Major difficulties‡	274	12	43.8	6.09 (2.85-13.01)†	7.93 (3.49-18.05)†
6MWT result					
≥ 561 m*	1005	5	5.0	1.00	1.00
496-560 m	1016	5	4.9	0.84 (0.24-2.88)	1.01 (0.29-3.51)
≤ 495 m	1027	12	11.7	1.84 (0.63-5.42)	2.47 (0.81-7.56)
No result§	590	16	27.1	4.79 (1.81-12.70)†	6.99 (2.46-19.86)†

Model 1: Adjusted for age;
Model 2: Adjusted for age and body mass index;
* Reference group;
† $p < 0.05$;
‡ Includes participants who need help or are unable to walk 2 km;
§ Examining physician did not grant permission to perform the test or participant was unwilling or unable to initiate or finalize the test



	0-2 yrs		2-4 yrs		4-6 yrs		6+ yrs	
	At risk	Deaths	At risk	Deaths	At risk	Deaths	At risk	Deaths
Group 1	296	2	294	1	293	5	288	7
Group 2	101	3	98	1	97	2	95	6
Group 3	37	1	36	3	33	6	27	2

FIGURE 1 Survival functions according to self-reported ability to walk 2 km in 63- to 76-year-old women.



	0-2 yrs		2-4 yrs		4-6 yrs		6+ yrs	
	At risk	Deaths	At risk	Deaths	At risk	Deaths	At risk	Deaths
Group 1	118	2	116	0	116	1	115	2
Group 2	118	0	118	0	118	2	116	3
Group 3	123	3	120	2	118	1	117	6
Group 4	63	1	62	2	60	9	51	4

FIGURE 2 Survival functions according to the six-minute walking test result in 63- to 76-year-old women. 6MWD = 6-min walking distance.

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