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Variations of cardiovascular disease associated genes exhibit sex-dependent influence on human longevity

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Abstract

This article investigates the relationship between the polymorphic variations in genes associated with cardiovascular disease and longevity in the Danish population. A new procedure that combines both demographic and the individual genetic information in determining the relative risks of the observed genetic variations is applied. The sex-dependent influences can be found by introducing sex-specific population survival and incorporating the risk of gene–sex interaction. Three genetic polymorphisms, angiotensinogen M/T235, blood coagulation factor VII (FVII) R/Q353 and FVII-323ins10, manifest significant influences on survival in males, with reduced hazards of death for carriers of the angiotensinogen M235 allele, the F VII Q353 allele, and the FVII-323P10 allele. The results show that some of these genotypes associated with lower risk of CVD could also reduce the carrier's death rate and contribute to longevity. However, the presence of sex-dependent effects and the fact that major CVD-associated genes failed to impose detrimental influence on longevity lead us to concur that the aging process is highly complicated.

Keywords: Gene; Cardiovascular disease; Sex-dependent influence; Longevity; Survival analysis

1. Introduction

Cardiovascular diseases (CVD) account for about 50% of all deaths worldwide. Genetic polymorphisms on a variety of genes have been studied to see if they are associated with

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Table 1
DNA polymorphism of CVD candidate genes. Table revised from Bladbjerg et al. (1999) 82:1100-5. FVII: blood coagulation factor VII; PAI: plasminogen activator inhibitor-1; t-PA: tissue plasminogen activator; GPIIb/IIIa: platelet receptor glycoprotein Iib/IIIa; prothrom: prothrombin; MTHFR: methylene tetrahydrofolate reductase; ACE: angiotensin converting enzyme; angiotens: angiotensinogen

Genotypes	Control group number (frequency)	Centenarian group number (frequency)	χ^2 (p-value)
Haemostasis genes			
FVII R/Q 353			0.82(0.66)
RR353	162(0.81)	157(0.84)	
RQ353	37(0.18)	28(0.15)	
QQ353	2(0.01)	2(0.01)	
FVII-323ins10			1.50(0.48)
P0P0	156(0.78)	153(0.82)	
P0P10	42(0.20)	30(0.16)	
P10P10	3(0.02)	3(0.02)	
FVII intron7 $(37bp)_n$			0.00(0.99)
H5H5	0(0.00)	1(0.005)	
H5H6	10(0.05)	6(0.03)	
H5H7	3(0.02)	6(0.03)	
H6H6	96(0.48)	86(0.47)	
H6H7	75(0.37)	67(0.37)	
H7H7	17(0.09)	15(0.08)	
H7H8	0(0.00)	1(0.005)	
Fibrinogen 455G/A			0.63(0.99)
455GG	132(0.66)	129(0.70)	
455GA	64(0.32)	53(0.28)	
455AA	4(0.02)	3(0.02)	
PAI-675(4G/5G)			2.15(0.34)
4G4G	55(0.27)	54(0.29)	
4G5G	106(0.53)	86(0.46)	
5G5G	40(0.20)	47(0.25)	
t-PA intron8ins311			1.85(0.39)
I/I	76(0.38)	71(0.39)	
I/D	94(0.47)	76(0.41)	
D/D	31(0.15)	37(0.20)	
GPIIb/IIIa L/P33			0.21(0.90)
LL	141(0.70)	133(0.71)	
LP	54(0.27)	49(0.26)	
PP	4(0.02)	5(0.03)	
Prothrom 20210G/A			0.84(0.36)
GG	193(0.97)	184(0.98)	
GA	6(0.03)	3(0.02)	
AA	0	0	
MTHFR A/V114			0.60(0.74)
AA	96(0.48)	85(0.46)	
AV	86(0.43)	79(0.43)	
VV	19(0.10)	22(0.12)	
BP regulating genes			
ACE intron16ins287			0.07(0.97)
I/I	46(0.23)	41(0.22)	
I/D	102(0.51)	95(0.51)	

Table 1 (*continued*)

Genotypes	Control group number (frequency)	Centenarian group number (frequency)	χ^2 (*p*-value)
D/D	51(0.26)	49(0.27)	
Angiotens M/T235			0.53(0.77)
MM	81(0.41)	77(0.37)	
MT	98(0.49)	85(0.41)	
TT	21(0.11)	23(0.11)	

cardiovascular disorders. For example, the ϵ4 allele of the apolipoprotein E gene has been related not only to an increased risk of atherosclerotic cardiovascular disease in Finnish (Kervinen et al., 1994) and Danish (Gerdes et al., 2000a) studies but also to the incidence of Alzheimer's disease (Corder et al., 1993). Further more, homozygosity of the e4 allele has been confirmed to show deleterious effect on mortality at old ages (Heijmans et al., 2000) and a significant depletion of the ϵ4 allele has been found in French (Schachter et al., 1994), Finnish (Louhija et al., 1994), Danish (Gerdes et al., 2000b), Chinese (Zhang et al., 1998) and Japanese (Asada et al., 1996) centenarians. Other genes that have been studied for polymorphisms associated with CVD and longevity include genes coding for apolipoprotein B (Hegele et al., 1986; Myant et al., 1989; Paulweber et al., 1990; Kervinen et al., 1994; De Benedictis, 1997, 1998), apolipoprotein A-I (Hoeg, 1996; Saha et al., 1995), apolipoprotein C-I (Galinsky et al., 1997), angiotensin converting enzyme (ACE) (Galinsky et al., 1997; Nakata et al., 1997; Alvarez et al., 1999; Schachter et al., 1994), angiotensinogen (Cong et al., 1998; Chen et al., 1998; Frossard et al., 1998), and the tissue plasminogen activator (TPA) (Macko et al., 1999; Thogersen et al., 1998). Sex-dependent effects on CVD prevalence and survival were reported in some of these studies (Galinsky et al., 1997; De Benedictis et al., 1998), which suggests that the penetrance of loci influencing CVD and survival may vary according to sex. To detect how the CVD-associated genetic variations are related to longevity in the Danish population, one study focused on polymorphisms of genes involved in regulating blood pressure and in blood coagulation was conducted (Bladbjerg et al., 1999). However, no significant associations were reported in the study, which used conventional statistical analysis, namely, the gene-frequency method (Table 1). In this paper, we apply a new approach derived by Vaupel and Yashin and developed in several recent articles (Yashin et al., 1998, 1999, 2000; Tan et al., 2001) on the previously published data (Bladbjerg et al., 1999), and combine both demographic and individual genetic information to determine the relative risks of the observed genetic variations. We incorporate important factors such as sex-dependent influences and also individual heterogeneity in unobserved frailty, which was previously ignored. With this strategy, we can detect polymorphisms that show potential sex-dependent effects. We estimated the relative risks of the observed genes as well as of gene–sex interaction and present sex-specific survival and the estimated proportions of the carriers of the observed gene alleles.

2. Materials and methods

2.1. Individual genotype data

Blood samples were taken from both young and old people in Denmark (Bladbjerg et al., 1999). The group of old people consists of two parts. The first set is from the first centenarian study carried out on Funen island. All individuals born before December 31, 1894 who were still alive on 1 May, 1994 are included in the study. They were interviewed the same day, and 39 out of 51 participants agreed to have blood sample taken. The second set of data comes from the Danish Longitudinal Centenarian Study, which covers all people who became centenarians between 1 April, 1995 and 31 May, 1996 throughout Denmark. Blood samples were collected from 148 out of 207 participants. Altogether, blood samples were collected from a total of 187 centenarians, among them 47 males and 140 females. The younger group consists of blood donors at the blood bank of Odense University Hospital. They are healthy people aged 20–64 years (75 females and 126 males).

Two kinds of candidate genes that influence the developments of CVD are investigated, haemostasis genes and blood pressure regulating genes (Table 1). Polymorphic variations of these genes have been associated with CVD either directly or through their association with blood levels of CVD risk factors as previously described (Bladbjerg et al., 1999).

2.2. Demographic data

Age-specific cohort population survival is calculated from cohort life tables for Danes born between 1894 and 1974, such that the survival for each age is taken from a cohort life table for people who reached that age in 1994.

2.3. Analytical strategy

We define the relative risk of one observed gene allele or genotype r as the ratio of hazard of death for carriers, $\mu(x|r)$, to that for the non-carriers or the baseline hazard, $\mu_0(x)$. Then according to the proportional hazard assumption, $\mu(x|r) = r\mu_0(x)$. The corresponding survival function for the carriers is:

$$s(x|r) = e^{-\int_0^x \mu(t,r)dt} = e^{-\int_0^x r\mu_0(t)dt} = e^{-r\int_0^x \mu_0(t)dt} = e^{-rH_0(x)} = s_0(x)^r.$$

Here, $s_0(x)$ is the survival distribution corresponding to the baseline hazard function, and $H_0(x)$ is the cumulative hazard at age x. Although r can take on any value greater than zero, a gene allele with r larger than 1 (frailty allele) increases the hazard of death, while a gene allele with r smaller than 1 (longevity allele) reduces it. Taking heterogeneity in an individual's unobserved frailty, which can also contribute to survival (Vaupel et al., 1979), into consideration, the average survival for a group of carriers can be derived as $\bar{s}(x|r) = (1 - \sigma^2 r \ln s_0(x))^{-1/\sigma^2}$ when the unobserved frailty follows a gamma distribution with mean 1 and variance σ^2. Accordingly, mean survival for non-carriers is $\bar{s}(x) = (1 - \sigma^2 \ln s_0(x))^{-1/\sigma^2}$.

Since all individuals can be grouped as carriers and non-carriers of a gene allele or genotype, one can introduce a simple two-point distribution for the allele or genotype (Vaupel and Yashin, 1985). Define the risk of gene–sex interaction, $r_{g\times s}$, as the risk of male carriers to that of female carriers. Then the average survival at age x for the mixed population consisting of both carriers and non-carriers is:

$$\bar{\bar{s}}_m(x) = p\bar{s}_{1,m}(x) + (1-p)\bar{s}_{0,m}(x),$$
$$\bar{\bar{s}}_f(x) = p\bar{s}_{1,f}(x) + (1-p)\bar{s}_{0,f}(x).$$

Here, p is the proportion of carriers at birth, and $\bar{\bar{s}}_m(x)$ and $\bar{\bar{s}}_f(x)$ are survival rates at age x for males and females, obtainable from population statistics, $\bar{s}_{1,m}(x) = (1 - \sigma^2 r r_{g\times s} \ln s_{0,m}(x))^{-1/\sigma^2}$ is mean survival for males carriers and $\bar{s}_{1,f}(x) = (1 - \sigma^2 r \ln s_{0,f}(x))^{-1/\sigma^2}$ is mean survival for female carriers, $\bar{s}_{0,m}(x) = (1 - \sigma^2 \ln s_{0,m}(x))^{-1/\sigma^2}$ is mean survival for male non-carriers and $\bar{s}_{0,f}(x) = (1 - \sigma^2 \ln s_{0,f}(x))^{-1/\sigma^2}$ is mean survival for female non-carriers. The proportion of carriers at age x is

$$p_m(x) = \frac{p\bar{s}_{1,m}(x)}{\bar{\bar{s}}_m(x)}$$

for males and:

$$p_f(x) = \frac{p\bar{s}_{1,f}(x)}{\bar{\bar{s}}_f(x)}$$

for females. Based on the binomial distribution of the genotype, a likelihood function for the data containing both male and female individuals can be constructed as:

$$L \propto \prod_x p_m(x)^{n_m(x)}(1-p_m(x))^{N_m(x)-n_m(x)} p_f(x)^{n_f(x)}(1-p_f(x))^{N_f(x)-n_f(x)}$$

where $n_m(x)$, $n_f(x)$ are the numbers of male and female carriers at age x, $N_m(x)$, and $N_f(x)$ are the total number of participants at age x. In the estimation, a single σ^2 is restricted to all the alleles in order to reduce the amount of the parameters to be estimated. Parameters that maximize the likelihood function are estimated by the maximum likelihood procedure in Gauss software (Apotech System, 1996).

3. Results

The results of parameter estimates are shown in Table 2. The significance levels for risk parameters (relative risks for the genes and for gene–sex interaction) are determined by testing the statistical differences between the estimated risks and one with null hypothesis $H_0 : r = 1$. The p-values for frequency parameters are obtained by testing $H_0 : p = 0$. The highest likelihood was achieved when σ^2 is about 0.4. There is no significant risk for each of the single gene allele, which is consistent with Bladbjerg et al. (1999). However, three polymorphic variations, angiotensinogen M/T235, FVII R/Q353, and FVII-323ins10, isplay significant sex-dependent effects that reduce the hazards of death for male carriers of angiotensinogen M235, FVII Q353, and FVII–323P10 by factors of

Table 2
Parameter estimates for the CVD associated genes with heterogeneity

Gene allele	Frequency	Risk		Risk of gene–sex interaction	
		Est.	*p*-value	Est.	*p*-value
MTHFR-A	0.91	1.15	0.44	0.84	0.44
MTHFR-V	0.52	0.99	0.91	0.95	0.75
Angiotens-M	0.59	1.12	0.30	0.67	0.00
Angiotens-T	0.89	1.10	0.59	0.91	0.69
ACE-I	0.75	1.05	0.70	0.92	0.63
ACE-D	0.77	0.95	0.67	1.13	0.52
FVIIR353	0.99	0.86	0.77	1.65	0.54
FVIIQ353	0.20	1.25	0.16	0.68	0.01
t-PA-I	0.85	1.15	0.34	0.99	0.98
t-PA-D	0.63	1.04	0.70	0.93	0.62
PAI-675-4G	0.80	1.16	0.27	0.96	0.83
PAI-675-5G	0.73	1.02	0.88	1.04	0.81
GPIIbIIIa-L	0.98	1.19	0.63	0.89	0.79
GPIIbIIIa-P	0.29	1.02	0.85	0.96	0.77
FVII-323P0	0.99	0.99	0.97	1.21	0.75
FVII-323P10	0.22	1.26	0.13	0.69	0.01
FVIIintron7-H6	0.90	1.10	0.60	1.15	0.55
FVIIintron7-H7	0.47	0.99	0.99	0.85	0.24
Fibrinogen-G	0.98	0.99	0.98	0.22	0.33
Fibrinogen-A	0.34	1.16	0.22	0.81	0.14

0.670, 0.682 and 0.688, respectively, when the risk of gene–sex interaction for females is set to one as reference. The result indicates that some gene polymorphisms that reduce the hazard of CVD are also favourable for male longevity but not for female longevity. In Fig. 1, sex-specific survivals for carriers and non-carriers of the angiotensinogen M235 allele are plotted. The male carriers have better survival than non-carriers but for females it is the opposite although the difference in survival between female carriers and non-carriers is very small. The observed and estimated proportions of carriers of the angiotensinogen M allele by age for males and for females are plotted in Fig. 2. While the frequency of carriers of the M-allele goes up with age in males, it tends to decline in females due to the sex-dependent influence of the gene.

In addition to the risk parameters, the model also provides estimates on the initial frequencies for carriers of the gene alleles. The *p*-values for the estimated initial frequencies are not shown since they are all significantly different from zero. Proportions of carriers of the three alleles are estimated as 0.591, 0.195 and 0.220 respectively.

4. Discussions

Incorporation of gene–sex interaction in the analysis produces results that were missed

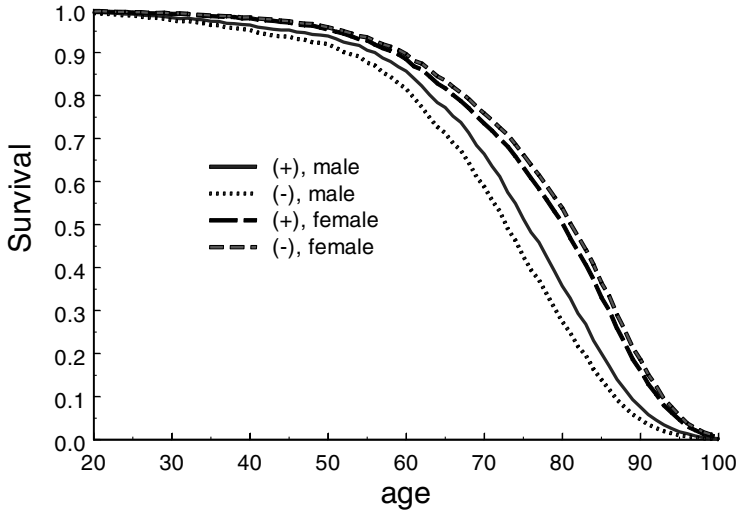

Fig. 1. The sex-specific survivals for angiotensinogen M allele carriers and non-carriers.

in the original analysis using the gene frequency method (Bladbjerg et al., 1999). The fact that the gene frequency method ignores interactions when comparing the gene frequency differences between the control group and the centenarians could be attributed to the lack of efficiency of the gene frequency method when applied to small samples. In the new model, the risk of gene–sex interaction is defined as an extra risk for male carriers in comparison to female carriers. Information from both sexes is used to determine the parameter of interaction. However, one important nontranditional epidemiologic approach, the case-only design, which was originally derived for analysing gene-environment interaction in disease prevalence (Piegorsch et al. 1994; Khoury and Flanders, 1996), can be introduced here to detect gene–sex interaction in human longevity when treating centenarians as cases. Taking the angiotensinogen M allele for example, there are 106 centenarian carriers (34 males, 72 females) and 76 non-carriers (12 males, 64 females) of the allele. The odds ratio comparing male carriers to female carriers is 2.519 with a 95% confident interval of 1.203–5.276. The statistical test showed a significantly higher proportion of carriers of the allele in male centenarians than that in female centenarians ($\chi^2 = 5384$, $p = 0.020$) which means that the allele favours male survival. Although this conclusion is compatible with our result, the case-only approach does not allow for evaluation of the independent effect of the allele alone, but merely involves a departure from multiplicative effects (Rothman and Greenland, 1998; Yang and Khoury, 1997). In the relative risk model exploited in this paper, our primary interest is not only to test for the gene–sex interaction but also to evaluate the risk of the allele or genotype as well as its initial frequency. In addition, we can also estimate the genotype specific survival distribution as shown in Fig. 1. Nevertheless, the easily applicable case-only approach is a useful tool for measuring gene–sex interaction in human longevity as it avoids the troubles originated from ordinary case-control design.

The detected sex-specific influences of the three gene polymorphisms are not totally

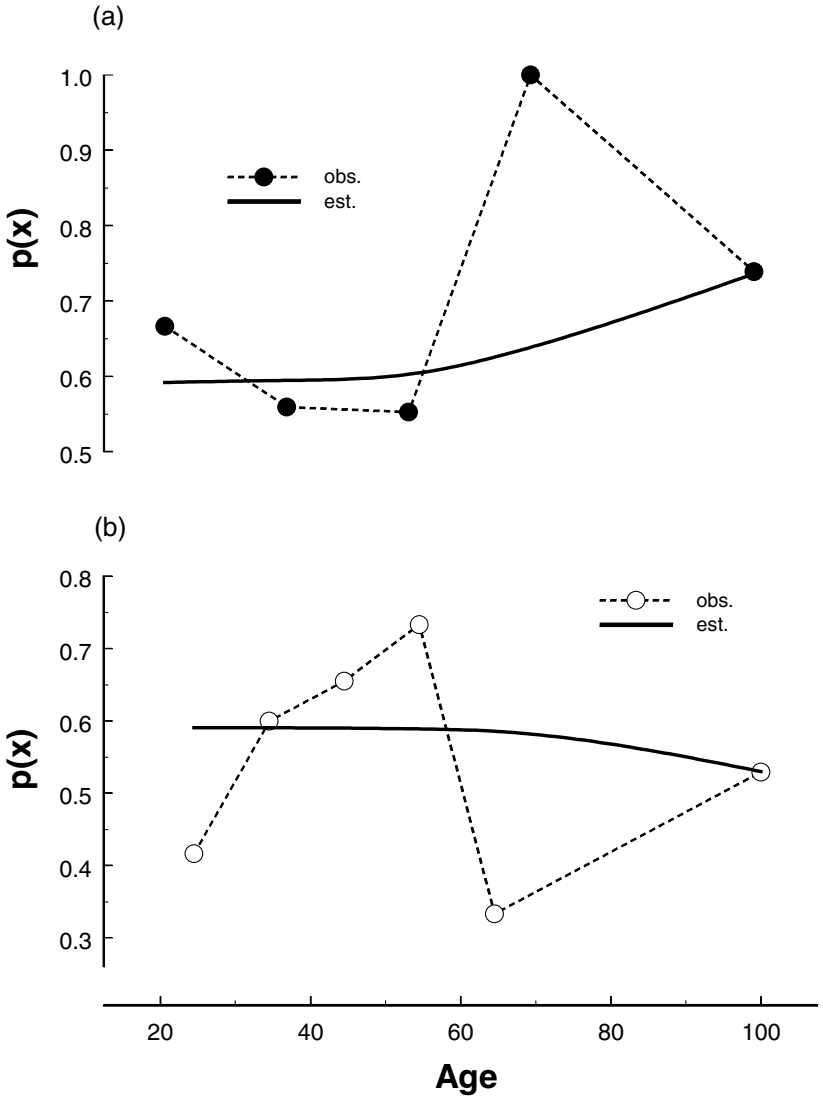

Fig. 2. The observed and estimated proportions of angiotensinogen M allele carriers by age for males (a) and females (b).

unexpected as previous studies have found sex-dependent effects of these polymorphisms on other phenotypical traits. For example, the genotypes of the angiotensinogen gene were found to be significantly associated with variation in systolic resting (Hegele et al., 1994) and exercise (Rankinen et al., 2000) blood pressure, but only in males. The results indicate that the genetic variation in the locus modifies the responsiveness of the blood pressure to endurance training, but the functional impact is related to differences in sex. The gender-related effect in the genetic modulation of FVII plasma levels has been studied

extensively. The FVII polymorphisms intron7(37bp)n and −323ins10 were reported to show stronger effect on plasma FVII level in male than in female Italians (Di Castelnuovo et al., 1998). The plasma FVII clotting activity has been suggested as a risk marker of cardiac death (Meade et al., 1993), and also the FVII polymorphisms are predictive of CVD in Italian studies (Iacoviello et al., 1998; Girelli et al., 2000). However, the putative relation between FVII polymorphisms and CVD are turned down in other studies (Lane et al., 1996; Feng et al., 2000), and it may be speculated that this discrepancy is, at least partly, caused by an effect of gender. In women, FVII levels are increased by menopause (Scarabin et al., 1990, 1996) and hormone replacement therapy (Cushman et al., 1999; Marchien van Baal et al., 2000), and elderly women have higher FVII levels than men of the same age (Scarabin et al., 1996; Ishikawa et al., 1997). Also, women with coronary artery disease have higher FVII levels than men with the disease (Kalaria et al., 2000; Ossei-Gerning et al., 1998). Perhaps the effect of hormones and lifestyle factors drown the effect of genotype on CVD risk in women. Although there have been many studies on disease association, the present study reveals, for the first time, the sex-dependent association of the polymorphisms with longevity.

The gene–sex interaction has been reported to be an important phenomenon in the genetic modulation of human life span in other studies on gene and longevity, such as studies of the HLA-DR alleles (Dorak et al., 1994; Ivanova et al., 1998), the HLA-Cw1, Cw7 alleles (Proust et al., 1982), and the large allele group at the tyrosine hydroxylase (THO) locus (De Benedictis et al., 2000; Tan et al., 2001). The sex-dependent influence indicates that the effect of a gene on a multifactorial trait depends on the physiological scenario changes in males and females differently, the effect of a certain gene on survival could vary between the sexes. The existence of gene–sex interaction shows not only the complexity of the aging process. It also indicates that the two sexes follow different trajectories toward extreme longevity (Franceschi et al., 2000).

The polygenic feature of life span makes it imperative to take into account the unobserved hidden heterogeneity in individual frailty for two reasons. First, there is unobserved heterogeneity in an individual's genetic make-up, which accounts for about 25% of the variation in life span according to previous studies (McGue et al., 1993; Herskind et al., 1996; Yashin and Iachine, 1995). Regarding the number of genetic variations that contribute to life span, Martin (1997) estimated that there could be up to about 7000. In such a highly polygenic situation, it is advisable to consider the influences from other unobserved genes when making an inference from the observed ones. Second, there are also non-genetic factors that have a predominant influence on life span, and these constitute another layer of heterogeneity in individual frailty. The existence of genetic and non-genetic heterogeneity in individual frailty affecting life span can influence an inference from an observed genetic polymorphism. In our analysis, we also tried to apply a simple model that does not take unobserved frailty into account. The estimated relative risks of gene–sex interaction for the three significant alleles were systematically weaker than they were in the heterogeneity model with risk parameters 0.856 for angiotensinogen M235, 0.857 for FVII Q353 and 0.860 for FVII–323P10. The differences in parameter inference present a good example of the difficult situation facing studies of longevity and of multifactorial diseases (Clerget-Darpoux, 2000). Individual heterogeneity in the unobserved attributes is an obstacle that prevents us from unraveling the genetic component in

longevity. The introduction of Gamma-distributed frailty in our analysis is a convenient and helpful way to deal with the problem in survival analysis (Tan et al., 2001).

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