

The Heritability of CHD Mortality in Danish Twins After Controlling for Smoking and BMI

Andreas Wienke,¹ Anne M. Herskind,² Kaare Christensen,³ Axel Skytthe,³ and Anatoli I. Yashin⁴

¹Institute of Medical Epidemiology, Biostatistics and Informatics, Martin-Luther-University Halle-Wittenberg, Halle, Germany

²Odense University Hospital, Odense, Denmark

³Danish Center for Demographic Research, and the Danish Twin Registry, University of Southern Denmark, Odense, Denmark

⁴Center for Demographic Studies, Duke University, Durham, United States of America

Cause-specific mortality data on Danish monozygotic and dizygotic twins are used to analyze heritability estimates of susceptibility to coronary heart disease (CHD) after controlling for smoking and Body Mass Index (BMI). The sample includes 1209 like-sexed twin pairs born between 1890 and 1920, where both individuals were still alive in 1966. The participants completed a questionnaire in 1966 which included questions on smoking, height and weight. The analysis was conducted with both sexes pooled due to the relatively small number of twin pairs. Follow-up was conducted from January 1, 1966 to December 31, 1993. The correlated gamma-frailty model with observed covariates was used for the genetic analysis of frailty to account for censoring and truncation in the lifetime data. During the follow-up, 1437 deaths occurred, including 435 deaths due to CHD. Proportions of variance of frailty attributable to genetic and environmental factors were analyzed using the structural equation model approach. Different standard biometric models are fitted to the data to evaluate the magnitude and nature of genetic and environmental factors on mortality. Using the best-fitting model without covariates, heritability of frailty to CHD was found to be 0.45 (0.11). This result changes only slightly to 0.55 (0.13) in a DE model after controlling for smoking and BMI. This analysis underlines the existence of a substantial genetic influence on individual frailty associated with mortality caused by CHD.

Twin studies are one of the most widely used methods for quantifying the influence of genetic and environmental factors on specific diseases. In the case of binary traits (where disease is either present or not), concordance analysis provides a powerful and widely accepted method in genetic epidemiology. Concordance rates are easy to calculate and allow a clear interpretation (Gatz et al., 2000; McGue, 1992). In practical applications, time-to-event data (time of onset of disease, age at death) is often available, but usually in a

truncated and/or censored form. Censoring of bivariate observations can be a complex problem as either or both individuals of a pair may be subject to censoring, and the censoring times need not be the same for both individuals. Additional problems arise through bivariate truncation, which implies a nonrandom selection of the study population from the total twin population. Furthermore, in many cases covariates are available. Unfortunately, it is difficult to manage truncated and censored time-to-event data and covariates within the context of concordance analysis. A large part of the motivation for the methodology of this article is exploring the potential for censored and truncated data and the inclusion of measured covariates. One key question here is whether the inclusion of covariates changes the heritability estimates of susceptibility to the disease under study.

We aim to study bivariate survival times T_1 , T_2 which depend, via proportional hazards models, on unobserved variables Z_1 , Z_2 (called frailties). That is, we seek to explain an association between two (non-negative) survival times. They have a continuous joint distribution through their common dependence upon unobserved random variables. Such models are particularly convenient in the context of survival data and they stem from the (univariate) concept of frailty introduced by Vaupel and colleagues (Vaupel et al., 1979). The univariate frailty model extends immediately to frailty models with a bivariate survival function $S(t_1, t_2) = P(T_1 > t_1, T_2 > t_2)$. The bivariate frailty model asserts that T_1 and T_2 are conditionally independent, given Z_1 , Z_2 , and that

$$S(t_1, t_2) = E(e^{-Z_1 H_{01}(t_1) - Z_2 H_{02}(t_2)})$$

Received 10 August, 2004; accepted 7 October, 2004.

Address for correspondence: Andreas Wienke, Martin-Luther-University Halle-Wittenberg, Medical Faculty, Institute of Medical Epidemiology, Biostatistics and Informatics, Magdeburger Strasse 27, 06097 Halle, Germany. E-mail: andreas.wienke@medizin.uni-halle.de

for some cumulative baseline hazard functions H_{01} and H_{02} . We will give more detailed information about this model presently.

We want to apply the bivariate frailty model to twin data on mortality due to coronary heart disease (CHD). The role of family aggregation of CHD is well established. Questions about the nature of the genetic effects (additive vs. nonadditive) are addressable. Former studies from the Danish (Harvald & Hauge, 1970) and Swedish twin registries (de Faire et al., 1975; Marenberg et al., 1994) found a genetic component in the risk of death from coronary heart disease. Recent studies (Wienke et al., 2001; Zdravkovic et al., 2002) have used approaches from survival analysis to account for truncation and censoring present in the data. Such methods have to be combined with genetic epidemiology methods. The heterogeneity of individuals with susceptibility to CHD as well as important covariates are included in the present model. For such a combined analysis, the correlated gamma-frailty model with observed covariates (Yashin et al., 1996; Yashin & Iachine, 1997) was applied in this paper, which takes into account the dependence of life spans of relatives (twins). This approach enables the combination of age at death data with data on the cause of death, smoking, and Body Mass Index (BMI); and further deals with truncated and censored observations. Individuals who died from causes other than CHD were considered as censored and combined with the censored individuals who did not die during the follow-up period. The advantages of the model were empirically demonstrated in the statistical analysis of lifetime data from Danish twins that were used by Herskind, McGue, Iachine and colleagues (1996), but with special focus on mortality caused by CHD and the application of a frailty model.

Materials and Methods

Twins' mortality data were provided by the Danish Twin Registry, founded in 1954 as the world's first nationwide twin registry. This population-based registry includes twins born in Denmark during the period 1870 to 1910 and all like-sex pairs born between 1911 and 1930. For detailed information about the Danish Twin Registry, see Hauge (1981).

In 1966, a questionnaire including questions about smoking, height and weight was mailed to all twins born between 1890 and 1920 who were alive and traceable on January 1, 1966 — 3709 individuals answered the questionnaire (response rate 65%). Questions about phenotypic similarities included in the questionnaire were used to assess the zygosity. This zygosity classification was compared with laboratory methods (serological markers). The misclassification rate was found to be below 5% (Holm, 1983; Lykken, 1978). Excluded from the study were 813 twins with nonresponding partners, four pairs with unknown zygosity and 212 pairs with incomplete or uncertain

Table 1

Study Population (Number of Individuals) by Gender, Zygosity and Cause of Death

	Males		Females	
	MZ twins	DZ twins	MZ twins	DZ twins
Deaths				
—CHD	96	153	76	110
—other	206	280	204	312
All causes	302	433	280	422
Alive	118	199	266	398
Total	420	632	546	820

information on height and weight. Twenty-three pairs were excluded because of incomplete information about cause of death, resulting in a study population of 1209 twin pairs.

Smoking status was divided into four groups: non-smokers, ex-smokers, current cigar or pipe smokers and current cigarette smokers. Nonsmokers were defined as individuals who had smoked less than 100 cigarettes, 50 cigars or cigarillos, or 5 packs of tobacco in their lifetime. Persons who gave no information on lifetime smoking status or current smoking status were pooled with the ex-smokers. Current cigarette smokers who also smoked other kinds of tobacco were included in the group of current cigarette smokers. As the association between BMI and CHD mortality is J- or U-shaped rather than linear, BMI was divided into three groups: < 22 kg/m², 22–28 kg/m², and > 28 kg/m².

Individuals were followed from January 1, 1966 to December 31, 1993. Those persons identified as deceased after that date are classified as living for our purposes. At the end of the follow-up, approximately 40% of the twins were still alive, resulting in right censored data. Two hundred and ten male monozygotic (MZ) and 316 dizygotic (DZ) twin pairs, and 273 female MZ and 410 DZ twin pairs participated. In addition to age at death, information on cause of death was also available for all individuals who died during the follow-up. For the study, only the underlying cause of death was considered. Detailed information about death status, gender, zygosity, smoking, and BMI of the study population is given in Table 1 and 2.

Mortality

After the age of 6 years, death rates for Danish twins born between 1870 and 1900 are almost the same as those for the same cohorts of the Danish population. The distributions of age at death for MZ twins are close to those of DZ twins for both sexes (Christensen et al., 1995; Christensen et al., 2001). Recent papers dealing with twin cohorts born during the period 1870 to 1930 found similar mortality patterns for Danish twins and the general Danish population with respect to CHD (Christensen et al., 2001; Wienke et al., 2001).

Table 2
Study Population (Number of Individuals) by Sex, BMI and Smoking

	Nonsmokers	Ex-smokers	Pipe/cigar smokers	Cigarette smokers	Total
Males					
BMI < 22	11	12	45	31	99 (9.4%)
BMI 22–28	78	141	333	208	760 (72.2%)
BMI > 28	24	35	91	43	193 (18.3%)
Total	113 (10.7%)	188 (17.9%)	469 (44.6%)	282 (26.8%)	
Females					
BMI < 22	105	46	47	99	297 (21.7%)
BMI 22–28	350	138	134	171	793 (58.1%)
BMI > 28	157	40	37	42	276 (20.2%)
Total	612 (44.8%)	224 (16.4%)	218 (16.0%)	312 (22.8%)	

This similarity suggests that it is possible to generalize genetic results from survival analysis of twins to the total population with respect to mortality due to CHD.

For the present study, CHD is grouped as ICD 420 in the sixth and seventh revision and as ICD 410–414 in the eighth ICD revision.

Statistical Methods

Univariate lifetime models cannot capture the association between the life spans of related individuals like twins. Consequently, bivariate distributions of dependent lifetimes are necessary. For genetic analysis of time-to-event data, associations between durations are needed. In this paper, genetic and environmental factors acting on susceptibility (frailty) to mortality due to CHD are analyzed when smoking and BMI are controlled for. The correlated gamma-frailty model with observable covariates can be used to fit bivariate lifetime data and to provide a specific parameter for correlation of frailty to death. It is interesting that individual frailties in twin pairs could not be observed but that their correlation could be estimated by application of the correlated gamma-frailty model.

More specific assumptions about the structure of the lifetimes would now want to be made. To include heterogeneity in the model, a correlated gamma-frailty model (Wienke et al., 2002; Wienke, Holm et al., 2003; Wienke, Lichtenstein et al., 2003; Yashin & Iachine, 1995; Yashin et al., 1995) is assumed. Let Z_j ($j = 1, 2$) be the frailties, and X_j ($j = 1, 2$) vectors of observable covariates of the two individuals of a twin pair. Assume that individual hazards are represented by the proportional hazards model $\lambda(t, Z_j, X_j) = Z_j \lambda_0(t) e^{\beta X_j}$ ($j = 1, 2$) with a baseline hazard function $\lambda_0(t)$ describing the risk of dying as a function of age and β denoting the vector of regression parameters. Let the lifetimes of the two twin partners be conditionally independent given their frailties Z_1 and Z_2 . Additionally, a decomposition $Z_1 = Y_0 + Y_1$ and $Z_2 = Y_0 + Y_2$, where Y_0, Y_1 , and Y_2 are independent gamma-distributed random variables with $Y_0 \sim \Gamma(k_0, \lambda)$, $Y_1 \sim \Gamma(k_1, \lambda)$ and $Y_2 \sim \Gamma(k_2, \lambda)$. Here k_0, k_1, k_2, λ are nonnegative parameters and $\Gamma(k, \lambda)$ denotes a

Gamma distribution with parameters k and λ . Obviously, Z_1 and Z_2 are correlated in view of the shared part of frailty Y_0 in both Z_1 and Z_2 . To force Z_1 and Z_2 to have the same distribution it is assumed that shape parameters k_1 and k_2 for the distributions of Y_1 and Y_2 are the same, $k_1 = k_2$. This condition is reasonable with respect to twins, because there is no reason to assume different distributions of frailty in twin partners. The standard assumption is employed that the mean frailty of individuals is 1 (at the beginning of the follow-up), which means that $E(Z_j) = (k_0 + k_1)/\lambda = 1$ ($j = 1, 2$). The common variance is given by $\sigma^2 = 1/\lambda$. Let ρ be the correlation coefficient of Z_1 and Z_2 , which is given simply by:

$$\rho(Z_1, Z_2) = \frac{k_0}{k_0 + k_1}$$

As frailties Z_j ($j = 1, 2$) are usually unobservable, the correlation coefficient used in the methods of quantitative genetics cannot be estimated from the empirical data directly. A bivariate lifetime model is therefore needed that allows indirect calculation of the parameters. The bivariate gamma distributed frailty with the above-mentioned properties was constructed in Yashin et al. (1995). The unconditional bivariate survival function is given by:

$$S(t_1, t_2 | X_1, X_2) = \frac{S(t_1 | X_1)^{1-\rho} S(t_2 | X_2)^{1-\rho}}{(S(t_1 | X_1)^{-\rho^2} + S(t_2 | X_2)^{-\rho^2} - 1)^{1/\rho^2}} \quad [1]$$

where $S(t|X)$ denotes the marginal univariate survival function, assumed to be equal for both partners in a twin pair. Using a parametric approach a Gamma-Gompertz model was fitted to the data, for example,

$$S(t | X) = (1 + [(1 + s^2 \frac{\alpha}{\gamma} (e^{\gamma t} - 1))^{\frac{\rho^2}{s^2}} - 1] e^{\beta X})^{-1/s^2}$$

where $\alpha, \gamma, s^2, \rho, \beta$ are parameters to be estimated.

The lifetimes T_{11}, T_{12} are assumed to be independently censored from the right. Starting from this model, the likelihood function of the data is able to be derived:

$$L(t_1, t_2, \delta_1, \delta_2) = \delta_1 \delta_2 S_{t_1 t_2}(t_1, t_2 | X_1, X_2) - \delta_1 (1 - \delta_2) S_{t_1}(t_1, t_2 | X_1, X_2) - (1 - \delta_1) \delta_2 S_{t_2}(t_1, t_2 | X_1, X_2) + (1 - \delta_1) (1 - \delta_2) S(t_1, t_2 | X_1, X_2)$$

$$- (1 - \delta_1) \delta_2 S_{t_2}(t_1, t_2 | X_1, X_2) + (1 - \delta_1) (1 - \delta_2) S(t_1, t_2 | X_1, X_2)$$

$$(1 - \delta_2) S(t_1, t_2 | X_1, X_2)$$

with δ_{ij} ($i = 1, \dots, n; j = 1, 2$) as censoring indicator. Partial derivatives of the marginal survival functions are given by

$$S_{t_j}(t_1, t_2 | X_1, X_2) = \frac{\partial S(t_1, t_2 | X_1, X_2)}{\partial t_j}$$

($j = 1, 2$) and

$$S_{t_1 t_2}(t_1, t_2 | X_1, X_2) = \frac{\partial^2 S(t_1, t_2 | X_1, X_2)}{\partial t_1 \partial t_2}$$

Because of the independence assumption between lifetimes and censoring times, the distribution of the censoring times does not enter the likelihood function.

As mentioned above, the twin pair data set used is not randomly selected from the total twin population. Since both members of a twin pair had to be still alive

on January 1, 1966, the survival times in the data set are sampled from specific conditional distributions. If a twin pair was born in year 1880 (or 1881, ..., 1920), the condition of survival of both twins until the year 1966 implies that both twins had to survive until the age of $t^* = 1966 - 1880$ in order to be included in the sample. If the survival times are denoted by T_1 and T_2 with survival function $S(t_1, t_2 | X_1, X_2)$, then the conditional survival function for a twin pair is:

$$S(t_1, t_2 | X_1, X_2, T_1 > t^*, T_2 > t^*) = \frac{S(t_1, t_2 | X_1, X_2)}{S(t^*, t^* | X_1, X_2)}$$

Consequently, the likelihood function of independently left truncated and right censored lifetime data is given by:

$$L(t_1, t_2, \delta_1, \delta_2, t^*) = \frac{\delta_1 \delta_2 S_{t_1, t_2}(t_1, t_2 | X_1, X_2) - \delta_1 (1 - \delta_2) S_{t_1}(t_1, t_2 | X_1, X_2) + (1 - \delta_1) \delta_2 S_{t_2}(t_1, t_2 | X_1, X_2) + (1 - \delta_1)(1 - \delta_2) S(t_1, t_2 | X_1, X_2)}{S(t^*, t^* | X_1, X_2)}$$

For a combined analysis of MZ and DZ twins, two correlation coefficients ρ_{MZ} and ρ_{DZ} were included respectively. The correlations between MZ and DZ twins provide information about genetic and environmental influences on frailty within individuals.

Quantitative Genetics of Frailty

In studies of MZ and DZ twin pairs, the intrapair-correlations of the trait under study (in this case frailty on mortality due to CHD) play the key role for analysis of genetic and environmental factors. Using these coefficients, five standard genetic models of frailty are fitted to the data that correspond to five different assumptions about its structure. Resemblance in twins (completely for MZ twins and partly for DZ twins) is caused by three factors: additive genetic factors (A), genetic factors due to dominance (D) and shared environmental factors (C). Nonshared environment (completely for MZ twins and partly for DZ twins) is responsible for intrapair differences in twins. From the estimation point of view, three parameters could be included into the model simultaneously as there are data from two different groups of relatives (MZ and DZ twins). Models that are more complex need data from additional groups of relatives. Each additional group allows for an additional parameter in the model. The following biometric models were fitted to the data: ACE, ADE, AE, DE and CE. In these notations, an ACE model refers to the decomposition of frailty $Z = A + C + E$. ADE, AE, DE and CE models are defined similarly. Small letters a^2, c^2, e^2, d^2 are used to refer to the respective proportions of variance. For example, the relation $1 = a^2 + c^2 + e^2$ corresponds to the decomposition of variance in the ACE model of frailty. Standard assumptions about of the quantitative genetics yields in the following relations:

$$\begin{aligned} \rho_{MZ} &= a^2 + d^2 + c^2 \\ \rho_{DZ} &= 0.5a^2 + 0.25d^2 + c^2 \\ 1 &= a^2 + d^2 + c^2 + e^2 \end{aligned} \tag{2}$$

Here $a^2 + d^2$ denotes (broad sense) heritability of the trait under study. For detailed information about these genetic models and deriving the upper equations, see the monograph by Neale and Cardon (1992). Note that the AE model and the CE model are not nested. Consequently, the likelihood ratio test could not be used to define the model with the best fit to the data. The Akaike Information Criterion (AIC; Akaike, 1987) is used to compare nonnested models.

To combine the approach of quantitative genetics with the methods of survival analysis the correlated gamma-frailty model is used with genetic and environmental components of frailty. In this approach, the decompositions in [2] must be substituted into survival model [1]. This model must be used to estimate the parameters a^2, d^2, c^2, e^2 by the maximum likelihood method directly. Analysis was made using the standard statistical software package GAUSS.

Results

As not all models are nested, the likelihood ratio test can not be applied to compare all five biometric models. Applying the correlated gamma-frailty model with and without observed covariates, the Akaike Information Criterion prefers the AE and DE model respectively. There is not much difference in the fit between the AE and the DE model. Using these two models, heritability changes from 0.45 (0.11) without covariates to 0.48 (0.12) with covariates (AE model) and from 0.50 (0.13) in the model without covariates to 0.55 (0.13) in the model with covariates (see Table 3). Standard errors for the ACE model are not shown in Table 3 since $c^2 = 0$ is the boundary of the parameter space.

Using the best-fitting DE model, the likelihood ratio test indicates a significant influence of BMI on CHD mortality ($\beta_1 = 0.53$ [0.25] and $\beta_2 = 0.47$ [0.24]). Here β_1 and β_2 describe individuals with BMI less than 22 kg/m² and more than 28 kg/m² respectively. The reference group are individuals with BMI between 22 kg/m² and 28 kg/m². Smoking shows a significant influence on CHD mortality ($\beta_3 = 0.57$ [0.24], $\beta_4 = 0.32$ [0.23] and $\beta_5 = 0.48$ [0.26]). Here β_3, β_4 and β_5 denote cigarette smokers, pipe/cigar smokers and former smokers respectively. The reference group is the nonsmokers. $\beta_6 = 1.44$ (0.28) denotes the risk of males compared to females. The results with respect to the (second best) AE model are similar.

Discussion

The method presented in this article with its suitability for censored and truncated data and the possibility to include observed covariates overcomes the well-known drawbacks of the traditional concordance analysis in twin studies with time-to-event data. An important question arising from the genetic analysis

Table 3Estimates of the Components of Variance in Frailty to Mortality from CHD ($n = 1209$)

Model	σ^2	a^2	d^2	c^2	e^2	β_1	β_2	β_3	β_4	β_5	β_6	AIC
ACE	7.74	0.45		0.00	0.55							4566.1
	(—)	(—)		(—)	(—)							
	4.54	0.48		0.00	0.52	0.54	0.47	0.57	0.32	0.49	1.47	4494.6
	(—)	(—)		(—)	(—)	(—)	(—)	(—)	(—)	(—)	(—)	
AE	7.74	0.45			0.55							4564.1
	(4.02)	(0.11)			(0.11)							
	4.54	0.48			0.52	0.54	0.47	0.57	0.32	0.49	1.47	4492.6
	(1.70)	(0.12)			(0.12)	(0.26)	(0.25)	(0.24)	(0.24)	(0.26)	(0.29)	
ADE	7.73	0.44	0.01		0.55							4566.1
	(4.04)	(0.20)	(0.20)		(0.12)							
	4.38	0.17	0.36		0.47	0.53	0.47	0.57	0.32	0.49	1.45	4494.0
	(1.65)	(0.41)	(0.47)		(0.14)	(0.26)	(0.25)	(0.24)	(0.23)	(0.26)	(0.28)	
DE	7.16		0.50		0.50							4565.9
	(4.07)		(0.13)		(0.13)							
	4.28		0.55		0.45	0.53	0.47	0.57	0.32	0.48	1.44	4492.2
	(1.65)		(0.13)		(0.13)	(0.25)	(0.24)	(0.24)	(0.23)	(0.26)	(0.28)	
CE	8.77			0.30	0.70							4567.4
	(4.49)			(0.08)	(0.08)							
	4.83			0.31	0.69	0.55	0.45	0.57	0.32	0.50	1.50	4497.2
	(1.81)			(0.08)	(0.08)	(0.27)	(0.25)	(0.25)	(0.24)	(0.27)	(0.30)	

Note: σ^2 = variance of frailty; a^2 = additive genetic effects; d^2 = genetic effects as a result of dominance; c^2 = common environment; e^2 = nonshared (individual) environment (including measurement errors); β_1 = BMI < 22 kg/m²; β_2 = BMI > 28 kg/m²; β_3 = cigarette smokers; β_4 = pipe/cigar smokers; β_5 = former smokers; β_6 = males; AIC = Akaike Information Criterion.

of models with observed covariates (in this case smoking and BMI) is whether genes that are responsible for variation in observed covariates also contribute to a variation in susceptibility to CHD. Traditional biometrical methods of regression analysis can lead to spurious effects of covariates. In extreme cases, common genes not covariates may be responsible for variation in life span.

Both smoking and BMI are influenced by genes with heritability estimates 0.35–0.75 (smoking) and 0.5–0.8 (BMI; Bouchard, 1994; Heath & Madden, 1995; Herskind, McGue, Sørensen et al., 1996). Whether common genes influence these phenotypic traits as well as susceptibility to CHD, however, is an open question. In 1958, Fisher suggested that the association between smoking and lung cancer is spurious and reflects only the circumstance that the same genes influence both smoking habits and lung cancer. This was the starting point for a long debate on genetic confounding.

The conclusion of this paper is that the inclusion of smoking and BMI do not cause any substantial changes in the heritability estimates. Hence, no evidence was found for common genetic factors acting on smoking and susceptibility to CHD, or BMI and susceptibility to CHD. This study confirms the earlier finding by Zdravkovic et al. (2004) that the genetic influence on susceptibility to CHD is not mediated through genetic influence on smoking and BMI.

As expected, the inclusion of observable covariates decreases the heterogeneity in the study population that can be seen in the decline of variance in frailty from $\sigma^2 = 7.74$ to $\sigma^2 = 4.54$ (AE model) and from $\sigma^2 = 7.16$ to $\sigma^2 = 4.28$ in the DE model. It is therefore clear that frailty is not a phenotypic trait. Frailty depends on the model; it describes factors not included in the model.

When observed covariates are included in the model, the relative importance of environmental factors (shared environment, C, and nonshared environment, E) is reduced, leading to an increase in the heritability estimates observed in the present analysis. This may appear strange, although use of the heritability coefficient when computed as a proportion of variance may be misleading. The variance in the trait can be decomposed as follows: $\sigma^2 = \sigma^2_{\text{genes}} + \sigma^2_{\text{environment}}$. One does not know whether the heritability increases due to an increase in the genetic variance or due to a decrease in the environmental variance. In the present case, the (moderate) increase was largely due to the influences of the latter. By including smoking and BMI, the genetic variance was reduced from $\sigma^2_{\text{genes}} = 3.48$ to $\sigma^2_{\text{genes}} = 2.18$ in the AE model and from $\sigma^2_{\text{genes}} = 3.58$ to $\sigma^2_{\text{genes}} = 2.35$ in the DE model respectively. The reduction of the environmental heterogeneity in frailty is more pronounced, for example, from $\sigma^2_{\text{environment}} = 4.26$ to $\sigma^2_{\text{environment}} = 2.36$ in the AE model and from $\sigma^2_{\text{environment}} = 3.58$ to $\sigma^2_{\text{environment}} = 1.93$ in the DE model. Thus, the focus should not be on the increase in heritability but

rather on the decrease in the environmental variance. These results help us understand that the risk factors studied represent primarily environmental sources of variation for CHD-death, despite the role that genetic influences may play in specific risk factors. For a more detailed discussion of variance components, see Hopper (1993).

This point is also important when reviewing why the AE model could be replaced by the DE model when observed covariates are taken into account. For example, this could occur when the additive genetic component of observed covariates contains a substantial portion of the additive genetic component of a trait (as well as a part of independent environmental component). The resulting genetic and environmental variances of the trait may be reduced substantially as in this case. The sizes of these parts will determine the best-fitting model and the new value of heritability estimate. It may well be then that the residual proportion of genetic variation in the trait (frailty) in the presence of observed covariates is mostly due to dominant genetic effects.

The analysis underlines the importance of genetic factors on individual susceptibility to CHD. The heritability estimate in this study (0.45 for both sexes combined in an AE model without covariates) is lower than those found in a previous analysis of an extension of the presented data set (without covariates) with heritability estimates of 0.53 and 0.58 for males and females respectively (Wienke et al., 2001). The lower estimates in this subsample may be a consequence of a decline in the heritability of CHD with increasing age as found in Marenberg et al. (1994) and Zdravkovic et al. (2002). The twin population in this study is much older, the time of truncation was 1966 compared with 1943 in the paper by Wienke et al. (2001). Furthermore, the youngest cohorts (birth years 1921 to 1930) are not included in the present analysis.

The main limitation of the present study is that the risk factor information was obtained from a self-report questionnaire in 1966. It cannot be ruled out that self-reported information, especially regarding weight, is biased downwards. Smoking information appears reliable, as smoking was widely accepted in the 1960s. Alternatively, people reported as smokers in the 1960s may have since stopped smoking, but still be in the smoking category, which may result in a downward risk bias for smokers. Detailed information about tobacco smoked per day over the follow-up period is not available. Consequently, the group of smokers consists of both heavy and light smokers, which may result in an additional downward risk bias for smokers.

The proposed method allows the censored and truncated time-to-event data handling of related individuals in the case of observed covariates. The hypothesis of genetic confounding can be checked and the influence of observable covariates on heritability analyzed.

Acknowledgments

The authors wish to thank the Danish Twin Register for providing the twin data and Susann Backer for help in preparing the paper for publication. The research was partly supported by NIH/NIA grant 7PO1AG08761-09. A. Wienke and A. I. Yashin would like to thank the Max Planck Institute for Demographic Research in Rostock (Germany) for providing excellent research facilities during their visit at the institute in summer 2004.

References

- Akaike H. (1987). Factor analysis and AIC. *Psychometrika*, 52, 317–322.
- Bouchard, C. (1994). Genetics of obesity: Overview and research directions. In C. Bouchard (Ed.), *The genetics of obesity* (1st ed., pp. 223–233). Boca Raton, FL: CRC Press.
- Christensen, K., Vaupel, J. W., Holm, N. V., & Yashin, A. I. (1995). Mortality among twins after age 6: Fetal origins hypothesis versus twin method. *British Medical Journal*, 310, 432–436.
- Christensen, K., Wienke, A., Skytthe, A., Holm, N. V., Vaupel, J. W., & Yashin, A. I. (2001). Cardiovascular mortality in twins and the fetal origins hypothesis. *Twin Research*, 5, 344–349.
- de Faire, U., Friberg, L., & Lundman, T. (1975). Concordance for mortality with special reference to ischaemic heart disease and cerebrovascular disease: A study on the Swedish Twin Registry. *Preventive Medicine*, 4, 509–517.
- Fisher, R. A. (1958) Cancer and smoking. *Nature*, 182, 596.
- Gatz, M., Pedersen, N. L., Crowe, M., & Fiske, A. (2000). Defining discordance in twin studies of risk and protective factors for late life disorders. *Twin Research*, 3, 159–164.
- Harvald, B., & Hauge, M. (1970). Coronary occlusion in twins. *Acta Geneticae Medicae et Gemellologiae*, 19, 248–250.
- Hauge, M. (1981). The Danish twin register. In S. A. Mednick, A. E. Baert, & B. P. Bachmann (Eds.), *Prospective longitudinal research* (pp. 217–222). Oxford, UK: Oxford Medical Press.
- Heath, A. C., & Madden, P. A. F. (1995). Genetic influences on smoking behavior. In J. R. Turner, L. R. Cardon, & J. K. Hewitt (Eds.), *Behavior: Genetic approaches in behavioral medicine* (pp. 45–63). New York: Plenum Press.
- Herskind, A. M., McGue, M., Iachine, I. A., Holm, N., Sørensen T. I. A., Harvald, B., & Vaupel, J. W. (1996). Untangling genetic influences on smoking, body mass index and longevity: A multivariate study of 2464 Danish twins followed for 28 years. *Human Genetics*, 98, 467–475.

- Herskind, A. M., McGue, M., Sørensen, T. I. A., & Harvald, B. (1996). Sex and age specific assessment of genetic and environmental influences on body mass index in twins. *International Journal of Obesity*, *20*, 106–113.
- Holm, N. V. (1983). *The use of twin studies to investigate causes of diseases with complex etiology with a focus on cancer*. Unpublished doctoral dissertation [in Danish], Odense University, Denmark.
- Hopper, J. L. (1993). Variance components for statistical genetics: Applications in medical research to characteristics related to human diseases and health. *Statistical Methods in Medical Research*, *2*, 199–223.
- Lykken, D. T. (1978). The diagnosis of zygosity in twins. *Behavior Genetics*, *8*, 437–473.
- Marenberg, M. E., Risch, N., Berkman, L. F., Floderus, B., & de Faire, U. (1994). Genetic susceptibility to death from coronary heart disease in a study of twins. *The New England Journal of Medicine*, *330*, 1041–1046.
- McGue, M. (1992) When assessing twin concordance, use the probandwise not the pairwise rate. *Schizophrenia Bulletin*, *18*, 171–176.
- Neale, M. C., & Cardon, L. R. (1992). *Methodology for genetic studies of twins and families*. Dordrecht, the Netherlands: Kluwer Academic.
- Vaupel, J. W., Manton, K. G., & Stallard, E. (1979). The impact of heterogeneity in individual frailty on the dynamics of mortality. *Demography*, *16*, 439–454.
- Wienke, A., Holm, N. V., Skytthe, A., & Yashin, A. I. (2001). The heritability of mortality due to heart diseases: A correlated frailty model applied to Danish twins. *Twin Research*, *4*, 266–274.
- Wienke, A., Christensen, K., Skytthe, A., & Yashin, A. I. (2002). Genetic analysis of cause of death in a mixture model with bivariate lifetime data, *Statistical Modelling*, *2*, 89–102.
- Wienke, A., Holm, N., Christensen, K., Skytthe, A., Vaupel, J., & Yashin, A. I. (2003). The heritability of cause-specific mortality: A correlated gamma-frailty model applied to mortality due to respiratory diseases in Danish twins born 1870–1930. *Statistics in Medicine*, *22*, 3873–3887
- Wienke, A., Lichtenstein, P., & Yashin, A. I. (2003). A bivariate frailty model with a cure fraction for modeling familial correlations in diseases, *Biometrics*, *59*, 1178–1183.
- Yashin, A. I., & Iachine, I. A. (1995). How long can humans live? Lower bound for biological limit of human longevity calculated from Danish twin data using correlated frailty model. *Mechanisms of Ageing and Development*, *80*, 147–169.
- Yashin, A. I., & Iachine I. A. (1997). How frailty models can be used for evaluating longevity limits: Taking advantage of an interdisciplinary approach. *Demography*, *34*, 31–48.
- Yashin, A. I., Manton, K. G., & Iachine, I. A. (1996). Genetic and environmental factors in duration studies: Multivariate frailty models and estimation strategies. *Journal of Epidemiology and Biostatistics*, *1*, 115–120.
- Yashin, A. I., Vaupel, J. W., & Iachine, I. A. (1995). Correlated individual frailty: An advantageous approach to survival analysis of bivariate data. *Mathematical Population Studies*, *5*, 145–159.
- Zdravkovic, S., Wienke, A., Pedersen, N. L., Marenberg, M. E., Yashin, A. I., & de Faire, U. (2002). Heritability of death from coronary heart disease: 36 years follow-up of 20,966 Swedish twins. *Journal of Internal Medicine*, *252*, 247–254.
- Zdravkovic, S., Wienke, A., Pedersen, N. L., Marenberg, M. E., Yashin, A. I., & de Faire, U. (2004). Genetic influences on CHD-death and the impact of known risk factors: Comparison of two frailty models. *Behavior Genetics*, *34*, 585–591.