STATISTICS IN MEDICINE
Statist. Med. 2000; **19**:1217–1235

Genetic analysis of the age at menopause by using estimating equations and Bayesian random effects models

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SUMMARY

Multi-wave self-report data on age at menopause in 2182 female twin pairs (1355 monozygotic and 827 dizygotic pairs), were analysed to estimate the genetic, common and unique environmental contribution to variation in age at menopause. Two complementary approaches for analysing correlated time-to-onset twin data are considered: the generalized estimating equations (GEE) method in which one can estimate zygosity-specific dependence simultaneously with regression coefficients that describe the average population response to changing covariates; and a subject-specific Bayesian mixed model in which heterogeneity in regression parameters is explicitly modelled and the different components of variation may be estimated directly. The proportional hazards and Weibull models were utilized, as both produce natural frameworks for estimating relative risks while adjusting for simultaneous effects of other covariates. A simple Markov chain Monte Carlo method for covariate imputation of missing data was used and the actual implementation of the Bayesian model was based on Gibbs sampling using the freeware package BUGS.

INTRODUCTION

The informativeness of traditional epidemiologic studies is often enhanced by the incorporation of information about genetic risk. Twin data have proven very useful in assessing the relative importance of genetic and familial factors in the aetiology of complex phenotypes [1, 2]. The basis

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Contract/grant sponsor: Australian National Health and Medical Research Council
Contract/grant sponsor: NIH; contract/grant numbers: AA07535, A07728

Received September 1998
Accepted July 1999

of the analyses is a comparison of the phenotypic covariance in monozygotic (MZ) twins who are genetically identical to that in dizygotic (DZ) twins who, on the average, have half their genes in common. This contrast permits the decomposition of the phenotypic variance into a part due to additive genetic factors (A), and one due to environmental effects. The environmental variance, in turn, may be split into a component reflecting shared (C) and unique environmental factors (E). This decomposition of variance (ACE) is similar to that used in much of current linkage analytic methods.

There have been multiple extensions to the basic ACE model, for example, to handle multivariate observations, covariates and various degrees of gene by environment interaction. Concurrently, there has been much progress in the adaptation of logistic and Cox models to the analysis of age-of-onset using data from larger, more informative but costlier, families [3–7]. However, the current analytical methods for twin data still have limited ability to handle censored observations. At one extreme, researchers will exclude from the analyses those twin pairs where one of the twins' phenotype is censored. Depending on the degree of censoring, this practice may lead to significant biases in the parameter estimates and to decreases in statistical power. As an alternative, Pickles *et al.* [8] proposed to model age-at-onset as a gradation disease liability. However, his approach assumes a simple censoring mechanism, where all subjects are observed until a maximum censoring time, greater than all observed failure times. Complications will arise when the censored observation on one twin is much lower than the observed age-of-onset in the other twin. This could be due, for example, to premature deaths or dropouts from a longitudinal study.

In the present paper, we will adapt two recent methods for the analysis of censored data in twins. The first method [9, 10] models hazard rates with the Cox proportional hazard model. First, we use a bivariate survivor function, proposed by Clayton [11], that requires only one additional parameter, to model the correlation in the survival times of twins. Parameter values are obtained using estimating equations. This is an extension of earlier work in the complete-case correlated outcomes arena [12–14]. It has two desirable features: (i) robustness – no higher-order distributional assumptions are required beyond pairwise ones; and (ii) computational efficiency.

Second, we develop a Bayesian method for the analysis of censored data in twins, using Markov chain Monte Carlo (MCMC) methods. We begin with a Bayesian representation of a mixed effects model, where genetic and environmental contributions are treated as random effects, while allowing for adjustments for observed covariates. A complete likelihood analysis of censored data in twins would be computationally intractable, a problem that is avoided when using MCMC methods. The Gibbs sampler [15] is the most popular algorithm used in MCMC applications to correlated data. MCMC methods have been used for linkage analysis [16–18], for the estimation of parameters in the mixed model with and without covariates [19, 20], for estimation of gene–smoking interaction and covariate imputation [21], for performing combined linkage and segregation analysis [22, 23] and for mixed models of large complex pedigrees [24]. We perform the Bayesian analysis using the BUGS program [25]. Some recent computer packages that implement Gibbs sampling for analysis of pedigree data include BUGS [25–27], Genetic Analysis Package [28] and MIXD [29, 30].

We then apply these two methods to twin data on menopausal age and obtain complementary results. Recent work has highlighted the relationships between age at natural menopause and factors such as reproductive, sociodemographic and certain behavioural influences (for example, birth year, parity, smoking, education and age at menarche) [31–34]. Standard failure time regression analyses as used in earlier studies have limitations in unravelling the complex

mechanisms leading to the cessation of reproductive capability, because these methods do not sufficiently address the interrelationships between certain independent variables which may share parameters of the same maturation and ageing process. There has been limited work on the genetic synchronization of menopause with various aspects of reproductive and behavioural factors. Snieder *et al.* [35] investigated this relationship using methods that required multivariate normality and did not allow censored data.

MATERIALS AND METHODS

The data on age at menopause

To illustrate the methods, we have used data from successive mail surveys of twins in Australia (1980 to 1996). Twin pairs were recruited from the Australian Twin Registry (National Health and Medical Research Council). The Australian Twin Registry is a volunteer registry with more than 27 000 twin pairs enrolled, about 10–20 per cent of the estimated number of twin pairs in the population. Mailed questionnaires obtained information on health histories and behaviours, life-style factors, family structure and personality. The subjects in this study were born between 1893 and 1964. Zygosity was determined from self-report items shown to give at least 95 per cent agreement with true zygosity as determined by genotyping [36].

Age at menopause was defined as age at last menstrual period, determined retrospectively, after a woman had stopped menstruating for 12 months not due to pregnancy, lactation, or ill health (Figure 2). As covariates, we considered birth year and age at menarche (both continuous), and binary variables including smoking (0 = non-smokers), parity (0 = fewer than 2 children) and education (0 = no university education). We excluded women who had commenced hormone replacement therapy (HRT) before reaching menopause. A total of 5593 women had a valid endpoint age for analyses and data on covariates as well. Censoring of menopausal age (58 per cent) was due to: (i) hysterectomies or bilateral oophorectomies before natural menopause; (ii) cessation of menses for other reasons (for example, pregnancy, lactation, ill health); and (iii) continued menstruation at the last time of follow-up. 2182 pairs remained for genetic analysis after removal of the singletons (1355 MZ and 827 DZ).

The hypothesized covariates of age at menopause were birth year, education, social class, occupation, annual income, smoking habits, alcohol use, body mass index (BMI), age at menarche, and age at full-term pregnancy. Selection of these variables was based on previous studies of determinants of menopausal age. Where information was collected in more than one survey, the value provided at the time closest to age at menopause was used, except for age at menarche, which was taken from the earliest survey, and quantities of smoking and drinking, which were accumulated over the woman's life span until age at menopause.

Birth year and age at menarche were treated as continuous variables. Education data were categorized as: 1, high school or less (including apprenticeship and diploma); 2, technical or teacher's college; 3, university or postgraduate degree. Participants were asked to classify themselves into the working or middle class. Information on occupation was based on the Australian Bureau of Statistics (ASCO) coding system, consisting of nine standard classifications. We regrouped these into four major classes: 1, upper white-collar workers; 2, lower white-collar workers; 3, trade and sales service workers; and 4, blue-collar factory workers. Women who described their major lifetime occupation as homemakers, all married, were assigned their

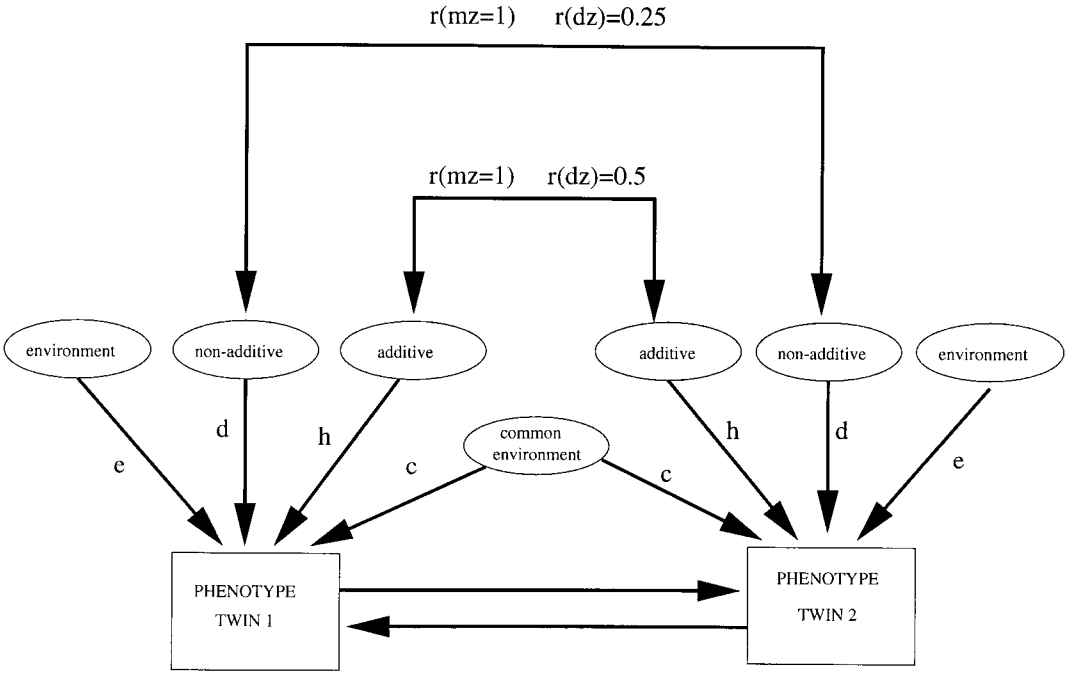

Figure 1. Path diagram of twin resemblance indicating different sources of shared and non-shared variation: d, e, h and c are the corresponding path coefficients for these effects. The MZ additive and dominance genetic correlations are both equal to 1.0, while the DZ additive and dominance genetic correlations are equal to 0.5 and 0.25, respectively.

husband's occupation status. Average annual income of twins and their spouses were calculated and classified into: 1, income less than AU$10 000; 2, $10 000 to less than $30 000; 3, $30 000 to less than $50 000; or 4, $50 000 and more. A woman was classified as a non-smoker if she reported that she had never smoked in all surveys, started smoking after menopause, or smoked only once or twice in her lifetime before menopause. Smokers were all those who reported more than occasional smoking in any survey and started before menopause. Alcohol use was assessed by typical intake in the past 12 months, number of drinks in a typical week, and number of specific drinks (beer, wine, spirits, sherry, other) consumed each day of the past week. The combined information is used to classify women into non-drinkers and drinkers (light, moderate, heavy). Body mass index was computed using the formula $\text{BMI} = \text{weight(kg)}/[\text{height(m)}]^2$. Age at full-term pregnancy was calculated from a woman's year of birth and her first child's date of birth. Parity was represented by the number of live-born children reported in the latest survey.

Genetic model

Covariance between twins can provide useful information about genetic and environmental contributions to variation within individuals. Human genetic research in the biometrical or quantitative genetic tradition [37, 38] has shown how familial resemblance for qualitative traits

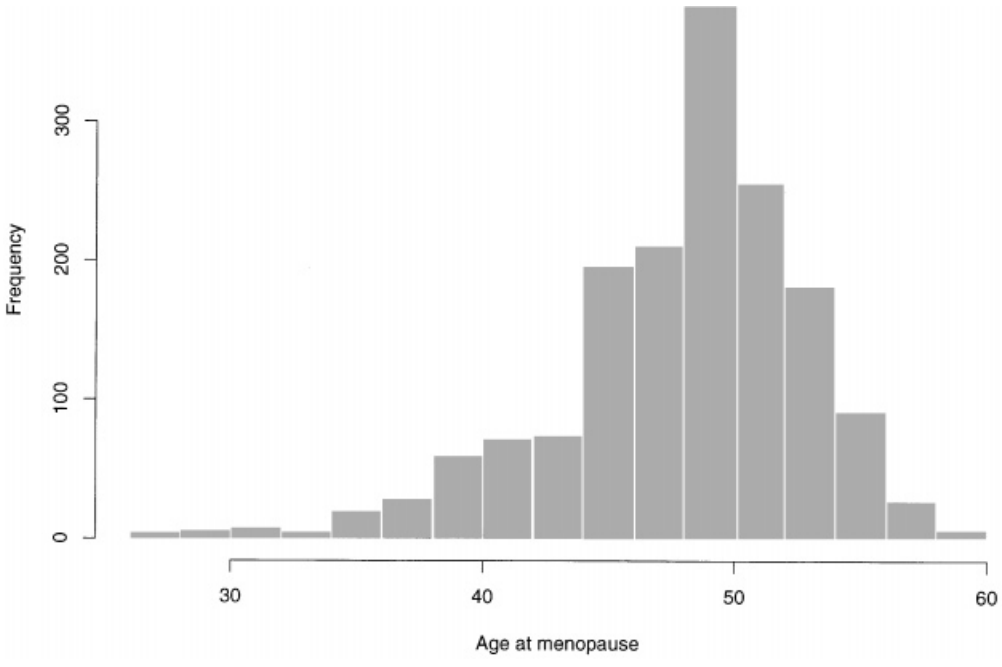

Figure 2. Histogram for the age at natural menopause in 1693 post-menopausal Australian female twins born between 1893 and 1962.

could be modelled using genetic and environmental variance components. Specifically, the decomposition is based on the probabilities that a pair of relatives will have zero, one or two alleles at any autosomal genetic locus that are identical by descent. Two alleles are said to be identical in descent if one of them has been derived by direct replication from the other or if both are copies of the same gene in a common ancestor. The path diagram (Duncan [39]) in Figure 1 illustrates the univariate model for decomposing variance. The total phenotypic variance can be decomposed into two genetic and two environmental components. Additive genetic factors (A) are the effects of genes taken singly and added over multiple loci, whereas genetic dominance (D) represents genetic interaction (within loci). Shared environmental effects (C) are the environmental effects that are shared by twins, and specific environmental effects (E) are the environmental influences that are unique to each individual. The diagram indicates how each type of factor contributes to the covariance within an MZ or DZ twin pair. Additive genetic factors and genetic dominance are perfectly correlated in MZ twins, whereas DZ twins, like ordinary siblings, share only half of the additive genetic effects and one quarter of the genetic dominance effects. Shared environmental effects are perfectly correlated in both MZ and DZ twins. Lower case letters (path coefficients) represent the genetic and environmental loadings on the trait. The model assumes negligible effects of assortative mating, epistasis, genotype-environment correlation and/or interaction. It is also assumed that shared environmental influences are similar for MZ and DZ twins. Analyses of genotype frequencies and expected MZ and DZ twin correlations may be found in standard texts [38, 40, 41] and Neale and Cardon [2]. Throughout this paper, we will assume that the phenotype has been rescaled to unit variance, so that $\sigma_T^2 = 1$. On the further

assumption of random mating in the population the expected phenotypic correlations for monozygotic and dizygotic twins are respectively $r_{MZ} = h^2 + c^2 + d^2 = \sigma_A^2 + \sigma_C^2 + \sigma_D^2$ and $r_{DZ} = 1/2h^2 + c^2 + 1/4d^2 = 1/2\sigma_A^2 + \sigma_C^2 + 1/4\sigma_D^2$. Since estimates of c^2 and d^2 are confounded in data for twins reared together [1, 42], the MZ and DZ phenotypic correlations may be parameterized as an ACE or ADE model. In either case, the difference between a variance of unity and the expected MZ correlation yields an estimate of e^2. Maximum-likelihood estimates of model parameters can be obtained by fitting models to summary covariance matrices in multigroup analyses using standard software for structural equation modelling [2]. The proportion of variance in the trait attributable to genetic effects is defined as the heritability of the trait. The classical definition of heritability is $h^2 = 2(\rho_{MZ} - \rho_{DZ})$, twice the difference of the intraclass correlation coefficients of MZ and DZ twins.

Estimating equations approach

Let $y = (\delta_{ki}, t_{ki}, \mathbf{Z}_{ki})$ denote the data collected for the ith twin in the kth family ($k = 1, \ldots, K$ and $i = 1, 2$) where $\delta_{ki} = 0$ if the observation is censored, t_{ki} is either the recorded age at menopause or the age at the most recent follow-up for pre-menopausal women, and $\mathbf{Z}_{ki}$ is a vector of measured covariates. We assume that censoring time, age at menopause and the covariates are independently distributed. These assumptions can be relaxed in more general models, subject to identification constraints. The hazard rate for menopause is the instantaneous probability that menopause occurs immediately after time t, given that the woman is pre-menopausal at time t. The hazard rate under the Cox proportional hazards model [43] is given by

$$\lambda(t_{ki}) = \lambda_0(t_{ki}) \exp(\beta' \mathbf{Z}_{ki})$$

where $\lambda_0(\ )$ is the baseline hazard function, and β is a vector of regression coefficients.

We follow Clayton [11] in modelling the bivariate survivor function

$$F(t_{k1}, t_{k2}) = \left[\left\{ \frac{1}{F_1(t_{k1})} \right\}^{\theta} + \left\{ \frac{1}{F_2(t_{k2})} \right\}^{\theta} - 1 \right]^{-1/\theta}$$

where F_1 and F_2 are univariate survivor functions, θ is a scalar parameter that measures the degree of dependence between the twins' times at onset, independence being implied by $\theta = 0$, and positive association by $\theta > 0$. The Clayton model allows negative dependencies and has the property that failure times are absolutely continuous for $\theta > -0.5$. In addition, the cross-ratio (or odds-ratio) function as studied by Oakes [44] is

$$c(t_{k1}, t_{k2}) = \lambda(t_{k1} | T_{k2} = t_{k2}) / \lambda(t_{k1} | T_{k2} \geqslant t_{k2}) = 1 + \theta.$$

This is equivalent to assuming that the odds ratio is invariant over the grid region that supports the data. Heuristically, the parameter $1 + \theta$ is an odds ratio that depends on the degree of dependence between the menopausal ages of the two twins. If genetic factors do influence menopausal age, we would expect to see a higher concordance in the age of onset in MZ twins who are genetically identical, than in DZ twins who, on the average, share half their genes in common. Under the current model, this translates as $\theta_{MZ} > \theta_{DZ}$. We may use a standard method to estimate within pair correlations for 2×2 tables from odds ratios. Estimates of twin correlations ρ_{MZ} and ρ_{DZ} are recovered from using the relationships $r_{MZ} = \min(1, \ln(1 + \theta_{MZ}))$ and $r_{DZ} = \min(1, \ln(1 + \theta_{MZ}))$. Testing for the presence of genetic factors underlying age at menopause is equivalent to testing $H_0: \rho_{MZ} = \rho_{DZ}$. We test this hypothesis using a z-transform

(Reference [45], p. 315) of the point estimates of the correlation coefficients. Let z_1 and z_2 denote the transformed statistics of the correlations. Specifically, we reject H_0 when $E/D > Z_{1-\alpha}$, where

$$E = E(z_1 - z_2) = \frac{1}{2}\log\left[\left(\frac{1+r_{MZ}}{1-r_{MZ}}\right)\left(\frac{1-r_{DZ}}{1+r_{DZ}}\right)\right]$$

$$D^2 = V(z_1 - z_2) = \frac{1}{(n_{MZ}-3)} + \frac{1}{(n_{DZ}-3)}$$

and $Z_{1-\alpha}$ is the standard normal deviate corresponding to the one-sided α significance level.

This approach has the advantage of providing a test for the presence of genetic effects through a single parameter (θ). However, it is limited in its ability to attribute the phenotypic variance to specific effects (for example, additive gene action).

Mathematical details of the GEE model and the iterative procedure to estimate the regression coefficients β, θ_{MZ} and θ_{DZ} are summarized in Appendix A.

MCMC analysis using BUGS

The Bayesian paradigm and Gibbs sampling. Markov chain Monte Carlo (MCMC) is an alternative Bayesian approach that provides estimates of likelihoods and associated parameter values when exact computation is not feasible [46, 47]. MCMC methods can be used to draw samples from the underlying joint distribution of major genotypes and polygenic values, conditional on the observed data. From these samples, desired parameters and likelihoods can be estimated without the need to resort to exact computation. MCMC methods have been used for linkage analysis [16, 17], for estimation of parameters in the mixed model with and without covariates [19, 20], for estimation of gene–smoking interaction and covariate imputation [21], for performing combined linkage and segregation analysis [22, 23], and for mixed models of large complex pedigrees [24].

In a general setting, let y be the observed data, and θ be everything not observed including parameters and latent variables. The implementation of Bayesian methods [57] using realistic models and priors is computer-intensive and relies heavily on cunning computational tools to approximate integrals. The problem, in general terms, is to obtain the expected value of a function of interest $s(.)$ under the posterior density $p(\theta|\mathbf{x})$

$$E[s(\theta)] = \frac{\int_\Theta s(\theta)p(\theta)p(\mathbf{x}|\theta)\mathrm{d}\theta}{\int_\Theta p(\theta)p(\mathbf{x}|\theta)\,\mathrm{d}\theta}$$

which cannot generally be found analytically. One method to carry out the integration on the RHS is to perform simulation of exact Bayesian posterior distributions using Markov chain Monte Carlo techniques such as Gibbs sampling. The Gibbs sampler [5] is the most popular algorithm used in MCMC applications to correlated data. Gibbs sampling was introduced to the main statistical community by Gelfand and Smith [48], and has since been applied in even a wider array of problems. The Gibbs sampler is easy to implement because it only depends on the local neighbourhood structure. In the context of pedigree analysis [30], the basic procedure is a sequential updating of missing and latent data including the underlying and unobserved major genotypes, polygenic effects and environmental effects. Values for the missing or latent data are sampled from the local conditional distribution, a function of the observed individual data, the current sampled values of other missing/latent data for this particular individual such as

polygenic and environmental effects, and the values for the sampled genetic effects in the immediate neighbours of an individual. Gibbs sampling basically consists of three main steps:

1. *Step* 1. Setting initial values for unobserved quantities (parameters and latent variables).
2. *Step* 2. For each parameter or latent variable θ_j, sample from its 'full conditional distribution' given the current values of all other quantities in the model.
3. *Step* 3. Examine sampled values of parameters and latent variables to monitor convergence and to provide summary measures.

Some of the most recent and popular packages that implement Gibbs sampling for analysis of pedigree data include BUGS [25–27], Genetic Analysis Package [28] and MIXD [29, 30]. We have used BUGS mainly because it is a freeware product.

The Model. For a simple sequence of independently identically distributed failure times t_i with covariate vector z_i, a Weibull distribution may be used to model time to failure as

$$f(t_i, \mathbf{z}_i) = \mathrm{e}^{\beta' \mathbf{z}_i} t_i^{\gamma - 1} \exp(-\mathrm{e}^{\beta' \mathbf{z}_i} t_i^{\gamma}) \tag{1}$$

where β is a vector of unknown regression coefficients. This leads to a baseline hazard of the form

$$\lambda_0(t_i) = \gamma t_i^{\gamma - 1}.$$

Reparameterize by letting $\mu_i = \mathrm{e}^{\beta' \mathbf{z}_i}$, the conditional distribution of t_i given μ_i is then Weibull (γ, μ_i).

In survival models, unobserved or unmeasured explanatory variables, some of which may be genetic, are often referred to as frailties. The frailties take values restricted to the positive line and may be assumed to act multiplicatively on the hazard. Extending the above model to twin data, a multiplicative individual heterogeneity or frailty term representing the latent genetic and common environment variables may be modelled as random effects simultaneously with the effects associated with observed covariates. Consider right censored time menopause data $\{(T_{ij}, \delta_{ij}, \mathbf{z}_{ij}); 1 \leqslant j \leqslant n\}$ from n pairs; here T_{ij} denotes true menopausal age of the jth twin or her censored time depending on whether $\delta_{ij} = 1$ or 0, respectively, and $\mathbf{z}$ denotes a $p \times 1$ vector of covariates. The aim here is to model the correlation structure within twin pairs to satisfy the fundamental ACE genetic model [38, 40, 49]

$$\mathrm{var}(\mathrm{MZ}) = \mathrm{var}(\mathrm{DZ}) = \sigma_{\mathrm{T}}^2 = \sigma_{\mathrm{A}}^2 + \sigma_{\mathrm{C}}^2 + \sigma_{\mathrm{E}}^2$$

$$r_{\mathrm{MZ}} = \sigma_{\mathrm{A}}^2 + \sigma_{\mathrm{C}}^2$$

$$r_{\mathrm{DZ}} = \frac{1}{2}\sigma_{\mathrm{A}}^2 + \sigma_{\mathrm{C}}^2$$

so that the variation in an individual response is the sum of additive genetic effects (σ_{A}^2), common environmental effects (σ_{C}^2) and residual environmental effects (σ_{E}^2). We formulate a mixed model to represent the conditional distribution of t_{ij} given covariate effects, random additive genetic and common environment effects as

$$t_{ij} | \mu_{ij} \sim \mathrm{Weibull}(\gamma, \mu_{ij}) \qquad i = 1, \ldots, n; \quad j = 1, 2$$

where

$$\log \mu_{ij} = \begin{cases} \alpha + \beta' \mathbf{z} + m_i & \text{for MZ twin} \\ \alpha + \beta' \mathbf{z} + d_i + d_{ij} & \text{for DZ twin} \end{cases} \tag{2}$$

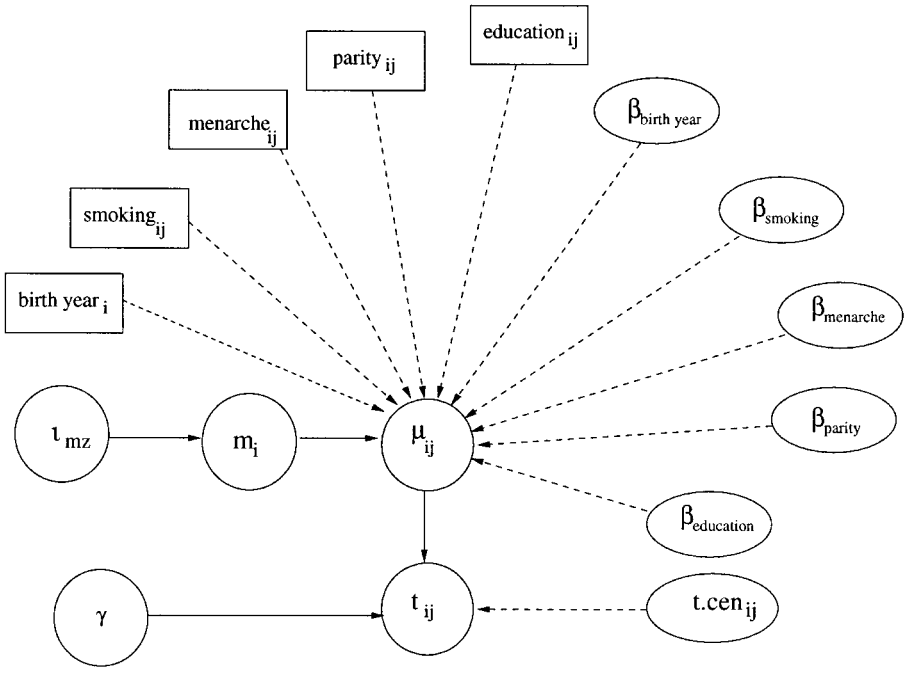

Figure 3. Graphical model of covariate and random family effects for an MZ twin, t_{ij} represents the observed failure time for the jth twin in the ith pair with $t.\text{cen}_{ij}$ being an indicator variable of censoring status. Full arrows indicate stochastic links to which a probability is attached; broken arrows denote deterministic relationships; β's are regression coefficients, τ is the precision of the prior distribution and equals the inverse of the variance; m_i is an independent additive random effect modelled as $m_i|t \sim \text{N}(0, \sigma_A^2 + \sigma_C^2)$. Rectangles represent actual data values for the covariates; γ and μ_{ij} are scale and shape parameters for the underlying Weibull distribution.

and m_i, d_i, d_{ij} are independent additive random effects or latent variables. If we model $m_i|t \sim \text{N}(0, \sigma_A^2 + \sigma_C^2)$, $d_i|t \sim \text{N}(0, \frac{1}{2}\sigma_A^2 + \sigma_C^2)$ and $d_{ij}|t \sim \text{N}(0, \frac{1}{2}\sigma_A^2)$, then the ACE covariance model for twin data is satisfied. We may interpret m_i as a random MZ family effect if the pair is an MZ twin pair, d_i as a random DZ family effect if the pair is a DZ twin pair and d_{ij} as a residual DZ effect for the jth twin. For censored observations, the time to menopause distribution is a *truncated* Weibull, with lower bound corresponding to the censoring time. The regression coefficients and the precision of the random effects (τ_{MZ}, τ_{DZ}) are given 'non-informative' Normal and Gamma priors, respectively. The shape parameter of the time to menopause, r, is also given a non-informative Gamma prior which is slowly decreasing on the positive real line.

The overall model (including random effects) may be described in a Bayesian graph (Figure 3) which simplifies sampling from full conditional distributions by exploiting partial independence properties [27]. In this graph, each random quantity is represented by a node, which may be connected by directed or undirected links. Conditional independence assumptions are represented by the absence of such links.

We implemented the Gibbs sampler using the BUGS program [25] (code in Appendix B). The efficiency of this implementation is increased when continuous variables are rescaled to have

means near the minimum observed value. In the current application, we thus subtracted 7 from age at menarche, 16 from age at menopause, and 1892 from birth year. Imputation of missing data is handled naturally in the Gibbs sampling framework by treating missing values as additional unknown quantities and randomly sampling values from their full conditional distributions. We chose simple prior distributions for imputation, since the number of missing values for covariates is small (< 1 per cent for menarche, education and parity) and there is no indication of non-random missingness in our data. Rescaled age at menarche (after subtracting 7) was assumed to follow a Gaussian prior distribution with mean 5.95 and standard deviation 0.49, values estimated from complete data. The observed proportion of women with university education is 12 per cent, while women having at least two children make up 85 per cent of the sample; therefore, imputation for missing education and parity covariates were based on Bernouilli prior distributions with these respective parameter values. We performed an initial 25 000 burn-in iterations followed by an additional 5000. Parameter estimates are the mean and standard deviation (SD) of all post-convergence Gibbs samples; confidence intervals are computed as the lower and upper $\alpha/2$ percentiles from the last 5000 iterations. Convergence to the posterior distribution was confirmed by using the different criteria provided by the add-on CODA package including those of Gelman and Rubin [50], Geweke [51] and Raftery and Lewis [52, 53].

RESULTS

Preliminary analyses

Not accounting for correlation within twins, the median age at menopause was 51 for twins. This estimate is identical to that found in a sample of 855 women randomly selected from the Australian electoral roll. Kaplan–Meier curves for age at menopause did not significantly differ between controls and twins (logrank $p = 0.63$, Figure 4). We then selected one twin (proband) from each pair at random, assigned them to one of two groups depending upon whether their menopausal age was before 50, and plotted Kaplan–Meier [54] survival curves for the twins of these individuals (Figure 5). The MZ twins or probands reached menopause earlier than the DZ twins if their respective twins reached menopause at or before the age of 50. As the age at menopause in one's twin increased, the difference between the survival probability of the MZ twins and that of the DZ twins became smaller. This suggests that there may be a genetic component to reaching menopause before the age of 50.

We used multivariable Cox regression to screen covariates for inclusion into the model [32]. Later birth year and no smoking were significantly associated with a later age at menopause after adjustment for other variables in the model. In addition we also found factors for later menopause were: parity of two or more; university education; and menarche age before 14 years (just significant).

First, a standard genetic analysis was performed using only twin-pairs where both twins were post-menopausal at the time of the survey (267 MZ pairs and 159 DZ pairs) using the program Mx [55]. The estimates of correlation in age-at-onset were $r_{MZ} = 0.49$ and $r_{DZ} = 0.33$. Since r_{MZ} did not exceed twice r_{DZ}, the ACE model was the most appropriate to fit first. In subsequent analyses, we found no evidence for the presence of shared environmental effects ($5249.2–5248.6 = \chi^2_1 = 0.6$, $P > 0.2$). We thus re-estimated the parameters for a model which allowed only additive genetic and unique environmental factors. The heritability estimate

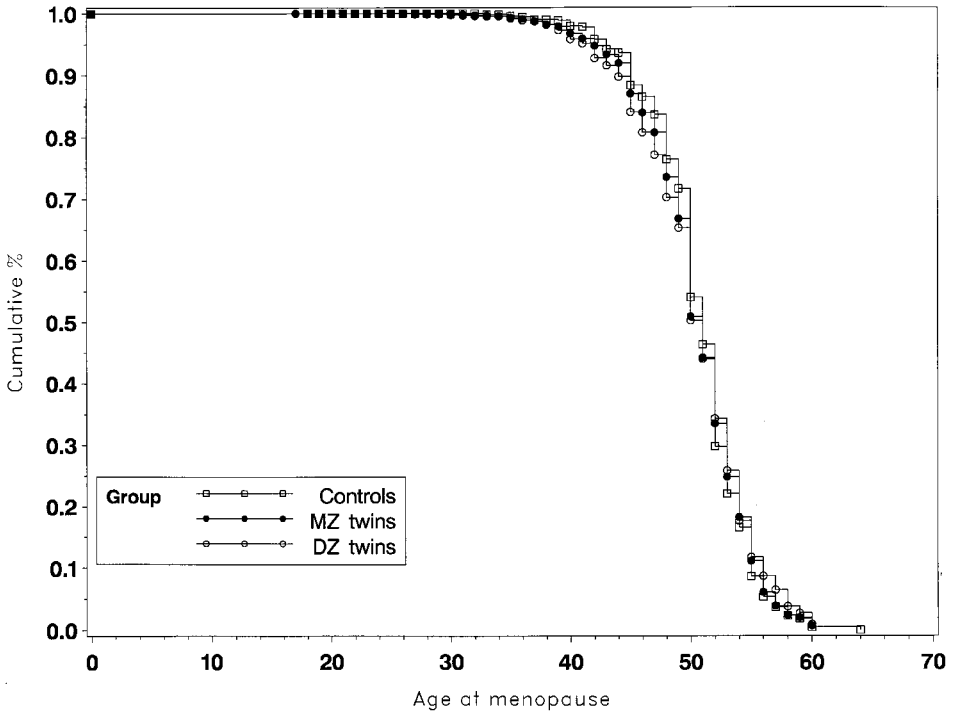

Figure 4. Kaplan–Meier curves for age at natural menopause in 2980 monozygotic (MZ), 2981 dizygotic (DZ) Australian female twins and 837 controls.

produced by Mx was 0.505. These analyses were conducted on a complete data pairs only, due to the limited capability of Mx to handle censored data, resulting in reduced sample size and power.

GEE approach

We then re-analysed the data using the GEE approach. An inspection of residual plots following model fitting provided no evidence for the failure of proportional hazards assumption and did not detect influential observations. The results (Table I) suggest that later birth year, university education, and having at least two children are associated with later age at menopause. Table I also presents the odds ratio for each zygosity type which can be used to investigate patterns of familial aggregation. The odds ratios for MZ and DZ twin pairs are 1.764 and 1.355, respectively. Both are significantly different from independence (odds ratio = 1, $P < 0.01$). From these odds ratios, we estimated the within-pair correlations to be $r_{MZ} = 0.568$ and $r_{DZ} = 0.304$. The test statistic for the equality of these two correlations was 7.47, indicating a highly significant difference between MZ and DZ twin correlations. The classical estimate of heritability was $2(0.568 - 0.304) = 0.528$. The proportion of the phenotypic variance attributed to the common environment was an insignificant 0.040 ($= r_{MZ} - h^2$).

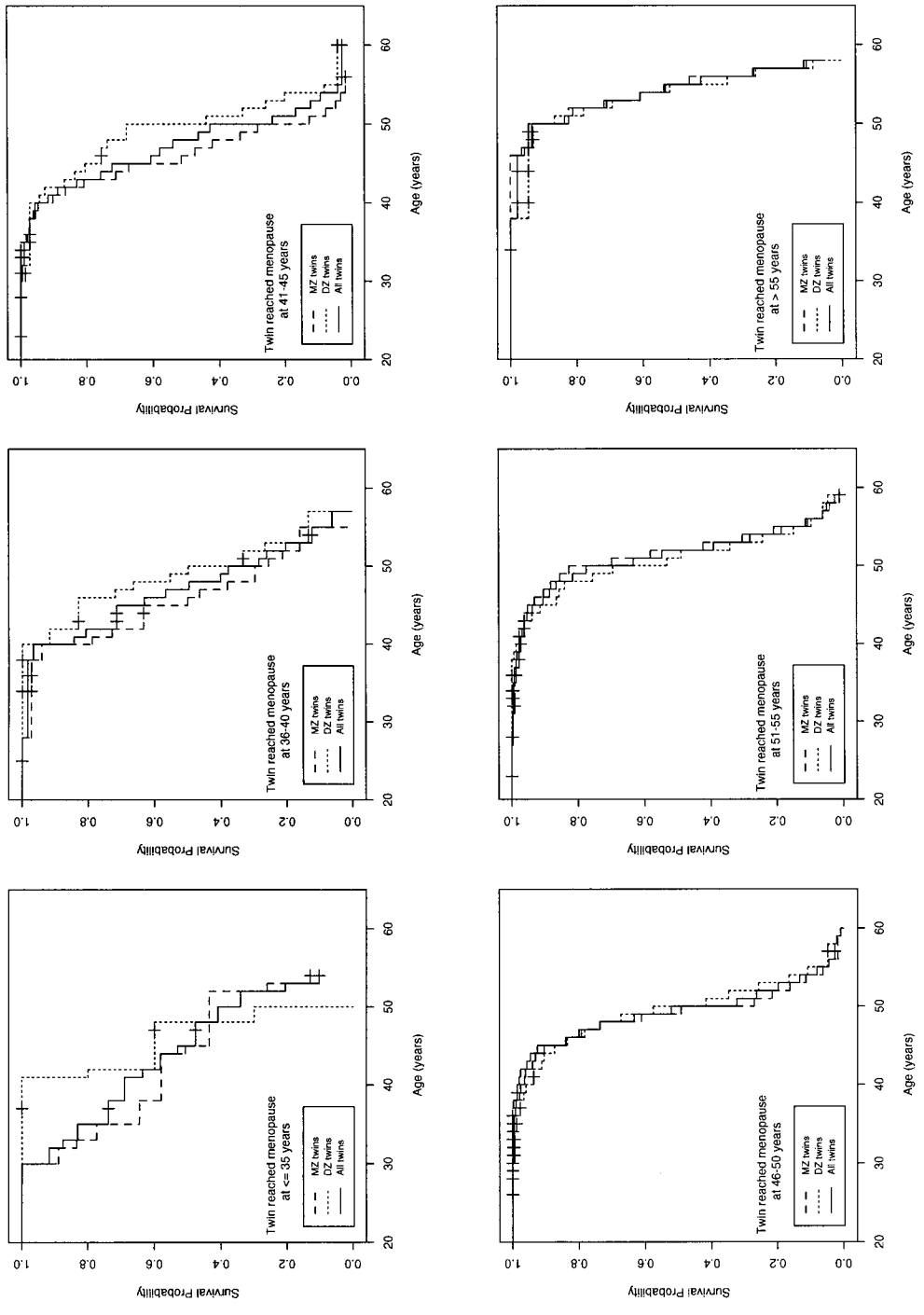

Figure 5. Age-specific probabilities of reaching menopause in subjects whose twins were menopausal.

Table I. GEE approach: estimated regression coefficients in the proportional hazards model and estimated odds ratios for quantifying familial aggregation in a longitudinal study of time to menopause in Australian twins (* indicates significance).

Covariate	$RR = e^{\beta}$	Coefficient β	Robust SE(β)	Z-statistic
(*a*) *Mean effects*				
Year of birth	0.978	− 0.022	0.0038	− 5.79*
Smoking	1.123	0.116	0.0680	1.72
University education	0.643	− 0.442	0.1077	− 4.10*
Menarche	0.984	− 0.016	0.0226	0.71
Parity	0.624	− 0.471	0.0960	− 4.90*
(*b*) *Patterns of familial aggregation*				
Zygosity		$1 + \theta$	SE(θ)	Z-statistic
MZ		1.764	0.1480	5.16*
DZ		1.355	0.1270	2.79*

Bayesian approach

We re-analysed using Gibbs sampling to impute missing menarche, parity and education and to estimate subject-specific covariate effects and random genetic (σ_A^2) and common environment (σ_C^2) effects on the log scale. The estimated shape parameter r was 9.81 with a 95 per cent confidence interval of (9.65, 10.10). The results are summarized in Table II. The residual plots did not indicate a gross departure from the underlying Weibull model and revealed no influential observation. We checked the sensitivity of the analyses to initial parameter values by re-running the Gibbs sampler five more times using different starting values. The resulting estimates did not differ by more than 5 per cent from the values reported here. The mean estimate for (σ_A^2) was 0.73 (95 per cent CI: 0.13–1.41) and for (σ_C^2) was 0.01 (95 per cent CI: − 0.456–0.469). Again, the results suggest that the effect of a common environment is negligible. We then compared models that incorporate (i) covariate effects only, (ii) random genetics effects only (ACE, AE and CE), (iii) a mixture of covariate effects and random genetics effects (ACE, AE and CE), using the Akaike information criterion (AIC) whenever non-nesting of two models invalidated the use of likelihood ratio tests. The estimated log-relative risks due to birth year, parity, age at menarche, smoking and education were essentially unchanged when comparing the ACE model (Table II) to the AE or CE model.

DISCUSSION

We applied three methods – path analysis on complete data, generalized estimating equation and Bayesian analysis – to the genetic analysis of age at menopause using twin data. Under all three approaches, the results suggest that additive genetic factors play an important role in menopause age and that shared environmental factors do not. We focused attention on the latter approaches

Table II. Gibbs sampling approach: estimated regression coefficients and estimated variance components in a longitudinal study of time to menopause in Australian twins (* indicates significance).

Covariate	$RR = e^{\beta}$	Coefficient β	Robust SE(β)	95% CI of β
(*a*) *Mean effects*				
Year of birth	0.971	− 0.029	0.0035	(− 0.036, − 0.023)*
Smoking	1.148	0.138	0.0788	(− 0.187, 0.293)
University education	0.672	− 0.397	0.1400	(− 0.676, − 0.123)*
Menarche	0.976	− 0.024	0.0204	(− 0.063, 0.015)
Parity	0.556	− 0.586	0.1260	(− 0.830, − 0.033)*
(*b*) *Variance components*				
σ_A^2		0.730	0.329	(0.129, 1.410)
σ_C^2		0.011	0.240	(− 0.456, 0.489)

because they are more appropriate for modelling age-to-onset data and they allow the inclusion of covariates in the analyses. Under both approaches, there were suggestions that age at menopause was influenced by later birth year, having a university degree, and having two or more children. The principal difference between the two approaches is in the interpretation of the regression coefficients. The GEE method uses a marginal approach resulting in regression coefficients that describe the average population response to changing covariates, whereas the Bayesian approach produces subject-specific coefficients. A secondary distinction is in the nature of the within-pair dependence. The GEE model only describes a common covariance among twin pairs of a particular zygosity, whereas the Bayesian approach can explicitly describe the source of this covariance. A third advantage of the Bayesian method is its flexibility in incorporating prior information, if available for the covariates or latent effects by modifying their prior distributions. Further, the Bayesian method would permit a more accurate decomposition of the genetic variance into additive and dominant components and thus provide the means for a direct assessment of the no-dominance assumption. Finally, it is also interesting to record the amount of CPU time required for each method: 20 seconds for the GEE approach and approximately 30 hours for the Bayesian analysis on an Ultra-SPARC Workstation. Since years of work and millions of dollars have been dedicated to collecting, maintaining and updating the twin data, this extra CPU time requirement by the MCMC method is well worth the additional genetic information and flexibility that it provides.

The analyses performed here associated a somewhat different level of significance to some covariates than did a previous analysis of the same data that treated observations from twin pairs as if they were independent [32]. In the former paper, we found that year of birth, smoking, university education, later menarche and parity were all significant contributors to the variation in age at menopause. However, to account for genetics effects in this paper, we could only use information from complete twin pairs, thereby reducing the power to detect all the covariate effects. Specifically, the negative effects of smoking and abnormally late menarche are no longer significant here even though the trends are still in the same directions. However, the previous analysis included an additional 1597 singletons to the data set used in the present analysis. Further, these singletons tended to be older: 45 per cent were born before 1940, versus 36 per cent in the sample used here. It is thus possible that the differences were

due less to the analytical techniques than to a loss in power and to differences in the samples being studied.

There is also a need to examine the possibility of age interaction effects. In several diseases (for example, familial breast cancer and Alzheimer's disease) 'genetic' forms occur earlier in life than sporadic cases. Therefore we might expect that twins might be more concordant for premature ovarian failure (say due to occult leiomyomatosis or endometriosis) than later menopause. This can be tested via a conditional approach [56], contrasting the MZ and DZ odds ratios in a simple contingency table that splits cells into early and late menopausal ages for twin 1 versus twin 2, or by calculating age-specific risks using straightforward and graphical life-table analysis that constructs survival curves to examine the risk of menopause at every age point among the subjects whose twins had reached menopause within a prespecified age interval (Figure 5). We found no significant age interaction effect. However, this result should be interpreted with caution since our study may have limited power due to the high censoring proportion (58 per cent).

Our analyses of the menopause data have several limitations. First, it is reasonable to surmise the existence of common genetic influences on age at menopause and age at menarche. Therefore, treating these covariates as fixed effects (GEE) or subject-specific effects (Bayesian) may in fact obscure these common genetic effects. The solution to this problem would be in the development of multivariate MCMC methods which can incorporate a mixture of censored and non-censored observations. Second, it is not possible from twin data alone to separate shared environment from dominant genetic effects. Extensions to the methods presented here should then allow the inclusion of data on other forms of relatives besides the twins, which would enable the estimation of additive and dominant effects simultaneously. Lastly, we are currently performing simulation work to determine the robustness of MCMC methods in the genetic decomposition of censored observations, to, for example, misspecification of the hazard function or the genetic model.

APPENDIX A: THE GEE MODEL AND ESTIMATION OF PARAMETERS

We applied the iterative method of Hsu and Prentice [9] and Hsu and Zhao [10] to obtain simultaneous estimates of β and θ. Consistent estimates of the regression coefficients from a partial likelihood and non-parametric estimates of the baseline can be obtained using methods by Breslow [58] or Efron [59]. As the model does not require the specification of a baseline hazard function, we estimate it at each iterative step using the relationship between baseline hazard and survival rates ($S_0(t)$)

$$S_0(t) = \Pr(\text{pre-menopausal at } t) = \exp\left(- \int_0^t \lambda_0(t) \right) = \exp(- \Lambda_0(t))$$

The survival function for the ith twin in the kth pair is

$$S(t_{ki}|\mathbf{Z}_{ki}) = [S_0(t_{ki})]^{\exp(\beta' \mathbf{Z}_{ki})}$$

in which S_0 can then be estimated non-parametrically.

The iterative procedure for the estimation of β and $\Theta = (\theta_{MZ}, \theta_{DZ})'$ is based on the Newton–Raphson algorithm. Let (β_i, Θ_i) denote the values of the estimates at the ith iteration. These values

are updated using

$$\begin{pmatrix}\beta_{i+1}\\ \Theta_{i+1}\end{pmatrix}=\begin{pmatrix}\beta_i\\ \Theta_i\end{pmatrix}+\Sigma_1^{-1}\begin{pmatrix}U_1\\ U_2\end{pmatrix}$$

where U_1 and U_2 are estimating equations for parameters β and Θ respectively, and Σ_1 is the partial derivative matrix of $(U_1, U_2)'$ with respect to the vector of parameters $(\beta, \Theta')'$. Explicitly

$$U_1=\sum_{k=1}^{K}\mathbf{Z}_k'[\delta_k-\hat{\Lambda}_k(T_k;\beta)]$$

$$U_2=\sum_{k=1}^{K}\eta_k'[(a_k(T_k)-\alpha_k(T_k)]$$

where $T_k=(t_{k1}, t_{k2})$, $\hat{\Lambda}_k$ is the estimated cumulative hazard for the kth family, $a_k(T_k)$ and $\alpha_k(T_k)$ are observed and expected twin covariances for age-of-onset. The matrix η_k allows estimation of dependence parameters that are relationship specific [60]. Note that Σ_1^{-1} is the naive covariance matrix that does not take into account the correlation in the twins. A robust covariance matrix is given by

$$\Sigma_1^{-1}\begin{pmatrix}\Sigma^{(11)} & \Sigma^{(12)}\\ \Sigma^{(21)} & \Sigma^{(22)}\end{pmatrix}\Sigma_1^{-1}$$

where $\Sigma^{(11)}$ is the information on the marginal parameters β from the mean function, $\Sigma^{(22)}$ is the information on the dependence parameter Θ from the covariance function, and $\Sigma^{(21)}$ is the information of the marginal parameter β from the covariance function.

APPENDIX B: A BUGS PROGRAM TO IMPLEMENT THE BAYESIAN MCMC APPROACH IN MODELLING AN ACE MODEL WITH COVARIATE EFFECTS AND RANDOM GENETICS/ENVIRONMENTAL EFFECTS

```
# Model A + C + E
model menopause:
const   M = 2,
        NUMFAMS = 2182,                 # number of families
        MZFAMS = 1355,                  # number of MZ twin pairs
        DZFAMS = NUMFAMS-MZFAMS,        # number of DZ twin pairs
var     mz[MZFAMS],                     # MZ effect with precision tauMZ
        dz[DZFAMS],                     # DZ effect with precision tauDZ
        d[DZFAMS,M],                    # Individual DZ effect with precision tauE
        tauE, tauMZ, tauDZ,             # precisions
        VAmz, VAdz,                     # Variance component for MZ and DZ twins
        VAe,                            # Variance of extra DZ effect
        VA,                             # Variance due to additive genetics
        VC,                             # Variance due to common environment
        mu[NUMFAMS, M],                 # Weibull mean for subject
        t[NUMFAMS,2],
        t.cen[NUMFAMS,2],               # Censored observations
        byr[NUMFAMS,2], smoke[NUMFAMS,2], menarce[NUMFAMS,2], parity[NUMFAMS,2],
        zyg[NUMFAMS], educat[NUMFAMS,2], alpha, r, beta.byr, beta.smoke, beta.men,
        beta.parity, beta.educat;
```



```
data zyg, t, t.cen, byr, smoke, menarce, parity, educat in ''menop.dat'';

inits in ''menop.in'';
{
for (i in 1:NUMFAMS){
       for(j in 1:M){
            menarce[i, j] ~ dnorm(5.95,0.49);
            parity[i, j] ~ dbern(0.846);
            educat[i, j] ~ dbern(0.124);}}

for(i in 1:MZFAMS){
  for(j in 1:M){
    t[i, j] ~ dweib(r,mu[i,j])I(t.cen[i,j],);
    log(mu[i,j]) <- alpha + beta.byr*byr[i,j] + beta.men*menarce[i,j]
       + beta.smoke*smoke[i,j] + beta.parity*parity[i,j]
       + beta.educat*educat[i,j] + mz[i];}
    mz[i] ~ dnorm(0.0, tauMZ);}

for(i in 1:DZFAMS){
  for(j in 1:M){
    t[i + MZFAMS,j] ~ dweib(r,mu[i + MZFAMS,j])I(t.cen[i + MZFAMS,j],);
    log(mu[i + MZFAMS,j]) <- alpha + beta.byr*byr[i + MZFAMS,j]
       + beta.men*menarce[i + MZFAMS,j] + beta.smoke*smoke[i + MZFAMS,j]
       + beta.parity*parity[i + MZFAMS,j]
       + beta.educat*educat[i + MZFAMS,j] + dz[i] + d[i,j];
    d[i,j] ~ dnorm(0.0,tauE);}
  dz[i] ~ dnorm(0.0,tauDZ);}
#Priors
alpha ~ dnorm(0.0,0.001);
beta.byr ~ dnorm(0.0,0.001);
beta.smoke ~ dnorm(0.0,0.001);
beta.men ~ dnorm(0.0,0.001);
beta.parity ~ dnorm(0.0,0.001);
beta.educat ~ dnorm(0.0,0.001);
tauMZ ~ dgamma(0.001,0.001);
tauDZ ~ dgamma(0.001,0.001);
tauE ~ dgamma(0.001,0.001);
r ~ dgamma(0.001,0.001);

VAmz <- 1/tauMZ;
VAdz <- 1/tauDZ;
VA <- 2*(VAmz-VAdz);
VC <- VAmz-VA;
}
```

ACKNOWLEDGEMENTS

We appreciate helpful suggestions and discussion with Kerrie Mengersen, Katrina Tiller and Paul Burton. Special thanks to Li Hsu who sent us her FORTRAN programs for implementation of the estimating equations technique. Helpful assistance is acknowledged for data collection from S. Maria, Pamela Saunders, John Pearson, Olivia Zheng and Bradley Christiansen. Data collection was supported by grants from the Australian National Health and Medical Research Council (NHMRC) and NIH grants AA07535 and A07728. We also thank the regional director and two referees for their valuable comments.

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