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Full title: Familial concordance for age at natural menopause: results from the Breakthrough Generations Study

Running title: Familial concordance for age at menopause

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ABSTRACT
Objective. Existing estimates of the heritability of menopausal age have a wide range. Furthermore, few studies have analysed to what extent familial similarities might reflect shared environment, rather than shared genes. We therefore analysed familial concordance for age at natural menopause and the effects of shared genetic and environmental factors on this concordance.

Methods. Participants were 2060 individuals comprising first-degree relatives, aged 31-90 years, and participating in the UK Breakthrough Generations Study. Menopause data were collected by a self-administered questionnaire and analysed using logistic regression and variance-components models.

Results. Women were at an increased risk of early menopause (≤45 years) if their mother (odds ratio = 6.2, P < 0.001) or non-twin sister (odds ratio = 5.5, P < 0.001) had had an early menopause. Likewise, women had an increased risk of late menopause (≥54 years) if their relative had had a late menopause (mother: odds ratio = 6.1, P < 0.01; non-twin sister: odds ratio = 2.3, P < 0.001). Estimated heritability was 41.6% (P < 0.01), with an additional 13.6% (P = 0.02) of the variation in menopausal age attributed to environmental factors shared by sisters.

Conclusions. We confirmed that early menopause aggregates within families and showed, for the first time, that there is also strong familial concordance for late menopause. Both genes and shared environment were the source of variation in menopausal age. Past heritability estimates have not accounted for shared environment, and so the effect of genetic variants on menopausal age may previously have been over-estimated.

KEYWORDS. Environment; Epidemiology; Family; Gene; Heritability; Menopause.

WORD COUNT. Abstract: 250; Main article: 3397.

ABBREVIATIONS. BGS, Breakthrough Generations study; DZ, Dizygotic; MZ, Monozygotic; SES, Socio-economic status.
INTRODUCTION
Age at menopause is a marker of reproductive ageing, with fertility ending approximately ten years before the onset of menopause. Furthermore, menopausal age relates to subsequent disease risk; a late menopause is associated with an increased risk of breast and endometrial cancers, but a decreased risk of cardiovascular disease.

The menopausal ages of family members are among the few known predictors of a woman’s own menopausal age. For example, there is an estimated 6-fold increase in risk of early menopause in women with a family history of the trait. Familial concordance is also present among women who reach menopause within the normal age range. However, in these studies, women reported the menopausal ages of their family members, which could potentially bias the results.

A small number of studies have examined familial concordance in more detail by trying to identify the causes of this resemblance; specifically, whether it is a consequence of shared genes, environment/behaviour, or a mixture of both. The effects of genetic factors are usually quantified by estimating the narrow-sense heritability ($h^2$) of age at menopause, which is the proportion of variation in age at menopause explained by additive genetic effects. Unlike the correlation studies discussed above, published heritability studies have included all family members under consideration as study participants, and so did not require women to report the menopausal ages of their family members. Heritability estimates from these studies have a wide range, between 31% and 87%. The lower estimates imply that most of the variation is due to environmental factors or non-additive genetic effects but the highest estimate, which is from the largest study to date ($N \sim 5800$ singleton and twin sisters), implies that most of it is due to additive genetic variants, and putatively these genes are discoverable.

The effect of shared environment (also known as common environment) on familial concordance for menopausal age has been investigated in only a small number of studies. If shared environment contributes to variation in menopausal age and is not taken into account, then it will generally cause heritability to be over-estimated. Two twin studies, however, have not found a significant effect of shared environment.

The aim of the present study was to investigate the familial concordance for age at natural menopause using data from a large number of related individuals. We also investigated the extent to which the familial aggregation was due to shared environmental and behavioural factors.

METHODS
Participants
The participants were nested within the Breakthrough Generations Study (BGS), a cohort study designed to investigate breast cancer aetiology. The study has received approval from the South Thames Multicentre Research Ethics Committee. All women aged 16 or older who lived in the United Kingdom were eligible and more than 111,000 joined during the recruitment period (2003-2010). The initial participants were registered supporters of the charity Breakthrough Breast Cancer, and women who referred themselves through the recruitment website and telephone line. Participants nominated other women to join the study, and the majority of participants were recruited through this method. Consequently, there are a large number of study participants who have other family members in the study.

BGS participants were potentially eligible for the current analyses if they had experienced a natural menopause (i.e. permanent menses cessation for at least six months not caused by medical intervention) and had at least one full sister, mother or daughter who was also a BGS participant. Of these participants, we restricted analyses to those women without a history of breast cancer or ductal carcinoma in situ, who reported their age at natural menopause and who had at least one relative who also reported their age at natural menopause. We excluded...
the small number of women who reported natural menopause before age 30 if their menses had only ceased for two years or less, because it was possible that menses might re-commence in these women.

Participants were asked to report their age at menopause at two points in the study; first, on the baseline questionnaire that was completed by all participants when they joined, and again on the second follow-up questionnaire that has currently only been completed by the participants who joined earliest. For most \((N = 2020, 98.1\%)\) the menopause age analysed here was reported at study entry, but a few \((N = 40, 1.9\%, \text{mean follow-up} = 5.8 \text{years})\) were reported only on the second questionnaire. Both questionnaires were self-administered, postal questionnaires. Women were asked to write their menopausal age to the last completed year. Multiple options were available for them to indicate the cause of their menopause, including natural, surgical, and chemotherapy-induced. Furthermore, there were detailed questions about ovarian and uterine surgery, and hormone replacement therapy and other hormone treatment.

Statistical analysis
For each type of relative pair (i.e. mother-daughter, non-twin sisters, monozygotic [MZ] twins, dizygotic [DZ] twins), logistic regression models were fitted in SAS 9.2\(^{15}\) to determine the risk of an early menopause \((\leq 45 \text{ years})\) if an older relative had had an early menopause. Analogous models were fitted for ‘usual-age’ menopause \((46-53 \text{ years})\) and late menopause \((\geq 54 \text{ years})\). It was not possible to determine from our data which of the twins was younger. The twin who was younger at study entry was therefore taken as the younger relative for analytic purposes.

Similarly, linear regression models were fitted with the younger relative’s menopausal age as the outcome and the older relative’s menopausal age as the explanatory variable. The linear regression models show the estimated effect of a year’s delay of an older relative’s menopause on the younger relative’s age at menopause, and so a value close to one year implies that the relatives are concordant for age at menopause.

The logistic and linear regression analyses were repeated with adjustment for both relatives’ smoking status at menopause (non-smoker, former or current), parity (continuous), decade of birth (ordinal categories) and socio-economic status (SES; ordinal categories). These factors were all self-reported by the participant on the baseline questionnaire, with the exception of SES for which we used Acorn scores,\(^{16}\) a residential measure of SES based on the woman’s postcode at entry.

We fitted variance-components models in SOLAR 4.2,\(^{17}\) which partition the total variance in age at menopause into its separate sources. The initial model included terms for variance due to additive genetic effects, sibling shared/common environment and unique environment including random error. The best fitting, most parsimonious model was then chosen using backward selection. Terms were removed from the model if the difference in \(-2\ln(\text{likelihood})\) of the full and nested models was non-significant at the 5% level in a distribution that is a 50:50 mixture of a \(X^2_1\) distribution and a point mass at zero.\(^{17}\) In order to estimate the shared environment component, it was assumed that both twin and non-twin sisters shared an environment while mothers and daughters did not. Narrow-sense heritability is defined as additive genetic variance/total variance.

Additional variance-components models were fitted with adjustment for smoking status, parity, decade of birth and SES. The adjusted models are similar to the unadjusted models except that, instead of partitioning the total variance, they partition the residual variance after removing the variance attributable to the known risk factors.
RESULTS

Summary of participants
There were 9995 participants who were potentially eligible for these analyses because they had reached natural menopause and had a mother, daughter or sister who was also a study participant. Of these, 2195 (22.0%) women were ineligible because they had a history of breast cancer or ductal carcinoma in situ, 310 (3.1%) because their age at menopause was unknown and 7 (0.1%) because their reported menopause occurred before age 30 and their menses had not ceased for more than two years. Subsequently, a further 5423 (54.3%) participants were ineligible because their relative(s) did not meet the inclusion criteria. This left 2060 individuals who were included in these analyses. Of these, 2017 (97.9%) provided information on all covariates and hence were included in the adjusted analyses.

The 2060 individuals were comprised of 998 families, including 157 mother-daughter pairs, 23 MZ twin pairs, 21 DZ twin pairs and 925 non-twin sister pairs. Table 1 presents descriptive characteristics of these individuals. The mean age at the time of reporting menopausal age was 60.1 years (range = 31 to 90 years) and nearly all of the participants were of white ethnicity (99.6%). The average age at natural menopause was 50.4 years (standard deviation = 4.1 years, range = 22 to 62 years); 237 (11.5%) women reported an early menopause and 427 women (20.7%) reported a late menopause.

Early menopause
Women were approximately six times more likely to have an early menopause if their mother had had an early menopause than if she had not (odds ratio [OR] = 6.2, \( P < 0.001 \), Table 2). There was a similar risk if their older sister had had an early menopause (OR = 5.5, \( P < 0.001 \)). Both estimates were similar after adjustment for the proband and her relative’s SES, smoking status, parity and decade of birth (not shown).

Women whose MZ twin had had an early menopause were at greatly increased risk of early menopause (OR = 18.0, \( P = 0.04 \)), but the confidence interval was wide, based on small numbers. It was not possible to estimate the OR for DZ twins because there were no DZ pairs where both had had an early menopause.

‘Usual-age’ menopause
Women whose mother reached menopause within the usual age range were nearly four times as likely to also reach menopause within this range, compared with women whose mother had had an early or late menopause (OR = 3.8, \( P < 0.001 \), Table 2). This OR increased to 7.2 after adjustment for the mother’s and daughter’s SES, smoking status, parity and decade of birth (95% CI = 2.7 to 19.6, \( P < 0.001 \), \( N \) pairs = 149). Non-twin sisters also showed concordance within the normal age range (OR = 1.8, \( P < 0.001 \)), and this estimate was unchanged after adjustment (not shown).

The corresponding ORs were 16.3 (\( P = 0.02 \)) for MZ twins and 1.9 (\( P = 0.47 \)) for DZ twins. Adjusted estimates for twins could not be calculated due to small numbers.

Late menopause
Risk of late menopause was increased six-fold if a woman’s mother had had a late menopause (OR = 6.1, \( P = 0.01 \), Table 2) and two-fold if a woman’s older sister had had a late menopause (OR = 2.3, \( P < 0.001 \)). After adjustment for SES, smoking status, parity and decade of birth, the OR for sisters was essentially unchanged (not shown), but the OR for mothers increased to 9.0 (95% CI = 1.2 to 67.2, \( P = 0.03 \), \( N \) pairs = 149). Again, estimates for twins were based on small numbers. The increased unadjusted OR for DZ twins was borderline significant (OR = 10.8, \( P = 0.05 \)). It was not possible to estimate the OR for MZ twins.
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Regression analyses
A year’s increase in the age at natural menopause of an MZ twin was associated with an average increase of 8.3 months ($P < 0.001$, Table 2) in her co-twin’s menopausal age. The corresponding increases were 4.1 months ($P = 0.09$) for a DZ twin, 3.4 months ($P < 0.001$) for a sister and 2.1 months ($P = 0.04$) for a mother. After adjusting for smoking status, parity, decade of birth and SES, the MZ twin and non-twin sister associations were attenuated but remained significant (not shown). The mother-daughter association was however no longer significant (increase = 0.5 months, $P = 0.63, N$ pairs = 149).

Only women whose menopause occurred before study entry or first follow-up were included in these analyses, and consequently sister pairs who both had a relatively early menopause and hence were more concordant for age at menopause might potentially have been over-represented causing our results to be biased. However, we repeated the analyses restricted to non-twin sisters who were both aged 60 or older at study entry, for whom such a bias would not apply, and results were similar ($N = 280$, unadjusted increase = 3.5 months, $P < 0.001$). There were too few pairs to restrict the analyses for other types of relative but since there is no bias for non-twin sisters, it is unlikely that a bias would be present for other relatives.

Heritability analyses
Table 3 presents the results of the unadjusted variance-components models. The best fitting model was the ACE model, which includes terms for genes, environment shared by siblings and unique environment. In this model, 41.6% ($P = 0.001$) of the total variance in age at menopause was attributed to additive genetic effects (heritability), 13.6% ($P = 0.02$) to shared environment and the remainder to unique environment.

Together smoking status, parity, decade of birth and SES explained 5% of the total variation in age at menopause (not shown). After accounting for these variables, the effect of other shared environmental factors was not significant ($P = 0.11$) in the ACE model. In this model, 47.6% ($P < 0.001$) of the residual variance in age at menopause was attributed to additive genetic effects and 8.4% ($P = 0.11$) to shared environment.

DISCUSSION
We found strong evidence that age at natural menopause was similar within families, and this familial aggregation was due to both shared genes and shared environment. To our knowledge, this is the first study to show a significant effect of shared environment. Our sample size is comparable with the only other large study that included relatives in different generations ($N \sim 2500$),

Previous research on familial concordance for menopausal age has tended to focus on early menopause, in part due to its importance in family planning, and has not considered late menopause, despite it being a risk factor for many chronic diseases, including breast cancer. In the present study, a family history of late menopause conferred a high risk of the trait. To our knowledge, this is the first study to produce an estimate of the risk of late menopause given a family history of the trait, but it has been shown previously that both early and late menopause are affected by genetic factors.

We defined menopause as late if it occurred at age 54 years or later, instead of the more common definition of 55 years or later, because including women who had reached menopause at age 54 years substantially increased the number of late menopause cases and improved the precision of our estimates. Age 55 is a somewhat arbitrary cut-off point for a continuous trait and so menopause at age 54 is unlikely to have a different aetiology to menopause at age 55 or older.

As with late menopause, the risk of early menopause was increased in women with a family history of the trait, and we found an increased risk of a similar order to existing estimates. In addition to the extremes, familial concordance was also shown within the usual range of
menopausal ages. The following discussion explains how further analysis of our data suggested that the family concordance for age at menopause could be attributed to both the behavioural/environmental and genetic factors that family members have in common.

As shown in the linear regression analyses, concordance for menopausal age was highest for MZ twins, followed by DZ twins and sisters; the association between mothers’ and daughters’ ages at menopause was small. It was however of a similar order to previous findings. Specifically, we found that for each year’s delay in the mother’s menopause, the daughter’s menopause was delayed by an average of 2.1 months, which is similar to Torgerson et al’s estimate in the only other study that produced such an estimate. It therefore appears that mother-daughter concordance is strong at the extremes of menopausal age, as shown by the high odds ratios for early and late menopause, but not for the middle ground.

A genetic component to age at menopause was suggested in the linear regression analyses because MZ twins were roughly twice as concordant for menopausal age as DZ twins. Indeed, in the best fitting variance-components model, a term for additive genetic effects was significant and the heritability of age at menopause was 42%. This is within the range of previous estimates (31-87%) but lower than those from other large studies (50-70%). These studies assumed, however, that shared environmental factors did not contribute to the variance, and if we also did not include the contribution of shared environment then heritability in our data would be 64%, which is similar to these previous estimates. It is therefore probable that variance attributed to common environment in our study was attributed to genetic factors in other studies, causing their heritability estimates to be artefactually higher.

Environmental factors shared by family members explained 14% of the total variance in menopausal age, and part of this was due to known factors, as shown by the reduction in the shared environment effect after adjusting for these factors. Environmental factors were the source of more than 50% of the residual variation after adjusting for these known risk factors, which suggests that there are important environmental or behavioural risk factors for menopausal age, with a substantial combined effect, that remain to be identified.

The significant shared environment effect in this study differs from the findings of twin studies, which found no evidence that part of the variation was due to shared environment. Both the type of analyses that we have presented here and those performed in twin studies require an assumption about who shared an environment, otherwise it is not possible to fit the statistical models. We applied the frequently used assumption that twin and singleton sisters shared an environment whereas mothers and daughters did not, since it is likely that relatives of the same generation would have more similar exposures than relatives in different generations. Furthermore, the results of our regression analyses showed that DZ twins and singleton sisters were more concordant for age at menopause than mothers and daughters. Since all three of these types of relatives share the same proportion (50%) of their genes on average, this difference is probably due to differences in shared environment.

It is a limitation of this study, and most other studies of this type, that it relied on recalled menopausal age. Recall errors could lead to misclassification when the data were grouped as early, usual or late. If such misclassification occurred non-differentially then it would be expected to reduce the odds ratios. Another potential source of bias was that study
participants were self-selected, and the participants in these analyses were those where at least two family members were motivated to join. It is unlikely however that either of these factors would have affected our heritability estimates because, although they might reduce the total variance in age at menopause, there is no reason that the variance would be attributed differently to genes or environment because of them. Furthermore, results were similar after we adjusted for factors such as SES. Like all heritability studies, our results describe the variation in the population in which they were measured.

A further limitation was that it was not possible to perform subgroup analyses by either ethnicity or region because too great a proportion of participants were of white ethnicity and lived in Southern England.

CONCLUSION
We found that 42% of the variation in age at natural menopause was attributable to additive genetic effects and 14% to environmental factors shared by sisters. The significant shared environment effect suggests that previous heritability estimates might have been over-estimated, and so the unidentified genetic variants that affect menopausal age might explain less of the variability in menopausal age than was previously suggested.

ACKNOWLEDGEMENTS
We thank the women who participated in the study, and our colleagues in the Breakthrough Generations Study Team. Breakthrough Breast Cancer, the Sir John Fisher foundation and the Institute of Cancer Research, who acknowledge NHS funding to the NIHR Biomedical Research Centre, supported this work.
Reference List


SUMMARY FOR TABLE OF CONTENTS
This study, based on 2060 women in the UK, showed that both early and late menopause aggregated within families, and that this was due to both shared genes and shared environment.
Table 1. Descriptive characteristics of the study subjects.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at menopause, <em>years</em></td>
<td>50.4</td>
<td>4.1</td>
</tr>
<tr>
<td>Age at time of reporting about their menopause, <em>years</em></td>
<td>60.1</td>
<td>7.7</td>
</tr>
<tr>
<td>Parity</td>
<td>2.1</td>
<td>1.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>N</th>
<th>%</th>
</tr>
</thead>
</table>

Age at menopause
- Early, ≤45 *years*  237 11.5
- Normal, 46-53 *years*  1396 67.8
- Late, ≥54 *years*  427 20.7

Socio-economic status\textsuperscript{b}
- 1 (Highest)  1052 51.1
- 2  197 9.6
- 3  577 28.0
- 4  112 5.4
- 5 (Lowest)  98 4.8

Year of birth
- Before 1925  29 1.4
- 1925-1934  160 7.8
- 1935-1944  534 25.9
- 1945-1954  1124 54.6
- 1955 onwards  213 10.3

Smoking status\textsuperscript{c}
- Non-smoker  1341 65.1
- Former smoker  500 24.3
- Current smoker  200 9.7

Geographical region
- Southern England  1107 53.7
- Central England  311 15.1
- Northern England  362 17.6
- Scotland  152 7.4
- Northern Ireland  19 0.9
- Wales  92 4.5
- Isle of Man  1 0.1
- Jersey  16 0.8

Ethnicity\textsuperscript{d}
- White  2051 99.6
- Indian  2 0.1

SD = Standard deviation.
\textsuperscript{a} 2060 individuals in total.
\textsuperscript{b} 24 subjects were of an unclassified socio-economic status.
\textsuperscript{c} 19 subjects did not report their smoking status.
\textsuperscript{d} 7 subjects did not report their ethnicity.
Table 2. The effect of an older relative’s age at natural menopause on a woman’s own age at natural menopause.

<table>
<thead>
<tr>
<th>Proband’s age at menopause</th>
<th>Relative’s age at menopause</th>
<th>Type of relative (Number of individuals)</th>
<th>Odds ratio (95% CI)</th>
<th>Average increase in menopausal age for each year increase in the relative’s menopausal age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mother</td>
<td>Older sister</td>
<td>Monozygotic twin*</td>
</tr>
<tr>
<td>Early menopause (≤45 years)</td>
<td>Early</td>
<td>10</td>
<td>31</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Not early</td>
<td>16</td>
<td>61</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Early</td>
<td>12</td>
<td>71</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Not early</td>
<td>119</td>
<td>762</td>
<td>18</td>
</tr>
<tr>
<td>Odds ratio (95% CI)</td>
<td></td>
<td>6.20 (2.31, 16.65)***</td>
<td>5.46 (3.32, 8.96)***</td>
<td>18.00 (1.08, 298.99)*</td>
</tr>
<tr>
<td>Usual-age menopause (46-53 years)</td>
<td>Usual</td>
<td>89</td>
<td>446</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Not usual</td>
<td>31</td>
<td>207</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Usual</td>
<td>16</td>
<td>148</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Not usual</td>
<td>21</td>
<td>124</td>
<td>5</td>
</tr>
<tr>
<td>Odds ratio (95% CI)</td>
<td></td>
<td>3.77 (1.75, 8.12)***</td>
<td>1.81 (1.35, 2.41)***</td>
<td>16.25 (1.44, 183.09)*</td>
</tr>
<tr>
<td>Late menopause (≥54 years)</td>
<td>Late</td>
<td>6</td>
<td>69</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Not late</td>
<td>5</td>
<td>111</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Late</td>
<td>24</td>
<td>160</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Not late</td>
<td>122</td>
<td>585</td>
<td>18</td>
</tr>
<tr>
<td>Odds ratio (95% CI)</td>
<td></td>
<td>6.10 (1.72, 21.61)**</td>
<td>2.27 (1.61, 3.22)***</td>
<td>10.80 (1.00, 117.00)</td>
</tr>
</tbody>
</table>

CI = Confidence Interval.
* P < 0.05; ** P < 0.01; *** P < 0.001.
* The twin who was younger at study entry was, arbitrarily, taken as the proband and the twin who was older at study entry was taken as the predictor relative.