Human Molecular Genetics, 2011, Vol. 20, No. 1 **186–192**
doi:10.1093/hmg/ddq417
Advance Access published on October 17, 2010

Common genetic variants are significant risk factors for early menopause: results from the Breakthrough Generations Study

Anna Murray[1,*], Claire E. Bennett[1], John R.B. Perry[1], Michael N. Weedon[1], ReproGen Consortium, Patricia A. Jacobs[2], Danielle H. Morris[3], Nicholas Orr[4], Minouk J. Schoemaker[3], Michael Jones[3], Alan Ashworth[4] and Anthony J. Swerdlow[3]

[1]Peninsula Medical School, University of Exeter, St Lukes, Exeter EX1 2LU, UK, [2]Wessex Regional Genetics, Salisbury District Hospital, Salisbury SP2 8BJ, UK, [3]Section of Epidemiology, The Institute of Cancer Research, Sutton, Surrey SM2 5NG, UK and [4]Breakthrough Research Centre, The Institute of Cancer Research, 237 Fulham Road, London SW3 6JB, UK

Received May 24, 2010; Revised and Accepted September 20, 2010

Women become infertile approximately 10 years before menopause, and as more women delay childbirth into their 30s, the number of women who experience infertility is likely to increase. Tests that predict the timing of menopause would allow women to make informed reproductive decisions. Current predictors are only effective just prior to menopause, and there are no long-range indicators. Age at menopause and early menopause (EM) are highly heritable, suggesting a genetic aetiology. Recent genome-wide scans have identified four loci associated with variation in the age of normal menopause (40–60 years). We aimed to determine whether theses loci are also risk factors for EM. We tested the four menopause-associated genetic variants in a cohort of approximately 2000 women with menopause ≤45 years from the Breakthrough Generations Study (BGS). All four variants significantly increased the odds of having EM. Comparing the 4.5% of individuals with the lowest number of risk alleles (two or three) with the 3.0% with the highest number (eight risk alleles), the odds ratio was 4.1 (95% CI 2.4–7.1, $P = 4.0 \times 10^{-7}$). In combination, the four variants discriminated EM cases with a receiver operator characteristic area under the curve of 0.6. Four common genetic variants identified by genome-wide association studies, had a significant impact on the odds of having EM in an independent cohort from the BGS. The discriminative power is still limited, but as more variants are discovered they may be useful for predicting reproductive lifespan.

INTRODUCTION

Earlier menopause is associated with a decreased risk of breast cancer, but an increased risk of osteoporosis and cardiovascular disease (1). There is also a significant impact on fertility associated with early menopause (EM), which is particularly relevant to current populations where delaying childbearing has become more prevalent. The number of births per 1000 British women is now greater for those in their early 30s than it is for women in their early 20s (2,3). Fertility decreases long before the onset of menopause, beginning on average at about the age of 30 years. It is estimated that natural fecundity ceases at a mean age of 41 years, i.e. 10 years before menopause, and therefore women who are destined to have an EM and who delay childbearing until their 30s are more likely to have problems conceiving (4).

Natural, non-surgical, menopause occurs at a mean age of 51 years in Caucasian populations, with a roughly normal distribution between 40 and 60 years, but a tail below 40 years (5,6). Menopause before the age of 40, or premature ovarian failure (POF), occurs in 1% of the population (6). Menopause before 45 years occurs in ∼5% of women and is often termed 'EM'. Menopause is initiated by a fall in the number of oocytes in the ovary below a threshold level of about 1000

*To whom correspondence should be addressed. Tel: +44 1392262976l; Fax: +44 1392262926; Email: anna.murray@pms.ac.uk

Table 1. Association of GWAS menopause SNPs with age of natural menopause in BGS controls, i.e. menopause >45 years, excluding those with surgical menopause ($n = 1261$)

SNP	chr	Minor allele	Allele 2	MAF in controls (%)	ReproGen GWAS, per-allele effect	BGS controls Per-allele effect (se)	*P*-value
rs4806660	19	C	T	36.5	−0.406 (0.03)	−0.257 (0.12)	0.027
rs16991615	20	A	G	7.0	0.971 (0.0624)	0.924 (0.23)	0.000056
rs9379896	6	C	T	18.5	0.242 (0.0377)	0.121 (0.14)	0.39
rs244715	5	A	G	45.9	0.291 (0.0334)	0.059 (0.12)	0.64

Effect sizes are in years and are per copy of the minor allele.

(7). However, loss of oocytes occurs throughout female life with maximal numbers present before birth: approximately 6 million oocytes are present at 6 months gestation. By puberty, the number has decreased to ~400 000. Only a small proportion of oocytes are lost through ovulation, and the majority of the reduction is by atresia. The rate of atresia increases with age, particularly in the 10 years prior to menopause (8). The current methods for predicting age at menopause are reliant on detecting the peri-menopausal changes in oocyte number and are therefore poor long-range predictors (9). Hormonal serum levels alter prior to menopause, including follicle-stimulating hormone (FSH), antimullerian hormone (AMH) and inhibin B. Of these, AMH is the best long-term predictor, with levels decreasing approximately 10 years before menopause (10,11). In addition to endocrine markers, other markers of ovarian reserve are antral follicle count and ovarian volume (9). Genetic predictors of menopausal age have the obvious advantage of being present from birth and thus have the potential to offer women advice about their reproductive lifespan from an early age, enabling them to make informed reproductive choices.

The heritability of menopausal age has been estimated to be between 30 and 85%, indicating a substantial genetic component to this complex trait, and a significant proportion (15–30%) of POF cases are familial, suggesting a genetic aetiology (12–15). Recent genome-wide association studies (GWAS) have been very successful in identifying genetic loci for many complex traits. Two independent studies, investigating age at menopause, published their data in 2009 and between them identified four loci associated with normal age of menopause variation, on chromosomes 19, 20, 6 and 5 (16,17). In each of the GWAS, women with menopause before 40 years were excluded. Although genes involved in POF may also regulate menopausal age in the normal range, it is possible that there is a different genetic aetiology for women with EM. Hence, we tested the newly identified genetic variants associated with normal variation in menopausal age, in a population of approximately 2000 women from the Breakthrough Generations Study (BGS) who had EM, defined as menopause before the age of 46 years, plus 2000 matched controls. The BGS has not been included in any of the discovery of GWAS and thus represents a completely independent cohort. We included estimates of the effects of combining information from all four variants to calculate the cumulative genetic effect and assess the usefulness of these variants as predictors of menopausal age and their potential for assisting reproductive choice for young women, prior to oocyte depletion.

RESULTS

Four common genetic variants influence menopausal age by between 0.7 and 11 months per allele

Using menopause age as a quantitative trait, the directions of association and effect sizes of all four single nucleotide polymorphisms (SNPs) were consistent with those published in the GWAS (Table 1). The effect sizes for all SNPs were slightly lower than that in the discovery GWAS, as expected due to 'winners curse', but the chromosome 19 and 20 hits in particular have substantial effects on age at menopause with a reduction in the menopausal age of 3 months (0.257 years) and an increase of 11 months (0.924 years) per allele, respectively. Using the adjusted R^2 from the regression model, we calculated that together the four variants explained 1.4% of the variance in menopause age in controls.

Menopause variants are associated with EM

All four SNPs were associated with an increased risk of menopause before the age of 46, with the risk allele consistent with the GWAS, i.e. being the allele associated with decreasing menopausal age. The odds ratios (ORs) per allele for each SNP ranged from 1.13 to 1.85, the non-synonymous SNP on chromosome 20 having the largest effect (Table 2**)**. When comparing homozygote groups, the ORs ranged from 1.35 to 2.8. Three percent of women were homozygous for all four risk variants; of these 97 women, 66 (68%) were in the EM group and 31 (32%) were controls. We calculated the expected ORs for EM based on the quantitative trait estimates from the ReproGen GWAS and the BGS controls, and there was evidence that rs4806660 had a larger OR for EM than predicted [observed OR = 1.45 (CI 1.32–1.59) versus expected OR = 1.20 (CI 1.17–1.23), $P = 0.0001$] (Supplementary Material, Table S2).

Menopause variants are associated with POF

There were 260 women in the BGS cohort with POF and we determined the association with the menopause SNPs in these women. We were not well powered to detect the effects, but for all four SNPs there was evidence that the odds of being a POF case, per risk allele, were not significantly different from the odds of being an EM case excluding POF ($P > 0.05$) (Table 2). The smallest *P*-value in this analysis was for rs16991615, with $P = 0.051$. There was also nominal evidence that the rs16991615 SNP had a lower OR for POF than would be expected from the quantitative trait estimates [observed

Table 2. Association of GWAS menopause variants in EM and POF cases versus controls

SNP	chr	Risk allele	Number of controls	Risk allele frequency in controls	EM including POF (menopause <46) versus controls				EM excluding POF (menopause 40–45) versus controls				POF (menopause <40) versus controls			
					N	OR	95% CI	*P*-value	*N*	OR	95% CI	*P*-value	*N*	OR	95% CI	*P*-value
rs4806660	19	C	1898	0.365	2067	1.45	1.32–1.59	8.88×10^{-16}	1810	1.44	1.31–1.58	2.35×10^{-14}	257	1.54	1.27–1.85	6.48×10^{-6}
rs16991615	20	G	1879	0.930	2041	1.85	1.51–2.25	1.45×10^{-9}	1783	1.96	1.59–2.42	4.38×10^{-10}	258	1.30	0.88–1.92	0.18
rs9379896	6	T	1902	0.815	2072	1.13	1.01–1.27	3.8×10^{-2}	1813	1.14	1.01–1.29	0.03	259	1.06	0.84–1.35	0.61
rs244715	5	G	1606	0.541	1733	1.20	1.09–1.32	1.7×10^{-4}	1520	1.22	1.10–1.35	0.0001	213	1.12	0.91–1.37	0.29

ORs are per risk allele.

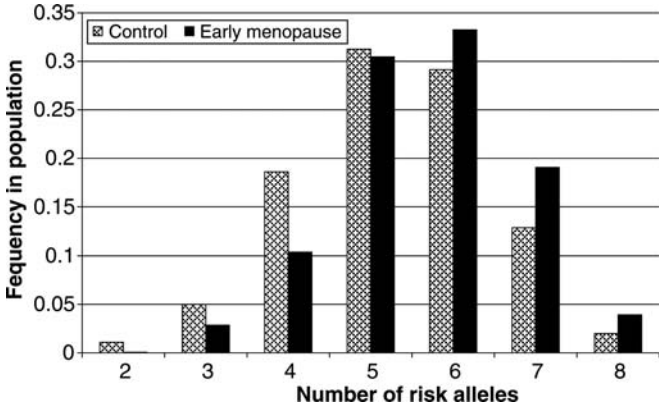

Figure 1. Number of EM risk alleles for all four SNPs in women with menopause at 45 and below (EM) compared with women who had menopause after the age of 45 years (controls).

OR = 1.3 (CI 0.88–1.92) versus expected OR = 2.08 (CI 1.90–2.29), $P = 0.02$] (Supplementary Material, Table S2).

Increased odds of having EM when menopause risk alleles are combined

Combining all risk alleles into an allele score gave a per-allele increased odds of being in the case group of 1.34 (95% CI 1.26–1.43, $P = 2.2 \times 10^{-20}$) (Fig. 1). Weighting each risk allele by effect size did not appreciably alter the OR (OR = 1.38, 95% CI 1.29–1.48, $P = 1.1 \times 10^{-19}$); therefore, results are presented for the unweighted alleles.

When risk alleles were combined, there were significantly increased odds of having EM in individuals with eight risk alleles compared with the median number in the control population of five risk alleles (OR = 2.02, 95% CI 1.30–3.15, $P = 0.002$) (Fig. 2). We also compared individuals at the extremes of the risk allele distribution and determined the increased likelihood of being a case: comparing the 4.5% of individuals with the lowest number of risk alleles (two or three) with the 3.0% with the highest number (eight risk alleles), the OR was 4.1 (95% CI 2.4–7.1, $P = 4.0 \times 10^{-7}$). Comparing the 18.8% of women with less than five risk alleles with the 19.1% with more than six, the difference was also significant (OR = 2.9, 95% CI, 2.3–3.6, $P = 4.6 \times 10^{-19}$).

Combined risk alleles have 60% discriminatory power

The discriminative power of the four menopause SNPs for EM was calculated by determining the area under the curve (AUC) in a receiver operator characteristic (ROC) analysis. Combining all four SNPs gave an AUC of 0.6 (Fig. 3).

DISCUSSION

It is well established that EM can have a genetic aetiology, but few loci have been identified. Four loci associated with variation in the normal age of menopause were recently identified by two independent GWAS with very robust statistical evidence (16,17) and we investigated the role of these common variants in EM.

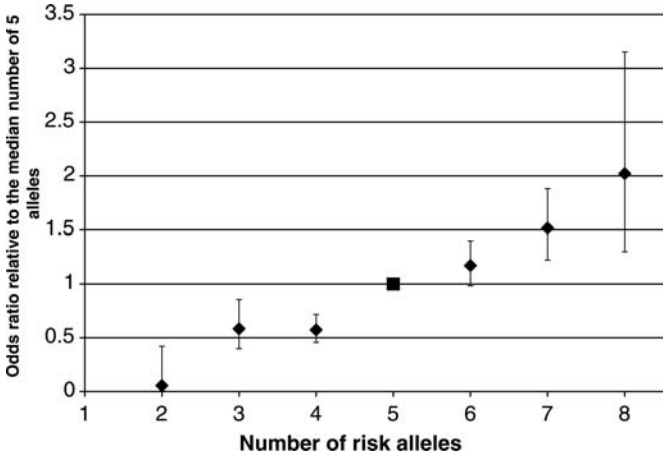

Figure 2. ORs of EM for each number of risk alleles compared with the median number of five risk alleles (indicated by a square). ORs are plotted as diamonds and 95% confidence intervals are indicated with vertical lines.

The four published variants had similar effects on age at menopause as observed in the two published discovery studies and our unpublished data from ReproGen, although in each case the effect was smaller, which is consistent with other GWAS, where the discovery effect is larger than in replication cohorts due to over-inflation or 'winners curse'. Two of these variants, on chromosomes 19 and 20, were significant at $P < 0.05$, but we had <20% power to detect the observed effect sizes at $P < 0.05$ for the SNPs on chromosomes 5 and 6. Together, the four SNPs explain about 1.4% of the variation in age at menopause. This is comparable to other complex traits such as height and BMI: 20 variants explain about 3% of the variation in height (18) and the 17 variants published to date for BMI only explain about 1% of the variation (19).

Our data suggest that variants associated with normal menopausal age are also significant risk factors for EM. The non-synonymous SNP in *MCM8* (rs16991615) increased the risk of EM by 85% per allele, the rare allele being protective for EM. The chromosome 19 variant near *TMEM224* (rs4806660) also has a substantial effect on risk of EM, increasing the OR by 45%. These ORs are higher than many reported for complex traits, which generally have per-allele ORs less than 1.5, with the exception of autoimmune disorders, macular degeneration and pigmentation loci (20) (http://www.genome.gov/gwastudies); however, further replication in additional EM cohorts would be beneficial in order to confirm the effects.

It has been hypothesized that EM cases may have a different aetiology from normal menopause. Our data suggest that there is at least some common aetiology between EM and normal menopause as the same genetic loci are associated with both traits. However, the OR for the chromosome 19 SNP was significantly greater than predicted, based on the effect on menopause age in the normal range in the quantitative trait analysis, suggesting that this locus may have a non-linear effect on menopause age. We investigated the aetiology of EM further in women at the extreme of the menopause distribution, i.e. menopause before 40 years. Women who have menopause before 40 are clinically classified as having POF and are often investigated for a genetic aetiology, most commonly by cytogenetic screening and *FMR1* mutation testing. The published menopause variants were originally identified in women excluding those with POF. The chromosome 20 SNP was the only variant where there was a suggestion that the effect might be different in POF cases compared with other EM cases, as the OR for POF cases was lower than predicted from the quantitative trait estimates and did not overlap the 95% confidence interval for EM, but with the caveat that this SNP is rare (MAF = 7%) and the number of POF cases was relatively small. However, these data suggest that the effect of the chromosome 20 SNP is not as strong in POF cases and suggest that POF may have a different aetiology. Our data therefore provide evidence that while some EM cases represent the tail of the normal distribution, some may have a different aetiology. The role of menopause variants in POF requires replication in additional independent studies, but these preliminary data support the necessity to look for EM-specific genes as these may not overlap with genes for menopause in the remainder of the age distribution.

EM has a significant impact on female health and results in early infertility. Current techniques have good predictive power for the end of female reproductive life, but only in the immediate pre-menopausal period when ovarian reserve is already diminished and natural conception is likely to be difficult or impossible. Commercial over-the-counter tests are available that measure hormone levels, usually FSH and/or AMH, but they need to be repeated every 2 years and are not good long-range predictors. It would be beneficial for women to be able to predict the timing of the end of their reproductive life in their early 20s, so that they can decide whether they want to risk the chance of infertility by delaying childbearing. The high heritability of menopausal age makes the potential for a genetic test extremely attractive. Very few genes have been demonstrated to be common causes of EM, with the possible exception of the *FMR1* premutation gene which accounts for ~5% of idiopathic POF cases (21), but the utility of *FMR1* as a genetic predictor has yet to be proved (22). We therefore studied the discriminative power of the four menopause loci identified by GWAS. The AUC for the ROC analysis was 0.6 for the four variants in combination, which is still some way from the power of tests such as the Framingham risk score for predicting coronary heart disease, where the AUC is typically near 0.8. The genetic power to discriminate EM cases is, however, comparable to other genetic risk predictors, e.g. recent estimates for breast cancer, where 10 variants have an AUC of 59.7% (23) and diabetes, where the AUC is 55–60% (24,25). Although the predictive power of the current variants is limited, as more variants are discovered this should increase, and in the absence of other good predictors for menopause, a genetic score would have value, because it could be carried out early and would be relatively inexpensive.

MATERIALS AND METHODS

Study population

We selected 2118 women with natural menopause before the age of 46 years and 1941 controls with menopause after 45 years, from the BGS. The BGS is a prospective

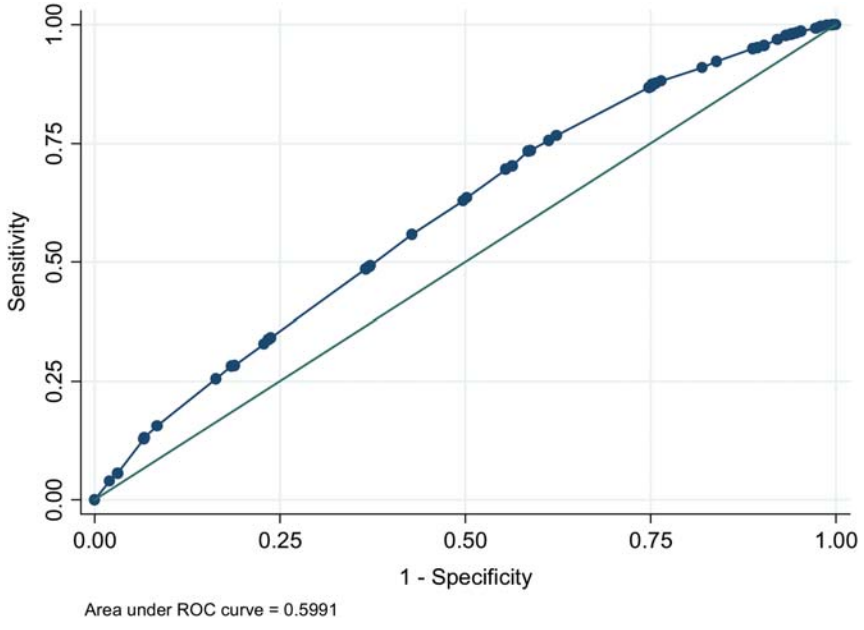

Figure 3. ROC plot modelling the discriminatory power of all four menopause SNPs, for EM (≤45). On the *y*-axis is the true-positive rate or sensitivity of the test for predicting EM and on the *x*-axis is the false-positive rate or specificity of the SNPs for predicting EM.

epidemiological cohort study launched in September 2004, the primary objective of which is to investigate the environmental, behavioural, hormonal and genetic causes of breast cancer and which is also investigating the causes of other cancers and diseases (http://www.breakthroughgenerations.org.uk/). The cohort consists of over 110 000 women from the general population of the UK aged 16 and older at the date of entry. Recruitment is through volunteers connected with the charity Breakthrough Breast Cancer, volunteers responding to publicity and via them their friends, family members and other contacts. Each participant completes a questionnaire and most provide a blood sample for analysis of genomic, hormonal and other blood factors. Participants are asked questions that include detailed menstrual histories, thus enabling identification for the present analyses of a group. Natural menopause was defined as absent menstruation for at least 6 months without known cause. Women were excluded if periods stopped because of pregnancy, breastfeeding, surgery, hormonal contraceptive use and other types of medical treatment or if there was a medical condition or illness that could have caused amenorrhoea (e.g. polycystic ovary syndrome). We selected one control for each EM case, matched for date of birth (within 12 months), ethnicity, year of questionnaire completion and source of recruitment. Women were eligible as controls if they were post-menopausal at entry to the study with a menopausal age of 46 or over (74.3%) or if they were pre-menopausal and entered the study aged 46 and over (25.7%). Menopause could be natural or surgically induced provided there was evidence they were still menstruating after age 45. There were 182 women who had surgical menopause in the control group. There were 126 cases who were <46 years old at entry to the study and controls aged 46 at entry were selected for each of these. We excluded women with a history of breast cancer as cases and controls. When multiple individuals from one pedigree were available, we included only one individual—the youngest who met the above criteria. Details of the final genotyped population are given in Table 3.

Genotyping

Following the publication of the four variants associated with age at menopause, the research groups involved formed a consortium and have pooled data and meta-analysed results. Thus, the signals have been refined, and for three of the four signals, a different SNP became the strongest association signal in the region and was selected for this analysis (ReproGen Consortium, unpublished data). The linkage disequilibrium between the tested and published SNPs was as follows: rs244715 and rs365132: $R^2 = 0.677$, rs4806660 and rs1172822: $R^2 = 0.965$, rs9379896 and rs2153157: $R^2 = 0.194$. All samples were therefore typed for the following four SNPs: rs16991615 (chr 20, position 5 896 227) rs9379896 (chr 6, position 10 994 935), rs4806660 (chr 19, position 60 516 446), rs244715 (chr 5, position 176 436 169). Genotyping was performed in-house using TaqMan PCR assays designed by Applied Biosystems. Genotype frequencies were in Hardy–Weinberg equilibrium ($P > 0.1$), call rates were >93%, with >99% concordance of 288 duplicates.

Analysis

In order to determine the effect size of the published genetic variants (16,17) on menopausal age without the bias of the 'winner's curse' (26), we analysed the association of the SNPs with normal menopausal age as a quantitative trait, in the BGS controls (menopausal age >45 years), who had a natural menopause. Thus, we excluded the 182 women who had surgical menopause >45 years and the 498 women who were not yet menopausal, leaving a cohort of 1261 women

Table 3. Individuals selected from the BGS cohort for inclusion into the study

	Controls (menopause >45 years)	EM Menopause 40–45 years inclusive	Menopause <40 years
n	1941	1858	260
Mean menopausal age	51.5 years (sd = 2.9)	43.3 years (sd = 1.6)	35.7 years (sd = 4.3)
Mean age at recruitment	58.7 (sd = 8.5)	59.0 (sd = 8.4)	53.3 (sd = 11.0)
Smoking at menopause (%)	159 (8.2%)	336 (18.1%)	64 (24.6%)
White ethnicity (%)	1921 (99%)	1841 (99%)	256 (98%)
Risk allele frequency for rs4806660	0.365	0.453	0.469
Risk allele frequency for rs16991615	0.930	0.962	0.944
Risk allele frequency for rs9379896	0.815	0.834	0.824
Risk allele frequency for rs244715	0.541	0.589	0.568

with natural menopause from the control group. Linear regression was used to determine the effect of the minor allele for each SNP on menopausal age. A combination of all four variants in one model was used to estimate the variance in menopause age explained by the SNPs, using the adjusted R^2 value.

We performed additional analysis by subdividing the EM cohort group into: (i) POF, i.e. women with menopause <40 years; (ii) women with menopause between 40 and 45 years inclusive (Table 3). We compared each case group with the controls, i.e. women who were either non-menopausal but over 45 years at entry into study or had gone through menopause aged over 45. Logistic regression was used to determine the effect of menopause-lowering alleles of each SNP on the odds of being in the case group, assuming an additive genetic model. We repeated the logistic regression excluding non-white individuals ($n = 39$). In addition, we performed conditional logistic regression to account for matched pairs of case–control samples. The ORs were very similar in the conditional regression and excluding non-whites, when compared with the unconditional regression including non-whites (Supplementary Material, Table S1); we therefore present results for unconditional logistic regression and included all individuals in subsequent analyses.

We estimated the expected OR for each of the four variants for both the EM (<46 years) and POF (<40 years) groups based on the beta estimate from the ReproGen Consortium GWAS (unpublished data) and compared with the ORs we observed in the BGS. We also calculated the expected ORs based on the quantitative trait analysis in BGS controls, but as the menopause distribution is truncated at 45 years in this cohort and the sample size is relatively small, we consider the GWAS estimate to be more accurate. We calculated the expected ORs for both the point estimate quantitative trait beta and the upper and lower 95% CI intervals, by using the 'Case–Control for threshold-selected quantitative traits' analysis on the Genetic Power Calculator website (http://pngu.mgh.harvard.edu/~purcell/gpc/). Using the proportion of variation explained by an SNP and the allele frequency, the program generates expected allele frequencies in cases and controls, where cases and controls are defined by standard deviation thresholds. We then tested for heterogeneity (using Cochrans's *Q*-test in StatsDirect) between the expected and observed ORs.

The total number of risk alleles for EM, across all four SNPs, was calculated and those with menopause below 46 years were compared with controls. Individuals were only included if they were successfully genotyped for all four SNPs ($n = 3242$, of which 48% were controls). We determined the increased likelihood of being a case depending on the number of risk alleles using logistic regression. In addition, we compared individuals at the extreme 5 and 20% of the risk allele distribution. We also compared ORs for the number of risk alleles compared with the median number of alleles in controls, i.e. 5. In a further analysis, we calculated a weighted risk score based on the effect size of each variant: the number of risk alleles at each locus was multiplied by its per-allele effect size calculated from the quantitative trait analysis in controls. The weighted score was then re-scaled to reflect the number of SNPs tested, by dividing the weighted score by the sum of the effects (1.348) and multiplying by the number of SNPs (i.e. 4).

The power of the SNPs to discriminate EM cases was calculated by determining the AUC in an ROC analysis in Stata, including all SNPs as separate linear terms in the model, against menopause status, i.e. case (≤45) versus control. The AUC measures how well the model discriminates between cases and controls, such that a perfect test gives an AUC of 1.0 and a test with no predictive power gives an AUC of 0.5.

Adjusting by smoking status, a significant predictor of menopause status (27,28), did not influence our results; therefore, all data are presented without correction for smoking status. Data were analysed in Stata v10.1 (http://www.stata.com/).

SUPPLEMENTARY MATERIAL

Supplementary Material is available at *HMG* online.

ACKNOWLEDGEMENTS

We thank the women who participated in the study, our colleagues in the BGS Team, particularly the research nurses Alison Butlin, Alison Hart, Margo Pelerin and Jill Wood who collected blood. We would like to acknowledge the contribution of the participants and investigators from the ReproGen Consortium for providing data from unpublished meta-analyses. ReproGen includes the following studies: AGES, ARIC, CHS, DeCode, EGPUT, ERF, FHS, HAPI,

InCHIANTI, NHS, NTR, QIMR, RSI,II,III, SardiNIA, SHIP, TWINS, WGHS. For a full list of investigators, please see Supplementary Material.

Conflict of Interest statement. None declared.

FUNDING

We thank Breakthrough Breast Cancer and The Institute of Cancer Research for funding the BGS and, with the Sir John Fisher foundation, Danielle Morris. The Institute of Cancer Research acknowledges NHS funding to the NIHR Biomedical Research Centre. The study was funded by the Wellcome Trust (grant number 085943). Funding to pay the Open Access publication charges for this article was provided by the Wellcome Trust.

REFERENCES

1. Hartge, P. (2009) Genetics of reproductive lifespan. *Nat. Genet.*, **41**, 637–638.
2. Carolan, M. (2003) The graying of the obstetric population: implications for the older mother. *J. Obstet. Gynecol. Neonatal Nurs.*, **32**, 19–27.
3. Steiner, A.Z. (2009) Clinical implications of ovarian reserve testing. *Obstet. Gynecol. Surv.*, **64**, 120–128.
4. Broekmans, F.J., Soules, M.R. and Fauser, B.C. (2009) Ovarian aging: mechanisms and clinical consequences. *Endocr. Rev.*, **30**, 465–493.
5. Luoto, R., Kaprio, J. and Uutela, A. (1994) Age at natural menopause and sociodemographic status in Finland. *Am. J. Epidemiol.*, **139**, 64–76.
6. Coulam, C.B., Adamson, S.C. and Annegers, J.F. (1986) Incidence of premature ovarian failure. *Obstet. Gynecol.*, **67**, 604–606.
7. Perheentupa, A. and Huhtaniemi, I. (2009) Aging of the human ovary and testis. *Mol. Cell. Endocrinol.*, **299**, 2–13.
8. de Bruin, J.P., Bovenhuis, H., van Noord, P.A., Pearson, P.L., van Arendonk, J.A., te Velde, E.R., Kuurman, W.W. and Dorland, M. (2001) The role of genetic factors in age at natural menopause. *Hum. Reprod.*, **16**, 2014–2018.
9. Lambalk, C.B., van Disseldorp, J., de Koning, C.H. and Broekmans, F.J. (2009) Testing ovarian reserve to predict age at menopause. *Maturitas*, **63**, 280–291.
10. van Rooij, I.A., Tonkelaar, I., Broekmans, F.J., Looman, C.W., Scheffer, G.J., de Jong, F.H., Themmen, A.P. and te Velde, E.R. (2004) Anti-mullerian hormone is a promising predictor for the occurrence of the menopausal transition. *Menopause*, **11**, 601–606.
11. Onland-Moret, N.C., Peeters, P.H., van Gils, C.H., Clavel-Chapelon, F., Key, T., Tjonneland, A., Trichopoulou, A., Kaaks, R., Manjer, J., Panico, S. *et al.* (2005) Age at menarche in relation to adult height: the EPIC study. *Am. J. Epidemiol.*, **162**, 623–632.
12. van den Berg, S.M. and Boomsma, D.I. (2007) The familial clustering of age at menarche in extended twin families. *Behav. Genet.*, **37**, 661–667.
13. Towne, B., Czerwinski, S.A., Demerath, E.W., Blangero, J., Roche, A.F. and Siervogel, R.M. (2005) Heritability of age at menarche in girls from the Fels Longitudinal Study. *Am. J. Phys. Anthropol.*, **128**, 210–219.
14. Snieder, H., MacGregor, A.J. and Spector, T.D. (1998) Genes control the cessation of a woman's reproductive life: a twin study of hysterectomy and age at menopause. *J. Clin. Endocrinol. Metab.*, **83**, 1875–1880.
15. Murabito, J.M., Yang, Q., Fox, C., Wilson, P.W. and Cupples, L.A. (2005) Heritability of age at natural menopause in the Framingham Heart Study. *J. Clin. Endocrinol. Metab.*, **90**, 3427–3430.
16. He, C., Kraft, P., Chen, C., Buring, J.E., Pare, G., Hankinson, S.E., Chanock, S.J., Ridker, P.M., Hunter, D.J. and Chasman, D.I. (2009) Genome-wide association studies identify loci associated with age at menarche and age at natural menopause. *Nat. Genet.*, **41**, 724–728.
17. Stolk, L., Zhai, G., van Meurs, J.B.J., Verbiest, M.M.P.J., Visser, J.A., Estrada, K., Rivadeneira, F., Williams, F.M., Cherkas, L., Deloukas, P. *et al.* (2009) Loci at chromosomes 13, 19 and 20 influence age at natural menopause. *Nat. Genet.*, **41**, 645–647.
18. Weedon, M.N. and Frayling, T.M. (2008) Reaching new heights: insights into the genetics of human stature. *Trends Genet.*, **24**, 595–603.
19. Hofker, M. and Wijmenga, C. (2009) A supersized list of obesity genes. *Nat. Genet.*, **41**, 139–140.
20. Hindorff, L.A., Sethupathy, P., Junkins, H.A., Ramos, E.M., Mehta, J.P., Collins, F.S. and Manolio, T.A. (2009) Potential etiologic and functional implications of genome-wide association loci for human diseases and traits. *Proc. Natl Acad. Sci. USA*, **106**, 9362–9367.
21. Murray, A., Webb, J., Grimley, S., Conway, G. and Jacobs, P. (1998) Studies of FRAXA and FRAXE in women with premature ovarian failure. *J. Med. Genet.*, **35**, 637–640.
22. Gleicher, N., Weghofer, A. and Barad, D.H. (2009) A pilot study of premature ovarian senescence: I. *Correlation of triple CGG repeats on the FMR1 gene to ovarian reserve parameters FSH and anti-Mullerian hormone. Fertil. Steril.*, **91**, 1700–1706.
23. Wacholder, S., Hartge, P., Prentice, R., Garcia-Closas, M., Feigelson, H.S., Diver, W.R., Thun, M.J., Cox, D.G., Hankinson, S.E., Kraft, P. *et al.* (2010) Performance of common genetic variants in breast-cancer risk models. *N. Engl. J. Med.*, **362**, 986–993.
24. Talmud, P.J., Hingorani, A.D., Cooper, J.A., Marmot, M.G., Brunner, E.J., Kumari, M., Kivimaki, M. and Humphries, S.E. (2010) Utility of genetic and non-genetic risk factors in prediction of type 2 diabetes: Whitehall II prospective cohort study. *BMJ.*, **340**, b4838.
25. Lango, H., Palmer, C.N., Morris, A.D., Zeggini, E., Hattersley, A.T., McCarthy, M.I., Frayling, T.M. and Weedon, M.N. (2008) Assessing the combined impact of 18 common genetic variants of modest effect sizes on type 2 diabetes risk. *Diabetes*, **57**, 3129–3135.
26. Kraft, P. (2008) Curses—Winner's and otherwise—in genetic epidemiology. *Epidemiology*, **19**, 649–651.
27. Kinney, A., Kline, J. and Levin, B. (2006) Alcohol, caffeine and smoking in relation to age at menopause. *Maturitas*, **54**, 27–38.
28. Santoro, N.M.D., Brockwell, S.P., Johnston, J.P., Crawford, S.L.P., Gold, E.B.P., Harlow, S.D.P., Matthews, K.A.P. and Sutton-Tyrrell, K.D. (2007) Helping midlife women predict the onset of the final menses: SWAN, the Study of Women's Health Across the Nation. *Menopause*, **14**, 415–424.