Genetic evidence that lower circulating FSH levels lengthen menstrual cycle, increase age at menopause, and impact reproductive health: a UK Biobank study

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Abstract

Study question: How does a genetic variant altering follicle stimulating hormone (FSH) levels, which we identified as associated with length of menstrual cycle, more widely impact reproductive health?

Summary answer: The T allele of the *FSHB* promoter polymorphism (rs10835638) results in longer menstrual cycles and later menopause and, while having detrimental effects on fertility, is protective against endometriosis.

What is known already: The *FSHB* promoter polymorphism (rs10835638) affects levels of *FSHB* transcription and, as a result, levels of FSH. FSH is required for normal fertility and genetic variants at the *FSHB* locus are associated with age at menopause and polycystic ovary syndrome (PCOS).

Study design, size, duration: We conducted a genetic association study using cross-sectional data from the UK Biobank.

Participants/materials, setting, methods: We included white British individuals aged 40–69 years in 2006–2010, included in the May 2015 release of genetic data from UK Biobank. We conducted a genome-wide association study (GWAS) in 9,534 individuals to identify genetic variants associated with length of menstrual cycle. We tested the FSH lowering T allele of the *FSHB* promoter polymorphism (rs10835638) for associations with 29 reproductive phenotypes in up to 63,350 individuals.

Main results and the role of chance: In the GWAS for menstrual cycle length, only variants near the *FSHB* gene reached genome-wide significance ($P<5\times10^{-8}$). The FSH-lowering T allele of the *FSHB* promoter polymorphism (rs10835638G>T; MAF 0.16) was associated with longer menstrual cycles (0.16 s.d. (approx. 1 day) per minor allele; 95% CI 0.12–0.20; $P=6\times10^{-16}$), later age at menopause (0.13 years per minor allele; 95% CI 0.04-0.22; $P=5.7\times10^{-3}$), greater female nulliparity (OR=1.06; 95% CI 1.02-1.11; $P=4.8\times10^{-3}$) and lower risk of endometriosis (OR=0.79; 95% CI 0.69–0.90; $P=4.1\times10^{-4}$). The FSH-lowering T allele was not associated more generally with other reproductive illnesses or conditions and we did not replicate associations with male infertility or PCOS.

Limitations, reasons for caution: The data included might be affected by recall bias. Women with a cycle length recorded were aged over 40 and were approaching menopause, however we did not find evidence that this affected the results. Many of the illnesses had relatively small sample sizes and so we may have been under-powered to detect an effect.

Wider implications of the findings: We found a strong novel association between a genetic variant that lowers FSH levels and longer menstrual cycles, at a locus previously robustly associated with age at menopause. The variant was also associated with nulliparity and endometriosis risk. We conclude that lifetime differences in circulating levels of FSH between individuals can influence menstrual cycle length and a range of reproductive outcomes, including menopause timing, infertility, endometriosis and PCOS.

Introduction

Follicle stimulating hormone (FSH) is a key pituitary expressed hormone, which stimulates maturation of oocytes and is a biomarker of ovarian reserve. FSH is a heterodimer comprised of a hormone specific β-chain (FSHB) associated with an α-chain shared by other members of the glycoprotein hormone family (Nagirnaja, et al., 2010). The anterior pituitary produces FSH with transcription of FSHB rate-limiting for FSH production. FSH stimulates target cells by binding to the FSH receptor (FSHR), a G-protein coupled receptor (Fan and Hendrickson, 2005), promoting follicle maturation and oestrogen production in women, and Sertoli cell proliferation and spermatogenesis in males (Nagirnaja, et al., 2010).

Rare mutations in the *FSHB* gene cause truncation of the FSHB protein and result in hypogonadism and primary amenorrhea in females (Kottler, et al., 2010, Layman, et al., 1997, Matthews and Chatterjee, 1997) and, in a male, delayed puberty with azoospermia (Phillip, et al., 1998). Mouse models suggest that FSH is required for normal fertility. Female *Fshb* knockout mice are infertile and fail to complete normal folliculogenesis while male knockouts remain fertile but have reduced sperm counts, and infertility is observed in both male and female transgenic mice overexpressing human FSH (Kumar, et al., 1999, Kumar, et al., 1997).

A polymorphism in the promotor of *FSHB* (rs10835638G>T) -211 bp upstream of the FSHB transcription start site is associated with reduced FSHB *in vitro* and in human genetic studies. *In vitro*, the T allele of the promotor polymorphism reduces expression of a luciferase reporter gene (Hoogendoorn, et al., 2003) and decreases *FSHB* transcription in gonadotrope cells as a result of reduced LHX3 homeodomain transcription factor binding (Benson, et al., 2013). The T allele of rs10835638 is associated with lower FSH levels in males and females, and is associated with infertility in males (Grigorova, et al., 2008, Grigorova, et al., 2010, La Marca, et al., 2013, Ruth, et al., 2015, Schuring, et al., 2013, Simoni and Casarini, 2014, Tuttelmann, et al., 2012). Genetic association studies have identified signals at the FSHB locus associated with age at menopause (Day, et al., 2015,

Stolk, et al., 2012), polycystic ovary syndrome (PCOS) (Hayes, et al., 2015) and levels of luteinising hormone (LH) (Hayes, et al., 2015, Ruth, et al., 2015).

In the first genome-wide association study for menstrual cycle length, we identified the *FSHB* locus as the only signal associated with this trait. Using the unique resource of the UK Biobank (Allen, et al., 2014), we show that a common genetic variant known to alter FSH levels impacts a wide range of traits important to reproductive health, including fertility, endometriosis and menstrual cycle length.

Methods

Source of data

The UK Biobank includes 503,325 people aged 40–69 years recruited in 2006–2010 from across the UK (Allen, et al., 2014). We analysed data from the May 2015 interim release of imputed genetic data from UK Biobank which contains 73,355,667 SNPs, short indels and large structural variants in 152,249 individuals (http://www.ukbiobank.ac.uk/wp-content/uploads/2014/04/imputation_documentation_May2015.pdf). UK Biobank invited 9.2 million people to participate, giving a response rate of 5.47% (Allen, et al., 2012). Participants were registered with the NHS and lived within 25 miles of one of the 22 assessment centres. Participants answered detailed questions about themselves, had measurements taken and provided samples.

Phenotypes

We derived reproductive phenotypes from the UK Biobank data (Supplementary methods). Continuous phenotypes were age at birth of first and last child (females only), age at menarche, age at natural menopause, length of menstrual cycle, number of live births and number of children

fathered. To test assumptions of linearity, we analysed the binary outcomes early menarche (lower 5% tail), early menopause (20–44 years), long menstrual cycle (>31 days), short menstrual cycle (<20 days), and multiple pregnancy loss (>1 case).

We defined two infertility-related binary phenotypes; never pregnant (females) and never fathered a child (males). We analysed medical conditions as binary outcomes comparing people reporting a condition (case) with those who did not (control). Medical conditions included dysmenorrhea, endometriosis, fibroids, irregular menstrual cycles, menopausal symptoms, menorrhagia, ovarian cysts, polycystic ovary syndrome (PCOS), uterine polyps, vaginal/uterine prolapse and breast, endometrial and ovarian cancer. As more general indicators of gynaecological health, we included the medical interventions bilateral oophorectomy or hysterectomy in our analysis. Summary statistics for the reproductive traits are presented in Tables 1 and 2.

Participants

In our analysis, we included individuals who both self-identified as white British and were confirmed as ancestrally Caucasian by UK Biobank from genetic information (n=128,266). We calculated principal components (PCs) for inclusion as covariates in our analyses using FlashPCA (Abraham and Inouye, 2014). PCs were calculated in 120,286 unrelated participants (as identified by UK Biobank) based on 95,535 independent, directly genotyped SNPs (pairwise r^2 <0.1). These SNPs had MAF≥2.5% and missing-ness<1.5% across all participants in the May 2015 interim release of genetic data, and had HWE $P>1\times10^{-6}$ within the white British participants.

GWAS of length of menstrual cycle

We conducted a genome-wide association study (GWAS) to identify genetic variants associated with length of menstrual cycle (n=9,534) using BOLT-LMM to account for relatedness and population structure (Loh, et al., 2015). We transformed length of menstrual cycle by adjusting for recruitment centre and age at recruitment prior to inverse-normalisation, and performed association testing while adjusting for genotype chip. We filtered results on imputation quality>0.4, Hardy-Weinberg equilibrium $P>1\times10^{-5}$, and minor allele frequency >0.1%, resulting in approximately 14 million variants that were tested.

Testing for associations of the FSHB promoter polymorphism with reproductive phenotypes

We tested the FSH lowering T allele of the *FSHB* promoter polymorphism (rs10835638) for associations with reproductive phenotypes (total n=120,286). SNP rs10835638 was well-imputed in the data (imputation quality 0.995; HWE P=0.16; missing rate=0.3%). All analyses were carried out in males or females as appropriate (based on self-defined sex) using Stata (v13).

For continuous phenotypes, we transformed the phenotype by adjusting for recruitment centre, age at recruitment and first five PCs prior to inverse-normalisation. We performed linear regression of imputed minor-allele dosages at SNP rs10835638 on transformed phenotype with genotyping chip as a covariate. We carried out sensitivity analysis of the effect of different transformations, e.g. inverse normalising the trait prior to calculating the residuals, however this did not materially affect our results. For age at menopause and age at menarche, we also ran analysis using the *ReproGen* definition of the phenotype (untransformed age at menopause between 40 and 60 years not adjusted for age, untransformed age at menarche) to allow comparisons with published data (Day, et al., 2015, Perry, et al., 2014, Stolk, et al., 2012). For binary outcomes, we performed logistic

regression of the phenotype on minor-allele dosages at SNP rs10835638 including the first five PCs, recruitment centre, age at recruitment and genotyping chip as covariates.

Results

A common allele in the FSHB gene, known to lower FSH levels, is associated with longer length of menstrual cycle

The FSH lowering T allele of the FSHB promoter polymorphism (rs10835638G>T; MAF 0.16) was associated with longer menstrual cycles (0.16 s.d. (approx. 1 day) per minor allele; 95% CI 0.12–0.20; $P=6\times10^{-16}$). Of the reproductive traits tested, length of menstrual cycle was the most strongly associated with rs10835638 (Figure 1). The SNP was also associated with cycle length when we dichotomised into women reporting a cycle length of less than 28 days compared to those reporting the average length of 28 days (OR=0.70; 95% CI 0.54–0.90; $P=5.1\times10^{-3}$) (Figure 1). There was no evidence for an association with longer than 28 days compared to the average (OR=1.16; 95% CI 0.92–1.47; $P=0.21$). We also conducted analysis excluding cycle length values more than 3 s.d. from the mean (n=8,792; mean 26.6; s.d. 3.33; range 9-45) and results remained consistent.

Variants in or near the *FSHB* gene were the only ones that reached genome-wide significance in the GWAS for menstrual cycle length (Figure 2). The strongest association was for rs564036233G>GA, a 1 b.p. insertion which was associated with longer cycles by 1 day (0.16 s.d.) per minor allele (95% CI 0.12–0.20; $P=1.30\times10^{-16}$). The rs564036233 variant is in strong linkage disequilibrium (LD) with the promoter polymorphism rs10835638 ($r^2=0.82$) and conditional analysis indicated that rs564036233 and rs10835638 represent the same signal.

The FSHB allele associated with longer cycle length is associated with later menopause

The FSH lowering T allele of rs10835638G>T was associated with later age at menopause in UK Biobank (0.13 years per minor allele (*ReproGen* definition); 95% CI 0.04-0.22; P=5.7×10^{-3}). There was no association between rs10835638G>T and menopause age when we dichotomised the phenotype into early menopause compared to later menopause (Table 3). The *FSHB* locus is known to be associated with timing of menopause: in *ReproGen* consortium data, the signal at this locus (rs12294104) increases age at menopause by 0.23 years (95% CI 0.16-0.29; P=1.5×10^{-11}) (Stolk, et al., 2012). Later menopause is associated with later age at last birth and rs10835638G>T was also associated with later age at last birth (0.02 s.d. (approx. 0.1 years) per T allele; 95% CI 0.00-0.04; P=4.2×10^{-2}).

Longer cycle length is not a general feature of alleles associated with later age at menopause

We next tested the role of all other genetic variants associated with age at menopause. Only one of the other 55 published age at menopause signals was nominally associated with cycle length (p>0.05): rs10734411 was associated at p=0.005 (Day, et al., 2015, Perry, et al., 2014, Stolk, et al., 2012). For the 56 published menopause SNPs there was no correlation between the published effect estimates for age at menopause and the effect estimates from the GWAS for menstrual cycle length (R=0.064, p=0.63) (Figure 3). The *FSHB* SNP was an outlier in this plot, but removing it did not substantially affect the correlation (R=-0.027; p=0.84).

The FSHB allele associated with lower FSH is also associated with an indicator of female infertility

The FSH lowering T allele of the *FSHB* promoter polymorphism (rs10835638G>T) was associated with female nulliparity, i.e. greater odds of never being pregnant (OR=1.06; CI 1.02-1.11; P=4.8×10^{-3})

(Figure 1). The FSH lowering allele was not associated with other possible indicators of female infertility (later age at first birth and fewer live births) or male infertility (number of children fathered) (p>0.05) (Table 3).

The FSHB allele associated with higher FSH is also associated with higher odds of endometriosis and surgical intervention

The more common G allele was associated with increased odds of endometriosis (OR=1.27; CI 1.11-1.45; P=4.1×10^{-4}) (Figure 1). Of the seven published GWAS variants associated with endometriosis risk (Nyholt, et al., 2012), the variant on chromosome 12 was nominally associated with cycle length, with the allele associated with an increased risk of endometriosis also associated with shorter cycles (p=0.02).

The G allele of rs10835638G>T was also associated with increased odds of having the medical interventions bilateral oophorectomy (OR=1.12; 95% CI 1.06-1.19; P=1.4×10^{-4}) and hysterectomy (OR=1.13; 95% CI 1.06-1.20; P=1.0×10^{-4}), which are used as treatments for a range of gynaecological conditions including endometriosis.

The common FSHB variant, associated with FSH levels, is not associated with reproductive traits more generally

There was no consistent evidence that the *FSHB* variant was associated with age at menarche. There was a 0.03 year increase in age at menarche (*ReproGen* definition) per T allele of rs10835638G>T (95% CI 0.01-0.05; P=1.4×10^{-2}) and the binary phenotype of early menarche was associated at p>0.05 (Table 3). None of 122 published GWAS signals for menarche (Perry, et al., 2014) were associated with length of menstrual cycle at p<0.008.

The *FSHB* promoter polymorphism (rs10835638G>T) was not associated with other reproductive illnesses or conditions at *P*<0.05 (Table 3), except for menopausal symptoms (OR=0.62; 95% CI 0.41-0.93; p=0.02) (Figure 1). No associations were found with dysmenorrhea, fibroids, irregular menstrual cycles, menorrhagia, multiple pregnancy loss, ovarian cysts, PCOS, uterine polyps or vaginal/uterine prolapse, or with female breast, ovarian or endometrial cancer.

Discussion

In the first GWAS of menstrual cycle length we found a strong association between an FSH lowering, likely functional, variant in the *FSHB* promoter and longer cycles (Benson, et al., 2013, Grigorova, et al., 2008, Grigorova, et al., 2010, Hoogendoorn, et al., 2003, La Marca, et al., 2013, Ruth, et al., 2015, Simoni and Casarini, 2014, Tuttelmann, et al., 2012). This locus has been previously robustly associated with age at menopause in the *ReproGen* GWAS of menopause timing (Day, et al., 2015, Stolk, et al., 2012) and the allele associated with longer cycle length is associated with later age at menopause. We did not observe associations for the majority of age at menopause GWAS signals with length of menstrual cycle, including the four signals with effects of over one-third of a year per allele on menopause timing, implying that the association is specific to *FSHB:* Either FSHB has independent effects on both cycle length and menopause, or changes in cycle length are causally influencing menopause timing.

Our results are consistent with the observed epidemiological relationship between longer menstrual cycles and later age at menopause (Kaczmarek, 2007, Whelan, et al., 1990). It is possible that there is a biological limit on the lifetime number of menstrual cycles, hence women with longer cycles would have later menopause. Alternatively, they may have reduced follicle recruitment per cycle, depleting their ovarian reserve more slowly. Women with longer cycles have more waves of folliculogenesis during each cycle (Baerwald, et al., 2003, Baerwald, et al., 2012) but may recruit fewer antral follicles

per wave. Oocyte loss due to ovulation is unlikely to be driving the relationship, since this contributes much less to overall oocyte depletion than atresia, there is no robust evidence that preventing ovulation by use of the combined oral contraceptive pill influences menopause timing (Ayatollahi, et al., 2003, de Vries, et al., 2001, Dorjgochoo, et al., 2008, Gold, et al., 2001, Gold, et al., 2013, Kaczmarek, 2007, OlaOlorun and Lawoyin, 2009, Palmer, et al., 2003, Pokoradi, et al., 2011, Stepaniak, et al., 2013, van Noord, et al., 1997) and both longer and shorter cycles are more likely to be anovulatory (Mihm, et al., 2011). More work is needed to understand the molecular mechanism that explains the association between cycle length and menopause timing.

The FSH-reducing allele was associated with nulliparity, indicating increased female infertility. Although we were unable to distinguish those unable to have children from those not wishing to, nulliparious women will be enriched for both female and male factor infertility. The FSH-lowering allele has previously been found to be associated with male infertility (Grigorova, et al., 2008, Grigorova, et al., 2010, Simoni and Casarini, 2014, Tuttelmann, et al., 2012), but we found no association with males who had never fathered a child suggesting a female-specific effect. Using nulliparity as a proxy for infertility is unlikely to generate a false-positive association, but may have reduced our power to detect a true association. The relationship between FSH and fertility over a woman's lifetime may differ from the age-related changes in FSH around menopause. In contrast to our genetic association between lower FSH and infertility, women nearing menopause have higher FSH concentrations, poorer ovarian reserve and decreased fertility (Mihm, et al., 2011, Waller, et al., 1998). FSH is required for follicle development and it is proposed that an FSH threshold is required to achieve ovulation (Kumar, et al., 1999, Kumar, et al., 1997). Ovulation increases with increasing FSH in transgenic mice with FSH levels that increase with age independently of follicle depletion (McTavish, et al., 2007). A high baseline level of FSH, determined by genetic variation, may promote ovulation and explain our association with parity.

The FSH-increasing allele increased the risk of endometriosis in our study. Several GWAS of endometriosis have been performed, however none have reported a signal at the 11p14.1 locus and there was no evidence that the genome-wide significant endometriosis variants were associated with cycle length in our study (Adachi, et al., 2010, Albertsen, et al., 2013, Nyholt, et al., 2012, Painter, et al., 2011, Uno, et al., 2010). Drug treatments for endometriosis aim to prevent ovulation and menstruation, and to stabilise hormone levels, since oestrogens fuel ectopic endometrial growth (Vercellini, et al., 2014). The FSH-increasing allele may similarly stimulate abnormal growth of endometrium. Endometriosis is associated with earlier menopause (Pokoradi, et al., 2011, Yasui, et al., 2011) and shorter menstrual cycles (Vercellini, et al., 2014), consistent with our findings. The FSH-increasing variant associated with increased risk of endometriosis was also associated with parity, however endometriosis can cause infertility as a result of endometriotic lesions and chronic pelvic inflammation. Therefore, the association of the *FSHB* polymorphism with infertility appears to be independent of the association with endometriosis.

We found a modest association of the FSH-lowering allele with increased age at menarche, but the published age at menarche GWAS signals were not associated with length of menstrual cycle. The closest GWAS menarche signal to *FSHB* (rs16918636) is 1.13Mb away and is not in LD (r^2=0.001) with the *FSHB* promoter polymorphism SNP (Perry, et al., 2014). Although FSH is important for normal puberty, the role of variation in baseline FSH levels on puberty timing is uncertain.

UK Biobank recruited individuals over 40 years old, and many of the women still cycling will be approaching menopause, however if the association with cycle length was being driven by peri-menopausal changes we would expect all menopause-associated variants to be associated with cycle length. We were unable to replicate an association between the FSH-lowering allele and increased odds of PCOS (Hayes, et al., 2015). However, we had only a small number of cases (n=153) limiting our power to detect this association. Other illnesses had relatively small sample sizes and we may have been similarly under-powered. We might have also under-ascertained cases, as most illnesses

will be subject to recall bias as they are self-reported and collected retrospectively, while controls might include people not reporting an illness.

Our study provides evidence that a likely functional variant in the *FSHB* promoter is strongly associated with longer menstrual cycles, and to a lesser extent with female infertility and lower risk of endometriosis. There is considerable evidence that the T allele of the *FSHB* promoter polymorphism decreases FSH levels (Benson, et al., 2013, Grigorova, et al., 2008, Grigorova, et al., 2010, Hoogendoorn, et al., 2003, La Marca, et al., 2013, Ruth, et al., 2015, Simoni and Casarini, 2014, Tuttelmann, et al., 2012), but it has also been associated with increased LH levels (Hayes, et al., 2015, Ruth, et al., 2015). While we cannot rule out that the variant may be having direct or indirect effects on other hormone levels, a change in FSH is the most likely primary mechanism. In conclusion, we suggest that lower FSH levels result in longer menstrual cycles and as a consequence later menopause and, while having detrimental effects on fertility, are protective against endometriosis.

Authors' Roles

A.M. and K.S.R. designed the study, carried out analysis and drafted the article. All authors were involved in designing and performing analysis of the UK Biobank data, revising and approving the manuscript.

Acknowledgements

We thank Dr A.M. Godwin (DRCOG, MRCGP) for identifying medications influencing length of menstrual cycle.

This research has been conducted using the UK Biobank Resource.

Funding Information

A.R.W., H.Y., and T.M.F. are supported by the European Research Council grant: 323195:GLUCOSEGENES-FP7-IDEAS-ERC. R.M.F. is a Sir Henry Dale Fellow (Wellcome Trust and Royal Society grant: 104150/Z/14/Z). R.B. is funded by the Wellcome Trust and Royal Society grant: 104150/Z/14/Z. J.T. is funded by the ERDF and a Diabetes Research and Wellness Foundation Fellowship. S.E.J. is funded by the Medical Research Council (grant: MR/M005070/1) M.A.T., M.N.W. and A.M. are supported by the Wellcome Trust Institutional Strategic Support Award (WT097835MF). (323195). The funders had no influence on study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflicts of Interest

None to declare.

Table 1. Description of cohort of unrelated individuals for continuous outcome measures.

Phenotype	**N**	**Min**	**Max**	**Mean**	**S.D.**	**Lower quartile**	**Median**	**Upper quartile**
Age at first birth (years)[1]	43,066	10	50	25.1	4.6	22	25	28
Age at last birth (years) [1]	43,008	15	50	30.0	4.8	27	30	33
Age at menarche (years) [1]	61,306	9	17	12.9	1.6	12	13	14
Age at natural menopause (years) [1]	27,996	18	65	49.9	4.5	48	50	53
Length of menstrual cycle (days) [1]	8,870	7	300	26.8	6.2	25	28	28
Number of children fathered[2]	56,508	0	28	1.8	1.2	1	2	2
Number of live births[1]	63,306	0	22	1.8	1.2	1	2	2

[1]Females only; [2]Males only.

Table 2. Number of people included in binary outcome measures.

Phenotype	Description	Cases	Controls	N
Bilateral oophorectomy[1]	Yes vs no	5,118	57,177	62,295
Dysmenorrhea[1]	Yes vs none recorded	78	63,272	63,350
Breast cancer[1]	Breast cancer recorded on cancer registry vs none recorded	2,810	60,540	63,350
Early menarche[1]	Youngest 5% age at menarche vs oldest 5%	3,050	3,050	6,100
Early menopause[1]	Natural menopause at 20-45 years vs 50-60 years	3,058	17,805	20,863
Endometrial cancer[1]	Endometrial cancer recorded on cancer registry vs none recorded	342	63,008	63,350
Endometriosis[1]	Yes vs none recorded	993	62,357	63,350
Fibroids[1]	Yes vs none recorded	1,819	61,531	63,350
Hysterectomy[1]	Yes vs no	4,753	50,932	55,685
Irregular menstrual cycles[1]	Irregular menstrual cycles vs regular cycle	2,490	10,316	12,806
Long menstrual cycle (vs average)[1]	Menstrual cycle >31 days vs 28 days	237	3,889	4,126
Menopausal symptoms[1]	Yes vs none recorded	126	63,224	63,350
Menorrhagia[1]	Yes vs none recorded	348	63,002	63,350
Multiple pregnancy loss[1]	More than one pregnancy loss vs none	4,047	33,191	37,238
Never fathered child[2]	Never fathered a child vs one or more children fathered	11,729	56,310	44,779
Never pregnant[1]	Never pregnant vs one or more pregnancies	9,247	52,966	62,213
Ovarian cancer[1]	Ovarian cancer recorded on cancer registry vs none recorded	247	63,103	63,350
Ovarian cysts[1]	Yes vs none recorded	1,015	62,335	63,350
Polycystic ovary syndrome[1]	Yes vs none recorded	153	63,197	63,350
Short menstrual cycle (vs average)[1]	Menstrual cycle <20 days vs 28 days	288	3,889	4,177
Uterine polyps[1]	Yes vs none recorded	359	62,991	63,350
Vaginal/uterine prolapse[1]	Yes vs none recorded	653	62,697	63,350

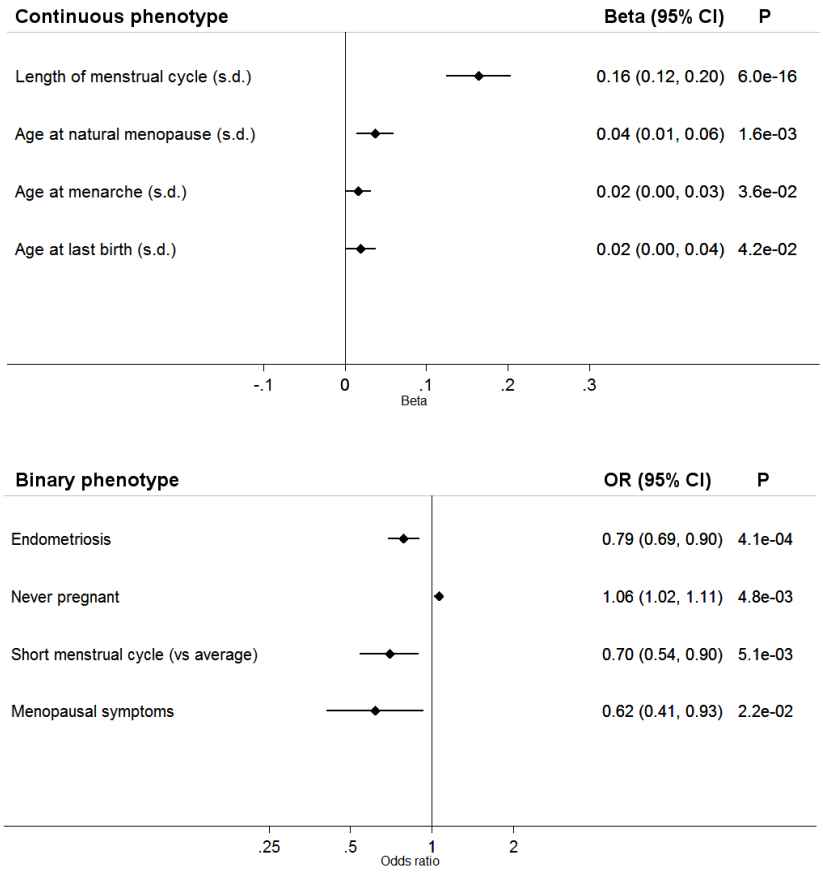
[1]Females only; [2]Males only.

Table 3. Associations with the FSH lowering T allele of rs10835638G>T. For continuous variables, effects (beta) are in standard deviations of the inverse-normally transformed variable to enable effect size comparisons.

Phenotype	**Statistic**	**Effect(95% CI)**	**SE**	**P**
Length of menstrual cycle (s.d.)	Beta	0.16(0.12,0.20)	0.02	6.0E-16
Endometriosis	OR	0.79(0.69,0.90)	0.05	4.1E-04
Age at natural menopause (s.d.)	Beta	0.04(0.01,0.06)	0.01	1.6E-03
Never pregnant	OR	1.06(1.02,1.11)	0.02	4.8E-03
Short menstrual cycle (vs average)	OR	0.70(0.54,0.90)	0.09	5.1E-03
Menopausal symptoms	OR	0.62(0.41,0.93)	0.13	2.2E-02
Age at menarche (s.d.)	Beta	0.02(0.00,0.03)	0.01	3.6E-02
Age at last birth (s.d.)	Beta	0.02(0.00,0.04)	0.01	4.2E-02
Age at first birth (s.d.)	Beta	0.02(0.00,0.03)	0.01	7.9E-02
Number of live births (s.d.)	Beta	-0.01(-0.03,0.00)	0.01	8.1E-02
Never fathered a child	OR	1.03(0.99,1.08)	0.02	1.2E-01
Early menopause	OR	0.95(0.88,1.02)	0.04	1.6E-01
Early menarche	OR	0.94(0.85,1.04)	0.05	2.1E-01
Fibroids	OR	0.94(0.86,1.03)	0.04	2.1E-01
Long menstrual cycle (vs average)	OR	1.16(0.92,1.47)	0.14	2.1E-01
Polycystic ovary syndrome	OR	1.18(0.88,1.59)	0.18	2.7E-01
Ovarian cysts	OR	0.94(0.83,1.07)	0.06	3.6E-01
Number of children fathered (s.d.)	Beta	0.01(-0.01,0.02)	0.01	4.1E-01
Menorrhagia	OR	0.92(0.74,1.13)	0.10	4.2E-01
Irregular menstrual cycles	OR	0.97(0.89,1.06)	0.04	4.6E-01
Multiple pregnancy loss	OR	0.98(0.91,1.04)	0.03	4.6E-01
Dysmenorrhea	OR	0.87(0.56,1.38)	0.20	5.6E-01
Breast cancer	OR	1.02(0.95 ,1.10)	0.04	6.4E-01
Ovarian cancer	OR	0.94(0.74,1.21)	0.12	6.4E-01
Vaginal/uterine prolapse	OR	0.97(0.83,1.13)	0.08	6.7E-01
Uterine polyps	OR	0.98(0.80,1.20)	0.10	8.6E-01
Endometrial cancer	OR	1.00(0.81,1.23)	0.11	9.7E-01

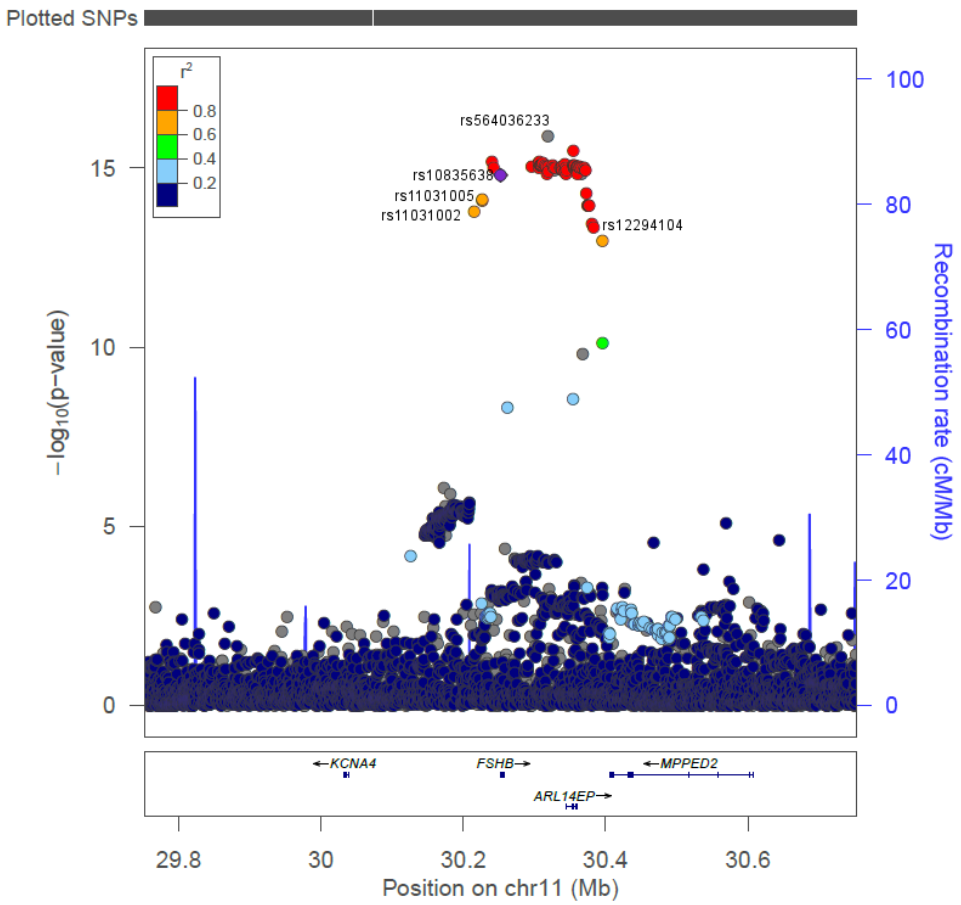
CI=confidence interval; OR=odds ratio; s.d.=standard deviations.

Figure 1. Phenotypes associated (p<0.05) with the FSH lowering allele of rs10835638G>T. For continuous variables, effects (beta) are in standard deviations of the inverse-normally transformed variable to enable effect size comparisons.



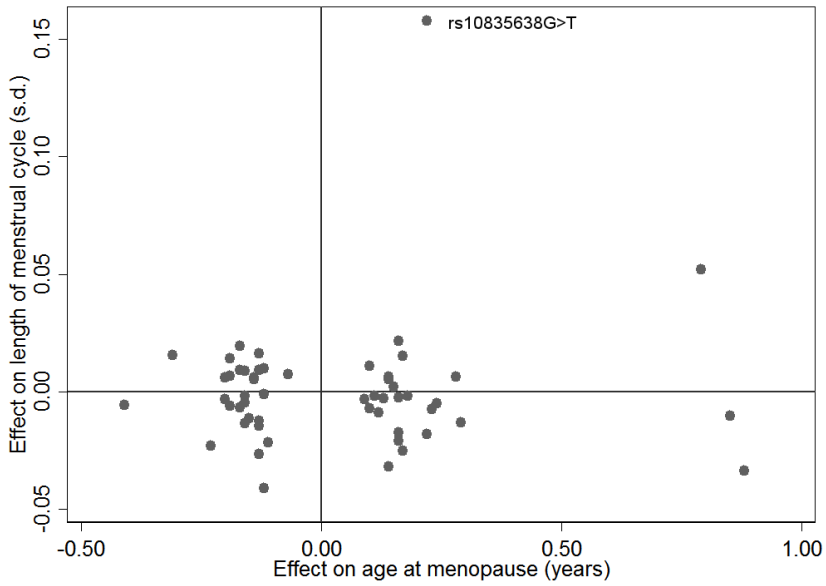
CI=confidence interval; OR=odds ratio.

Figure 2. LocusZoom plot showing variants associated with length of menstrual cycle. The most strongly associated variant for cycle length is rs564036233. Linkage disequilibrium (1000 Genomes Nov 2014 EUR) shown is with rs10835638, the FSHB promoter polymorphism. Other SNPs indicated were the variants most significantly associated with FSHB (rs11031005) and LH (rs11031002) in a GWAS of hormone levels (Ruth, et al., 2015), and with age at natural menopause (rs12294104) in a meta-analysis (Stolk, et al., 2012).



Note: Linkage disequilibrium values are not available for all SNPs since they are not included in 1000 Genomes Nov 2014 EUR.

Figure 3. Comparison of the published effect size of the 56 known age at menopause variants (Perry, et al., 2014, Stolk, et al., 2012) and their effect size in the GWAS for menstrual cycle length. There was no significant correlation between the effects on age at menopause and cycle length (R= 0.064, p=0.63). The FSHB promoter polymorphism (rs10835638G>T) is indicated.



References

Abraham G, Inouye M. Fast principal component analysis of large-scale genome-wide data. *PLoS One* 2014;9: e93766.

Adachi S, Tajima A, Quan J, Haino K, Yoshihara K, Masuzaki H, Katabuchi H, Ikuma K, Suginami H, Nishida N *et al.* Meta-analysis of genome-wide association scans for genetic susceptibility to endometriosis in Japanese population. *Journal of human genetics* 2010;55: 816-821.

Albertsen HM, Chettier R, Farrington P, Ward K. Genome-wide association study link novel loci to endometriosis. *PloS one* 2013;8: e58257.

Allen N, Sudlow C, Downey P, Peakman T, Danesh J, Elliott P, Gallacher J, Green J, Matthews P, Pell J *et al.* UK Biobank: Current status and what it means for epidemiology. *Health Policy and Technology* 2012;1: 123-126.

Allen NE, Sudlow C, Peakman T, Collins R, Biobank oboU. UK Biobank Data: Come and Get It. *Science Translational Medicine* 2014;6: 224ed224.

Ayatollahi SM, Ghaem H, Ayatollahi SA. Menstrual-reproductive factors and age at natural menopause in Iran. *International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics* 2003;80: 311-313.

Baerwald AR, Adams GP, Pierson RA. A new model for ovarian follicular development during the human menstrual cycle. *Fertil Steril* 2003;80: 116-122.

Baerwald AR, Adams GP, Pierson RA. Ovarian antral folliculogenesis during the human menstrual cycle: a review. *Hum Reprod Update* 2012;18: 73-91.

Benson CA, Kurz TL, Thackray VG. A human FSHB promoter SNP associated with low FSH levels in men impairs LHX3 binding and basal FSHB transcription. *Endocrinology* 2013;154: 3016-3021.

Day FR, Ruth KS, Thompson DJ, Lunetta KL, Pervjakova N, Chasman DI, Stolk L, Finucane HK, Sulem P, Bulik-Sullivan B. Large-scale genomic analyses link reproductive aging to hypothalamic signaling, breast cancer susceptibility and BRCA1-mediated DNA repair. *Nat Genet* 2015;In press.

de Vries E, den Tonkelaar I, van Noord PA, van der Schouw YT, te Velde ER, Peeters PH. Oral contraceptive use in relation to age at menopause in the DOM cohort. *Hum Reprod* 2001;16: 1657-1662.

Dorjgochoo T, Kallianpur A, Gao YT, Cai H, Yang G, Li H, Zheng W, Shu XO. Dietary and lifestyle predictors of age at natural menopause and reproductive span in the Shanghai Women's Health Study. *Menopause* 2008;15: 924-933.

Fan QR, Hendrickson WA. Structure of human follicle-stimulating hormone in complex with its receptor. *Nature* 2005;433: 269-277.

Gold E, Bromberger J, Crawford S, Samuels S, Greendale G, Harlow S, Skurnick J. Factors associated with age at natural menopause in a multiethnic sample of midlife women. *American Journal of Epidemiology* 2001;153: 865 - 874.

Gold EB, Crawford SL, Avis NE, Crandall CJ, Matthews KA, Waetjen LE, Lee JS, Thurston R, Vuga M, Harlow SD. Factors related to age at natural menopause: longitudinal analyses from SWAN. *Am J Epidemiol* 2013;178: 70-83.

Grigorova M, Punab M, Ausmees K, Laan M. FSHB promoter polymorphism within evolutionary conserved element is associated with serum FSH level in men. *Human Reproduction* 2008;23: 2160-2166.

Grigorova M, Punab M, Poolamets O, Kelgo P, Ausmees K, Korrovits P, Vihljajev V, Laan M. Increased Prevalance of the -211 T allele of follicle stimulating hormone (FSH) beta subunit promoter polymorphism and lower serum FSH in infertile men. *J Clin Endocrinol Metab* 2010;95: 100-108.

Hayes MG, Urbanek M, Ehrmann DA, Armstrong LL, Lee JY, Sisk R, Karaderi T, Barber TM, McCarthy MI, Franks S *et al.* Genome-wide association of polycystic ovary syndrome implicates alterations in gonadotropin secretion in European ancestry populations. *Nat Commun* 2015;6.

Hoogendoorn B, Coleman SL, Guy CA, Smith K, Bowen T, Buckland PR, O'Donovan MC. Functional analysis of human promoter polymorphisms. *Hum Mol Genet* 2003;12: 2249-2254.

Kaczmarek M. The timing of natural menopause in Poland and associated factors. *Maturitas* 2007;57: 139-153.

Kottler ML, Chou YY, Chabre O, Richard N, Polge C, Brailly-Tabard S, Chanson P, Guiochon-Mantel A, Huhtaniemi I, Young J. A new FSHbeta mutation in a 29-year-old woman with primary amenorrhea and isolated FSH deficiency: functional characterization and ovarian response to human recombinant FSH. *European journal of endocrinology / European Federation of Endocrine Societies* 2010;162: 633-641.

Kumar TR, Palapattu G, Wang P, Woodruff TK, Boime I, Byrne MC, Matzuk MM. Transgenic models to study gonadotropin function: the role of follicle-stimulating hormone in gonadal growth and tumorigenesis. *Molecular endocrinology (Baltimore, Md)* 1999;13: 851-865.

Kumar TR, Wang Y, Lu N, Matzuk MM. Follicle stimulating hormone is required for ovarian follicle maturation but not male fertility. *Nat Genet* 1997;15: 201-204.

La Marca A, Papaleo E, Alviggi C, Ruvolo G, De Placido G, Candiani M, Cittadini E, De Michele F, Moriondo V, Catellani V *et al.* The combination of genetic variants of the FSHB and FSHR genes affects serum FSH in women of reproductive age. *Hum Reprod* 2013;28: 1369-1374.

Layman LC, Lee EJ, Peak DB, Namnoum AB, Vu KV, van Lingen BL, Gray MR, McDonough PG, Reindollar RH, Jameson JL. Delayed puberty and hypogonadism caused by mutations in the follicle-stimulating hormone beta-subunit gene. *N Engl J Med* 1997;337: 607-611.

Loh PR, Tucker G, Bulik-Sullivan BK, Vilhjalmsson BJ, Finucane HK, Salem RM, Chasman DI, Ridker PM, Neale BM, Berger B *et al.* Efficient Bayesian mixed-model analysis increases association power in large cohorts. *Nat Genet* 2015;47: 284-290.

Matthews C, Chatterjee VK. Isolated deficiency of follicle-stimulating hormone re-revisited. *N Engl J Med* 1997;337: 642.

McTavish KJ, Jimenez M, Walters KA, Spaliviero J, Groome NP, Themmen AP, Visser JA, Handelsman DJ, Allan CM. Rising follicle-stimulating hormone levels with age accelerate female reproductive failure. *Endocrinology* 2007;148: 4432-4439.

Mihm M, Gangooly S, Muttukrishna S. The normal menstrual cycle in women. *Animal reproduction science* 2011;124: 229-236.

Nagirnaja L, Rull K, Uuskula L, Hallast P, Grigorova M, Laan M. Genomics and genetics of gonadotropin beta-subunit genes: Unique FSHB and duplicated LHB/CGB loci. *Mol Cell Endocrinol* 2010;329: 4-16.

Nyholt DR, Low S-K, Anderson CA, Painter JN, Uno S, Morris AP, MacGregor S, Gordon SD, Henders AK, Martin NG *et al.* Genome-wide association meta-analysis identifies new endometriosis risk loci. *Nat Genet* 2012;44: 1355-1359.

OlaOlorun F, Lawoyin T. Age at menopause and factors associated with attainment of menopause in an urban community in Ibadan, Nigeria. *Climacteric : the journal of the International Menopause Society* 2009;12: 352-363.

Painter JN, Anderson CA, Nyholt DR, Macgregor S, Lin J, Lee SH, Lambert A, Zhao ZZ, Roseman F, Guo Q *et al.* Genome-wide association study identifies a locus at 7p15.2 associated with endometriosis. *Nat Genet* 2011;43: 51-54.

Palmer JR, Rosenberg L, Wise LA, Horton NJ, Adams-Campbell LL. Onset of natural menopause in African American women. *American journal of public health* 2003;93: 299-306.

Perry JR, Day F, Elks CE, Sulem P, Thompson DJ, Ferreira T, He C, Chasman DI, Esko T, Thorleifsson G *et al.* Parent-of-origin-specific allelic associations among 106 genomic loci for age at menarche. *Nature* 2014;514: 92-97.

Perry JR, Hsu YH, Chasman DI, Johnson AD, Elks C, Albrecht E, Andrulis IL, Beesley J, Berenson GS, Bergmann S *et al.* DNA mismatch repair gene MSH6 implicated in determining age at natural menopause. *Hum Mol Genet* 2014;23: 2490-2497.

Phillip M, Arbelle JE, Segev Y, Parvari R. Male hypogonadism due to a mutation in the gene for the beta-subunit of follicle-stimulating hormone. *N Engl J Med* 1998;338: 1729-1732.

Pokoradi AJ, Iversen L, Hannaford PC. Factors associated with age of onset and type of menopause in a cohort of UK women. *Am J Obstet Gynecol* 2011;205: 34.e31-13.

Ruth KS, Campbell PJ, Chew S, Lim EM, Hadlow N, Stuckey BG, Brown SJ, Feenstra B. Genome-wide association study with 1000 genomes imputation identifies signals for nine sex hormone-related phenotypes. 2015.

Schuring AN, Busch AS, Bogdanova N, Gromoll J, Tuttelmann F. Effects of the FSH-beta-subunit promoter polymorphism -211G->T on the hypothalamic-pituitary-ovarian axis in normally cycling women indicate a gender-specific regulation of gonadotropin secretion. *J Clin Endocrinol Metab* 2013;98: E82-86.

Simoni M, Casarini L. Mechanisms in endocrinology: Genetics of FSH action: a 2014-and-beyond view. *European journal of endocrinology / European Federation of Endocrine Societies* 2014;170: R91-107.

Stepaniak U, Szafraniec K, Kubinova R, Malyutina S, Peasey A, Pikhart H, Pajak A, Bobak M. Age at natural menopause in three central and eastern European urban populations: the HAPIEE study. *Maturitas* 2013;75: 87-93.

Stolk L, Perry JR, Chasman DI, He C, Mangino M, Sulem P, Barbalic M, Broer L, Byrne EM, Ernst F *et al.* Meta-analyses identify 13 loci associated with age at menopause and highlight DNA repair and immune pathways. *Nat Genet* 2012;44: 260-268.

Tuttelmann F, Laan M, Grigorova M, Punab M, Sober S, Gromoll J. Combined effects of the variants FSHB -211G>T and FSHR 2039A>G on male reproductive parameters. *J Clin Endocrinol Metab* 2012;97: 3639-3647.

Uno S, Zembutsu H, Hirasawa A, Takahashi A, Kubo M, Akahane T, Aoki D, Kamatani N, Hirata K, Nakamura Y. A genome-wide association study identifies genetic variants in the CDKN2BAS locus associated with endometriosis in Japanese. *Nat Genet* 2010;42: 707-710.

van Noord PA, Dubas JS, Dorland M, Boersma H, te Velde E. Age at natural menopause in a population-based screening cohort: the role of menarche, fecundity, and lifestyle factors. *Fertil Steril* 1997;68: 95-102.

Vercellini P, Vigano P, Somigliana E, Fedele L. Endometriosis: pathogenesis and treatment. *Nat Rev Endocrinol* 2014;10: 261-275.

Waller K, Swan SH, Windham GC, Fenster L, Elkin EP, Lasley BL. Use of urine biomarkers to evaluate menstrual function in healthy premenopausal women. *Am J Epidemiol* 1998;147: 1071-1080.

Whelan EA, Sandler DP, McConnaughey DR, Weinberg CR. Menstrual and reproductive characteristics and age at natural menopause. *Am J Epidemiol* 1990;131: 625-632.

Yasui T, Hayashi K, Mizunuma H, Kubota T, Aso T, Matsumura Y, Lee JS, Suzuki S. Association of endometriosis-related infertility with age at menopause. *Maturitas* 2011;69: 279-283.