Supplementary Note

“Genome-wide analysis identifies 12 loci influencing human reproductive behaviour”

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1. **HUMAN REPRODUCTIVE BEHAVIOUR MOTIVATION AND PHENOTYPE DEFINITION**

1.1 **Phenotype Motivation**

Human reproductive behaviour – measured by age at first birth (AFB) and number of children ever born (NEB) – is a core topic of research across the medical, social and biological sciences.\(^1\) Two central indicators are the tempo of childbearing of age at first birth (AFB) and the quantum or number of children ever born (NEB). NEB is also often referred to in biological research as life-time reproductive success,\(^2\) number of offspring\(^3\) or as ‘fitness’ in evolutionary studies, which is the function of the number of children of a person in relation to the number of children of peers of the same birth cohort.\(^4,5\) Due to improvements in hygiene and the reduction in prenatal, infant and child mortality in industrialized societies, NEB has emerged as the gold standard to measure lifetime reproductive success indicating biological fitness.\(^5\)

AFB and NEB are complex phenotypes related not only to biological fecundity, but also behavioural in that they are driven by the reproductive choice of individuals and their partners, and shaped by the social, cultural, economic and historical environment. Genetic factors influence the first two factors of biological fecundity and choice, with the social and historical environment filtering the types of behaviour that are possible (e.g., via contraceptive legislation, social norms).

Although interrelated, AFB and NEB, but also childlessness, are distinct phenotypes. Late AFB, low NEB or remaining childless is not only due to ‘involuntary’ infertility or factors outside of the individual’s control (e.g., inability to find a partner), but also ‘voluntary’ choices to remain ‘childfree’.\(^6\) In the past four decades there has been a rapid postponement by around 4-5 years in the AFB to advanced ages in many industrialized societies\(^7\) and a growth in childlessness, with around 20% of women born from 1965-69 in Southern and Western European countries having no children.\(^8\) The biological ability to conceive a child starts to steeply decline for some women as of age 25, with almost 50% of women sterile by the age of 40.\(^9\) Birth postponement and a lower number of children has been largely
attributed to social, economic and cultural environmental factors (i.e., individual and partner characteristics, socioeconomic status). Not surprisingly, this delay has led to an unprecedented growth in infertility (i.e., involuntary childlessness), which impacts between 10-15% of couples in Western countries, with men and women affected equally. An estimated 48 million couples worldwide are infertile, with a large part of subfertility, particularly in men, remaining unexplained. Although therapeutic options for infertility in the form of Assisted Reproductive Technology (ART) are available, they are highly ineffective at later ages and older mothers have considerably more problems during gestation and delivery, also associated with low birth weight and preterm delivery. Recent studies have also linked advanced maternal age to a higher risk of schizophrenia in offspring.

Childless individuals (and those with a low NEB) are a heterogeneous group consisting of the involuntary childless (e.g., infertility, sterility) and voluntarily childless or ‘childfree’ (e.g., out of choice). Although primarily related to biological fecundity, involuntary childlessness may also be due to circumstantial socio-environmental reasons outside of the individual’s control, including a lack of ability to find a stable partner, divorce and lack of housing, employment or material resources to start a family. Those who are voluntarily childless are generally considered to have made an active choice or to be endowed with an underlying preference or personality traits that pull individuals towards or away from parenthood. It is difficult to disentangle the voluntary from the involuntary, however, since fertility intentions can be adjusted in relation to circumstances and these modifications are age-related.

A better understanding of the genetic architecture of human reproductive behaviour and its relation to the environment would enable the discovery of predictors of infertility, which would in turn greatly improve family planning but also reduce costly and invasive ART treatments. Examination of AFB and NEB may also produce a better understanding of the biology of human reproduction, which in turn may give insight into fundamental biological mechanisms and could have ramifications for the study of many health outcomes, especially the etiology of diseases related to the reproductive tract. Furthermore, it is important to understand whether and which proportion of these traits are driven by genetic, behavioural and environmental factors. Relatively little is known about the relationship between
indicators of women’s reproductive lifespan (menarche, menopause) and reproductive success. A smaller and recent study has produced some evidence of the link between age at first sexual intercourse (AFS) with AFB and NEB.\textsuperscript{23} The focus of this study, however, was on puberty and development and how the timing of puberty was linked with AFS.

By systematically investigating the relationship with genetic variants for a multitude of phenotypes related to human reproduction we can establish to what extent diseases related to the reproductive tract play a role in human reproduction and vice versa, and begin to chart the complex biological and related mechanisms that drive human reproduction. It is therefore crucial to not only examine genetic determinants of more biologically proximate phenotypes (e.g. age at menarche, endometriosis, PCOS) but also human reproductive behaviour and success. AFB and NEB represent more accurate and concrete measures of observed reproductive success in comparison with proxies which capture the reproductive life span (e.g., age at menarche, menopause) or infertility measures (e.g., endometriosis, PCOS).

To our knowledge, the current study is the largest meta-GWAS effort on human reproductive behaviour, which we launched in early 2012. A recently published smaller and related study of cohorts also involved in our study focused on age at first sex (AFS), also linking it to AFB and NEB (among other traits).\textsuperscript{23} The AFS study examined how individual variation in pubertal timing and personality characteristics related to high risk-taking and low neuroticism related to reproductive activity and success with AFS measures integrated into our examination of genetic correlations (see Supplementary Note, section 7).

Several studies have shown promising results for fertility-related outcomes related to both infertility and the reproductive life span. Previous research has uncovered a genetic component to reproduction with over 70 genome-wide association studies (GWAS) published for 32 traits and diseases associated with reproduction (for a review see ref.\textsuperscript{24}). This includes identification of genes such as those related to age at menarche\textsuperscript{25,26,27}, menopause\textsuperscript{28–32}, endometriosis\textsuperscript{33–36} and polycystic ovary syndrome\textsuperscript{37}. This study is the first step towards understanding the pathways between genes and the complex relationship between reproduction and other phenotypes and the environment.
1.2 Evolutionary causes of genetic variance in fertility

Given the diminishing child mortality rate in contemporary societies, evolutionary biologists have used NEB as a proxy for fitness.\textsuperscript{2,5,38} Additive genetic variance in fitness implies natural selection within populations: alleles that lead to higher reproductive success will have a higher frequency in future generations.\textsuperscript{39} Researchers have until now arguably given less attention to NEB than it deserves,\textsuperscript{1} perhaps due to a frequent erroneous interpretation of Fisher’s\textsuperscript{40} Fundamental Theorem of Natural Selection. The theorem states that the increase in population mean fitness ascribable to changing allele frequencies is equal to the additive genetic variance in fitness. It has often been misinterpreted, however, to mean that the additive genetic variance in fitness itself should always be close to zero. A close reading of the text shows that Fisher actually argued that fitness is moderately heritable in human populations. The misinterpretation of Fisher’s theorem is likely repeated so often due to its intuitive appeal. Naively, it may seem that genes that reduce fitness should have been less frequently passed on, leading to the elimination of genetic variability in traits such as fertility.\textsuperscript{40,41} Nevertheless, we find that fitness traits such as NEB and AFB have significant narrow-sense heritabilities – yet these traits are still not as heritable as morphological traits such as height.\textsuperscript{38,42–44} Several reasons have been put forward to explain the persistent genetic variance in fertility. One argument is that new mutations suffice to restore any genetic variance lost to selection.\textsuperscript{45} For the current study design, additional aspects to consider are sexual antagonistic genetic effects, non-additive genetic effects, environment and gene-environment interaction. As discussed in more detail in the Supplementary Note (Section 5), the current GWAS was conducted separately for both sexes, with a detailed examination explored within that section.

1.3 Additive genetic variation in fertility

Several twin and family studies provide evidence for a genetic component underlying both the tempo (AFB) and quantum (NEB) of human fertility.\textsuperscript{1,3} Heritability – the proportion of the variance in a trait explained by genetic variance – is typically assessed by a comparison of the phenotypic correlations of family members of different genetic relatedness (for example genetically identical or monozygotic and genetically fraternal dizygotic twins). The genetic component is reflected in the extent to which genetically identical twins are more similar in their fertility behaviour. As summarized in Fig. S1.1, heritability estimates for AFB
(for women) are around 0.25 and for NEB ranging from 0.15 to 0.45.\textsuperscript{1} A recent meta-analysis of all twin studies conducted until 2012\textsuperscript{44} shows average heritability of 0.45 (SE = 0.027, N = 50,265) among 64 reproductive disease traits of women and of 0.36 (SE= 0.054, N = 9,376) among 25 reproductive disease traits of men. These mainly pertain to diseases of the genitourinary system, endocrine, nutritional and metabolic diseases, and only few directly pertain to pregnancy, childbirth and the puerperium.

With the advent of molecular genetic data and complementary analytical tools,\textsuperscript{46} it has become feasible to go beyond twin models to produce heritability estimates to apply the same logic to unrelated individuals based on the genetic relatedness matrix across all individuals estimated from common SNPs from the whole genome.\textsuperscript{47,48} A recent study combined data from the Lifelines Cohort Study and the TwinsUK to estimate this so called SNP-based heritability as the lower bound of narrow sense heritability.\textsuperscript{38} Results show that 10% of the variance in NEB and 15% of the variance in AFB are associated with common additive genetic variance. Given that SNP-based heritability is estimated from the same genomic information as utilized in GWAS studies, these results suggest that we should be able to find genetic variants associated with human fertility when conducting GWAS meta-analyses of sufficient sample size.

1.4 Dominant genetic variation in fertility

GWAS typically assume additive genetic effects. Dominant models, however, are in principle also applicable.\textsuperscript{49} Dominant genetic effects and overdominance (heterozygote advantage) are mechanisms which potentially maintain non-additive genetic variation in fertility and other fitness related outcomes.\textsuperscript{40} Dominant genetic effects result if the conditional phenotypic mean of the heterozygote is not exactly intermediate between those of the homozygotes. Overdominance refers to the special case of the heterozygote possessing a fitness advantage over both homozygotes. At the equilibrium under selection, overdominance leads to an absence of additive genetic variance. Any deviation from strict additivity within a locus, however, should lead to dominance variance that is in principle detectable.\textsuperscript{45}
Previous studies approaching the genetic architecture of human fertility almost exclusively relied on twin designs. Dominant genetic effects are detectable in twin studies if the correlation in a trait among identical twins exceeds twice the correlation of fraternal twins. Correlations amongst family members, however, can by inflated by shared environmental factors and therefore hide dominant effects – a potential reason why previous twin studies did not find effects.49

Recently, Zhu and colleagues49 developed a method to estimate dominant genetic effects based on the genetic relatedness of unrelated individuals. This is a complementary approach to the established GREML analysis, which estimates additive genetic effects on traits. In the article of Zhu and colleagues, they quantify dominant relative to additive variance components for 79 quantitative traits and find little evidence for dominant effects. We applied the GREML model to investigate additive genetic effects on NEB and AFB in combined cohorts of women from the TwinsUK and the Lifelines study in the Netherlands.38 On a slightly larger sample – with a relaxed relatedness cut-off of 0.0550 and the exclusion of women younger than 45 for AFB – we replicated previous findings with a SNP-heritability of 0.09 for NEB and 0.17 for AFB. However, we find no evidence for dominant genetic effects δ²_{SNP} for either NEB (0.1x10⁻⁶, SE 0.07, P=0.45) nor AFB (0.02, SE=0.08, P=0.43, see Supplementary Table 28 for results). We can therefore conclude that due to this lack of evidence of dominant genetic effects, it is not problematic that we have excluded dominant models in our GWAS.

1.5 Environmental variations in fertility

Social scientists, such as demographers and sociologists, have attributed later ages of first birth, lower NEB and growing levels of childlessness in many industrialized societies almost exclusively to socio-environmental factors.7,10 First, the introduction of efficient and reliable contraceptives in the early 1960s revolutionized human reproductive behaviour.7 The diffusion of the pill in the late 1960s in the United States resulted in an almost immediate postponement in the age of first marriage for college-educated women.51 Contraception allowed women and couples to avoid pregnancy and delay entry into parenthood. Contraceptives were generally widely introduced across Western and Northern Europe,
Australia and North America in the late 1960s, which is where the majority of cohorts are located in the current study.

Second, there is a well-documented association between female education and later AFB. Early research demonstrated a strong inverse relationship between education and fertility, with women’s increased participation in higher college and University degrees resulting in a significant shift to later AFB. A central argument driving childbearing delay was the difficulty to balance student and mother (parent) roles, but also women’s opportunity costs in terms of wages and career progression that they forego when having children early. A third factor, interdependent with educational level, is women’s labour force participation and attachment. Research has demonstrated an incompatibility of early AFB and high NEB with paid labour force participation, largely due to work-family conflict and the high motherhood ‘wage penalty’. In fact, the postponement of AFB results in substantial increases in earnings, particularly for higher-educated women. It is estimated that there is a 7% motherhood wage penalty per child, with a year delay in motherhood increasing career earnings by 9%. A fourth factor is the Second Demographic Transition, which largely refers to cultural and ideational changes surrounding the preferences for and role of children, which is coupled with a shift to more individualistic desires for personal development. Since infant mortality rates have fallen sharply in modern societies, extra births are not required for insurance against death and children no longer provide the economic support and labour to support parents that they once did, which dramatically changes preferences and the need to have children. Research has also demonstrated that multiple national institutional factors are related to the delay of AFB and the decrease in NEB. This includes changes in the educational systems, labour market regulations, gender equity, but also economic uncertainty, the housing market, influence by friendship networks, family networks and social capital, and changes in partnering and mating practices. The empirical relationship of these factors – namely birth cohort and educational level – with genetic risk scores of AFB and NEB is elaborated upon in section 10.
1.6  Phenotype definition

The current study measures human reproductive choice by the two phenotypes of: age at first birth (AFB) and number of children ever born (NEB). AFB is the self-reported age when subjects had their first child. In most cohorts this was asked directly (e.g. “How old were you when you had your first child?”). Alternatively, it could also be calculated based on several survey questions (such as the date of birth of the subject and date of birth of the first child). Supplementary Table 2 describes in detail the exact question asked for each cohort and if applicable, whether and how it varies in the way it was asked to men and women. Often these questions were part of a medical questionnaire about women’s reproductive health, so for a large number of cohorts, only women were asked. For this reason, the sample size for AFB for women is considerably larger than for men. Note that only people who have had at least one child (parous) are eligible to be included for the analysis of this phenotype.

Number of children ever born (NEB) was the self-reported number of children. This phenotype was either asked directly (e.g. “How many children do you have?” or “How many natural (biological) children have you ever had, that is, all children who were born alive?”, or “How many children have you had - not counting any step, adopted, or foster children, or any who were stillborn?”) or it was calculated based on several survey questions (such as pregnancy histories and outcomes, number of deliveries). In most cases it was possible to distinguish between biological (live born or stillborn) and adopted or step-children. When it was possible to distinguish between cases, we used the number of live born biological children. We included cases for NEB if they finished their reproductive career (aged at least 45 for women and 55 for men at time of study) and were thus unlikely to have future biological children.

1.7  Instructions for contributing cohorts

The instructions given to cohorts who agreed to participate in our study are described in detail in the original Analysis Plan that was posted on the Open Science Framework preregistration site, described in detail in Supplementary Note Section 2.1 and uploaded December 9, 2013 at: https://osf.io/53tea/. AFB was advised to be treated as a continuous measure. When possible, we asked analysts to use the more direct question: How old were
you when you had your first child? Another variant is: What is the date of birth of your first child? In the case of the latter, we advised them to impute this variable to get the AFB by subtracting the date of birth of the first child from the date of birth of the subject.

Analysts were then asked to normalize the raw measure of the age at first birth for sex/birth cohort specific means and standard deviations. In other words, we asked them to compute a mean and standard deviation separately for men and women by birth cohort category (generally ten-year intervals) and then subtract the mean value for that group from the respondent’s value. They should then divide the result by the standard deviation. This was the final AFB variable measured in sex/cohort specific Z-score that is our regressand.

Analysts were asked to include birth year of the respondent (represented by birth year – 1900), its square and cubic to control for non-linear birth cohort effects. Combined analyses that included both men and women also needed to include interactions of birth year and its polynomials with sex. Some cohorts only used birth year and not its polynomials because of multi-collinearity issues/convergence of the GWA analysis.
2. PRIMARY GWAS OF HUMAN FERTILITY

2.1 Overview of human fertility analyses

The genome-wide association study (GWAS) of human fertility is based on the summary statistics that were uploaded to a central server by cohort-level analysts. As outlined in more detail in Section 1 of the Supplementary Note, our analysis includes the two phenotypes of age at first birth (AFB) and number of children ever born (NEB), with analysts producing results for women, men and combined analyses of both sexes, also including birth cohort as a covariate. The summary statistics were then subsequently quality-controlled and meta-analyzed by two separate independent centers at the University of Oxford and Erasmus University Rotterdam.

We follow the QC protocol of the GIANT consortium’s recent study of human height and employed the software packages QCGWAS and EasyQC, which allowed us to harmonize the files and identify possible sources of errors in association results. This procedure entailed that diagnostic graphs and statistics were generated for each set of GWAS results (i.e., for each file). In the case where apparent errors could not be amended by stringent QC, cohorts were excluded from the meta-analysis (see the bottom of Supplementary Table 1 for a list of excluded cohorts).

The lead PI of each cohort confirmed that the results in this study were based on analyses that had been approved by the local Research Ethics Committee and/or the relevant Institutional Review Board. All participants fell under the written informed consent protocol of each participating study. The entire project was also approved by the local Research Ethics Committee of the PI.

We first circulated three documents to interested cohorts at the end of April 2012, which included: (a) Rationale for a GWAS of Fertility Behaviour, (b) GWAS Fertility Behaviour Analysis Plan; and, (c) Collaboration Agreement for Fertility GWAS Meta-analyses. This was after a meeting and approval from the REPROGEN working group of the CHARGE consortium on Dec. 9, 2011 that we were not competing with existing efforts. Preliminary
results were presented at various CHARGE meetings between the years of 2012-2015. This study was initially set up as a two-stage GWAS with a large discovery and smaller replication phase. Due to an increasing influx of new data, we opened the participation to cohorts that had genome-wide data, but also to cohorts that had Metabochip data. We also included a list of 15 independent SNPs with $P<10^{-6}$ for cohorts that did not have genome-wide data available but could perform de-novo replication on a limited number of SNPs. Agreements also came through at a later stage from RPGEH (Kaiser Permanente Research Program on Genes, Environment, and Health), $N$(AFB women)=31,898, $N$(NEB women)=39,576, deCODE ($N$(AFB pooled)=60,602, $N$(NEB pooled)=65,228), and UK Biobank ($N$(AFB women)=40,082, $N$(NEB pooled)=88,094). Given the resulting well-powered total sample size of $N\approx 250k$ for AFB and $N\approx 340k$ for NEB we chose to merge the discovery and replication cohorts into a single large discovery phase, as is done in other recent well-powered GWAS efforts.\textsuperscript{72,75,76} We also decided to include in the meta-analysis only cohorts with genome-wide data, leaving the remaining cohorts that performed de-novo replication for follow-up analysis.

### 2.2 Participating Cohorts

A total of 62 cohorts contributed to this study. Cohorts with acceptable measures of AFB and/or NEB were eligible to participate. Some measured one or both of the phenotypes, and there was also variation by whether the question was asked to women and/or also men. Supplementary Table 1 provides an overview of the study-specific details of all analyses conducted for the traits of interest. Cohorts of unrelated individuals uploaded separate results for men and women. In addition to sex-specific association results, family-based cohorts uploaded pooled results. As described in the Supplementary Note (Section 1), particularly AFB is asked less frequently to men. The total number of association-result files per trait is as follows. We have 28 files for AFB men, 57 for AFB women, 72 for AFB pooled, 50 for NEB men, 67 for NEB women, and 102 for NEB pooled.

As Supplementary Table 1 shows, most cohorts were included in the meta-analysis (i.e., 62 cohorts are included, constituting 26 files for AFB men, 50 for AFB women, 64 for AFB pooled, 47 for NEB men, 60 for NEB women, and 91 for NEB pooled) and some only in the
follow-up analyses (9 cohorts, constituting 2 files for AFB men, 5 for AFB women, 6 for AFB pooled, 3 for NEB men, 5 for NEB women, and 9 for NEB pooled). We had to exclude the association results of two cohorts – ABCFS (AFB women, N=410, NEB women, N=410) and Longenity (AFB women, N=285; NEB women, N=352) – from the meta- and follow-up analyses due to unresolvable issues with the cohort’s association results that came up in the quality control procedures. For more details regarding the reasons for exclusion, see SI Section 2.6.

2.3 Genotyping and Imputation

Supplementary Table 1 gives an overview of the study-specific details on pre-imputation quality control filters applied to the genotype data, subject-level exclusion criteria, imputation software used, and the reference sample for imputation. Due to the fact that we started our study in 2012 before 1000G imputation, our analysis plan recommended using resulted imputed using the HapMap 2 CEU (r22.b36) reference sample.\textsuperscript{77}

2.4 Association analyses

Cohorts were asked to only include participants of European ancestry, with no missing values on all relevant covariates (sex, birth year, and cohort specific covariates), who were successfully genotyped genome-wide (e.g., genotyping rate greater than 95%), and who passed cohort-specific quality controls (e.g., no genetic outliers).

Cohorts were asked to use the fully imputed set of HapMap Phase 2 autosomal SNPs, and to estimate an additive linear model, including top principal components to control for population stratification and cohort specific covariates if appropriate. They were specifically instructed to control for population stratification for ancestry principal components with reference to Price et al. (2006).\textsuperscript{78} In addition, cohorts were requested to include birth year of the respondent (represented by birth year – 1900), its square and cubic to control for non-linear birth cohort effects. Analyses pooling data across sexes also needed to include interactions of birth year and its polynomials with sex. Some cohorts only used birth year and not its polynomials because of multi-collinearity issues/convergence of the GWA analysis.
Omission of these nonlinear birth year effects is unlikely to lead to biased inferences, since genotypes are not usually considered to be truly associated with birth year. However, inferences might be less accurate (i.e., have larger standard errors), since omission of nonlinear birth year effects can lead to larger residual variation.

2.5 Quality Control

In this section, we summarize the main steps and diagnostic tests of the Quality Control (QC) procedure. The quality control was conducted in two separate independent analysis centers (Oxford/Groningen and Rotterdam). Once data were submitted, each study was independently subjected to quality control in the two analyses centers according to standard protocols. We followed the QC protocol of the GIANT consortium’s recent study of human height\textsuperscript{72} and the SSGAC’s study of educational attainment.\textsuperscript{79,80}

Since this study began QC procedures have become more stringent. Recently, a comprehensive set of guidelines for GWAS QC was released.\textsuperscript{7} For the cohorts initially included in the study a first round of QC was performed using the R package QCGWAS.\textsuperscript{73} We updated the QC protocol based on the GIANT consortium’s and SSGAC’s protocols. The updated QC protocol was applied to all cohorts using the R package EasyQC.\textsuperscript{74} Findings of the first round of QC were used as a starting point for the updated QC.

In the QC procedure diagnostic graphs and statistics were generated for each set of GWAS results (i.e., for each result file uploaded by the cohort analysts). Most errors (e.g., coded allele reported as other allele and vice versa) could easily be addressed. In case apparent errors could not be amended by combining stringent QC with file-specific inspections and corrections, cohorts were excluded from the meta-analysis. For details on cohort inclusion and exclusion, see Supplementary Table 1.

Filters

We harmonized base pair positions of the markers across files using NCBI build 37. For each result file a given marker was excluded in case:

1. The combination of chromosome and base-pair position could not be uniquely linked to the HapMap Phase II CEU panel.
2. The marker had missing or incorrect values. Specifically,
a. the effect allele and other allele were missing,
b. the association p-value was missing or outside the unit interval,
c. the effect estimate was missing or reported to have infinite magnitude,
d. the standard error (SE) of the effect estimate was missing, negative, or infinite,
e. the allele frequency was missing or outside the unit interval,
f. the sample size was not reported, or zero or below,
g. the reported callrate was outside unit interval,
h. the reported imputation quality was negative, and
i. the reported imputed dummy was not binary.

3. The marker was not a SNP, not biallelic, non-autosomal, and/or monomorphic.

4. The sample size was below 30.

This filter is to guard against spurious associations due to overfitting of the model.

5. The minor allele count was 6 or below.

This filter is to guard against spurious associations with low-frequency SNPs in small samples. The risk of spurious associations has shown to be particularly high for SNPs that are extremely rare7.

6. Minor allele frequency (MAF) was below 1%.

For all the cohorts, we dropped SNPs with a MAF below 1%. For small cohorts we applied more stringent filters based on diagnostic tests and figures.

7. The SE of the effect estimate was greater than \(100/\sqrt{N}\).

Based on the approximation to the expected standard error by Winkler et al.7, we calculated that an SE greater than \(100/\sqrt{N}\) is at least 40% greater than the expected SE of the estimated effect of a SNP with a MAF of 1% for a trait with standard deviation of 10. Since in our analyses we only consider SNPs with MAF\(\geq 1\)% and traits with a standard deviation below 10, an effect estimate with an SE greater than \(100/\sqrt{N}\) can be considered to be unreasonably large.

8. The \(R^2\) of the marker with respect to the phenotype was greater than 10%.

We excluded SNPs for which the estimated \(R^2\) was greater than 10% (Supplementary Information in Rietveld et al.78) because such an \(R^2\) would defy all upper bounds on reasonable effect sizes of SNPs.

9. The marker was imputed while imputation quality was missing.

10. The marker was imputed while imputation quality was below 0.4.

For all the cohorts, we dropped imputed SNPs with an imputation quality below 0.4. For several cohorts we apply more stringent filters based on diagnostic tests and figures.

11. The callrate was below 95%.

12. The SNP was genotyped and not in Hardy–Weinberg Equilibrium (HWE).

We excluded genotyped SNPs if they fail the HWE chi-squared test. Violation of HWE will lead to lower chi-squared p-values as sample size increase, the threshold is therefore sample-size dependent. We applied an HWE p-value threshold of \(10^{-3}\) in case \(N < 1,000\), \(10^{-4}\) in case \(1,000 \leq N < 2,000\), \(10^{-6}\) in case \(2,000 \leq N < 10,000\), and no filter in case \(N \geq 10,000\).
**Diagnostic checks**

For the SNPs remaining after applying the filters of steps 1 – 12, we generated five key diagnostic graphs:

1. Allele frequency (AF) plots – to identify errors in allele frequencies and strand orientations.

   The AF plot shows the expected AF (based on the HapMap II CEU2 reference panel or the 1000 Genomes Phase 1 European panel in case of 1000 genomes imputed data) versus the reported AF.

2. Reported P-values versus P-values of the Z-scores (PZ) plots – to assess the consistency of the reported P-values with respect to those implied by the effect estimates and the corresponding standard errors.

3. Quantile-Quantile (QQ) plots – to check for evidence of unaccounted population stratification.

4. Reported Standard Error versus Expected Standard Error (SE) plots – to assess whether the reported standard errors behave in line with the approximation of the expected standard errors provided by Winkler et al., implemented as a QC step by Okbay et al.

These diagnostic plots were examined by two independent analysts. If problems were detected which could be resolved by more stringent thresholds, we applied the following *ad hoc* filters (descending order in terms of frequency used).

1. MAF filters more stringent than the generic MAF filter (e.g., 5% instead of 1%).

2. Imputation quality filters more stringent than the generic filter (e.g., 0.8 instead of 0.4).

3. Filter on the absolute difference between expected (based on the HapMap II CEU2 reference panel or the 1000 Genomes Phase 1 European panel in case of 1000 genomes imputed data) and reported allele frequencies. This filter helps to remove clear outliers in the AF-plots (e.g., strand-ambiguous SNPs that are likely to have been reverse-coded).

4. Filter on the absolute difference between the reported log(P-value) and the log(P-value) derived from the report Z-score. This filter helps to remove clear outliers in the PZ-plots. Such outliers can arise when software such as SNPTEST switches to another estimation method, for reasons such as poor convergence of the estimates.

For a list of the filters used per cohort, per association file, see Supplementary Table 27 which reports the total number of markers prior and post-QC when applying the described generic and specific filters, for each set of association results.

The AF plots for ABCFS (N=410 for AFB and NEB) shows a strong anti-diagonal that persists when considering only genotyped markers, implying that reverse-coded SNPs are likely to have been used for imputation, thereby yielding poorly imputed SNPs. Consequently, we exclude the ABCFS result files from the meta-analyses. In addition, for Longenity (N=285 for AFB and N=352 for NEB) many SNPs have far greater standard errors
for the effect estimates than expected, as well as callrates substantially below 95%. When applying QC to Longenity, only several hundreds of SNPs are left after QC. Consequently, we also exclude Longenity results from the meta-analyses.

2.6 Meta analyses

Cohort association results (after applying the QC filters) were combined using sample-size weighted meta-analysis, implemented in METAL. Sample-size weighting is based on Z-scores and can account for different phenotypic measurements among cohorts. The two QC centers agreed in using sample-size weighting to allow cohorts to introduce study-specific covariates in their cohort-level analysis. Only SNPs that were observed in at least 50% of the participants for a given phenotype-sex combination were passed to the meta-analysis. SNPs were considered genome-wide significant at P-values smaller than $5 \times 10^{-8}$ (α of 5%, Bonferroni-corrected for a million tests. The meta-analyses were carried out by two independent analysts. Comparisons were made to ensure concordance of the identified signals between the two independent analysts. The PLINK clumping function was used to identify the most significant SNPs in associated regions (termed “lead SNPs”).

The total sample size of the meta-analysis is $N=251,151$ for AFB pooled and $N=343,072$ for NEB pooled. Although considered to be separate from our main pooled results, we also performed separate meta-analyses for

- AFB women ($N=189,656$),
- AFB men ($N=48,408$),
- NEB women ($N=225,230$),
- NEB men ($N=103,909$)

The sex-specific results are discussed in more detail in Supplementary Note, Section 5. To understand the magnitude of the estimated effects, we used an approximation method to compute unstandardized regression coefficients based on the Z-scores of METAL output obtained by sample-size-weighted meta-analysis, allele frequency and phenotype standard deviation. Further details of the approximation procedure are available in the Supplementary Information of Rietveld et al.
Figure S2.1.1. to Figure S2.13.2 contains the forest plots and regional association plots of all genome-wide significant SNPs, the latter created by LocusZoom plots. The forest plots provide a visualization of the effect size estimates for each cohort and the summary meta-analysis (red rectangle) in addition to the 95% confidence intervals. As would be expected, small cohorts have larger confidence intervals. LocusZoom plots provide a graphic depiction of the local association results and include information about the locus, the location and orientation of the genes it includes, LD coefficients and the local estimates of recombination rates.
3. **BIVARIATE AND CONDITIONAL ANALYSIS OF THE TWO FERTILITY-RELATED TRAITS**

As joint analysis of correlated traits may boost power for mapping functional loci, we applied a recently developed multi-trait analysis method\(^{86}\) to test the association between each variant and the two correlated traits AFB and NEB simultaneously using multivariate analysis of variance (MANOVA). The analysis was performed based on the genome-wide meta-analysis summary statistics of each single trait. The joint analysis did not reveal additional genome-wide significant loci \((\lambda=0.995)\), however, such bivariate analysis, accounting for the correlation between the two phenotypes, improved the strength of two signals on chromosomes 1 and 5, indicating possible pleiotropic architecture between the AFB and NEB (Supplementary Fig. 30).

The analysis also provides a conditional association test of the genetic effect of each variant on AFB including NEB as a covariate, and that on NEB including AFB as a covariate. The conditional analysis also did not reveal additional genome-wide significant loci (Supplementary Fig. 31). Nevertheless, adjusting for NEB eliminated the three genome-wide significant loci on chromosomes 1, 2 and 6 for AFB, and adjusting for AFB eliminated the two genome-wide significant loci on chromosomes 1 and 14 for NEB, which may indicate underlying pleiotropic effects on both traits across these loci.
4. TESTING FOR POPULATION STRATIFICATION

Population stratification can severely bias GWAS estimates for causal variants and lead to false positives. This can occur if a particular variant of a SNP is more common in a particular subpopulation and if there are mean differences in the phenotype of interest between subpopulations due to factors that do not involve that SNP. As described in section S.2, all cohorts in the GWAS of AFB and NEB included the top principal components in their analyses to account for population stratification. Even despite this inclusion, residual stratification could still remain and affect the results.

To test the extent of this problem, we used two methods to assess if our GWAS results for AFB and NEB exhibit signs of population stratification. First, we used the LD Score intercept method described in Bulik-Sullivan et al. Second, we conduct a series of individual and within-family (WF) regressions using polygenic scores (PGS) as predictors on a dataset of DZ twins (STR and TwinsUK). Within-family regressions are based on family differences in PGS for AFB and NEB and are therefore are not affected by population stratification. We compare the coefficients of individual and WF regression using different p-value thresholds for the construction of PGS. Polygenic scores are based on independent results (i.e. meta-analysis results excluding STR and TwinsUK). Additional information on how we computed PGS are available in section 7 of the Supplementary Note.

4.1 LD Score Intercept Test

The LD Score intercept test uses GWAS summary statistics for all measured SNPs. LD Score regression is a method that can disentangle inflation in the chi-square statistics that is due to a true polygenic signal throughout the genome from inflation that is due to confounding biases such as cryptic relatedness and population stratification. The inflation due to a true polygenic signal impacts the slope of the LD regression, whereas inflation due to population stratification and other confounding biases affects the intercept of the regression.

We used the LDSC software to estimate the intercepts in LD Score regressions with the summary statistics of our GWAS of: (i) AFB (pooled sample), (ii) NEB (pooled sample), (iii)
AFB (women), (iv) AFB (men), (v) NEB (women), and, (vi) NEB (men). We estimated a separate LD Score regression for each of the phenotypes using the summary statistics from the meta-analyses based on all available data.

For each phenotype, we used the “eur_w_ld_chr” files of LD Scores computed by Finucane et al.\(^\text{92}\) and made available on https://github.com/bulik/ldsc/wiki/Genetic-Correlation. These LD Scores were computed with genotypes from the European-ancestry samples in the 1000 Genomes Project using only HapMap3 SNPs. Only HapMap3 SNPs with MAF > 0.01 were included in the LD Score regression.

Because genomic control (GC) will tend to bias the intercept of the LD Score regression downward, we did not apply GC to the summary statistics we used to estimate the LD Score regression. Furthermore, we excluded the deCODE cohort from the data for the estimation of the LD Score intercept for AFB and NEB, since the cohort-level regression estimates for deCODE did not directly correct for the high level of relatedness in the sample (their standard procedure is to apply GC). Our intercept estimates from the LD Score regressions are thus unbiased measures of the amount of stratification there is in the data (excluding deCODE) that we used for the GWAS of each phenotype.

Figures S4.1 and S4.2 show LD Score regression plots based on the summary statistics from the GWAS of AFB, and NEB. For AFB, we estimated a LD Score intercept of 1.0216 (SE=0.008) and for NEB, 1.009 (SE = 0.006). In all six cases, the intercept estimates are not significantly different from 1. By comparison, the mean \(\chi^2\) statistics for all the SNPs in the LD Score regressions are 1.239 for AFB and 1.141 for NEB. Under the null hypothesis that there is no confounding bias and that the SNPs have no causal effects on the phenotypes, the mean \(\chi^2\) statistics would be one, thus mean \(\chi^2\) statistics greater than one indicate that some SNPs are associated with the phenotypes. These estimates imply that about 9% of the observed inflation in the mean \(\chi^2\) statistics for AFB and about 6% of the inflation for NEB is accounted for by confounding bias (due to relatedness, or other confounds) rather than a polygenic signal.

As described in Section 2 of the Supplementary Information, we applied the standard single GC correction to produce our main estimates. Once a single GC is applied, the LD score regression estimates indicate negligible confounding bias due to population stratification. The LD score intercept for AFB is 0.9618 (SE= 0.0077) and for NEB 0.9763 (SE=0.0068).
can therefore conclude that the amount of inflation due to confounding bias is likely to be negligible in our final results.

4.2 Statistical Significance of the Polygenic Scores in a WF regression

To test the robustness of our all-SNP polygenic scores calculated with a set of SNPs meeting several different threshold P-values (5e-08, 5e-07,5e-06, 5e-05, 5e-04, 5e-03, 5e-02, 5e-01, all SNPs), we estimated WF regressions of AFB and NEB on each polygenic score in samples that are independent from those used to construct the scores. For each WF regression, we also compared the estimated coefficient on the polygenic score to the corresponding coefficient from individual-level regression.

For both the individual-level and WF regression, we standardized NEB and AFB on birthyear, birthyear squared, birthyear cubic, sex and the first 10 PCAs. Our regressions are based on 7,944 twin couples for AFB and 9,220 twin couples for NEB. Figures S4.1, S4.2 and Supplementary Tables 30, 31 report the results.

The regression analyses show that WF regression coefficients for both AFB and NEB are statistically different from zero when the p-value threshold is sufficiently far from zero. When including all SNPs, both coefficients for AFB and NEB are larger than zero, confirming that the GWAS analyses uncovered true polygenic signals. Overall, these results indicate a minimum effect of population stratification and the existence of polygenic signals.

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a Details on the construction of polygenic scores is available in section 6 of the Supplementary Note.
5. SEX-SPECIFIC GENETIC EFFECTS IN FERTILITY

Sex-specific genetic effects have been proposed as an important source of variation for complex human traits.\textsuperscript{93,94} For this reason we also ran sex-specific GWAS meta-analyses for both AFB and NEB and examined the genetic overlap among sexes using LD score bivariate regression and GCTA. Sex-specific effects refer to large differences in average phenotypes or biological processes known to differ between the sexes (e.g., hormonal effects). Since AFB and NEB are not only biological but also socio-behavioural phenotypes, it is likewise important to make a distinction between sex- versus gender-specific effects. Sex refers to biological differences between males and females, which often have their underpinnings in human reproduction.\textsuperscript{95} Gender refers to the socially-constructed differences between men and women that may give rise to particular behavioural outcomes (e.g., gender-specific social norms regarding alcohol consumption). There is growing evidence that biological (sex) and social (gender) processes are interrelated, which in turn impacts the phenotypes we are examining.\textsuperscript{96} Although we recognize the importance of these distinctions, it is beyond the scope of the current study to disentangle sex- versus gender-effects. Rather in this section we emphasize similarities and differences in the sex-specific GWAS results and examine the sex-specific genetic overlap of these traits.

There are several key sex-specific differences in AFB and NEB. Women in contemporaneous populations have a comparatively lower age at first birth than men, which is attributed factors such as the persistent age gap between partners.\textsuperscript{97} Fecundability is strongly influenced by sex-specific hormonal processes and gender-specific diseases. Sex can modify both penetrance and expressivity of a wide variety of traits.\textsuperscript{98,99} Sex-genotype interactions can also theoretically act to maintain genetic variation in a population.\textsuperscript{100} The existence of opposite genotypic effects among sexes (also called sexual antagonism) has been often theorized as one of the possible explanations for genetic differences in fertility.\textsuperscript{101} In other words, particular genes might influence men and women differently and will therefore still be transmitted to the next generation. Genes that contribute to the fecundability of men may therefore be inherited via women’s lineage and those for women via men’s lineage.\textsuperscript{102}
5.1 Sex-specific GWAS meta-analyses for AFB and NEB

In addition to the pooled GWAS results presented in the main text, we also ran sex-specific GWAS meta-analyses for AFB and NEB. The sample size for sex-specific analysis is: AFB women, N=189,656; AFB men, N=48,408; NEB women N=225,230; NEB men N=103,909.

Our results indicate 6 genome-wide significant (P<5x10^{-8}) independent SNPs for AFB women and 1 genome-wide significant independent SNP for NEB men. We do not find any genome-wide significant loci for AFB men and NEB women. Among the 6 hits for AFB women, 5 are also significant in the AFB pooled analysis, while 1 hit on chr8 (rs2721195; chr8: 145677011) is specific for women. We find a single independent SNP for NEB men (rs13161115; chr5:107050002) that reaches genome-wide statistical significance (P-value<5x10^{8}), which is not significant in the NEB pooled analysis. Supplementary Figure 34 shows the Miami plots for AFB and NEB sex-specific analyses. Supplementary Figure 35 depicts the QQ plots of men and women’s meta-analyses for AFB and NEB. The figure shows a noteworthy departure from the null hypothesis of no statistical association, in particular for the analysis of AFB women.

Table 1 (in the main text) shows the sex-specific signals respectively for AFB and NEB. The effects of all significant hits in AFB have the same direction for both men and women. The single locus found in NEB men (rs13161115) has an opposite effect on NEB for women, although the p-value associated with its effect size in NEB for women does not reach statistical significance.

5.2 Genetic overlap among sexes using LD score bivariate regression

We used LD score bivariate regression to estimate the genetic correlation among men and women based on the sex-specific summary statistics of AFB and NEB meta-analysis results. For each phenotype, we used the “eur_w_ld_chr/” files of LD Scores computed by Finucane et al. and made available on https://github.com/bulik/lsc/wiki/Genetic-Correlation. These LD Scores were computed with genotypes from the European-ancestry samples in the 1000 Genomes Project using only HapMap3 SNPs. Only HapMap3 SNPs with MAF>0.01 were included in the LD Score regression. Our estimates indicate a genetic correlation of r_e=0.86
(SE=\pm 0.052) among sexes for AFB and \( r_g = 0.97 \) (SE=0.095) for NEB. These results indicate a large genetic overlap among sexes for both AFB and NEB, which is statistically different from zero. We additionally test whether these genetic correlations support the null hypothesis of complete genetic overlap among sexes (\( r_g = 1 \)). We reject this null hypothesis for AFB, indicating sex-specific genetic variants for AFB. We do not find any evidence of sex-specific signals for NEB.

5.3 Genetic overlap among sexes using GCTA

We additionally estimate the degree of genetic overlap among sexes using Genomic-Relatedness-Matrix Maximum Likelihood (GREML)\(^{46}\) on six cohorts for which we have direct access to genotypic data.\(^{46,48,103,104}\) For the GREML analyses, we combine data from six cohorts: HRS, EGCUT, QIMR Lifelines Cohort Study, TwinsUK and STR (\( N_{\text{women}} = 20,966; N_{\text{men}} = 17,024 \), see Supplementary Table 33 for descriptive statistics). We used GCTA\(^{46}\) to construct a Genome-wide Relatedness Matrix (GRM) \( A^{n \times n} \) and estimate the models. For quality control (QC), we included in the analysis only HapMap3 SNPs with an imputation score larger than 0.6. We additionally excluded SNPs with a missing rate larger than 5%, MAF lower than 1% and which failed the Hardy-Weinberg equilibrium test for a threshold of \( 10^{-06} \). We applied these QC steps for each cohort and repeated again on the merged dataset. After QC, 847,278 SNPs could be utilized to estimate the GRM between individuals.

5.4 Bivariate GREML analysis

First, we fit a bivariate GREML model as proposed by Lee et al.\(^{104}\) treating the fertility traits of men and women as different traits.\(^{103}\) To account for potential country heterogeneity, we estimated genetic variation from within cohorts only (\( \sigma_{g_{wc}}^2 \)), setting the GRM between individuals from different cohorts equal to zero.\(^{50}\) This allows us to avoid the potential bias due to differences in allele frequency across different countries. The GRM can be depicted as a block matrix composed by six within-cohort GRMs (\( A_{g_{wc}} \)) containing only values for pairs of individuals within cohorts.

The variance-covariance matrix of the bivariate model is shown as:
whereas $f_{men}$ and $f_{women}$ are vectors of length $N_{men}$ and $N_{women}$ of fertility phenotypes (NEB or AFB), with N being the respective sample size of the subsets, $A_{wc,men}$ is the within population GRM for all individuals, $A_{wc,men}$ is the within cohorts GRM for men, and $A_{wc,women}$ for women. The parameter $\sigma^2_{g,wc,men}$ is an estimate of the genetic variance component for men and $\sigma^2_{g,wc,women}$ the genetic covariance across sexes. I is the identity matrix, and $\sigma^2_{g,wc,men}$, $\sigma^2_{g,wc,women}$ the respective, sex-specific residual variances within cohorts. We present the variance components standardized for the phenotypic variance $\sigma^2_p$. The correlation of the genetic factors are estimated as:

$$r_{\sigma^2_{g,wc,men},\sigma^2_{g,wc,women}} = \frac{\sigma^2_{g,wc,men}}{\sqrt{\sigma^2_{g,wc,men} \cdot \sigma^2_{g,wc,women}}}$$

We find significant heritability for NEB and both sexes $\sigma^2_{g,wp}/\sigma^2_p = 0.13$ (SE=0.057, $P=0.01$) for men, and 0.08 (SE=0.04, $P=0.01$) for women (see Supplementary Table 34 for full results). This means that around 10% of the variance in NEB is explained by common SNPs for both sexes. The estimated genetic correlation across sexes is 0.98 (SE=0.44) and a likelihood ratio-test against a perfect genetic correlation across sexes has a $p$-value of 0.5. We therefore cannot reject the null-hypothesis that genetic effects are the same across sexes.

For AFB we find a very similar pattern of sex specific SNP-based heritabilities of around 0.10 and a genetic correlation of 1.00 (SE=0.67, $P=0.5$ when testing against 1). These results also cannot reject the null-hypothesis that genetic effects on AFB are the same across sexes.

### 5.5 Analysis of differences between sample and effect sizes

Table 1 in the main text did not include the Ns of the sex-specific analyses. It is, however, important to place the $p$-value of women and men in context and clarify why the effect size for some loci is similar in men and women but the $p$-value is not. This could reflect a difference in sample size, or it may reflect a difference in variance. Supplementary Table 32 shows all of the sex-specific sample sizes, $p$-values, $z$-scores and the $p$-value differences.
between males and females by each SNP. It indicates sex-specific effects and a statistical test showing the differences between effect sizes.

The statistical is based on the differences between male and female Z-scores:

\[ Z_{\text{diff}} = \frac{Z_1 + Z_2}{\sqrt{\frac{1}{N_1} + \frac{1}{N_2}}} \sim N(0,1) \]

Supplementary Table 32 reports the P-value differences of this Z-score test. Despite the fact that p-values differ among the sexes, it seems plausible that the differences are mainly due to variation in sample size and not attributed to different effect sizes. Our results show that the only locus that has a statistically different effect between men and women after taking into account the number of test conducted is rs13161115 in chromosome 5, where the effect is significant only in men and the direction of the effect differs among sexes.

### 5.6 Discussion

Sex-genotype interactions and sexual antagonistic effects may affect the transmission of traits across generations and has been proposed as a possible source of genetic variation in fertility traits.\textsuperscript{102} Fecundability is strongly influenced by sex-specific hormones and infertility causes differ between men and women.\textsuperscript{105} Our results show little differences in the genetic architecture of the fertility traits (AFB, NEB) of our study between men and women. Out of 12 independent loci for AFB and NEB, only two have a sex-specific effect. Moreover, all the signals found for AFB and two out of three signals in NEB, have a consistent direction across the sexes. We found a high genetic correlation among men and women for both AFB and NEB, both using LDscore bivariate regression and GREML bivariate analysis. This suggests that most of the genetic effect of fertility due to common SNPs is shared across sexes. However, using LDscore regression, we reject the null hypothesis of \( r_g=1 \) for AFB (P=0.007).

A possible explanation of why we have not found more evidence for sex-genotype interactions may attributed to the fact that we analyzed only common variants and that we restrict our analysis to autosomal chromosomes. Moreover, our sex-specific meta-analysis may be underpowered to discover sex-specific loci.

When we compare Table 1 and 2 we note that in addition to the chr 5 locus for NEB, the chr
2 locus for AFB also shows a discrepancy between a sex-specific effect in the GWAS (women only) versus the (known) function of a candidate gene (AFF3). It would be premature to draw any firm conclusions since little is known about the role of AFF3 (chr 2) and EFNA5 (chr 5) in reproduction. For a substantial number of loci there are differences in the p-value between men and women, but the effect size suggests the association is present in both sexes. Only four loci seem to have a convincing null effect in men (rs1160544, rs10056247, rs2721195) or women (rs1316111). We would encourage functional follow-up studies on these points to further our understanding of human reproduction.

6. POLYGENIC SCORES PREDICTION

We performed out-of-sample prediction using cohorts for which we have direct access to genotypic data. We calculated polygenic scores for AFB and NEB, based on GWA meta-analysis results and used regression models to predict the same phenotypes in four independent cohorts: HRS, Lifelines, STR and TwinsUK. We ran ordinary least-squares (OLS) regression models and report the $R^2$ as a measure of goodness-of-fit of the model. In addition, we tested how well our polygenic scores for NEB could predict childlessness at the end of the reproductive period (using age 45 for women and 55 for men). Since age at first birth is observed only in parous women, we adopt an additional statistical model to account for censoring and selection. Finally, we also tested the predictive value of our polygenic scores for AFB for age at menarche (using TwinsUK) and age at menopause (using Lifelines).

6.1 Linear polygenic scores for AFB and NEB

We ran meta-analyses of the pooled AFB and NEB phenotypes, excluding each of the independent cohorts. Using these summary statistics, we constructed linear polygenic scores using the effect sizes from the original meta-analysis.\(^1\) We constructed all scores using the software PLINK and PRSice\(^2,3\) based on best call genotypes imputed to 1000G. For each phenotype, we calculated nine different scores using different p-value thresholds: 5e-08, 5e-07, 5e-06, 5e-05, 5e-04, 5e-03, 0.05, 0.5 and 1. Results are clumped using the genotypic data as a reference panel for LD structure.
We first regressed each phenotype on birthyear, its square and cubic to control for nonlinear trends in fertility, and the first 10 principal components, following the analysis plan distributed to the cohorts. If the cohort included both men and women, we included sex as a covariate in the regression models. Next, we regressed the residuals from the previous regression on the polygenic score. We performed a set of Ordinary Least Squares (OLS) regressions where we calculated $R^2$ as an indicator of goodness-of-fit of the regression model. For twin studies (STR and TwinsUK), we included only one MZ twin in the analysis and used clustered standard errors at the family level. To obtain 95% confidence intervals (CI) around the incremental $R^2$'s, bootstrapping was performed with 1,000 repetitions.

The results of the polygenic score analyses are depicted in Supplementary Figure 2. The sample-size-weighted mean predictive power of the AFB score constructed with all SNPs is 0.9%, while the NEB score predictive power is 0.2%.

### 6.2 Linear polygenic scores for infertility

We used the score for NEB in an additional analysis to predict the probability to remain childless at the end of the reproductive period. Despite its limited predictive power for number of offspring, our analysis shows that an increase of one standard deviation of the polygenic score is associated with a decrease of around 9% in the probability to remain childless for women, with no significant differences among men (see Supplementary Table 21). The results are consistent across different cohorts.

### 6.3 Additional statistical models for censoring and selection

There are two limitations when studying the genetic determinants of AFB. The first is that this measurement is assessed only for men and women who ever became parents and does not take into consideration that a proportion of respondents are still at risk of having a child (i.e., did not have a child yet by the date of the interview) or will remain childless. This problem is commonly referred in the statistical literature as ‘right censoring’, since the outcome is not observed for all respondents, despite the fact that part of the respondent are still ‘at risk’ of
experiencing childbirth. The second problem is statistical selection. Individuals with a measurement of AFB may be genetically different from individuals who remain childless. If childless individuals are different from the general population, the association results on AFB may be biased by selection problems. To investigate these two issues further, we estimated additional statistical models.

To control for right-censored data, we estimated semi-parametric Cox regression models in which we estimate the effect of the polygenic score (PGS) on increasing the hazard of having a child conditional at each age. In other words, it is a model that estimates the impact of AFB PGS on yearly AFB, which will allow us to assess whether an increase in the AFB PGS is associated with a reduced risk of childbearing at each yearly age interval. This class of models takes into account censoring and is widely used to study fertility timing. Our results show that the calculated PGS for AFB based on all SNPs is associated with an increased risk of childbearing at any age. The median AFB for men in the pooled sample is 28 and 26 for women. The hazard ratio of the PGS for AFB is 0.92 for women and 0.97 for men. This means that an increase of one standard deviation in the PGS is associated with an increase of 8% of AFB for women and 3% for men. Results for different cohorts and sex are depicted in Supplementary Table 22. Since this is a survival model that handles right-censoring (i.e., that the event of AFB did not occur by the observation time), the interpretation is that an increase in one standard deviation of the AFB PGS is associated with a reduction of 8% and 3% respectively for women and men in the hazard ratio of reproduction.

To control for selection, we estimated bivariate Heckman selection models in which we estimate the probability to be ‘eligible’ or at risk for AFB in a two-step procedure. Since we are interested in possible genetic differences among men and women who ever had children with respect to childless individuals, we used the PGS for NEB to model the probability to be at risk for AFB. The results from the Heckman selection models indicate slightly lower coefficients than OLS regression models but no substantial differences (see Supplementary Table 35 for details).
6.4 Linear prediction of age at menarche and age at menopause using AFB linear score

As an additional test, we examined whether the aforementioned PGS scores for AFB and NEB can predict related fertility traits such as age at menopause and age at menarche. We used the age at menopause measurement included in the Lifelines cohort. Age at menopause is measured with the question: “At which age have you had your last menstrual period?” We excluded women from the sample who reported to have had their last menstruation before age 30 or after age 60. The median age at natural menopause (ANM) in the sample is 45. Our results show that the PGS for AFB is associated with a later ANM. Since a substantive proportion of the sample of women in Lifelines is still in the pre-menopausal period, we estimated a proportional hazard model (Cox regression) in which we estimate ANM as a function of PGS for AFB. Our estimates indicate that having higher predisposition for AFB is associated with a later ANM. The hazard ratio estimate 0.97 indicates that an increase of one standard deviation of the PGS for AFB is associated with a decrease of ANM of about 3%. We used TwinsUK to model age at menarche. Our estimates indicate that an increase of one standard deviation on the PGS of AFB is associated with an increase of 0.06 years on age at menarche. Results are depicted in Supplementary Table 23.

6.5 Association of menopause variants with AFB

We also examined whether menopause variants are associated with AFB. We calculated a PGS of age at menopause based on the recent GWAS results from Day et al. (2015) and applied them to LifeLines and TwinsUK. The results for this analysis can be found in Supplementary Table 36 and shows no predictive power of the menopause genotype on AFB. This is consistent with the lookup exercise presented in S7.2, where none of our loci were significantly associated with age at Menopause. There might be several reasons why the LD score regression indicates a positive genetic correlation but we do not find evidence for specific loci. First, one or both of the studies may be underpowered and thus unable to identify a sufficiently large number of variants. Second, the correlation between the two traits may be spurious and mediated by other traits (e.g., age at menarche). We agree that it would be very interesting to pursue this in further research.
6.6 Discussion: The predictive power of polygenic scores

We acknowledge that the predictive power of the polygenic scores created from a meta-analysis of over 60 GWASs is only a fraction of what has been found in previous twin and family\(^1\) and even GREML studies.\(^{38}\) Several reasons have been noted for this ‘missing heritability’ problem,\(^{109}\) including non-additive genetic effects,\(^{49}\) epistatic effects,\(^{110}\) rare variants and inflated estimates from twin studies due to differential sharing of environmental factors in monozygotic and dizygotic twin pairs.\(^{111}\) Other factors that can explain the lower magnitude of effects are also plausible. Firstly, as we elaborate in Section S1.5, human reproductive behaviour is not only biological, but also strongly related to environmental factors, and we should therefore not expect to find large independent genetic effects. We do not expect the PGS score to explain part of the variance attributable to environmental factors (i.e., the C and E in twin studies), but rather argue that these environmental factors are likely much stronger than genetic factors for these behavioural outcomes. As argued recently elsewhere,\(^{39}\) it is vital to note that deep genetic analyses need to be united with strong and direct phenotypic measures. Although AFB and NEB are robustly measured, they inherently include a mix of voluntary (choice) and involuntary (infertility) measures. To overcome this problem, future innovations must unite rich genetic data with equally rich and precise phenotypic data collected precisely and continuously over several generations.

A second factor is that when studying phenotypes with behavioural component, GWAS discoveries are potentially limited by heterogeneity across birth cohorts and populations (e.g., countries) and particularly prone to gene-environment interaction. Fertility behaviour has been demonstrated to be strongly environmentally determined and modified (e.g., by the introduction of effective contraception).\(^{18}\) Although we examine gene-environment interaction across birth cohorts in Sweden in the Supplementary Note (section 10.1), in future research we will explore whether gene-environment interaction plays a role across birth cohorts and countries, with preliminary evidence suggesting that this is the case.\(^{112}\) This is in line with recent research that has shown cohort differences in the genetic influence on smoking over time.\(^{113}\)
7 GENETIC CORRELATIONS

7.1 Estimating genetic overlap using LD score regression

The estimates of the LD score regression reported in the main text was based on the LD-score regression method, which was developed by Bulik-Sullivan et al. (2015). Here we describe in more detail how these estimates were computed and the genetic correlation we estimated between AFB and NEB and 27 publicly-available GWAS results (Supplementary Table 25 and graphed in Figure 3 in the main text). We focus on infertility traits, developmental traits, anthropometric traits, neuropsychiatric conditions and selected behavioural traits. LD score regression works even in the presence of sample overlap and only requires summary statistics and a reference panel from which to estimate SNP’s “LD score”, which measures the amount of genetic variation tagged by a SNP.

The approach requires GWAS summary statistics for all SNPs in our GWAS and a reference sample from which the LD can be estimated in order to estimate the LD score regression. The method is written formally based on the following relationship:

\[ E[z_{1j}z_{2j}] = \frac{\sqrt{N_1N_2}}{M} \ell_j \rho_g + \text{intercept}, \]

Where \( z_{kj} \) is the z-score of SNP \( j \) from the GWAS of trait \( k \) \((k=1,\ldots,20)\), \( N_k \) is the sample size of the GWAS of trait \( k \), \( \ell_j \) is the LD Score of SNP \( j \), \( M \) the number of SNPs included in the GWAS, \( \rho_g \) the genetic covariance between traits 1 and 2, with the regression intercept represented by \( \text{intercept} \). The slope from the regression of \( z_{1j}z_{2j} \) on \( \sqrt{N_1N_2} \ell_j \) can be used to estimate the genetic covariance between the two traits. We are also able to estimate the heritabilities of the two traits, \( h_{g1}^2 \) and \( h_{g2}^2 \) from the univariate LD score regressions of traits 1 and 2. It therefore follows that an estimate of the genetic correlation is:

\[ r_g = \frac{\tilde{\rho}_g}{\sqrt{h_{g1}^2 h_{g2}^2}} \]
We use the file of LD scores computed by Finucane et al.\textsuperscript{92} using genotypic data from a European-ancestry population (eur_w_ld_chr). LD Scores are computed with genotypes from the European-ancestry samples in the 1000 Genomes Project using only HapMap3 SNPs. We additionally follow the common convention of restricting our analyses to SNPs with MAF > 0.01, thus ensuring that all analyses are performed using a set of SNPs that are imputed with reasonable accuracy across all cohorts that contributed towards meta-analyses.

The standard errors (SEs) produced by the LDSC python software package uses a block jackknife over the SNPs. This influences the interpretation. Conventional standard errors are interpreted as measuring the variability of the estimate holding the covariates constant, but drawing on a new set of individuals. In this technique, SEs are interpreted as the variability of the estimate holding the sample constant, but drawing a new set of SNPs.

### 7.2 Estimating the genetic correlation between AFB and NEB

The negative relationship of late AFB with lower NEB\textsuperscript{7,10,114} is well-established and consistent in advanced societies. Behavioural genetic models, based on twin or family studies show that this correlation is partially genetic, suggesting that natural selection favored a younger age at first birth over the Twentieth century.\textsuperscript{1,38,115} A recent study on genetic basis of fertility traits using molecular genetic data shows that common genetic variants influence NEB and AFB in a large sample of unrelated women.\textsuperscript{38} Their results indicate a significant negative genetic correlation ($r_g=-0.62$, SE=0.27) between AFB and NEB. This finding implies that individuals with genetic predispositions for an earlier AFB had a reproductive advantage. We replicated the analysis of Tropf et al.\textsuperscript{38} on a large sample of women from the Women General Health Study (WGHS, sample size N=40,120). We found a negative genetic correlation ($r_g=-0.26$, SE=0.13) between AFB and NEB. The results were limited to women and applied to a limited sample. We extend this work using LD score bivariate regression\textsuperscript{87,91} on AFB and NEB on both men and women to identify the extent of cross-trait genetic correlation.

The LD score bivariate estimates indicate high negative correlation $r_g=-0.66$ (SE=0.03, p-value=1.03x10\textsuperscript{-102}) between AFB and NEB. This result is consistent both in men and women.
and is in line with the phenotypic correlation. Genetic correlation of fertility traits among
women is slightly higher ($r_g=-0.66$, SE=0.04) than men ($r_g=-0.58$, SE=0.07). Overall these
results show a considerable genetic overlap between NEB and AFB (as found in section 3).
However, since the genetic overlap is statistically different from 1 for both men and women,
these results indicate the existence of trait-specific genetic components.

7.3 Results: phenotypic correlations with human reproductive behaviour

As discussed in the main text, we used information from 27 publicly-available GWAS results
to examine phenotypic correlations between AFB and NEB (Supplementary Table 25 and
Figure 3 in the main text). These included: nine developmental traits, some of which are
directly related to the reproductive span (age at menarche, age at menopause, Tanner
stage, age at voice breaking for males, polycystic ovary syndrome (PCOS), age at first
sexual intercourse, DZ Twinning, birth length, birth weight), four behavioural traits
(years of education, cigarettes per day, ever smoked, age onset smoking), seven
personality and neuropsychiatric traits (neuroticism, openness, schizophrenia, bipolar
disorder, subjective well-being, Alzheimer’s disease, autism), four cardiometabolic
traits (LDL cholesterol, triglycerides, type 2 diabetes, fasting insulin levels), and
three anthropometric traits (BMI, height, waist-hip ratio).

As shown in Fig. 3 and Supplementary Table 25 (P-values in bold indicate Bonferroni
correction (P-value<0.05/27=1.85x10^{-03})), AFB is positively correlated with years of
education, age at menarche, age at menopause, age at voice breaking, age at first sexual
intercourse and adult height, while it is negatively correlated with PCOS, adult BMI and
waist-hip ratio, triglycerides, diabetes and fasting insulin level. Once multiple testing is
controlled for, years of education and age at first sexual intercourse are the only traits
significantly correlated with NEB (P-value<2.25x10^{-03}), and the direction is negative for both
traits.
7.4 Discussion

7.4.1 Human development

AFB was shown to be positively correlated with the development measures of age at menarche, age at menopause, age at voice breaking and age at first sexual intercourse. A later age of menarche (AOM) has been associated with subfecundity and infertility in adulthood. A recent large cohort study of 73,107 women\textsuperscript{135} demonstrated that women who reached menarche later than 15 years (compared to a reference group of girls with an AOM at 13 years) had a higher risk of infertility. Women younger than 11 years at AOM had lower odds of subfecundity and all results remained significant also after adjusting for women’s age of pregnancy. Some studies, however, have also found a significant relationship between early AOM with diminished functional ovarian reserve later in life among infertile women.\textsuperscript{136} There is also evidence of a small increased risk of endometriosis associated with early AOM.\textsuperscript{137}

Stolk et al. (2012)\textsuperscript{138} linked age at menopause to genes implicated in DNA repair and immune function. A recent study reported genetic correlations indicating shared aetiologies in both sexes between the timing of puberty and BMI, lipid levels, type 2 diabetes and cardiovascular disease.\textsuperscript{139} Fertility timing has been positively associated with age at menarche and age at first intercourse. Although previous research has largely focused on identifying genes related to menopause and menarche that mark the end the beginning and end of the reproductive career, it is also possible that observed fertility (AFB, NEB) influences the subsequent age at menopause and ovarian aging. Exploring these overlaps and associations would be an interesting area for future research.

Results from a genetic study of age at first sexual intercourse (AFS) linked AFS to variation in pubertal timing, but also personality characteristics related to high risk-taking and low neuroticism.\textsuperscript{23} We examine the link with AFS and neuropsychiatric disorders in a later section (Section 7.4.5).

7.4.2 Cardiometabolic traits
Having more AFB-increasing alleles was also significantly associated with a lower genetic scores for triglycerides, Type 2 Diabetes and fasting insulin level. Pregnancy for women results in considerable alterations in the cardiovascular system. Reproductive events are associated with alterations in blood lipids and blood pressure and may therefore influence determinants of coronary heart disease. As with diabetes, there are mixed findings regarding the link between age at birth, parity and coronary heart disease (CHD). Some studies have linked the number of children and CHD risk with the prevalence lowest among those with 2 children with a linear increase with each additional child. These researchers have argued that it is not the pregnancy per se that has a biological impact but rather that the lifestyle risk factors associated with childrearing leads to obesity which in turn results in increased CHD in both sexes. Yet, they maintain the argument that biological responses of pregnancy may have additional adverse effects in women.

Other studies attempted to elucidate the mechanisms linking multiparity to cardiovascular disease demonstrating that repeated pregnancies induce long-term changes in cardiovascular regulation in women due to the changes in vascular compliance and endothelium-dependent vasoconstriction, which in turn increase the risk for CHD in multiparous women. A recent study related early puberty timing to higher risks for both Type 2 Diabetes and cardiovascular disease. It may be however, that just as with the studies on GDM (gestational diabetes mellitus) described shortly, retrospective and cross-sectional approaches may have limitations related to selectivity and unobserved confounding factors. A prospective study in the US found that a younger age at menarche was only weakly associated with CHD and that nulliparous women only had a slightly higher rate of CHD compared to parous women. They also found no change in the risk with an increasing number of births or any association with the age at first birth concluding that there is no clear link between reproductive history and risk of CHD. Further research is required to establish whether there is a true causal link and underlying genetic and biological mechanisms to explain the association between reproductive history and cardiometabolic traits.

There does, however, appear to be a link with the cardiometabolic traits that we measure in this study with infertility. Total cholesterol, triglycerides, LDL cholesterol levels and fasting insulin levels have been shown to be statistically higher in groups with endometriosis
compared to controls. Endometriosis is estimated to occur in 5-10% of premenopausal women with ~50% experiencing problems conceiving. A recent study also revealed a link between endometriosis and obesity-related traits. Other studies have also linked the impact of maternal cholesterol metabolism to ovarian follicle development and fertility. The role of the low-density lipoprotein receptor in cellular metabolism in inhibiting human reproduction has likewise been established. Others have linked metabolic syndrome, which is a compilation of symptoms such as a high BMI (obesity), type 2 diabetes, dyslipidemia, and hypertension with an increased prevalence of infertility in men.

A wide body of research links reproductive history to Type 2 Diabetes. Early studies found that nulliparity and multiparity or grand parity (5 or more children) was associated with higher levels of fasting glucose and insulin levels among nondiabetic women. Multiparity has been associated with higher risks of cardiovascular disease in both women and men and higher insulin resistance and type 2 diabetes. Other research found that high parity was associated with insulin resistance and type 2 diabetes, which even after adjusting for confounders (socioeconomic, higher obesity, inflammatory markers) grand parity is associated with a 27% increased risk for diabetes (95% CI, 1.02-1.57).

It is essential to note, however, that early cross-sectional and retrospective studies did not control for age, body size or socioeconomic status. Later cross-sectional studies that controlled for the abovementioned factors, continue to produce highly mixed results (for a review see ref ). A key limitation is that many of the previous studies lack universal GDM (gestational diabetes mellitus) screening and did therefore not measure preconcept glycaemia or glucose intolerance during pregnancy. A systematic review and meta-analysis demonstrated that women who had gestational diabetes had a seven-fold greater risk of developing Type 2 Diabetes. This suggests that once GDM status is accounted for, the direct parity effect will be very small or null. One the other hand, unobserved conditions such as PCOS, obesity or insulin resistance could in fact cause infertility (nulliparity) which would in turn lead to an underestimation of the association.

Gunderson et al. (2007) examined whether childbearing increased the incidence of Type 2 Diabetes after preconception glycaemia and gestational glucose intolerance were controlled
for. They concluded that childbearing did not elevate the incidence of diabetes among those without GDM (i.e., normal glucose tolerance during pregnancy). It was GDM rather that was associated with the highest risk of developing diabetes, which remained even after controlling for family history of diabetes, preconception glycaemia and obesity. Another study using GDM screening found that a woman’s age remained a strong predictor even after adjusting for prior GDM history, mirroring the general historical increase in GDM (and related levels of obesity) across time in certain groups. A logistic regression analysis also showed that mother’s age at birth (OR 95% CI per 5 years 1.6–1.8) was significantly associated with GDM. Parity was not significantly associated with GDM and had no effect on the GDM increase over time.\textsuperscript{154}

7.4.3 Anthropometric traits

A considerable body of literature links anthropometric traits (such adult height, BMI and increasingly waist-hip ratio) with fertility timing and success.\textsuperscript{133,155} Anthropological research argue that shorter women may have more reproductive success because of the trade-off between investing in energy in growth or reproduction.\textsuperscript{156} Moreover, taller women appear to become fertile at a later age (e.g., age at menarche) than shorter women, and women who have children at an early age reach a shorter adult height, which may result in a negative relationship between women’s height and reproductive success.\textsuperscript{155,157} The relationship between men’s height and fertility is more complex. One paper revealed a curvilinear association between men’s height and number of children in a nationally representative sample of US respondents.\textsuperscript{158} Men of average height appear to have a higher reproductive success than either short or tall men. The relationship between height and number of children in advanced societies is not always negative. A recent paper showed that in the Netherlands – the country with the highest average population height – the relationship is the opposite.\textsuperscript{155} A possible mechanism through which height may affect fertility is sexual selection and assortative mating. There is a certain degree of homogamy in anthropometric traits among spouses, even after controlling for a variety of socio-economic traits.\textsuperscript{159,160}
BMI and waist-hip ratio (WHR) is another area of research often linked with fertility success, particularly in couples seeking ART treatment.\textsuperscript{161} Both a very low and a very high BMI have been found to delay both the timing and number of children in both men and women.\textsuperscript{162} Waist-hip ratio measures body fat distribution and serves as a more accurate predictor of metabolic consequences independent of overall adiposity. A study locating new loci for WHR also found that seven of the loci exhibited marked sexual dimorphism, or in other words, that the genetic loci that modulate fat distribution have a stronger effect on WHR for women than men, suggesting strong gene-by-sex interactions.\textsuperscript{163}

7.4.4 AFB and educational attainment

As described already in detail in Section 1.5, the strong relationship between AFB and years of education is not surprising, since educational attainment is associated with higher AFB and a lower NEB in most advanced societies.\textsuperscript{54,164} As discussed previously, the study of the relationship between higher educational attainment and reproduction has been a central focus within demography and related social sciences.\textsuperscript{7,10,58,114,165} The majority of the research demonstrates that achieving higher education (particularly of women) operates to postpone AFB. Other studies have shown that fertility postponement may be related to higher cognitive ability,\textsuperscript{166} but additional research is required to separate cognitive scores from social environment (e.g., family environment, social class). Others have found that after controlling for age, physical maturity and mother’s education, there is a significant curvilinear relationship with intelligence and early sexual intercourse with both very low and very high intelligence operating as a protective factor against early sexual activity.\textsuperscript{167} Further careful research in this area would be necessary to understand the relationship.

7.4.5 AFB, personality and neuropsychiatric disorders

The results of the LD score regression did not find any significant association with neuroticism, openness, schizophrenia, bipolar disorder, well-being, Alzheimer’s disease or autism, so we will only touch upon this topic briefly. Personality has been demonstrated to be predictive of fertility intentions\textsuperscript{20,168} and the timing of childbearing.\textsuperscript{169,170} The finding that AFB is negatively correlated with neuroticism has also been found in previous non-genetic studies linking AFB to personality traits.\textsuperscript{171,172} A bidirectional effect between fertility and
psychological development has likewise been documented. This may suggest that the interaction between genetic and environment factors could be interpreted as genetic influences on fertility that have an effects on both fertility behaviour and psychological outcomes. Since personality, educational attainment and cognitive ability are largely formed before individuals enter into their childbearing years, it is plausible that personality and cognitive traits are likely causal and precede fertility variables. A recent study also demonstrated a genetic overlap between schizophrenia and AFB, showing a U-shaped relationship. The study confirmed that the schizophrenia risk profile score significantly predicted the relationship between maternal age and risk of schizophrenia in offspring.

7.4.6 Smoking behaviour

The strong negative correlation of a lower genetic risk of smoking (less cigarettes per day, lower chance to have ever smoked and later age of onset smoking) with a later AFB could operate via two mechanisms. First, it is well established that cigarette smoking has a detrimental biological effect on ovarian function and spermatozoa. There is an established link of a longer time to conception and decreased fertility with the increasing number of cigarettes smoked per day. Other studies have linked cigarette smoking to infertility such as problems with preimplantation, shrinking the size and quality of oocytes, and abnormal spermatozoa by decreasing sperm motility in smokers. A second potential mechanism is that the earlier onset of smoking and higher number of cigarettes smoked per day is also highly stratified by socioeconomic status. Smoking and low socioeconomic status are often linked to other environmental risk factors and a higher co-morbidity for other diseases. Smoking is thus often a marker for structural, health and material disadvantage in addition to being concentrated in groups with the lowest levels of education.

7.4.7 Limitations of LD score regression genetic correlations

Although LD score regression is a powerful tool to identify possible relationships between traits, we acknowledge that it does not allow us to establish causal directions or relationships or to adjust for potential mediating factors. The relationship between many of the traits discussed in this section is highly complex with potential bi-directional mechanisms. Further
studies are required to explore these relationships and establish whether the genetic risk
related to AFB and NEB are either partially or fully mediated by other factors.

URLs.

The LDSC software is available at the website: http://www.github.com/bulik/ldsc;


data on birth length, birth weight, Tanner stages have been contributed by EGG Consortium and has been downloaded from www.egg-consortium.org.; data on glycaemic traits have been contributed by MAGIC investigators and have been downloaded from www.magicinvestigators.org; DIAGRAM (type 2 diabetes) summary statistics, http://www.diagram-consortium.org/; SSGAC (educational attainment) summary statistics, http://www.thesgac.org/.
8. LOOK-UP OF LEAD SNPS IN AFB GWAS FOR AGE AT MENOPAUSE AND AGE AT MENARCHE

Following the results on genetic overlap with other phenotypes we tested – in a quasi-phenotype replication setting – whether the SNPs strongly associated with AFB in women are empirically plausible candidates SNPs for age at menarche and age at menopause. Our results reported in the previous section (SI, section 7) indicate a strong genetic correlation between these traits, suggesting a common genetic basis of reproductive behaviour and reproductive life span.

Here we use a two-stage approach that has been applied in other contexts.\textsuperscript{81,181} Since we are only looking at phenotypes measured among women with menarche and menopause, we started our analysis from the meta-analysis results from the AFB sample of women. In the first stage, we conduct a meta-analysis of age AFB excluding the cohorts that were part of the meta-analysis of the phenotype we intend to replicate. This step reduces the risk of overlap between the AFB sample from which the candidate SNPs are drawn and the sample used for testing the other phenotypes. We merged these SNPs with the publically available association results on the most recent GWAS on age at menarche\textsuperscript{116} and age at menopause\textsuperscript{117} from the Reprogen consortium website\textsuperscript{b}. We first merged the two association files and dropped SNPs that are not present in both the files. We aligned the alleles and the effects direction using the software package EasyStrata.\textsuperscript{182} We then selected the independent SNPs with a p-value<1x10^{-5}, using the clump procedure in PLINK\textsuperscript{84}, using the same settings described in section SI.2 (1000Kb window and LD threshold of R^2>0.1) to identify the most significant SNPs in associated regions included in both files. We define “prioritized SNP associations” as those that passed the Bonferroni correction for the number of SNPs tested (P=0.05/122=4.10x10^{-4}, both in age at menarche and age at menopause).

Supplementary Figure 36 shows the QQplots of the leading SNPs for AFB on age at Menarche (panel a) and age at menopause (panel b). Our results identified three SNPs after

\textsuperscript{b} Data downloaded in November 2015 from http://www.reprogen.org/data_download.html
Bonferroni-correction that can be used as good candidates for age at menarche. We do not isolate any clear “candidate SNP” for age at menopause. The three SNPs that we identified (rs9589; rs6803222; rs9858889) are all in Chromosome 3. Two of them (rs9589; rs6803222) lie in proximity (<500Kb) of rs2777888, which has been identified as the strongest signal in our AFB GWAS.
9. BIOLOGICAL ANNOTATION

9.1. Identifying potentially causal variants

We followed the post-GWAS pipeline reported by Vaez et al\textsuperscript{183} to shed light on the genomic context of the 12 independent genome-wide significant SNPs (Table 1 of the main text).

**In silico sequencing:** For *in silico* sequencing, we used the data of the 1000 Genomes Project phase3 release of variant calls. This data set is based on the 20130502 sequence freeze and alignments. We used version v5a (Feb. 20th, 2015), and included only the 503 subjects of European ancestry (accessed April 5, 2016)\textsuperscript{184}. The Variant Call Format (VCF)\textsuperscript{185} files for regions of 1 Mb at either side of each lead SNP were downloaded using the Tabix software package.\textsuperscript{186} Then, the $r^2$ between the lead SNPs and all other bi-allelic SNPs within the corresponding 2 Mb area was calculated as a metric of linkage disequilibrium (LD) using the Plink software package (v1.07).\textsuperscript{84} All SNPs in LD with any of the lead SNPs were then annotated by ANNOVAR software\textsuperscript{187} (version 1 Feb 2016, accessed April 9, 2016). We also used Sorting Intolerant From Tolerant (SIFT)\textsuperscript{188} and Polymorphism Phenotyping (PolyPhen)\textsuperscript{189} prediction scores to characterize the damaging impact of the nonsynonymous SNPs on the corresponding proteins. These scores were obtained from Ensembl release 83 (accessed April 11, 2016).\textsuperscript{190}

**In silico** pleiotropy analysis

To identify any other trait or outcome associated with these 12 independent loci, we used the publicly available data of the National Human Genome Research Institute (NHGRI) GWAS Catalog (Catalog of Published Genome-Wide Association Studies).\textsuperscript{191} We checked for pleiotropic effects of all lead SNPs as well as their linked variants (revealed in the previous phase of *in silico* sequencing) on other complex traits or diseases identified in previous GWAS studies and listed in the GWAS Catalog using ANNOVAR software\textsuperscript{187} (version 1 Feb 2016, accessed April 9, 2016).
9.2. **Gene-based GWAS analysis**

We performed gene-based testing with the full GWAS set (~2.5M HapMap-imputed SNPs) of both phenotypes using VEGAS.\(^1\)\(^,\)\(^2\)\(^,\)\(^3\) This software has the advantage of accounting for LD structure and the possibility to define a range beyond the gene bounds to include intergenic regions in the analysis. We defined a 50kb extra window surrounding the genes and considered every SNP for the gene-based analysis, ran the analyses per chromosome with up to \(10^6\) permutations and considered \(P<2.5\times10^{-06}\) as the threshold for significance (0.05/\(~20.000\) genes).

9.3. **eQTL and mQTL analyses**

eQTL\(^4\)\(^,\)\(^5\) and mQTL\(^6\)\(^,\)\(^7\) analyses performed by the BIOS consortium have been described previously. The methods described in these papers are summarized below.

**Genotype data**

The BIOS consortium used samples from five Dutch cohorts; genotype QC and generation was described previously for each cohort: The Leiden Longevity Study,\(^8\) The Rotterdam Study,\(^9\) The LifeLines-DEEP cohort,\(^10\) The Cohort on Diabetes and Atherosclerosis Maastricht (CODAM)\(^11\) and The Netherlands Twin Register.\(^12\) Genotype data were harmonized towards the Genome of the Netherlands (Genome of the Netherlands Consortium, 2014) (GoNL) using Genotype Hamonizer and subsequently imputed per cohort using Impute2 using the GoNL reference panel (v5). We removed SNPs with an imputation info-score below 0.5, a HWE \(P\)-value smaller than \(10^{-4}\), a call rate below 95% or a minor allele frequency smaller than 0.05.

9.3.2 **RNA data preparation, sequencing and quantification**

Total RNA from whole blood was deprived of globin using Ambions GLOBINclear kit and subsequently processed for sequencing using Illumina’s Truseq version 2 library preparation kit. Paired-end sequencing of 2x50bp was performed using Illumina’s Hiseq2000, pooling samples at 10 per lane, and aiming for \(>15M\) read pairs per sample. Finally, read sets per sample were generated using CASAVA, retaining only reads passing Illumina’s Chastity Filter for further processing. The quality of the raw reads was checked using FastQC. The adaptors identified by FastQC (v0.10.1) were clipped using cutadapt (v1.1) applying default settings (min overlap 3, min length). Sickle (v1.200) was used to trim low quality ends of the
reads (min length 25, min quality 20). Read alignment was performed using STAR 2.3.0e. To avoid reference mapping bias all GoNL SNPs with MAF > 0.01 in the reference genome were masked. Read pairs with at most 8 mismatches, mapping to at most 5 positions were used. Mapping statistics from the BAM files were acquired through Samtools flagstat (v0.1.19-44428cd). The 5’ and 3’ coverage bias, duplication rate and insert sizes were assessed using Picard tools (v1.86). We estimated expression on the gene, exon, exon ratio and polyA ratio levels using Ensembl v.71 annotation (which corresponds to Gencode v.16). Overlapping exons (on either of the two strands) were merged into meta-exons and expression was quantified for the whole meta-exon. To this end, custom scripts were developed which uses coverage per base from coverageBed and intersectBed from the Bedtools suite (v2.17.0) and R (v2.15.1). This resulted in base counts per exon or meta-exon. Expression data was first normalized using Trimmed Mean of M-values (TMM). Then expression values were log2 transformed, probe and sample means were centered to zero. To correct for batch effects, principal component analysis (PCA) was run on the sample correlation matrix and the first 25 PCs were removed. We saw that removing these PCs resulted in highest number of eQTLs detected. To ascertain that none of these 25 PCs are under genetic control, we ran separate QTL mapping on each principal component and ensured that there were no SNPs associated with them. After QC\textsuperscript{194} data was available from 2,116 samples.

9.3.3 Methylation data generation, mapping and normalization.

For the generation of genome-wide DNA methylation data, 500 ng of genomic DNA was bisulfite modified using the EZ DNA Methylation kit (Zymo Research, Irvine, California, USA) and hybridized on Illumina 450k arrays according to the manufacturer’s protocols. The original IDAT files were extracted from the HiScanSQ scanner. We remapped the 450K probes to the human genome reference (HG19) to correct for inaccurate mappings of probes and identify probes that mapped to multiple locations on the genome. Next, we removed probes with a known SNP (GoNL, MAF > 0.01) at the single base extension (SBE) site or CpG site. Lastly, we removed all probes on the sex chromosomes, leaving 405,709 high quality methylation probes for the analyses. Methylation data was directly processed from IDAT files resulting from the Illumina 450k array analysis. After QC,\textsuperscript{195} data was available from 3,841 samples.
9.3.4 eQTL and mQTL analysis

For each of the 12 SNPs identified in the GWAS, local (cis, exons/methylation sites < 1 MB from the SNP) and genome-wide (trans, exons/methylation sites > 5 MB from the SNP) effects were identified by computing Spearman rank correlations between SNPs and local or global exons/methylation sites. Bonferroni multiple testing correction was performed for the 12 SNPs tested ($P<2.5\times10^{-06}$ for cis mQTL analysis, $P<1\times10^{-08}$ for trans mQTL analysis, $P<1.2\times10^{-06}$ for cis eQTL analysis, $P<1.3\times10^{-08}$ for trans eQTL analysis). For each of the significant associations, the exons/methylation sites were selected, the strongest eQTLs were identified for these exons/methylation sites, and LD between these strongest eQTLs and the corresponding SNP identified in the GWAS were computed. LD was computed using BIOS genotypes (the genotypes used for eQTL and mQTL mapping).

9.4. Functional variant analysis using RegulomeDB

We used RegulomeDB\textsuperscript{201} to identify variants amongst the 322 SNPs that reached $P<5\times10^{-08}$ for association with AFB and/or NEB in the meta-analysis of GWAS that likely influence regulation of gene expression. RegulomeDB integrates results from RoadMap Epigenomics\textsuperscript{202} and the ENCODE project.\textsuperscript{203} SNPs that showed most evidence of being functional – defined as a RegulomeDB score $<4$ – were subsequently examined in more detail in terms of effects on gene expression (eQTLs) and their protein-binding capacity (Supplementary Supplementary Table 6).

9.4.1 Gene prioritization using four bioinformatics approaches

Potentially causal genes for the associations identified by GWAS were identified using four previously described bioinformatics tools: ToppGene,\textsuperscript{204} Endeavour,\textsuperscript{205} MetaRanker,\textsuperscript{206} and DEPICT.\textsuperscript{207} To this end, we first retrieved positional coordinates for all lead SNPs according to GRCh37/hg19 using Ensembl’s BioMart. These coordinates were used to subsequently extract all genes located within ±40kb of lead SNPs using the UCSC Supplementary Notebrowser. The identified genes then served as input for ToppGene and Endeavour. Genes with established roles in fertility served as training genes in this procedure, i.e. BRCA1, EGFR, ERBB2-4, HSD17B1, RBM5, ESR1, ESR2 and FSHB. All 10 genes were used in the
pooled and sex-specific analyses, while \textit{ESR1}, \textit{ESR2} and \textit{FSHB} were not used in the analyses in data from men only, for biological reasons. For MetaRanker we provided SNPs that reached $P<5 \times 10^{-04}$ and their chromosomal position as input, together with the previously mentioned set of training genes. Since ToppGene, Endeavour and MetaRanker are biased towards larger and well-described genes, we additionally performed a gene prioritization procedure using DEPICT. All SNPs that reached $P<5 \times 10^{-04}$ in the meta-analysis served as input, and information on prioritized genes, gene set enrichment, and tissue/cell type enrichment were extracted. Genes were subsequently prioritized that reached: 1) $P<0.05$ in DEPICT; or 2) $P<0.05$ in ToppGene, Endeavour and MetaRanker (Supplementary Tables 11, 12).

\subsection*{9.5. Functional network and enrichment analyses}

DEPICT was additionally used to identify gene set, cell type and tissue enrichment analyses, using the GWAS-identified SNPs with $P<5 \times 10^{-04}$ as input. Due to the relatively small number of identified loci, DEPICT was only able to perform these analyses for AFB and NEB pooled, and AFB women.

To construct a functional association network, we combined five prioritized candidate gene sets into a single query gene set: closest genes to the lead SNPs, closest genes to the nonsynonymous SNPs in high LD ($r^2>0.50$) with the corresponding lead SNP, closest genes to other types of SNPs in very high LD ($r^2>0.80$) with the corresponding lead SNP, and expression probe gene names of cis, and trans eQTLs. The single query gene set was then used as input for the functional network analysis. We applied the GeneMANIA algorithm together with its large set of accompanying functional association data. We used the Cytoscape software platform, extended by the GeneMANIA plugin (Data Version: 8/12/2014, accessed April 24, 2016). All the genes in the composite network, either from

\footnote{We initially used a threshold of $P<1 \times 10^{-5}$ for association with the respective outcomes in the meta-analyses of GWAS for SNPs to serve as input for the gene and tissue set enrichment analyses, as per the developers’ recommendations. We contacted the 1st author when this did not yield gene and tissue sets that were significantly enriched, and were advised to apply the slightly more lenient inclusion criterion of $P<5 \times 10^{-4}$.}
the query or the resulting gene sets, were then used for functional enrichment analysis against
Gene Ontology terms (GO terms)\textsuperscript{211} to identify the most relevant GO terms using the same
plugin.\textsuperscript{210}

10. GENE-ENVIRONMENT INTERACTIONS

Previous research based on twin studies shows differential heritability of fertility behaviour
across birth cohorts.\textsuperscript{212,213} With the exception of one recent mega-analysis,\textsuperscript{112} we are not
aware of any study that examines variation at the molecular level to understand whether the
genetic effect of AFB and NEB changes across birth cohort, level of education or other
environmental factors. There is an implicit assumption that the genes associated with
phenotypes are often constant across different historical, geographic or socio-economic
groups. In this section, we therefore examine gene-environment interaction by birth cohort
and educational attainment.

As elaborated upon already in detail in Section 1.5, there has been considerable
environmental variation over time and among groups that has influenced AFB and NEB. It is
plausible, therefore, that there are differences across birth cohorts (time) since individuals
born in different periods face diverse environmental conditions, such as the introduction and
availability of effective contraception, sexual norms and diversity in factors that ‘compete’
with fertility, such as the expansion of educational attainment and labor force participation of
women.\textsuperscript{7}

This builds upon research that has examined changes across cohorts on the genetics of
smoking. An early study adopted a twin design to demonstrate that genetic factors underlying
smoking desistance were more important after the introduction of a restrictive legislation on
smoking.\textsuperscript{214} A related study also showed strong genetic influences on smoking of cohorts
born in the 1920s, 1930s and 1950s, but not for those born in the 1940s and 1960s. They link
these differences to changes in legislation prohibiting smoking in public places.\textsuperscript{215} Using
GREML methods and a modified DeFries-Fulker approach, a recent study likewise
demonstrated that there were cohort differences in the genetic influence on smoking, which
increased over time.\textsuperscript{113}
It may also be the case that the PGS for AFB and NEB is moderated by educational attainment. If the genetic association operates differently by the level of educational attainment, it would provide additional insight into understanding how fertility preferences and education are transmitted across generations. A recent study using the HRS in the US suggested that natural selection has taken place in contemporary societies and that there has been slow selection of lower educational attainment for both sexes. In other words, the study argues that individuals endowed with genes predisposing them to more years of education are having fewer children and that natural selection (of those born from the 1930s to 1953) favors variants associated with less education. A commentary on this article emphasizes four main reasons to be tentative about the conclusions that can be drawn. First, selection on education is weak and evolutionary changes are slow. Second, the PGS for educational attainment is likely associated with many other (non)cognitive traits. Third, socio-environmental, cultural and economic factors often override genetic factors for this phenotype. Fourth, ‘years of education’ is not a precise measurement and finally, that there may be mortality selection in the HRS sample of genotyped individuals, who have a higher socioeconomic status.

10.1 Cohort analysis

We used the Swedish Twin Register (STR) to examine if the effect of a polygenic score (PGS) of AFB and NEB varies across birth cohort. We followed the analysis presented in the recent GWAS of education and divide the sample into six groups based on their year of birth. Each group spans five birth years, with the oldest ranging from 1929-1933 and the youngest born between 1954-1958. We then estimated the following regressions:

\[
AFB_i = \beta_0 + \beta_1 PGS_{AFB} + \beta_2 \text{Sex}_i + \sum_{c=1}^{6} \gamma_{c}^{AFB} \text{cohort}_{ci} + \sum_{c=1}^{6} \gamma_{c}^{PGS_{AFB}} \times \text{cohort}_{ci} + \sum_{k=1}^{10} \beta_k^{PC} C_{ki} + \varepsilon_i
\]

\[
NEB_i = \beta_0 + \beta_1 PGS_{NEB} + \beta_2 \text{Sex}_i + \sum_{c=1}^{6} \gamma_{c}^{NEB} \text{cohort}_{ci} + \sum_{c=1}^{6} \gamma_{c}^{PGS_{NEB}} \times \text{cohort}_{ci} + \sum_{k=1}^{10} \beta_k^{PC} C_{ki} + \varepsilon_i
\]
where $i$ indicate individuals and $k$ indexes principal components (PC) of the genetic data. We used a PGS standardized to have mean 0 and standard deviation 1 based on the GWAS meta-analysis results excluding the STR (details on how we constructed the PGS are available in Section 7 of the SI). The coefficients $\gamma_k$ estimate whether there is an interaction between the PGS and an individual’s birth cohort.

Supplementary Supplementary Table 38 reports the estimated coefficient from these regressions. The results indicate a U-shaped trend in AFB and a linear decline in NEB, but do not provide any clear evidence of interaction effects between the PGS’s and birth cohort. The only interaction coefficient that is significantly different from zero is the interaction between the PGS for NEB in the most recent birth cohort (those born 1954-1958). This analysis is a first descriptive attempt to examine GxE effects with birth cohorts. However, the PGSs are weighted by association coefficients of a GWAS where each cohort consists of individuals born in different years. Moreover, individual cohorts controlled for linear, quadratic and cubic trends in fertility behaviour in their analysis. It would be informative to extend these analyses to more recent cohorts and contexts and refine the approach.

10.2 Educational attainment

We tested the interaction effects between educational level and the PGS of AFB and NEB in three different samples (LifeLines, STR and HRS). To ensure out of sample prediction, the PGS excluded each respective sample as required.

For each cohort, we estimated the following regressions$^d$:

$$AFB_i = \beta_0 + \beta_1 PGS_{AFB_i} + \beta_2 Sex_i + \beta_3 education_i + \beta_4 PGS_{AFB_i} \times education_i + \sum_{k=1}^{10} \beta_k P C_k + \epsilon_i$$

$$NEB_i = \beta_0 + \beta_1 PGS_{NEB_i} + \beta_2 Sex_i + \beta_3 education_i + \beta_4 PGS_{NEB_i} \times education_i + \sum_{k=1}^{10} \beta_k P C_k + \epsilon_i$$

Where $education_i$ is measured as years of education. Supplementary Table 39 reports the estimated coefficient from these regressions. The results indicate that years of education are

$^d$ For HRS, we estimated only a PGS for NEB, since AFB is not collected in that data.
positively associated with AFB in both LifeLines and STR, and negatively associated with NEB in the HRS. With the exception of NEB in the HRS, we found no evidence of GxE effects with education. We can therefore conclude that it appears that education does not appear to moderate the effect of the PGS for AFB and NEB.

11. ROBUSTNESS CHECKS

To estimate the robustness of our results for AFB, we conducted two additional analyses. First, we estimated how the coefficients change if we control for Educational Attainment (EA). Using data from deCODE, we ran an additional association analysis using the 10 loci that were genome-wide significant in the meta-analysis (p-value<5x10^-8). The analysis has been restricted to individuals born between 1910 and 1975, who also had data available on completed education. The total sample size is 42,187 (17,996 males and 24,191 females). The analysis is adjusted for sex, year of birth (linear, squared and cubic), interaction between sex and year of birth and the first 10 PCAs. Education is measured by years of education, ranging between 10 and 20 years. Supplementary Table 40 reports the association results before and after adjusting for educational attainment. Our analysis shows that the effect sizes shrink after including educational attainment as a covariate, with an average reduction of around 15%. We also estimated the effect of a polygenic risk score of AFB calculated from meta-analysis data excluding the deCODE cohort. The polygenic score remains highly significant. The effect of 1SD of the AFB score decreases from 0.19 years (69 days) without controlling for education to 0.16 years (59 days) when we control for years of education. To summarize, this analysis shows that the coefficients are robust to the inclusion of educational attainment in the model.

Second, we estimated how the coefficients change after controlling for Education Attainment (EA) and Age at First Sex using the UKBiobank (N=50,954). We ran two association models: the first follows the GWAS analysis plan with no additional covariates and the second added years of education and age at first sexual intercourse as covariates. The results are presented in Supplementary Table 41 and Supplementary Figure 37. Our analysis shows that the effect sizes of our top hits are highly concordant (R^2=0.94). The inclusion of EA and
AFS as covariates weakens the effect sizes on average by 40% and increases the p-value of
the estimated coefficients. However, both EA and AFS have a significant genetic basis and
are highly genetically correlated with AFB. Therefore, possible genetic pleiotropy may affect
the results and capture a considerable proportion of the genetic effect. Nevertheless, 7 SNPs
out of 10 tested, have a p-value<0.05 in the model that controls for EA and AFS. Overall, we
interpret this additional analysis as a robustness test that confirm that the top hits from our
meta-analysis are robust to the inclusion of the confounding factors of EA and AFS.

12. POSITIVE SELECTION

We performed a Haploplotter analysis\(^{219}\) to examine if lead SNPs and/or functional variants
identified using RegulomeDB show evidence of positive selection. Three variants showed
standardized integrated haplotype scores <-2 or >2, indicating that these variants represent
the top 5% of signals in the population. These SNPs are: 1) rs7628058 on chromosome 3 for
AFB, an eQTLs for RBM6 in monocytes; 2) rs2247510 on chromosome 3 for AFB, an eQTL
for RBM6 and HYAL3 in monocytes and binding site for a range of transcription factors; 3)
rs2415984, the lead SNP in the chromosome 14 locus for NEB. Results are presented in
Supplementary Table 42.

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External scientists can apply to the Steering Committee of BASE-II for data access. Although the data are available for other parties are scientific data and not personal contact data, the scientific data are subject to a security level as if they were personal data to ensure that the BASE-II Steering Committee sufficiently protects the large volume of data collected from each BASE-II participant. All existing variables are documented in a handbook. Contact: Katrin Schaar, scientific coordinator, schaar@mpib-berlin.mpg.de.

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GOYA
The Danish National Research Foundation established the Danish Epidemiology Science Centre, which initiated and created the Danish National Birth Cohort. The cohort is a result of a major grant from this Foundation. Additional support for the Danish National Birth Cohort was obtained from the Pharmacy Foundation, the Egmont Foundation, the March of Dimes Birth Defects Foundation, and the Augustinus Foundation. Genotyping for the GOYA Study within the Danish National Birth Cohort was funded by the Wellcome Trust (Grant ref: 084762MA).

HBCS

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Health 2000

The Health 2000 Study was mainly funded from the budget of the National Institute for Health and Welfare (THL). Additional funding was received from the Finnish Centre for Pensions, the Social Insurance Institution of Finland, the Local Government Pensions Institution, the National Research and Development Centre for Welfare and Health, the Finnish Dental Association, the Finnish Dental Society, Statistics Finland, the Finnish Institute for Occupational Health, The Finnish Work Environment Fund, the UKK Institute for Health Promotion Research and the Occupational Safety and Health Fund of the State Sector. The data used for this study can be made available on request to the Health 2000/2011 scientific committee according to the ethical and research guidelines (www.terveys2011.info/aineisto) as well as Finnish legislation.

Health ABC

The Health ABC Study was supported by NIA contracts N01AG62101, N01AG62103, and N01AG62106 and, in part, by the NIA Intramural Research Program. The genome-wide association study was funded by NIA grant 1R01AG032098-01A1 to Wake Forest University.
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HRS (Health and Retirement Study): HRS is supported by the National Institute on Aging (NIA U01AG009740). The genotyping was funded separately by the National Institute on Aging (RC2 AG036495, RC4 AG039029). Our genotyping was conducted by the NIH Center for Inherited Disease Research (CIDR) at Johns Hopkins University. Genotyping quality control and final preparation of the data were performed by the Genetics Coordinating Center at the University of Washington. Data Access: HRS (Health and Retirement Study): Genotype data can be accessed via the database of Genotypes and Phenotypes (dbGaP, http://www.ncbi.nlm.nih.gov/gap, accession number phs000428.v1.p1). Researchers who wish to link genetic data with other HRS measures that are not in dbGaP, such as fertility data, must apply for access from HRS. See the HRS website (http://hrsonline.isr.umich.edu/gwas) for details.

HTO

We thank all the families who contributed to this study. Phenotyping and genotyping of the HTO cohort was funded by the Wellcome Trust, the UK Medical Research Council and the British Heart Foundation. Data are available upon request from the Principal Investigator, Bernard Keavney (bernard.keavney@manchester.ac.uk).

INGI-CARL

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INGI-Val Borbera
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LIFELINES
Lifelines is a multi-disciplinary prospective population-based cohort study examining in a unique three-generation design the health and health-related behaviours of 167,729 persons
living in the North of The Netherlands. It employs a broad range of investigative procedures in assessing the biomedical, socio-demographic, behavioural, physical and psychological factors which contribute to the health and disease of the general population, with a special focus on multi-morbidity and complex genetics. The Lifelines Cohort Study, and generation and management of GWAS genotype data for the Lifelines Cohort Study is supported by the Netherlands Organization of Scientific Research NWO (grant 175.010.2007.006), the Ministry of Economic Affairs, the Ministry of Education, Culture and Science, the Ministry for Health, Welfare and Sports, the Northern Netherlands Collaboration of Provinces (SNN), the Province of Groningen, University Medical Center Groningen, the University of Groningen, Dutch Kidney Foundation and Dutch Diabetes Research Foundation. The authors wish to acknowledge the services of the Lifelines Cohort Study, the contributing research centers delivering data to Lifelines, and all the study participant. Data availability: Lifelines is a facility that is open for all researchers. Information on application and data access procedure is summarized on www.lifelines.net.

Longevity
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MoBa
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Data availability

Data are available upon request from the NESDA data management bureau.

Nurses’ Health Study (NHS) and Health Professionals Follow-up Study (HPFS)

Supported by grants UM1 CA186107, UM1 CA167552, DK091718, HL071981, HL073168, CA87969, CA49449, CA055075, HL34594, HL088521, U01HG004399, DK080140, SP30DK46200, U54CA155626, DK58845, U01HG004728-02, EY015473, DK70756 and DK46200 from the National Institutes of Health, with additional support for genotyping from Merck Research Laboratories, North Wales, PA.

NTR (Netherlands Twin Register)

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OGP Ogliasta Genetic Park

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Rotterdam Study

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Sorbs

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THISEAS

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TwinGene (STR)

STR (Swedish Twin Registry) – The Jan Wallander and Tom Hedelius Foundation (P2012-0002:1), the Ragnar Söderberg Foundation (E9/11), The Swedish Research Council (421-2013-1061), the Ministry for Higher Education, The Swedish Research Council (M-2205-1112), GenomEUtwin (EU/QLRT-2001-01254; QLG2-CT-2002-01254), NIH DK U01-066134, The Swedish Foundation for Strategic Research (SSF). Researchers interested in using STR data must obtain approval from the Swedish Ethical Review Board and from the Steering Committee of the Swedish Twin Registry. Rietveld gratefully acknowledges funding
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Researchers using the data are required to follow the terms of an Assistance Agreement containing a number of clauses designed to ensure protection of privacy and compliance with relevant laws. For further information, contact Patrik Magnusson (Patrik.magnusson@ki.se).

TwinsUK

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Data availability: Data are available upon request from the TwinsUK data management bureau.

UKBiobank

This research has been conducted using the UK Biobank Resource

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Supplementary Figure 21 Regional association plot of rs293566 (chr20:31097877), a genome-wide significant SNP for AFB women.
Supplementary Figure 22  Forest plot for rs242997 (chr22:34503059), a genome-wide significant SNP for AFB pooled.

Supplementary Figure 23  Regional association plot of rs242997 (chr22:34503059), a genome-wide significant SNP for AFB women.

Supplementary Figure 24  Forest plot for rs10908474 (chr1:153753725), a genome-wide significant SNP for NEB pooled.

Supplementary Figure 25  Regional association plot of for rs10908474 (chr1:153753725), a genome-wide significant SNP for AFB women.

Supplementary Figure 26  Forest plot for rs13161115 (chr5:107050002), a genome-wide significant SNP for NEB men.

Supplementary Figure 27  Regional association plot of for rs13161115 (chr5:107050002), a genome-wide significant SNP for NEB men.

Supplementary Figure 28  Forest plot for rs2415984 (chr14:46873776), a genome-wide significant SNP for NEB pooled.

Supplementary Figure 29  Regional association plot of for rs2415984 (chr14:46873776), a genome-wide significant SNP for NEB pooled.

Supplementary Figure 30  Bivariate analysis of the two fertility-related traits, comparing to each of the single trait analysis.

Supplementary Figure 31  Conditional analysis of the two fertility-related traits, comparing to each of the single trait analysis.

Supplementary Figure 32  Assessing the extent to which population stratification affects the estimates from the GWAS of Age at first birth.

Supplementary Figure 33  Assessing the extent to which population stratification affects the estimates from the GWAS of number of children ever born.

Supplementary Figure 34  Miami plots for AFB and NEB sex-specific single genomic control meta-analysis.

Supplementary Figure 35  Quantile-quantile plots of SNPs for AFB (panel a) and NEB (panel b) in single genomic control, meta-analysis.

Supplementary Figure 36  Look-up of female AFB SNPs with p<1x10^{-04} for association with age at Menarche and Age at Menopause. Quantile-quantile plots.
Supplementary Figure 37  Comparison of effect size of 10 SNPs associated with AFB in the meta-analysis before and after controlling for educational attainment and age at first sexual intercourse.
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