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Molecular insights into the aetiology of reproductive ageing in females

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Abstract

As age at puberty declines and age at first pregnancy increases, the mechanisms that regulate reproductive lifespan become increasingly relevant to population health. The timing of menarche and menopause can have profound effects, not only on fertility, but also on the risks of diseases such as type 2 diabetes, cardiovascular disease and breast cancer. Genetic studies have identified dozens of highly penetrant rare mutations associated with reproductive disorders, and also ~175 common genetic variants associated with timing of puberty or menopause. These findings, alongside other functional studies, have highlighted a diverse range of mechanisms involved in reproductive ageing, implicating core biological processes such as cell cycle regulation and energy homeostasis. The aim of this article is to review the contribution of such genetic findings to understanding the molecular regulation of reproductive timing, and the biological basis for epidemiological links between reproductive ageing and disease risks.

Introduction

The timing of reproductive ageing in women varies substantially in normal populations and this has profound social, economic and clinical impacts^{1–4}. Reproductive lifetime in women can be defined as the time between the onset of puberty and oocyte depletion at menopause. The first signs of puberty typically occur around ages 8–13 years⁵, with a broader window of menopause timing between ages 40–60 years⁶. In rare cases, these timings are disrupted. Around 1% of women have Primary Ovarian Insufficiency (POI) defined as the occurrence of menopause before age 40 years⁷. Natural fertility declines on average 10 years before the menopause and the relevance of early menopause on infertility is increasing as maternal ages at child birth steadily rise in many Western populations^{8,9}. At the start of the reproductive lifetime, precocious or delayed/absent puberty are disorders with significant adverse physiological and psychological consequences^{10,11}. Within these extremes, normal

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variation in reproductive ageing is associated with the risks for a wide range of disease outcomes. These have been well-described by epidemiological studies that record the timing of well-recalled reproductive ageing milestones, such as the age at menarche (first menstrual bleed) and the age at natural menopause (in women without surgical or medical causes of oocyte depletion).

Until recently, there was little genetic overlap between the loci/genes identified for the timings of puberty and menopause. However, new statistical genetics techniques have estimated a modest shared genetic aetiology for puberty and menopause timings^{12,13}, and these estimates are supported by new epidemiological evidence linking these two traits¹⁴. Furthermore, a number of GWAS loci for age at natural menopause are located in/near genes that regulate the hypothalamic-pituitary reproductive axis (*CHD7*, *FGFR1*, *SOX10*, *KISS1* and *TAC3*)¹³, indicating that the same mechanisms may regulate both extremes of reproductive ageing.

The widely reported links between reproductive ageing and other health outcomes have prompted a number of research questions. Firstly, what biological processes and which genes contribute to the wide population variance in reproductive timing? In turn, which of these processes link reproductive ageing to disease? Lastly, can genetic tools help to identify those individuals at high risk of abnormal reproductive ageing in order to inform their health and reproductive choices? This issue is particularly relevant for early menopause, where accurate prediction is yet only possible within 5-years of the event. This review aims to describe how recent genetic studies have provided molecular insights, illustrating key findings that have transformed our understanding of reproductive ageing and its relevance to disease.

The genetic architecture of reproductive ageing

The identification of genes that regulate reproductive function has been informed by targeted DNA sequencing in individuals with rare disorders of reproductive timing and, more recently, by large-scale array genotyping of single nucleotide polymorphisms (SNPs) in population-based samples.

Rare Mendelian disorders—Clinical cases with Idiopathic Hypogonadotropic Hypogonadism (IHH; delayed/absent puberty) or POI have long been studied using DNA sequencing. For each condition, around 20-30 genes have been identified^{15–37} (Figure 1), exhibiting autosomal dominant, autosomal recessive, X-linked and oligogenic inheritance patterns. A sizable fraction of IHH cases (around 30-40%) are explained by such gene defects, however this proportion is much lower for POI (~10%), with expanded triplet repeats in *FMR1* or other X-chromosome abnormalities found in only ~5%. The identification of genetic causes in cases with central precocious puberty is even more difficult, with the recent report of paternally-inherited loss of function mutations in *MKRN38* by whole-exome sequencing adding to reported activating mutations in *KISS* and *KISSR39*. Recently, rare pathogenic mutations for IHH have been also been implicated in the aetiology of less severe, self-limiting delayed puberty⁴⁰ and this raises the question

whether such pathogenic mutations might contribute significantly to the normal population variance in puberty timing.

Population-based studies—In the complementary population-based approach, Genome-wide Association Studies (GWAS) have now identified ~150 distinct genomic loci associated with the timings of puberty or menopause. These studies are designed to identify common genetic variants, each with small effect, but in aggregate may contribute substantially to the wide variation in reproductive ageing in normal populations (Table 1). Individual effect sizes for common variants (minor allele frequency [MAF] >5%) range from ~2-5 weeks for age at menarche⁴¹, with similar estimates obtained from follow-up studies of timing of breast development and other physical changes of puberty⁴². Low-frequency variants (defined as MAF 1-5%) in *ALMS1* have the largest reported effect on puberty timing in normal populations – the rare allele delays menarche by ~3 months⁴³. For age at menopause, there are now 56 reported common or low-frequency variants ranging in effect size from 0.07-0.88 years per allele¹³. In aggregate these loci explain only a small proportion of the population variance for each trait (~5%), however statistical modelling suggest that common genetic variation, when eventually fully characterised, will explain a substantial component of the trait heritability. The genetic architecture of these traits likely involves tens of thousands of variants, potentially implicating hundreds if not thousands of genes.

GWAS benefits from a ‘hypothesis-free’ approach to gene identification, utilising the properties of Linkage Disequilibrium (LD), the high correlation between inherited variants, to ensure broad coverage across the genome. The drawback of LD is the uncertainty regarding the identity of the causal genes and variants in associated genomic regions (“loci”). This is compounded by the difficulty in experimentally testing large numbers of genes/variants using high-throughput methods sensitive enough to detect the likely small molecular effects. Despite these limitations, genomic and bioinformatics approaches can help to map plausible causal genes. In turn, pathway analyses across groups of plausible genes can powerfully implicate important aetiological mechanisms, whose significance remains even if a few of the mapped genes are incorrect. For example, 29 of the 44 genomic regions identified for age at menopause map in/near genes involved in DNA damage-response (DDR), including 18 loci where the DDR gene candidate is either the nearest, or is linked via altered mRNA expression levels (eQTL)¹³. This degree of enrichment far exceeds that expected by chance, and would remain significant even if some candidate DDR genes were incorrectly mapped.

Allelic heterogeneity—Considerable allelic heterogeneity and overlap exists across many of the genes identified for reproductive timing (Figure 2). Notable examples include the report of autosomal recessive loss of function alleles in *TACR3* as a relatively frequent finding in IHH cases⁴⁴, whilst haploinsufficiency of the same *TACR3* alleles is associated with a pubertal delay of ~1.25 years in the general population⁴³, and common variants (MAF ~15%) near this gene have a much smaller ~2.5 week impact⁴¹. Similarly, loss of function mutations in *KISS1R* cause IHH, whereas gain of function mutations cause the opposite phenotype of precocious puberty³⁹. Interestingly, common variants associated with

menopause timing map within ~50kb of this gene¹³. The DNA helicase B gene (*HELB*) locus contains multiple statistically independent common variants associated with menopause timing, in addition to harbouring lower-frequency and relatively higher penetrance missense alleles¹³. Common variants and rare alleles at the imprinted gene *MKRN3* cause physiological variation and rare clinical disorders, respectively, and both types exhibit paternal-specific parent-of-origin effects^{38,41}.

Furthermore, there is overlap between common loci for reproductive timing and genes disrupted in rare syndromes where disordered reproductive ageing is one of several clinical features, such as *IGSF145* and *PCSK146* (Figure 2).

As technology evolves, it is likely that the above two research study designs will yield further overlaps. The allelic heterogeneity observed at many loci suggests that targeted DNA sequencing at GWAS highlighted genes in cases with reproductive disorders will yield novel molecular diagnoses. Eventually, the comprehensive cataloguing of all genetic variation – common, rare and complex – in very large population-based samples will lead to better estimates of genetic penetrance, and will allow evaluation of the impact of common variation on extreme phenotypes, and the impact of rare variants on common phenotypes. Ultimately, better understanding of the genetic architecture for reproductive ageing across the full phenotypic spectrum may lead to accurate prediction and the classification of disease states informed by genotype.

Biological mechanisms inferred from molecular studies of reproductive ageing

Energy homeostasis and reproductive ageing—Epidemiological studies consistently report links between earlier age at menarche and higher BMI or obesity risk. A causal relationship between these traits is strongly supported by their strong genetic correlation¹². Almost all of the GWAS-reported BMI-increasing alleles have effects also on earlier timing of menarche^{41,47}, which are directionally concordant with the traditional epidemiological association⁴⁸, and this supports the presence of a general mechanism linking weight status to puberty timing and reproductive function (Figure 3). Leptin, an adipocytokine with potent appetite-suppressing central actions, has been proposed as such a key signal. Rare deleterious mutations in the leptin gene (*LEP*) cause severe early onset obesity associated with hypogonadotropic hypogonadism⁴⁹, however leptin therapy allows normal puberty timing rather than precocious puberty⁵⁰, indicating that leptin has permissive rather than direct actions. More recently, a low-frequency missense variant in *PRKAG1* was associated with earlier age at menarche⁴³ (rs1126930; MAF 3.4%; beta=-0.09 years/allele, P=9.6x10⁻¹¹). *PRKAG1* encodes the gamma-1 regulatory subunit of AMP-activated protein kinase, which senses and maintains cellular energy homeostasis by promoting fatty acid oxidation and inhibiting fatty acid synthesis. The identification of specific fatty acids that activate pituitary gonadotropes is a topic of ongoing investigation⁵¹. Insulin has various peripheral actions that promote sex hormone secretion (from gonads and adrenals), aromatisation (in adipocytes) and bioavailability and could therefore potentially link weight status to puberty timing.

Other evidence shows that many GWAS loci for adult BMI influence weight gain and adiposity from infancy and early childhood⁵², which is consistent with the established

dependence of the reproductive axis activity on energy balance, although the causal relationship between BMI and puberty timing might yet be bi-directional. Notable exceptions to this model of shared biological mechanisms include GWAS loci at *MC4R* and *LEPR*. Despite a relatively large effect on BMI, evident in children of both sexes, *MC4R* variants are unrelated to puberty timing in girls^{41,47}, although a possible association is reported in boys⁴². In contrast, the GWAS locus at the leptin receptor gene (*LEPR*) influences pubertal timing but not adult BMI, indicating a possible additional role for the leptin pathway outside of its established actions on energy homeostasis.

In contrast to puberty timing, the epidemiological relationship between BMI and timing of natural menopause has been less clearly described. Recent systematic reviews describe a potential mildly protective effect of overweight and obesity on risk of early menopause, although with moderate heterogeneity between studies and variable consideration of smoking and other potential confounders^{53,54}. The recent large GWAS reported significant inverse genome-wide genetic correlations between age at natural menopause and adult obesity ($r_g = -0.15$, $P = 0.0004$) and adult BMI ($r_g = -0.13$, $P = 0.003$)¹³, which support a shared aetiological basis, but the actual mechanisms linking higher BMI to later menopause are yet unclear.

Hypothalamic-pituitary-gonadal axis—Delineation of the central hypothalamic-pituitary axis that regulates gonadal sex steroid production and fertility has been largely informed by studies of monogenic reproductive disorders (Figure 3). Rare mutations that disrupt the embryonic migration from the nasal placode of hypothalamic gonadotropin-releasing hormone (GnRH) producing cells cause Kallman syndrome (hypogonadotropic hypogonadism associated with anosmia)¹⁵. Other rare mutations more specifically disrupt GnRH cell function, e.g. genes that encode kisspeptin (*KISS1*), neurokinin B (*TAC3*) and GnRH (*GNRH1*) and their receptors (*KISSR*, *TACR3* and *GNRHR*). Central hypogonadism is also a consequence of mutations that disrupt the development of pituitary gonadotropes, such as in *HESX1* and *SOX2*, or mutations in genes that encode the pituitary hormone follicle-stimulating hormone (FSH) β -subunit⁵⁵ or its receptor (*FSHR*)⁵⁶. Conversely, *MKRN3* has recently been identified as a paternally-expressed hypothalamic repressor of pulsatile GnRH secretion³⁸.

The (expected) concordance between the mechanisms that underlie rare reproductive disorders and those that contribute to population variance in reproductive timing has been recently demonstrated by the enrichment of age at menarche GWAS signals in or near to genes that underlie monogenic disorders of puberty⁴¹. These include age at menarche GWAS signals in or near to genes that regulate GnRH cell function (*MKRN3*, *GNRH1*, *TACR3*, *LEPR*), hypothalamus/pituitary development (*POU1F1*, *TENM2*) or pituitary hormone processing (*PCSK1*, *PCSK2*), as well as other signals with potential roles in hypothalamus/pituitary development (*FRS3*, *LGR4*, *TBX6*)⁴¹. Furthermore, a recent age at menarche GWAS signal was reported on the X chromosome in/near *IGSF1*⁴³. Rare X-linked mutations in *IGSF1* were recently described to cause central hypothyroidism, hypoprolactinemia, delayed puberty and macro-orchidism in males, but a yet uncertain reproductive phenotype in heterozygous females^{45,57}. Retinoic acid-related nuclear hormone receptors were implicated by GWAS signals for age at menarche in/near *RXR α*

and *RORA*, as well as sub-genome-wide signals in/near *RXRA*, *RORB* and *RXRβ*. These could mediate effects of retinoic acid signalling on GnRH secretion⁵⁸ or could regulate other hormone receptor sensitivities.

A more surprising genetic finding has been the relevance of components of the central hypothalamic-pituitary axis to the population variance in menopausal timing. GWAS loci for age at menopause are enriched in/near genes implicated in monogenic disorders of puberty, with five signals in/near genes disrupted in hypogonadotropic hypogonadism (*KISS1R*, *TAC3*, *CHD7*, *SOX10* and *FGFR1*). A further menopause signal in/near *FSHB* is also associated with circulating FSH levels. Previously, the menopausal rise in hypothalamic/pituitary activity was thought to be a passive consequence of the loss of sex steroid feedback inhibition⁵⁹. While it is possible that these components may also have peripheral actions directly on the ovary⁶⁰, there appears to be increasing evidence for a central neural control of reproductive ageing.

Gene silencing—The hypothalamic-pituitary-gonadal axis (Figure 3) is already active in utero and during early infancy – hence, as well as requiring the maturation of excitatory components, the onset of puberty may be portrayed by the silencing of repressive factors that are active during childhood⁶¹. Evidence to support this model has been reported by recent animal studies and human GWAS findings, described below.

The first GWAS for age at menarche identified common variants in *LIN28B*, encoding a potent and specific regulator of let-7 microRNA pre-processing. let-7 microRNAs are developmental stage-specific post-transcriptional silencers of numerous target genes. The *LIN28*-let-7 axis maintains cell pluripotency⁶² has been shown in experimental models to regulate pubertal development, glucose metabolism, tissue repair and cancer risk⁶³. Subsequently, a recent exonic-focussed study for age at menarche identified a low-frequency missense variant in *TNRC6A* associated with later age at menarche⁴³. *TNRC6A* encodes an Argonaute-navigator protein, which, akin to the *LIN28*-let-7 axis, is responsible for post-transcriptional gene silencing through a variety of RNA interference and microRNA pathways⁶⁴.

The most recent GWAS for age at menarche in women identified an enrichment of associated regions in or near known imprinted genes⁴¹. These are genes where expression is silenced by stable DNA methylation marks that are determined by the parental origin of the alleles. Specifically, genome-wide significant signals for age at menarche were located at three imprinted gene regions, *MKRN3-MAGEL2*, *DLK1*, and *KCNK9*, and at these signals the associations were dependent on the parental origin of alleles, consistent with expression patterns. Thus, at the maternally-imprinted (silenced) loci (*MKRN3-MAGEL2* and *DLK1*) only the paternally-inherited alleles but not the maternally-inherited alleles were associated with age at menarche, and vice versa for the paternally-imprinted locus, *KCNK9*. These findings suggest possible parental origin-specific selection advantages of alleles that regulate reproductive timing.

An elegant model of epigenetic repression of the hypothalamic-pituitary-gonadal axis was recently described in female rats⁶⁵. Towards the onset of puberty, DNA methylation

decreases the hypothalamic expression of two key Polycomb group genes, *Eed* and *Cbx7*, in key hypothalamic neurons. As well as decreasing the levels of repressive EED and CBX proteins, an increase in activating histone modifications likely also contributes to the upregulation of *Kiss1* expression and the onset of pulsatile GnRH secretion. The relevance of this epigenetic model in humans is supported by recent GWAS findings, which identified a menarche-associated locus at *CBX7* as well as strong enrichment of menarche-associated signals near genes that encode activating JmjC-domain-containing histone demethylases⁴¹.

Establishing the oocyte pool—The central dogma of reproductive biology is that the oocyte pool is finite and oocytes produced during fetal development steadily apoptose throughout life until menopause (Figure 4). This has been challenged by experiments that demonstrate the presence of ovarian stem cells in mice and humans, which can be experimentally manipulated to generate functional oocytes^{66–68}. Primordial germ cells migrate to the gonadal ridge during week 6 of development when the diploid germ cells are termed oogonia⁶⁹. Multiple rounds of mitosis generate a pool of ~7 million oogonia by 6 months gestation. Meiosis starts at around week 11–12, such that the fetal ovary contains a mixture of both mitotic and meiotic germ cells. Oocytes are arrested in the first meiotic division until ovulation, many years later⁷⁰. This process is controlled by cAMP secreted by the surrounding granulosa cells in the ovary. Atresia of oocytes begins during fetal life and continues until menopause, with only a few hundred oocytes lost through ovulation. Whether stem cells in the human ovary contribute to the physiological follicle pool has yet to be determined. It is possible that stem cells generate oocytes continually throughout life, but that this is a relatively small contribution to the overall pool and the process becomes inefficient with age and is therefore not sufficient to prevent menopause⁷¹. However the presence of stem cells in human ovaries has generated substantial interest in the possibilities for activating oogenesis in women with poor ovarian reserve, or for preserving fertility in women undergoing chemotherapy for example, where current techniques are inefficient.

Meiosis and non-disjunction—As women age the risk of chromosomal abnormalities in their offspring increases substantially, along with risk of miscarriage, as a result of errors in segregation of chromosomes (non-disjunction) resulting in aneuploidy. The majority of non-disjunction errors occur during maternal meiosis 1, when meiosis resumes following puberty and maternal age is a major risk factor⁷². However it is still not clear what underlying molecular mechanism explains why female meiosis is more error prone than male meiosis and why age has such a large impact on the likelihood of errors. In 1968 Henderson and Edwards proposed the ‘production line hypothesis’ of oogenesis, which suggests that oocytes are ovulated in the order in which they are produced and that recombination rates are higher in oocytes entering meiosis earlier compared to oocytes going through meiosis at a later stage⁷³. There is evidence in animal models and humans to support the first element of production line hypothesis, that oocytes are ovulated in the order in which they are produced^{74,75}. However the role of ovarian ageing is less clear. Recombination rate is certainly a risk factor for non-disjunction and is much more variable in females than in males, which might explain why female meiosis is more error prone. However its role in ovarian ageing is less clear as recent studies in human fetal ovarian tissue suggest that recombination rate does not vary substantially with age⁷⁶. Alternatively, the maternal age

effect may reflect the age-related decline in oocytes expression of cohesin, a complex which tethers sister chromatids during meiosis. Recessive mutations *STAG3*, encoding a component of this complex, and a mouse knockout model demonstrate that the cohesin complex is essential for oocyte development. Affected cases present with primary amenorrhea and mutant mice show meiotic arrest in leptotene with ovaries devoid of follicles by one week of age³⁴. The synaptonemal complex is also a key chromosome maintenance structure in meiotic recombination. It is composed of three different protein subunits and is involved in homologous chromosome pairing. Mutation in *SYCE1* which encodes a component of this complex has been described in a consanguineous family; both affected two sisters had primary amenorrhea, but one had normal pubertal timing and the other delayed puberty⁷⁷. Thus it appears that maintenance of chromosome pairs during meiosis is essential for both correct segregation of chromosomes and for oocyte viability.

Twinning and ageing—The rate of dizygous (but not mono-zygous) twinning increases with maternal age, peaking at 35-39 years⁷⁸. The mechanism is not understood. Five to ten follicles are recruited from the oocyte pool per menstrual cycle in response to the rise in FSH secretion. Selection of a dominant follicle is thought to be regulated by AMH secreted by maturing follicle granulosa cells to suppress the growth of other follicles^{79,80}. It is possible that the age-related rise in FSH and decline in AMH may disrupt mono-ovulation and increase the incidence of two oocytes being ovulated.

DNA damage repair—Recent genetic studies have highlighted the substantial role of DNA damage-response (DDR) mechanisms in ovarian ageing, reflecting the susceptibility of oocytes to DNA damage throughout life⁸¹. Double-strand DNA breaks accumulate with age in human and mouse ovarian follicles, with concomitant down-regulation of the key DNA repair genes *BRCA1*, *MRE11*, *Rad51* and *ATM*⁸². Double strand break repair also resolves the chromatid cross-overs that result from recombination in meiosis 1, thus inefficient repair could also result in oocyte loss during early development. About two thirds of GWAS loci for menopause timing contain DDR-related genes⁸¹. In addition there are several examples of rare monogenic mutations in DDR genes causing early menopause or primary ovarian insufficiency (POI)^{26,35,36}. Carriers of cancer predisposing mutations in the DDR genes *BRCA1* and *BRCA2* have reportedly earlier age at natural menopause than other women^{83,84}. Recessive mutations in two gene members of the minichromosome maintenance family reportedly segregate with the POI phenotype in three large consanguineous kindreds (MCM8³⁵ and MCM9³⁶). Concordantly, a GWAS locus at *MCM8* has a relatively large effect on normal menopause timing, with each minor allele increasing menopause age by ~1 year⁸¹. Fibroblasts and lymphocytes cultured from patients with POI and mutations in *MCM8/9* display chromosome instability. Both *MCM8* and *MCM9* mutations inhibited the formation of foci at the site of double strand breaks. MCM8 and MCM9 form a complex with each other at sites of double strand breaks and recruit RAD51, to promote homologous recombination repair⁸⁵. Mouse models of these two genes show gonadal failure and chromosome instability, demonstrating the key role of double strand break repair in oocyte survival⁸⁶.

Other DDR processes are implicated in ovarian ageing. Mitochondrial function may influence the ovarian reserve through accelerated apoptosis. Mutations in three genes that encode mitochondrial proteins result in Perrault syndrome, characterised by POI or primary amenorrhea plus sensorineural hearing loss^{87–89}. Increased oocyte apoptosis is also the proposed mechanism by which *FMR1* expansion limits reproductive lifespan. Carriers of 60–200 copies (“premutation alleles”) of the polymorphic CGG repeat sequence in *FMR1* have a fivefold higher risk of early menopause³⁷. Mouse models show that the CGG repeat expansion acts through a RNA-mediated mechanism resulting in atresia of developing follicles, rather than a limited primordial oocyte pool size^{90,91}.

Whether women with premature ovarian ageing have more generalised premature ageing is not known. Women with progeria syndromes, such as Hutchinson Gilford syndrome or Werner syndrome, often exhibit premature menopause or primary hypogonadism⁹². DDR mechanisms could potentially contribute to the epidemiological links between early age at menopause and risk of other age-related diseases.

Autoimmunity—10–30% of cases of POI may have an autoimmune aetiology, as indicated by detectable anti-ovarian antibodies or co-segregation with an established autoimmune disorder, such as thyroiditis or Type 1 diabetes⁹³. However, unlike most other autoimmune disorders, there is not yet robust evidence for an HLA haplotype association with POI or age at natural menopause^{94,95}. A GWAS locus for age at natural menopause is located in the HLA region on chromosome 6, but association with specific HLA haplotypes has yet to be confirmed⁸¹. Other GWAS loci implicate the immunity-related genes *IL11* and *NLRP11*. Female mice with null mutations in the *IL-11* receptor gene are infertile due to defective uterine decidualization⁹⁶, while the *NLRP* gene family regulates both innate immunity and reproduction. Several *NLRP* genes show an oocyte specific expression pattern⁹⁶, while *NLRP5* has been implicated in POI and encodes an autoantigen in a mouse model of autoimmune POI^{97,98}.

Genetic insights into mechanisms that link reproductive ageing to other health-related traits

Numerous epidemiological studies have linked the timing of reproductive ageing to various adverse health outcomes; however in many cases the causal nature of these associations and their underlying mechanisms are unclear. Supporting evidence from genetic analytical modelling can help to reduce confounding and avoid recall bias. Furthermore, the identification of shared biological mechanisms between two traits may also increase the priors that they are causally related. In addition to the strong genetic correlation between higher BMI or obesity risk and earlier puberty timing (described above), genetic findings support other mechanisms that could explain observed the links between reproductive ageing and other health-related traits.

Anthropometric traits—The shared genetic architecture between puberty timing and adult height reflects the complex relationships between these traits. Girls who develop puberty early are consistently taller than other girls during early and mid-childhood, but due to earlier cessation of growth are usually shorter as adults⁹⁹. A recent analysis using LD

Score regression identified an overall positive genome-wide genetic correlation between age at menarche and adult height ($r_g=0.11$, $P=6\times 10^{-5}$)¹², which supports the causal nature of the overall epidemiological association⁹⁹. Directionally consistent with that observation, the menarche age-raising alleles at two GWAS loci (in *LIN28B* and *SIX6*) were also associated with taller adult height⁴¹. These genes have plausible regulatory functions in reproductive maturity. The role of *LIN28B* in gene silencing and repressing developmental regulation is described above. *SIX6* encodes a homeodomain transcription factor that regulates the transcription of gonadotrope-specific genes in pituitary gonadotrope cells¹⁰⁰. Conversely, at the GWAS locus *CENPW*, which encodes an RNA-associated nuclear matrix protein, the menarche age-raising allele was associated with shorter adult height⁴¹, which is directionally discordant with the overall observed association. Furthermore, this variant was the only menarche signal to be associated with the autoimmune disorder type 1 diabetes⁴¹. These diverse associations may indicate multiple pleiotropic roles of this specific *CENPW* locus on growth, puberty timing and disease risk. By contrast, there is no overlap between GWAS loci identified for age at menopause and height and no genetic correlation between these traits ($r_g=0.006$; $P=0.88$)¹³.

The GWAS locus near *LEKRI* and *CCNLI* is common to puberty timing⁴¹, birth weight¹⁰¹ and central adiposity¹⁰² (waist-to-hip ratio adjusted for BMI). Here the menarche age-raising allele also confers higher birth weight and greater central adiposity, consistent with the epidemiological associations between these traits¹⁰³. *CCNLI* encodes a regulator of gene transcription and pre-mRNA splicing. The menarche age-raising allele has also been associated with lower insulin response and disposition index following an oral glucose challenge¹⁰⁴, possibly supporting a role of insulin secretion in promoting heavier birth weight and earlier puberty, but the mechanism remains unclear.

Type 2 diabetes and cardiovascular disease—Recent epidemiological evidence indicates non-linear relationships between puberty timing and risks for incident Type 2 diabetes (T2D)¹⁰⁵ and coronary heart disease (CHD)¹⁰⁶. Early menarche is associated with higher risks of T2D and CHD, while late menarche has neutral effects on T2D and confers higher risk of CHD^{105,106}. Some of these relationships might be explained by overweight and obesity which promote both earlier puberty and higher disease risks, however the disease associations remain evident after adjustments for BMI. Early menopause is a well-established risk factor for cardiovascular disease, due to early loss of the protective effects of oestrogen but also other mechanisms¹⁰⁷, and there appears to be a linear inverse association between age at menopause and risk of T2D, which is independent of BMI¹⁰⁸.

GWAS findings have implicated both BMI-dependent and BMI-independent mechanisms that could plausibly link menarche timing to T2D risk. These include direct actions of the *Lin28/let-7* axis on insulin sensitivity and glucose homeostasis¹⁰⁹, and GABA-B receptor signalling or cell-cycle efficiency impacting beta-cell mass¹¹⁰. There is also emerging evidence for a shared genetic aetiology for reproductive ageing and lipid regulation, highlighted by the implication of steroidogenic acute regulatory protein (*STAR*) genes in both menarche timing (*STARD4*)⁴¹ and menopause timing (*STAR* and *STARD3*)¹³. This protein family are key regulators of steroid hormone synthesis by facilitating the conversion of cholesterol into the androgen/estrogen precursor pregnenolone. Disease associations with

age at menopause might reflect both beneficial and adverse effects of sex steroid exposure. In addition, the GWAS loci for menopause timing at *GCKR*, could implicate a role for glucokinase regulation and glucose sensing in reproductive ageing as well as T2D, CVD and dyslipidaemia¹¹.

Breast cancer—There is strong epidemiological evidence linking a longer reproductive lifespan (indicated in women by earlier puberty and later age at menopause) to higher risks for sex hormone-sensitive cancers, particularly for hormone receptor positive breast cancer¹². These observational links are supported by recent genetic evidence for a causal relationship between later age at natural menopause and higher risk for breast cancer. These genetic findings also infer that women with later menopause have more efficient DDR mechanisms, therefore their higher susceptibility to breast cancer very likely indicates non-DDR mechanisms, the foremost candidate mechanism being a prolonged lifetime exposure to oestrogens and/or progesterone¹³.

Conclusions

Recent genetic findings, using GWAS designs that capture common variants and other genomic arrays that focus on exonic and other low frequency and rare variants, have identified and implicated hundreds of loci and genes that regulate the timing of reproductive ageing as well as other complex traits and diseases. While these new findings may have only incrementally explained the population variance in these traits, due to their relatively weak effect sizes or low allele frequencies, the wealth of genetic association signals provides substantially greater power to implicate the underlying biological mechanisms. These emerging analytical findings provide evidence for shared genetic aetiologies, and hence shared biological mechanisms, linking the (previously considered unrelated) milestones of reproductive ageing, and also linking the timing of reproductive ageing to various disease risks.

Biographies

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John Perry is a senior investigator scientist at the Medical Research Council Epidemiology Unit. Prior to this appointment, he was a Sir Henry Wellcome Fellow holding academic positions at the University of Oxford, Kings College London, University of Michigan and the University of Exeter. His current research interests include using large-scale genomic and genetic epidemiology approaches to better understand the aetiology of reproductive ageing and its relevance to broader metabolic health.

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Anna Murray is a senior lecturer in genetics at the University of Exeter Medical School and has a longstanding interest in the genetic causes of early menopause. Following her PhD, Anna worked at the University of Southampton, including as a Wellcome Trust Career Development Fellow. She led studies demonstrating for the first time, an association between *FMRI* premutation alleles and premature ovarian failure, an early example of a low

frequency genetic variant with large effect size. Her current research interests include polygenic and monogenic causes of variation in menopause age and the related health outcomes associated with reproductive ageing.

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Key Points

The genetic architecture of reproductive ageing, puberty timing and age at menopause, involves hundreds of rare, low-frequency and common variants, with allelic heterogeneity often apparent at the same loci.

Recent progress in localising these variants has shed light on the wide range of biological mechanisms that regulate these traits, including energy homeostasis, gene silencing, and DNA repair.

Population variability in ages at menarche and menopause is associated with a wide range of later life health outcomes, notably earlier timings on increased risks for type 2 diabetes and cardiovascular disease.”

Mechanisms that link reproductive ageing to metabolic diseases involve both obesity-related and BMI-independent pathways.

Sex hormone exposure is the likely overwhelming mechanism linking reproductive ageing to risks of breast cancer and other hormone-sensitive cancers.

Figure 1. The genetic architecture of female reproductive lifespan.

This schematic figure illustrates that variants with lower allele frequency (x-axis) have larger effect sizes (y-axis) on puberty or menopause timing. Font sizes of the names of the implicated genes is scaled to the relative effect size of the variant within the frequency categories.

Figure 2. Overlaps in implicated genes between menarche and menopause timing and between variant frequency groups.

Background colour shading indicates allele frequency of associated variants: red (rare), green (low-frequency) and blue (common). Lines indicate overlaps in the genes implicated by associated variants, between menarche and menopause traits, and also between variant frequency groups.

Figure 3. Schematic overview of the aetiological mechanisms governing reproductive ageing in women

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Figure 4. Changes in oocyte reserve throughout life.

This schematic figure indicates ovarian germ cell numbers throughout life and the mechanism that influence this. Histograms indicate the normal population variance in ages at menarche (puberty) and menopause.

Table 1
Selected GWAS implicated genes with strong evidence for a functional role in reproductive ageing.

Trait	Gene	Frequency class ¹	Effect size ²	Evidence ³
Menarche	<i>ALMS1</i>	LF	3 months	FMN
Menarche	<i>ASCC3</i>	Common	1.5 weeks	EFN
Menarche	<i>DLK1</i>	Common	2 weeks	BEN
Menarche	<i>FSHB</i>	Common	1.5 weeks	NB
Menarche	<i>IGSF1</i>	Common	3 weeks	BFMN
Menarche	<i>INHBA</i>	Common	3.5 weeks	BN
Menarche	<i>KDM4C</i>	Common	1.5 weeks	BFN
Menarche	<i>LAMB2</i>	Common, LF	2-4.5 weeks	FN
Menarche	<i>LEPR</i>	Common	1.5 weeks	BM
Menarche	<i>LIN28B</i>	Common	6 weeks	BEN
Menarche	<i>MKRN3</i>	Common	5 weeks	BM
Menarche	<i>PRKAG1</i>	Common, LF	2-4.5 weeks	FN
Menarche	<i>SIRT3</i>	Common	1.5 weeks	BEN
Menarche	<i>SMARCA1</i>	Common	1.5 weeks	EFN
Menarche	<i>STARD4</i>	Common	2 weeks	BEN
Menarche	<i>TACR3</i>	Common, LF, Rare	2.5 weeks - 1 year	BFN
Menarche	<i>TNRC6A</i>	LF	4 weeks	FN
Menarche	<i>TRIM66</i>	Common	2 weeks	EFN
Menarche	<i>ZNF131</i>	Common	1.5 weeks	BEN
Menarche	<i>ZNF654</i>	Common	2 weeks	EFN
Menopause	<i>APT</i>	Common	1.5 months	BEN
Menopause	<i>BRCA1</i>	Common	1.5 months	BEFN
Menopause	<i>CHD7</i>	Common	1.5 months	BEN
Menopause	<i>DIDO1</i>	Common	2 months	BEN
Menopause	<i>DMC1</i>	Common	2 months	BEN
Menopause	<i>EXO1</i>	Common	2 months	BFN
Menopause	<i>FSHB</i>	Common	2.5 months	BEN
Menopause	<i>HELB</i>	Common, LF	1 - 11 months	BEFN
Menopause	<i>INO80</i>	Common	1.5 months	BEN
Menopause	<i>MCM8</i>	Common	10.5 months	BFN
Menopause	<i>MSH5</i>	Common	2 months	BEN
Menopause	<i>MSH6</i>	Common	2 months	BEN
Menopause	<i>PRIM1</i>	Common	3.5 months	BEFN
Menopause	<i>SLCO4A1</i>	Common	2-10.5 months	FN
Menopause	<i>TLK1</i>	Common	2 months	BEN

Trait	Gene	Frequency class ¹	Effect size ²	Evidence ³
Menopause	<i>UIMC1</i>	Common	3 months	BEN

¹ Common refers to a population minor allele frequency > 5%, low-frequency (LF) 1-5%, rare < 1%.

² Per-allele effect size based on test statistics from GWAS discovery meta-analysis

³ Evidence type supporting this gene being causal at the GWAS highlighted locus. B = supporting biology, E = linked via altered expression to the associated SNP (eQTL), F = the lead SNP (or a strongly correlated proxy) is a missense variant in this gene, N = nearest gene to the association signal, M = reported monogenic mutations for associated disorders in the gene.