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The genetics of pubertal timing in the general population: recent advances and evidence for sex-specificity

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Abstract

Purpose of review—To overview advances in the genetics of puberty based on studies in the general population, describe evidence for sex-specific genetic effects on pubertal timing, and briefly review possible mechanisms mediating sexually dimorphic genetic effects.

Recent findings—Pubertal timing is highly polygenic, and many loci are conserved among ethnicities. A number of identified loci underlie both pubertal timing and related traits such as height and body mass index (BMI). It is increasingly apparent that understanding the factors modulating the onset of puberty is important because the timing of this developmental stage is associated with a wider range of adult health outcomes than previously appreciated. While most of the genetic effects underlying the timing of puberty are common between boys and girls, some effects show sex-specificity and many are epigenetically modulated. Several potential mechanisms, including hormone-independent ones, may be responsible for observed sex differences.

Summary—Studies of pubertal timing in the general population have provided new knowledge about the genetic architecture of this complex trait. Increasing attention paid to sex-specific effects may provide key insights into the sexual dimorphism in pubertal timing and even into the associations between puberty and adult health risks by identifying common underlying biological pathways.

Keywords

pubertal timing; sex specificity; genome-wide association; epigenetics

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Conflicts of interest

There are no conflicts of interest to declare.

Introduction

The onset of puberty is sexually dimorphic, occurring earlier in girls than in boys and exhibiting partly sex-specific impact on health. Recent large studies show strong evidence that pubertal timing is linked to a diverse range of adult health outcomes, some of which are shared while others are specific to either men or women (1). Investigation of the genetic underpinnings of pubertal timing within the general population may improve our understanding of male vs. female differences in onset and associations with health outcomes. Significant discoveries have been made in recent years by incorporating ever-larger sample sizes, samples with diverse ethnic backgrounds, and traits which also allow the inclusion of males. In particular, studies in both sexes allow investigation not only of the common genetic underpinnings, but also of the differences. In this review, we provide a brief update of the key developments in our understanding of the genetics of normal pubertal timing, explore some evidence for sex-specific genetic effects in pubertal traits, and speculate on potential mechanisms that may mediate these effects.

Genetic studies of age at menarche (AAM): larger sample sizes and diverse ethnicities

Recent studies have shed light on the genetic architecture underlying pubertal timing in the general population. Large-scale genome-wide association studies (GWAS) show that pubertal onset is highly polygenic, like other quantitative traits (2,3). However, known genome-wide significant variants for AAM (currently 123 SNPs at 106 loci, n > 180,000 women) still explain only 2.7% of the trait variance (2). Expanding the variants studied to include potentially functional low-frequency variants (with a minor allele frequency of 1–5%) and common variants on the X chromosome found an additional 5 and 7 associated loci, respectively, but these variants only explained 0.5% more of the variance (4). The small amount of explained variance suggests that other types of genetic variation, such as copy number changes and gene-gene and epigenetically mediated gene-environment interactions (5), should be investigated to help explain the missing heritability. It is also possible that variation in pubertal timing may be the result of hundreds or thousands of genetic variants with very small effect sizes.

Until recently, little overlap was seen between genes harboring rare, functionally damaging variants and genes near common variants known to influence puberty. However, with the publication of 106 AAM loci, a significant enrichment for signals in/near genes causing rare Mendelian puberty disorders was observed. Examples include *MKRN3*, underlying central precocious puberty (CPP), and genes associated with hypogonadism, such as *LEPR-LEPROT*, encoding the leptin receptor, and *TACR3*, encoding the neurokinin B receptor. In addition, a variant 10 kb away from *GNRH1* was suggestively associated with AAM (2).

Other recent studies have examined puberty in non-European populations, notably in African American (AA) and Asian women (Table 1). These studies mainly seek to replicate European loci, although not necessarily at the same SNPs. Candidate studies in AA women have had some success (6,7); in one study, 25 of the 42 loci known at the time contained variants that were significantly associated in AA women. However, de novo GWAS in these

samples failed to generate statistically significant novel loci, partly due to small sample sizes. Furthermore, the linkage disequilibrium (LD) structure differs between populations, with Europeans having longer stretches of LD than African populations (8), so widely used SNP-chips aimed at tagging common European haplotypes may miss important variation in other ethnicities. In other populations, including Koreans (9), Asians, Hispanics, and Native Hawaiians (10), and Filipinos (11), effect sizes for variants at the leading European loci (at *LIN28B* and 9q31/*TMEM38B*) do appear robust across populations, although effect sizes vary by ethnicity (Table 1). Taken together, these studies show that some known loci are important for pubertal onset regardless of ethnicity, and that larger sample sets are needed.

Epigenetics: imprinting and parent-of-origin effects

The role of epigenetics is a recent key advance in our understanding of the mechanisms regulating pubertal onset (5). In the large-scale GWAS of AAM, six loci fell in imprinted genomic regions and four of these had parent-of-origin effects, i.e. only the paternal or maternal allele was associated with AAM (2). Of these, a paternally-inherited variant at *MKRN3*, an imprinted gene in the Prader-Willi syndrome critical region (chr 15q11-q13), was associated at a similar magnitude as variants at *LIN28B* (> 0.1 yrs per allele). Concurrently, a whole-exome sequencing study of 40 individuals from 15 families with CPP found 4 heterozygous mutations in *MKRN3* (12). All individuals with CPP inherited the mutation from their fathers; individuals who inherited mutations from their mothers had normal puberty. Further studies revealed additional *MKRN3* mutations in Brazilian (13), Greek (14), German (15), Ashkenazi-Sephardic Jewish (16), and Korean CPP cases (17).

It is unknown whether *MKRN3* or another gene, *MAGEL2*, is responsible for the GWAS signal (2), as truncating mutations in *MAGEL2* affecting paternal alleles have been reported in Prader-Willi cases with hypogonadism or delayed puberty (18). In mice, however, levels of Mkrn3 decreased in the arcuate nucleus of the hypothalamus immediately before puberty (12), and circulating MKRN3 also declines preceding pubertal onset in girls (19). Collectively, these data indicate that MKRN3 is part of the inhibitory brake that restrains puberty. It is interesting in this regard that when prepubertal rodent models were treated with chemicals modifying genomic epigenetic marks, each of three approaches showed results that were consistent with a mechanism in which epigenetically-mediated suppression is lifted at the onset of puberty (5,20).

The closest gene at 19 out of 32 AAM loci published in 2010 is a regulator of the epigenome (3,5), and genes in the Jmj-domain-containing lysine-specific demethylase family were highly enriched for association with AAM (enrichment $P = 0.006$) in the most recent GWAS (2). Many genes in this family encode proteins that epigenetically modify specific methylation marks. In female mice, DNA methylation of Polycomb group silencing complex genes leads to the enrichment of activating H3 lysine modifications, triggering pubertal onset (20). AAM has been inversely associated with global DNA methylation (21), providing further evidence that an epigenetic switch plays an important role in the onset of puberty (22,23).

Sex differences and genetic effects on puberty

Several recent GWAS of pubertal traits investigated the genetic overlap in the regulation of pubertal phenotypes between boys and girls. These studies mainly focused on the commonalities between the sexes. However, differences also exist, and may prove important (Fig. 1). For example, in a GWAS of the timing of the pubertal growth spurt, several variants were associated in only one sex in gender-specific analyses (24), including rs960273 (*GNA12*) in males (*P* for sexual heterogeneity ($P_{sex\text{-}het}$) = 5.2×10^{-4}) and rs7628864 (*VGLL3*) in females ($P_{sex\text{-}het} = 6.8 \times 10^{-6}$). Of these, rs7628864 was also associated with pubertal timing, while rs960273 showed sex-specific associations with total postnatal linear growth but not pubertal timing. Additionally, another study found an association between an AAM-associated locus, rs480014 (*CABLES1*), and taller childhood height in boys ($P_{sex\text{-}het} = 0.04$) but not girls, as well as other race- and sex-specific associations with weight and BMI changes in adolescence (29).

Two additional studies explored the overlap between menarche-associated variants and male genital and female breast development assessed by Tanner puberty scales (2,26). These studies concluded that much of the genetic architecture underlying pubertal timing is shared between the sexes, as the majority of alleles have concordant effect directions in males and females. However, further investigation of AAM-associated loci in male and female Tanner data revealed that not all menarche-associated loci behave the same way (Table 2; Supplemental figure 1). Some variants have the same effect direction but significantly different effect sizes, such as rs7759938 and rs2153127 at *LIN28B* and rs10453225 at *TMEM38B*; some loci show a strong association in one sex only, such as rs17233066 at *SAT2B*; and others have opposite effects, like rs1324913 at *KLF12*. It should be noted that the Tanner dataset was quite small, which could have resulted imprecise estimates and random effects. Further in-depth studies of loci with sex-specific associations are necessary to understand sexual dimorphism in pubertal timing.

Sex-specific effects at *LIN28B* have been investigated in humans and mouse models. In humans, *LIN28B* variants have sex-specific associations with postnatal growth (25), and are associated with adiposity traits in adult women, but not men (27, 28). *LIN28A* and *LIN28B* participate in a negative feedback loop with the *let-7* family of microRNAs. In mouse models of this pathway, *Lin28b* loss of function (LOF) and reciprocal *let-7* gain of function (GOF) mice both displayed sex-specific effects on pubertal timing and growth (Corre, et al., submitted). Such data suggest that the *LIN28-let-7* system is likely to have complex, partly sex-specific influences on growth and pubertal timing and that further study of this pathway in humans is warranted.

Explaining sex differences

Sex-specific genetic effects are common in model organisms (30,31), and recent GWAS show that common genetic variation can influence traits or diseases in a sex-specific manner in humans (see (32) for a recent review). Puberty is a dimorphic trait that displays sex-specific genetic effects. The mechanisms underlying sex-specificity remain largely speculative. While sex hormones (testosterone and estrogen) are the primary drivers of

differential gene expression, resulting in different trait manifestations and disease risks (33), these hormones do not explain all sex differences. For example, in blastocysts, which are pre-gonadal and lack influence from sex hormones, almost a third of detected gene transcripts showed sexually dimorphic gene expression (34), pointing toward an alternative mechanism prior gonadal steroid synthesis.

The most fundamental difference between male and female cells is the sex chromosome complement (Fig. 2). The X and Y chromosomes are structurally heteromorphic (37) but share a pseudoautosomal region containing 29 genes (38); the human X and Y chromosomes have about 1400 (39) and 27 (40) unique genes, respectively. In humans, Turner (45-XO) and Kleinfelter (47-XXY) syndromes illustrate the implications of having the correct complement of sex chromosomes (41). In mice, the Four Core Genotypes (FCG) model has been used to test the contribution of the sex chromosomes versus the gonads (42). In this model, a normal XX female is mated with a XY^{-}*Sry* male (with the *Sry* region translocated from the Y chromosome to an autosome), creating offspring with four potential genotypes: XX females, XY^{-} females with normal autosomes and no *Sry*, XX*Sry* males, and XY^{-}*Sry* males with *Sry* on an autosome. In studies utilizing this model, X vs Y sex chromosome effects have been seen for many traits regardless of the hormonal milieu, including adiposity (43), metabolism (44), HDL cholesterol (45), food intake (46,47), hypothalamic neuronal development (48), brain structure and function (49), and juvenile behavior (50).

One way the sex chromosomes influence autosomal gene expression is through sex-linked genes acting as transcription factors, such as *SRY* (51,52) (Fig. 2a). Additionally, the sex chromosomes can reportedly affect epigenetic regulation of gene expression on the autosomes (53). In *Drosophila*, the large heterochromatic Y chromosome directly impacts autosomal gene expression through effects on the epigenetic status of other chromosomes (54–57). The heterochromatic inactive X may also bias autosomal expression (52,53).

Gene dosage is another contributor to sex-specific gene expression. Most genes on the second female X chromosome are randomly switched off, but around 15% escape X-inactivation and are expressed at twice the level found in males (58). Mammalian embryos may have sex-specific gene expression before random X-inactivation (33), which may have later-life effects on gene expression. In addition, 6–10% of autosomal genes are monoallelically expressed (59), and in these cases, parent-of-origin effects such as at *MKRN3* can occur where a variant is expressed when it comes from one parent and not the other.

Epigenetic marks can be sex-specific and have lasting impact on sex-differentiated gene expression. For example, an X-linked gene was found that had significantly lower expression in males and was reduced further by in utero maternal stress (60). This gene had long-term effects on metabolic and neurodevelopmental programming (61). Several studies showed long-lasting sex-specific epigenetic changes with transcriptional implications for nearby genes in the adult brain and liver, some of which are hormone-dependent (62,63). However, a small but significant increase in global autosomal methylation is associated with being male (64), apparently not driven by sex hormones (65). Epigenetic mechanisms are likely to play a large role in how certain genetic polymorphisms regulate gene expression

differently in males and females. In lymphoblast cell lines grown without sex hormones, 12–15% of autosomal expression quantitative trait loci (eQTLs), genomic variants associated with variation in mRNA expression levels, were expressed in a sex-specific manner (66) due to differential usage of regulatory elements or isoforms (67,68).

In GWAS, mechanisms underlying sex-specific genetic associations remain mostly unknown. In two cases, a sex-specific eQTL overlapped a previously reported disease SNP, one for eosinophilic esophagitis and one for Crohn's disease, two conditions more common in males (66). In other studies, epigenetic mechanisms, such as sex-specific DNA methylation in response to environmental cues, may be sequence-dependent and have been highlighted (63,64).

Conclusions

Genetic investigation of pubertal timing is an active field of research with implications for understanding the biological basis for how pubertal maturation is triggered. In future research, more emphasis should be placed on sex-specific effects, as these may provide keys to understanding sexual dimorphism in pubertal onset and perhaps the associations between timing and later life health outcomes, some of which are also sex-specific.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Key Points

- Significant discoveries into the genetic background of pubertal timing have been made in recent years by incorporating ever-larger sample sizes, samples with diverse ethnic backgrounds, and traits which also enable the study of puberty in males.
- Studies in both sexes show that many genetic effects are similar between boys and girls, but differences also exist that may be important for understanding sexual dimorphism in pubertal timing and the associations between the timing of puberty and adult health.
- Many mechanisms, including hormone-independent ones, may be responsible for sex-specific effects.

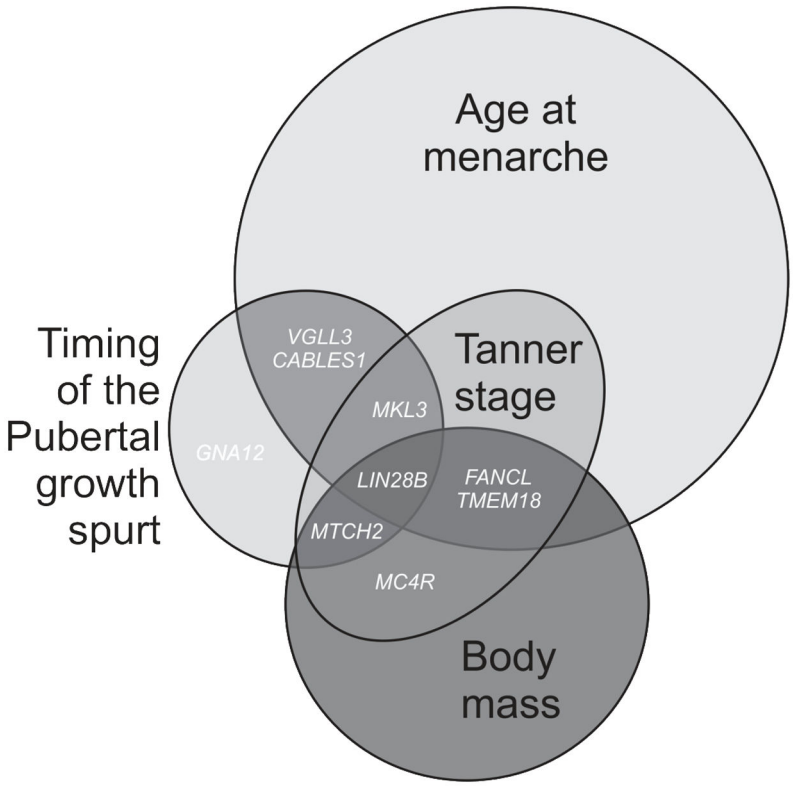

Fig. 1. (Original) Puberty-associated loci with sex-specific effects

Genome-wide association studies have resulted in over 100 genome-wide significant loci for the pubertal traits of age at menarche, the timing of the pubertal growth spurt, and Tanner puberty staging. Body mass is a related trait that shares a genetic component with puberty. For AAM, 106 autosomal loci (2), 2 X-chromosome loci, and 5 low-frequency loci (4) are currently identified. There are 5 known loci which are significantly associated with the pubertal growth spurt and pubertal timing (24), and an additional 2 significant loci for Tanner staging (26). Gene names are shown for loci at which sex-specific effects have been seen. For example, variants near *LIN28B* have been associated with age at menarche (2,3), the timing of the pubertal growth spurt and postnatal growth (24,25), and Tanner stage (26), with stronger associations seen in girls. Additionally, these *LIN28B* variants were also associated with body mass traits in adult women but not adult men (27,28). Other pubertal loci at which sex-specific effects have been seen include *GNA12* and *VGLL3* (24), *CABLES1* (29), and *MKL3*, *MTCH2*, *MC4R*, *FANCL*, and *TMEM18* (26).

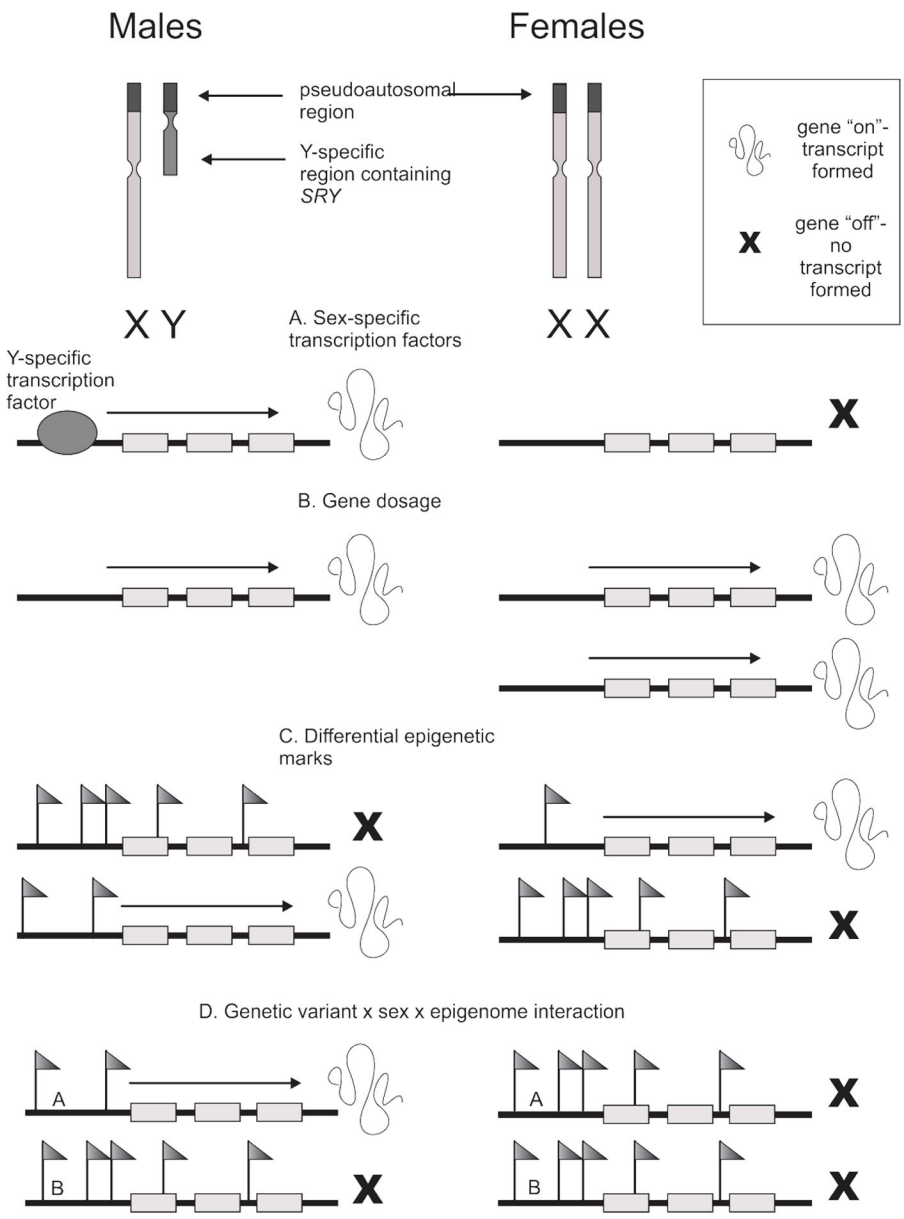

Fig. 2. (Original) Schematic representation of several mechanisms of sex-specific gene expression

The most fundamental difference between male and female cells is the sex chromosome complement; males possess an X and a Y chromosome, while females have two X chromosomes. Some examples of how male and female cells may experience differential gene expression include A) sex-specific transcription factors, such as the male-specific *SRY* gene on the Y chromosome; B) gene dosage due to incomplete X-inactivation in females, resulting in higher transcript levels in females for some genes; and C) sex-differentiated epigenetic marks, which may cause differential regulatory element usage, expression of different isoforms in each sex, or even differential gene expression entirely. In D) a real example of a genetic variant × sex × epigenome interaction is schematically represented. In (35,36), GWAS-associated genetic variants at 7q12-q21 were associated with childhood-

onset asthma in boys only, via sex-specific DNA methylation in response to smoking exposure. In the figure, A and B represent two DNA sequence variants in the associated region. Only boys with variant A have active gene expression in the presence of sex-specific DNA methylation.

Table 1

(Original) Reported effect sizes for the leading European menarche-associated locus at *LIN28B* in diverse ethnicities

Until 2013, only European-descent populations were assessed for age at menarche in GWAS studies. The leading locus in these studies at *LIN28B* has since been assessed in various ethnicities. In this table, the effect at the *LIN28B* locus is summarized for various ethnicities, including the effect size and *P*-value for the most recent European GWAS for reference (2). These studies show that while variants at *LIN28B* are the most robust in Europeans, heterogeneity exists in other populations. For example, a GWAS in Korean women (6) did not report variants at this locus as significantly associated, and many other ethnicities show varied effects.

First author	Year	Study Type	Population	Sample size	*LIN28B* SNP Studied	Reported allele	Effect size (years)	*P*	If GWAS, was LIN28B the leading locus?	Was rs7759938 leading SNP at LIN28B locus?
Perry	2014	GWAS	European	182,416	rs7759938	C	0.12	7.8×10^{-110}	Yes	
Pyun	2013	GWAS	Korean	3,437	Not reported	NA	NA	NA	No	Not reported
Croteau-Chonka	2013	Candidate	Filipino	827	rs7759938	T	−0.0118	0.019	NA	NA
Tanikawa	2013	GWAS + candidate	Japanese	15,495	rs364663 rs7759938	A T	−0.089 −0.087	5.49×10^{-7} 1.12×10^{-6}	Yes	No
Demerath	2013	GWAS	African American	18,089	rs9386427	T	0.079	0.0025	No	No
Carty	2013	Candidate	European	24,819	rs314280	T	0.08	2×10^{-8}		
			African American	7,999			0.05	0.16		
			Asians	3,934			0.13	0.0012		
			Hispanics	3751			0.001	0.98		
			American Indians	231			0.25	0.14	NA	NA
			Hawaiians	728			0.22	0.02		
Spencer	2013	GWAS + Candidate	African American	4,159	rs7759938	A	−0.02	0.61	No	No
Delahanty	2013	Candidate	Chinese	6,929	rs7759938	C	0.12	3.8×10^{-6}	NA	NA

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Table 2

(Original) Sex-specific puberty effects

123 menarche-associated variants were extracted from GWAS data on Tanner male genital stage and Tanner female breast stage (25). The effect sizes are shown for the menarche-delaying alleles. In the Tanner data, beta values are derived directly from the Tanner scale, where 1 is prepubertal and 5 is adult phenotype. Thus, a negative beta value corresponds to later-than-average pubertal timing, since it reflects a lower Tanner score. Only 4 variants had significant *P*-values for a difference between the sexes ($P_{sex\text{-}dif}$) at a Bonferoni-corrected significance threshold of 0.0004 (corrected for assessing 123 menarche-associated variants). A) Variants with a stronger effect in one sex versus the other. In some cases the variant appears to be associated with Tanner stage in both sexes but to a stronger degree in one versus the other, and in other cases the effect size is close to zero for one sex but higher in the other. B) Variants showing potentially opposite directions of effect. It should be noted that the dataset was relatively small (n males = 3769; n females =6147), meaning that random effects may exist.

SNP	Nearby gene[a]	Chr	Position[b]	Allele [c]	Male beta	Female beta	$P_{sex\text{-}dif}$[d]	$P_{sex\text{-}het}$[e]
A. Stronger effect in one sex								
rs2153127	*LIN28B*	6	105455237	T	−0.049	−0.067	**2.6×10^{-5}**	0.474
rs7759938	*LIN28B*	6	105485647	C	−0.063	−0.088	**1.5×10^{-7}**	0.344
rs10453225	*TMEM38B*	9	107960041	G	−0.074	−0.053	**9.7×10^{-5}**	0.411
rs246185	*MKL2*	16	14302933	C	−0.131	−0.019	**1.9×10^{-6}**	**4.2×10^{-4}**
rs2274465	*KDM4A*	1	43894144	C	−0.071	0.006	0.014	0.010
rs2947411	*TMEM18*	2	604168	A	0.014	−0.060	0.017	0.046
rs1400974	*SATB2*	2	199346935	A	0.005	−0.046	0.027	0.081
rs4895808	*CENPW*	6	126823127	C	0.001	−0.041	0.044	0.141
rs6964833	*GTF2I*	7	73739845	T	−0.021	−0.057	0.007	0.262
rs12915845	*DET1*	15	86843471	C	−0.073	−0.022	0.003	0.072
rs12446632	*GPRC5B*	16	19842890	A	−0.085	−0.045	0.006	0.303
rs8050136	*FTO*	16	52373776	C	0.001	−0.042	0.041	0.139
rs10423674	*CRTC1*	19	18678903	A	−0.002	−0.045	0.033	0.145
C. Opposite effects								
rs268067	*BCL11A*	2	59734549	A	0.034	−0.039	0.101	0.048
rs2687729	*EEFSEC*	3	129377916	G	−0.037	0.031	0.082	0.031
rs4840086	*SIM1*	6	100315159	A	0.031	−0.033	0.060	0.027
rs7821178	*PEX2*	8	78256392	C	−0.049	0.011	0.108	0.044
rs1324913	*KLF12*	13	73533589	G	0.050	−0.038	0.011	0.003

SNP	Nearby gene[a]	Chr	Position[b]	Allele [c]	Male beta	Female beta	$P_{sex\text{-}dif}$[d]	$P_{sex\text{-}het}$[e]
rs9635759	*CA10*	17	46968784	A	0.039	−0.046	0.014	0.007

[a] Best nearby candidate gene according to Perry, 2014.

[b] Position shown for genome build 37.

[c] Menarche-delaying allele.

[d] *P*-value for sex difference is defined as the combined P-value of both sexes assuming different effect sizes between them (2 degrees of freedom), according to http://www.well.ox.ac.uk/gwama/tutorial.shtml and Mägi, Lindgren, and Morris, 2010.

[e] *P*-value for sex heterogeneity is defined as the amount of heterogeneity in allelic effects between the sexes (1 degree of freedom), according to http://www.well.ox.ac.uk/gwama/tutorial.shtml and Mägi, Lindgren, and Morris, 2010 [31].