Neuroendocrinology: Cutting Edge review

Genetic regulation of puberty timing in humans

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Keywords:
puberty
genetics
menarche
imprinted genes
BMI
obesity

Running Head: Genetics of puberty timing

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Abstract

Understanding the regulation of pubertal timing has relevance to developmental and human biology, and to the pathogenesis of various diseases. Recent large-scale genome-wide association studies for puberty timing and adult height, body mass index (BMI) and central body shape provide evidence for shared biological mechanisms that regulate these traits. There is substantial genetic overlap between age at menarche in women and BMI, with almost invariable directional consistency with the epidemiological associations between earlier menarche and higher BMI. By contrast, the genetic loci identified for age at menarche are largely distinct from those identified for central body shape, while alleles that confer earlier menarche can be associated with taller or shorter adult height. The findings of population-based studies for age at menarche show increasing relevance for other studies of rare monogenic disorders and enrich our understanding of the mechanisms that regulate the timing of puberty and reproductive function.

Introduction

Puberty is the process of attainment of reproductive maturity and involves a range of physiological changes. The timings of specific pubertal milestones serve as markers of the tempo of development. Age at menarche, a woman's first menstrual bleed, is reasonably well-recalled and is widely recorded in epidemiological studies. It has an estimated heritability of ~50% in contemporary western settings[1] and is associated with later life risks for type 2 diabetes, cardiovascular disease and various other health outcomes [2, 3]. The lack of similar well-recalled pubertal measures in men means that similar data are yet sparse. Earlier puberty timing is generally, but not invariably, associated with higher risks for adverse health outcomes, and therefore the global declines in average ages of puberty in females[4–9] and males[10] have important relevance for health. These secular trends appear to be driven by increases in childhood BMI, which to some extent may underlie many of the later health risks[10,11]. Recent genetic studies have shed light on the mechanisms linking weight status to puberty timing, and also other biological pathways that might independently link puberty timing to later health.

Rare genetic disorders of puberty

Rare mutations in >20 genes that disrupt the gonadotropin-releasing hormone (GnRH) axis have been described[12]. Those that disrupt embryonic migration of GnRH producing cells from the nasal placode also cause anosmia (Kallman syndrome)[13]. Mutations in other genes that encode hypothalamic neurotransmitters or their receptors, rather than GnRH cell migration, typically cause normosmic hypogonadotropic hypogonadism; these include the genes for kisspeptin (*KISS1*), neurokinin B (*TAC3*) and GnRH (*GNRH1*) and their receptors (*KISSR*, *TACR3* and *GNRHR*). Hypogonadotropic hypogonadism associated with other pituitary hormone deficiencies also results from mutations that disrupt pituitary development, such as in *HESX1* and *SOX2.*

Rare mutations in the genes that encode the pituitary hormones follicle-stimulating hormone (FSH) and luteinizing hormone (LH) have differing effects in males and females. In females, absent puberty and ovarian failure are consequences of deleterious mutations in the *FSH* β-subunit[14] or FSH receptor genes (*FSHR*)[15]. By contrast, affected males have reduced spermogenesis without other features of gonadal failure[16]. Only one consanguineous kindred is reported with a deleterious mutation in the *LH* gene[17]. The homozygous proband had bioinactive LH and absent puberty at age 17, while heterozygous males reported variable infertility but normal puberty, and heterozygous females had normal sexual development and were fertile.

Few genetic disorders are described that result in precocious puberty. As a mirror image of the hypogonadotropic hypogonadism phenotype due to loss of function mutations in *KISS* or *KISSR*, activating mutations in these genes result in precocious puberty, at least in girls[18,19]. A recent study of 40 members of 15 families affected by precocious puberty used whole-exome sequencing to identify paternally-inherited mutations in *MKRN3*[20], an exclusively paternally-expressed gene located in the imprinted Prader–Willi syndrome region. Since that report of deleterious mutations in 15 affected members of 5 families from Brazil, USA and Europe[20], *MKRN3* mutations have also been described in 2/6 affected families[21] and in 8/215 apparently sporadic cases[22]. Studies in mice indicate that *MKRN3* may act as a hypothalamic repressor of puberty onset[20].

Recently, various genetic disorders of puberty associated with ataxia have been described. Rare mutations in *POLR3A* that disrupt RNA polymerase cause hypomyelinating leukodystrophy[23]. The combination of ataxia and hypogonadism is also seen in disorders of ubiquitination due to rare mutations in *RNF216* and *OTUD4*[24], and in individuals with Gordon Holmes syndrome as a result of mutations in *CHIP* (also called *STUB1*)[25,26].

Genetic disorders of both puberty and adiposity

The adipocyte-derived hormone leptin and its receptor link nutritional status to the GnRH axis. Rare deleterious mutations in the leptin gene (*LEP*) cause severe early onset obesity with hyperphagia[27]. Furthermore, as in the mouse model[28], affected individuals have hypogonadotropic hypogonadism[29,30]. Leptin therapy in children with leptin deficiency allows normal puberty timing rather than precocious puberty[31], suggesting that leptin is necessary for GnRH axis activity, but is not itself the trigger for puberty timing[32]. Rare deleterious mutations in *LEPR* cause severe, early-onset obesity, and an apparently less severe reproductive phenotype of abnormally delayed rather than absent puberty, with irregular menses from age 20 years and normal adult estradiol, luteinizing hormone, and follicle-stimulating hormone levels reported in the 3 affected women[33]. Converse to those disorders of delayed puberty and excess adiposity, rare mutations in *POLD1* were recently reported to cause hypogonadism in males associated with lipodystrophy (particularly lack of subcutaneous adipose tissue) and deafness[34]. Affected individuals have low BMI and limb muscle mass, but are insulin resistant. *POLD1* regulates DNA polymerase activity, but it is yet unclear how this relates to disruptions in gonadal function and adipose tissue development and metabolism.

Recent genome-wide association studies

In contrast to the long history of reports of mutations in cases and families affected by rare clinical disorders, the discovery of common genetic variants that contribute to the wide normal variation in puberty timing, and other complex physiological traits, has accelerated only in the last 5-10 years since the advent of genome-wide association studies (GWAS). This approach allows the concurrent analysis of millions of single nucleotide polymorphisms (SNPs) across the genome in very large individual-based study designs to provide the statistical power that is necessary to robustly identify genomic regions of association.

Four recent large-scale GWAS have been reported for puberty timing, adiposity and adult height (Table 1). A GWAS for age at menarche identified 123 independent signals at 106 genomic loci [35]. The strongest individual signal, a SNP near to *LIN28B*, altered age at menarche by 0.11 years per allele, and in total the top 123 SNPs explained 2.7% of the population variance in puberty timing. Secondly, a GWAS for BMI in adults identified 97 genomic loci, of which 56 had not been previously linked to any measure of adiposity; taken together the top 97 SNPs explained around 2.7% of the variance in adult BMI[36]. Thirdly, a parallel project by the same investigators identified 49 genomic loci, of which 33 were novel, robustly associated with measures of central-to-peripheral distribution of body shape and adiposity; the effects were larger in women than in men likely reflecting the greater heritability in hip circumference in women. Together the top SNPs explained 2.4% of the variance in 'waist-to-hip ratio adjusted for BMI' in women compared to only 0.8% of the variance in this trait in men[37]. Finally, a remarkable 697 independent signals at 423 loci were associated with adult height; together these top SNPs explained 16% of the variance in adult height and the investigators extrapolated that thousands of common variants across the genome account for ~50% of variance[38].

Notably, all of these recent GWAS relied on traits that were measured or self-reported by adults, reflecting the relative paucity of genetic data in children, and therefore the role of these genetic variants on detailed childhood phenotypes is mostly unclear. A recent GWAS for childhood timing of 'Tanner puberty stages' in 6,147 girls and 3,997 boys did not identify any new loci for puberty timing, but confirmed the relevance of the *LIN28B* locus[39] and the same dataset supported the likely relevance of the vast majority those loci recently identified for age at menarche in women to puberty timing in general in both boys and girls[35]. The reported menarche GWAS loci were enriched in/near genes that underlie rare disorders of puberty, including common variants in/near *LEPR*, *GNRH1*, *PCSK1*, *PCSK2*, *POU1F1*, *TENM2* and *TACR3*, as described above, which indicates their likely generic relevance to pubertal timing and reproductive function.

Genetic co-regulation of BMI and puberty timing

The existence of genetic co-regulation of BMI and age at menarche had been suggested by twin studies[40], and this prediction has been validated in those recent GWAS findings. The list of shared loci between these two traits includes the following 13 genomic regions in or near: *FTO, SEC16B, TMEM18, NEGR1, TNNI3K, GNPDA2, BDNF, BCDIN3D, GPRC5B, GALNT10, MAP2K5, TRIM66 and LRP1B.* At all of these loci, the BMI-increasing allele is also associated with earlier age at menarche, which is directionally concordant with the observed epidemiology association between BMI and age at menarche. In addition to these 13 'top signals' identified as passing stringent statistical criteria for each trait, our analysis across all of the 97 recently reported BMI loci shows an overall linear correlation between the reported effects on BMI[36] and those reported on age at menarche[35] (R-square=0.26), indicative of a general mechanism linking these two traits (Figure 2).

However, there are some exceptions to this pattern, which could indicate other specific mechanisms that act on BMI and puberty timing. Firstly, the BMI locus near *MC4R* has the fourth largest estimated effect size on BMI[41] (and has similar effects on BMI in men and women[42]), but shows no association at all with age at menarche (P=0.19[35]) (Figure 2), which suggest that it regulates BMI through a mechanism

different to most other genetic factors. Intriguingly, at this locus the BMI-*increasing* SNP is also associated with *later*-puberty in boys (P=0.0009)[39]. Further genetic studies in boys might shed light on the highly variable reportedly effects of BMI on puberty timing in observational epidemiological studies[43,44]. Secondly, a locus for age at menarche is located close to the BMI locus at *ETV5*; however the genetic signals for these traits are not correlated, which may indicate heterogeneity in downstream biological mechanisms[45]. Lastly, our analysis of these reported findings and other publically available datasets indicates that loci that regulate both BMI and age at menarche are also enriched for associations with fasting insulin levels, which could indicate a specific mechanism that potentially links these two traits (Figure 3).

In contrast to the reported BMI loci, the genetic loci reported for measures of central-to-peripheral distribution of body shape and adiposity show little relevance for age at menarche[37]. Only one locus for WHR-adjusted-for-BMI (at *LEKR1*) is also identified as a locus for age at menarche[35]. Notably, this *LEKR1* locus is also reported to regulate birth weight[46] and the menarche age-raising allele was also associated with higher birth weight, which is directionally concordant with observed epidemiological observation[47]. Therefore, while earlier age at menarche might predispose to greater central adiposity through its effects on promoting weight gain and BMI[48], GWAS findings do not yet support any distinct mechanisms linking these traits.

Genetic co-regulation of height and puberty timing

There is good evidence from observational studies in the general population that (as is seen in girls with the clinical disorder precocious puberty[49]) girls who develop puberty earlier than average are taller than other girls during childhood and adolescence but are shorter as adults due to earlier epiphyseal bone fusion[50] (in contrast there appears no association with adult height in men[51]). This complex relationship between height and puberty timing in females is reflected in the genetic co-regulation of these traits. The GWAS findings for age at menarche include three loci that were also reported as GWAS loci for adult height, however there was directional inconsistency in these overlapping findings. The menarche age-raising alleles at the *LIN28B* and *SIX6* loci are also associated with taller adult height, which is directionally-concordant with the phenotypic association. Conversely, at the *CENPW/ NCOA7* locus, the menarche age-raising allele is also associated with shorter adult height. Our analysis across all 123 signals identified for age at menarche shows distinct clusters of age at menarche alleles that are associated with either taller or shorter adult height (Figure 3). Clarification between such mechanisms, which promote both faster childhood growth and earlier puberty timing but have distinct effects on adult height potential, could have major clinical and public health benefits.

Epigenetic regulation of puberty timing

The recent GWAS for age at menarche in women identified loci at three imprinted gene regions, *MKRN3-MAGEL2, DLK1,* and *KCNK9*, which showed parent-of-origin specific associations that were concordant with the established parent-of-origin specific mRNA expression patterns. Thus, at the exclusively paternally-expressed loci (*MKRN3-MAGEL2* and *DLK1*) only the paternally-inherited alleles (but not the maternally-inherited alleles) showed associations with menarche timing, and vice versa for the exclusively maternally-expressed locus, *KCNK9*. The association pattern at the *MKRN3-MAGEL2* locus is also concordant with the paternal-specific inheritance of rare pathological mutations in *MKRN3*[20]. Notably, a recent parent-of-origin specific GWAS for BMI identified two signals, including one near to *KCNK9*[52]. Intriguingly, both paternally- and maternally-inherited alleles at *KCNK9* are associated with BMI, but in opposite directions, and this SNP (rs2471083) is completely unrelated to the SNP identified for age at menarche (rs1469039; linkage disequilibrium r^2= 0.001). Therefore, there may be distinct biological mechanisms regulated by this same imprinted region.

More widely, there was enrichment of the 106 menarche-associated regions in known imprinted gene regions[35]. The importance of imprinting on pathways relating to energy balance in animal models[53]

suggest that there may be further important motifs that also affect reproduction in humans. These findings indicate that the expression and phenotypic manifestation of some genetic factors that influence puberty timing are tightly regulated by stable epigenetic marks that reflect parental origin.

In addition to gene imprinting, the recent GWAS findings strongly indicate the potential involvement of other epigenetic mechanisms in puberty timing. These include JmjC-domain-containing demethylases that catalyse gene activating epigenetic changes specifically at lysine 9 histone H3 marks[35]. Furthermore, a menarche-associated locus was identified at *CBX7*, which encodes a component of the Polycomb group of transcriptional silencers[54]. These GWAS findings support the likely relevance in humans of the reported close epigenetic regulation of reproductive timing in animal models. Pubertal onset in female rats is reportedly triggered by DNA methylation and decreased hypothalamic expression of two key Polycomb group genes, *Eed* and *Cbx7*, which in turn leads to enrichment of activating lysine modifications on histone H3 and activation of expression of *Kiss1*[54]. Studies in rhesus monkeys suggest that methylation and histone modification of the *GnRH* gene itself affect expression[55]. Beyond the completion of puberty and attainment of sexual maturity, epigenetic mechanisms may also contribute to the regulation of reproductive function, with epigenetic fluctuations observed across the female ovulation cycle[56].

Other mechanisms inferred by GWAS

In addition to those menarche GWAS loci identified in/near genes that underlie rare disorders of puberty, other menarche loci point to potential new candidate genes or biological pathways for such disorders. A menarche locus on chromosome 5 lies near *PCSK1*, in which rare mutations cause severe early onset-obesity and delayed/absent puberty was described in the one reported adult case[57]. The further menarche locus on chromosome 20 near *PCSK2* strongly points to a common role of the encoded type 1 and 2 prohormone convertases in the regulation of pubertal timing and reproductive function. Other menarche loci indicate candidate genes for GnRH neuron migration and pituitary development not yet linked to Kallmann syndrome and other hypothalamic/pituitary disorders. rs9647570 on chromosome 5 lies within *TENM2*, which encodes a transmembrane protein expressed in developing brain and is induced by the product of the Kallmann syndrome gene *FGF8*[58]. rs2479724 on chromosome 6 lies near *FRS3*, which encodes Fibroblast growth factor receptor substrate 3 and is involved in the signalling of the product of the Kallmann syndrome gene *FGFR1*[59]. rs7103411 and rs1129700 are menarche signals strongly associated with expression levels of *LGR4* and *TBX6*, respectively, which are both genes that encode enhancers for the pituitary development factor *SOX2*. We anticipate that further overlap with GWAS findings will be uncovered, particularly as studies of rare disorders incorporate exome-wide and genome-wide sequencing approaches.

GWAS menarche loci were highly enriched in/near genes that encode nuclear hormone receptors, co-activators or co-repressors, including those receptors involved in retinoic acid (RA) signalling. The cluster of retinoid receptors comprises the RA receptors (RARA, RARB and RARG), the retinoic X receptors (RXRA, RXRB and RXRG), and also the RAR-related orphan receptors (RORA, RORB, and RORC). They bind to RA response elements, activate gene transcription and have diverse functions across embryonic development (including GnRH neuron migration[60]), cell differentiation and homeostasis (including GnRH expression and secretion[61]).

In addition to the substantial role of genetic determinants obesity susceptibility on pubertal timing, our findings indicate other mechanisms that could explain the epidemiological associations between early menarche and higher risks of adult disease, which are partially independent of BMI. The first studies for age at menarche identified variants intronic in *LIN28B*[62–64], which encodes a potent and specific regulator of *let-7* microRNA processing. Experimental overexpression of its homologue *Lin28a* promotes growth, delays pubertal onset and maturation and enhances insulin sensitivity, while fetal (but not neonatal or adult) deficiency of *Lin28a* and *Lin28b* impairs results in postnatal growth defects, insulin resistance and glucose intolerance[65]. Other possible mechanisms linking puberty timing to adult disease include actions

of $GABA_B$ receptor signalling on pancreatic beta cell function[66], mitochondrial sirtuin 3 on cell responses to oxidative stress[67], and DLK1/preadipocyte factor 1 on peripheral lipid oxidation and lipid storage[68].

Conclusions

Genetic studies have underpinned our understanding of biological mechanisms that regulate puberty timing. Both monogenic studies of patients with rare disorders and polygenic studies of large population-based samples support the key role of embryonic development and signalling pathways of the hypothalamic-pituitary axis, as well as important links between energy homeostasis, growth and development, which re-enforce the critical connection between energy balance and reproduction. While further evidence is needed to identify and confirm the causal genes at GWAS loci, pathway-based analyses provide strong evidence for mechanisms such as retinoid signalling and lysine-specific demethylases.

Finally, the timing of childhood growth and development has been much debated as a trait with putative selection advantages. In adverse settings characterised by extremely high child and early adult mortality, early puberty and onset of reproductive maturity may increase fecundity despite the side effect of short adult height[69]. Conversely, in a recent analysis genetic variants associated with later age at menarche show evidence of positive selection in both European and African populations[70]. The enrichment of menarche-associated SNPs in imprinted gene regions further supports puberty timing as a selective trait and indicates parental conflict in this evolutionary perspective. Genomic imprinting results in monoallelic expression exclusively from the maternal or paternal copy. It affects only around 100 mammalian genes and supposedly evolved to control the dosage of developmentally important genes, such as those that regulate placentation, embryonic growth, infant feeding or parenting[71]. It has been hypothesized that paternal fitness is enhanced by maximising resource allocation to his offspring; while maternal fitness is enhanced by reserving resources for her subsequent children[72,73]. In addition to such reflective insights, the increasing evidence implicating epigenetic mechanisms in puberty timing and reproductive function may inform future understanding of early life programming and therapeutic strategies.

Table 1: Recent genome-wide association studies (GWAS) for puberty timing and anthropometric outcomes.

Reference	Phenotype	Study size	Number of genomic loci (novel loci)	Number of independent signals	Variance explained by the top signals
Perry et al (2014)	Age at Menarche	182,427 (women only)	106 (65)	123	2.7%
Locke et al (2015)	Adult BMI	339,224	97 (56)	103	2.7%
Shungin et al (2015)	Adult Waist-to-hip ratio (adjusted for BMI)	224,459	49 (33)	65	2.4% (women) 0.8% (men)
Wood et al (2014)	Adult Height	253,288	423 (243)	697	16%

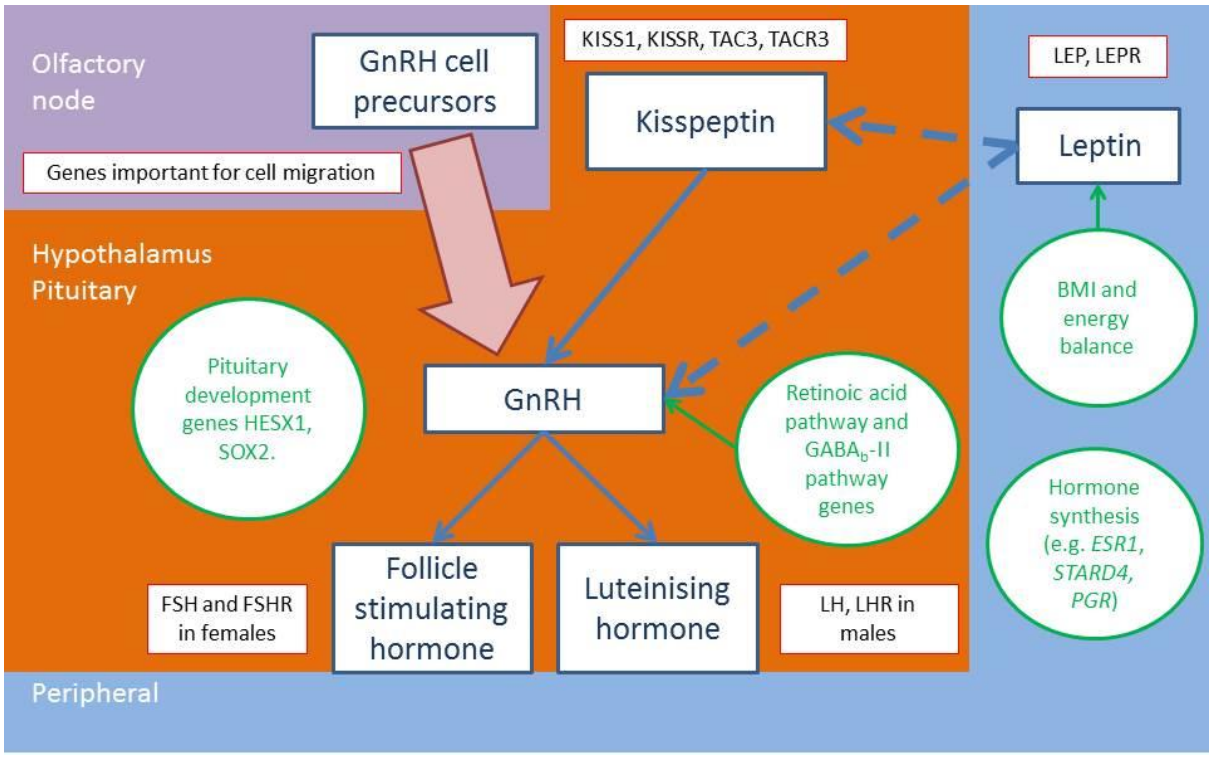

Figure 1. Schematic diagram of the biology of puberty initiation, knowledge from rare and mono-genetic studies is bounded in red, the suggested mechanisms of common variants are bounded in green.

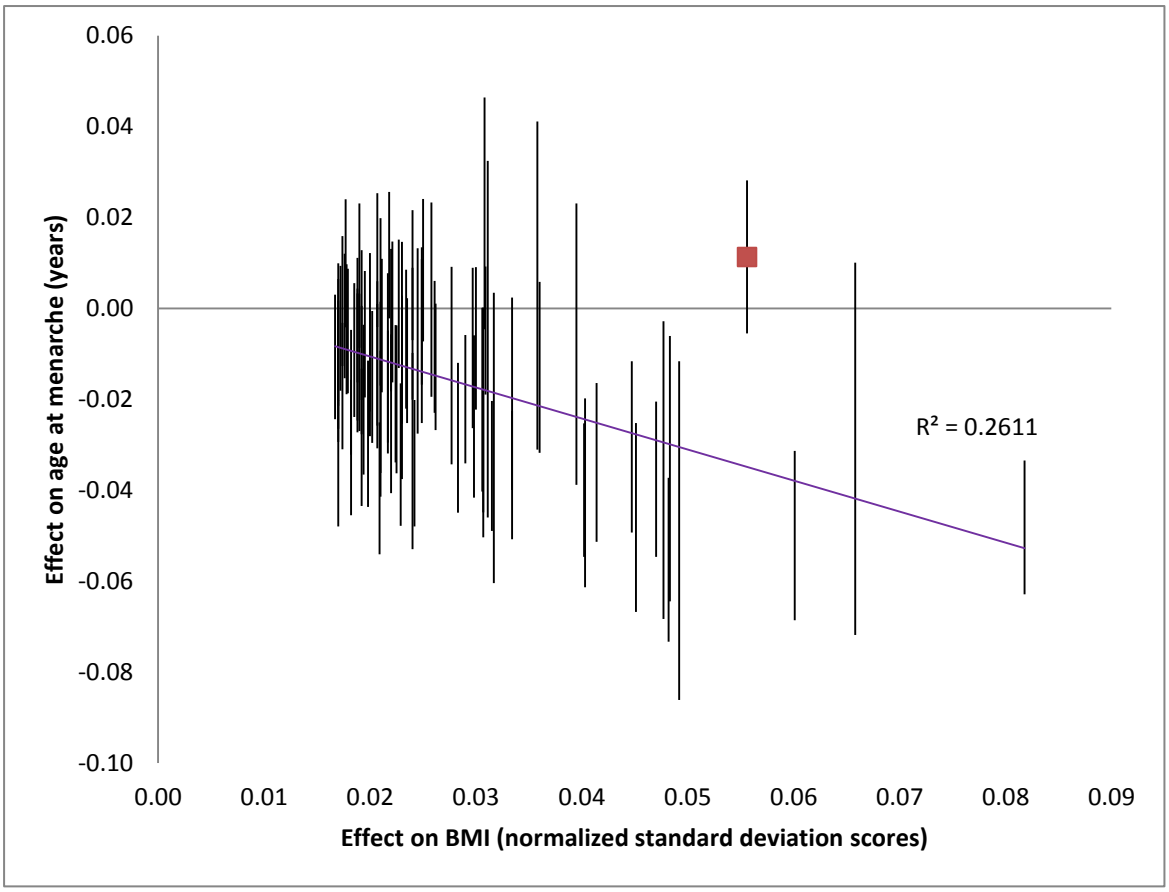

Figure 2 . Plot of the 97 common variants identified by genome-wide association studies for BMI, indicating their per-allele effect sizes on adult BMI (with 95% confidence intervals) compared to their effect sizes on age at menarche. The R^2 value indicates the strength of correlation between effect sizes. In general, BMI-increasing alleles also confer earlier menarche. The *MC4R*-region variant (highlighted in red) is a notable exception.

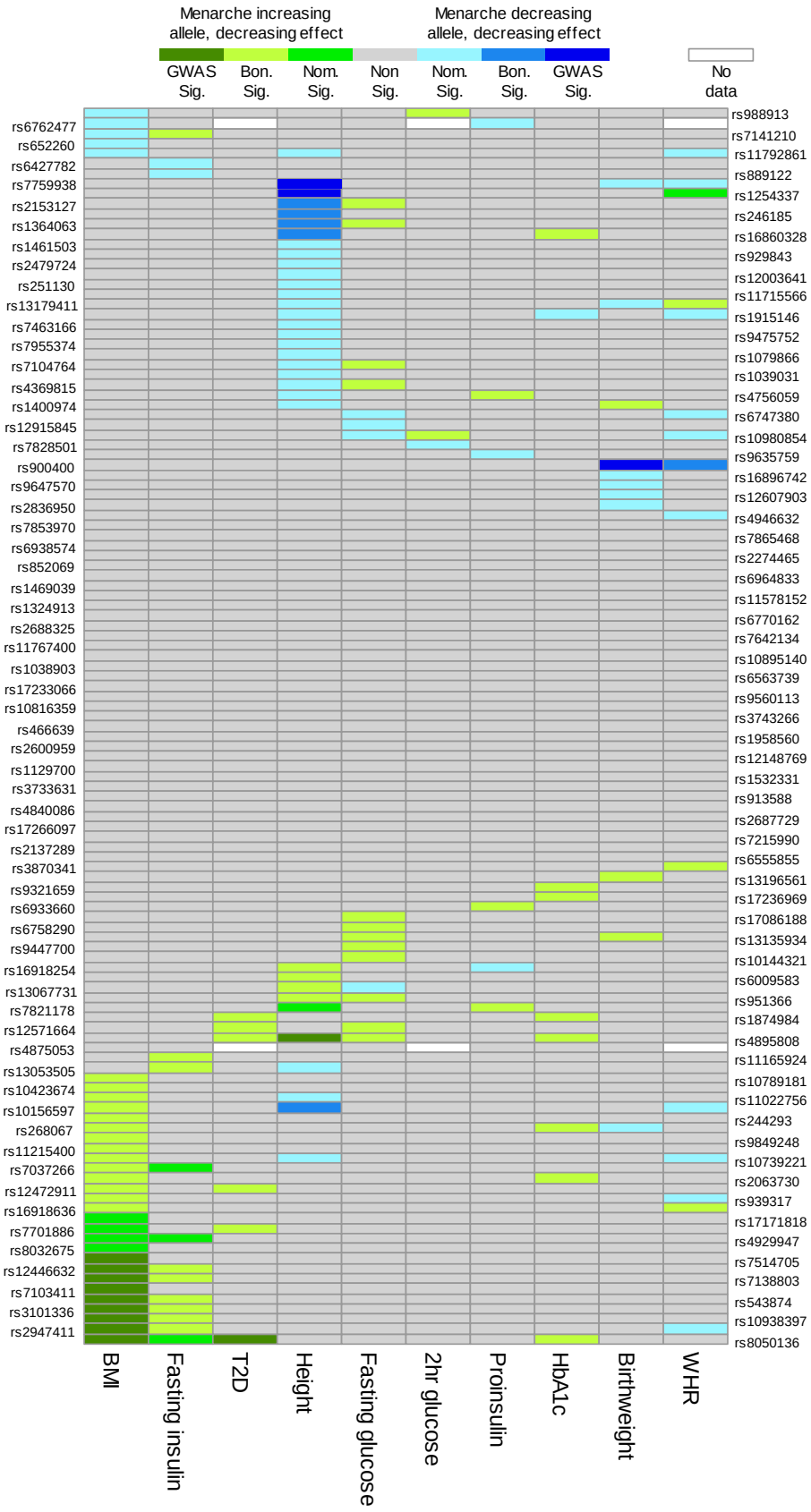

Figure 3. Heatmap indicating the effects of 123 common variants identified for age at menarche on a number of metabolic-related phenotypes (data derived from publically-available datasets[41,46,74–78]).

Green bars indicate menarche age-decreasing variants that are also associated with higher values of other phenotypes; blue indicates menarche age-decreasing variants also associated with lower values of other phenotypes.

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