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Genome-wide association study of age at menarche in African-American women

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African-American (AA) women have earlier menarche on average than women of European ancestry (EA), and earlier menarche is a risk factor for obesity and type 2 diabetes among other chronic diseases. Identification of common genetic variants associated with age at menarche has a potential value in pointing to the genetic pathways underlying chronic disease risk, yet comprehensive genome-wide studies of age at menarche are lacking for AA women. In this study, we tested the genome-wide association of self-reported age at menarche with common single-nucleotide polymorphisms (SNPs) in a total of 18 089 AA women in 15 studies using an additive genetic linear regression model, adjusting for year of birth and population stratification, followed by inverse-variance weighted meta-analysis (Stage 1). Top meta-analysis results were then tested in an independent sample of 2850 women (Stage 2). First, while no SNP passed the pre-specified $P < 5 \times 10^{-8}$ threshold for significance in Stage 1, suggestive associations were found for variants near *FLRT2* and *PIK3R1,* and conditional analysis identified two independent SNPs (rs339978 and rs980000) in or near *RORA*, strengthening the support for this suggestive locus identified in EA women. Secondly, an investigation of SNPs in 42 previously identified menarche loci in EA women demonstrated that 25 (60%) of them contained variants significantly associated with menarche in AA women. The findings provide the first evidence of cross-ethnic generalization of menarche loci identified to date, and suggest a number of novel biological links to menarche timing in AA women.

INTRODUCTION

The timing of the age at first menses (menarche) is one of the primary features shaping female reproductive history and is associated with a number of current and later life health outcomes (1). Earlier age at menarche is associated with increased risk for breast cancer (2–4), reduced stature and increased risk of obesity (5–7) and type 2 diabetes (8), whereas late menarche may be associated with an increased risk of Alzheimer's disease (9) and stroke (10), as well as lower fertility (11,12). Identification of genetic variants influencing variation in the age at menarche may thus shed light on mechanisms involved in a number of chronic diseases in women.

Age at menarche is under relatively strong genetic control, with heritability estimated at ~50% (13–16). Candidate gene association studies point to the involvement of a number of genetic pathways, most notably those involved in steroid hormone signaling and transport [e.g. estrogen receptor (ER) genes and sex hormone binding globulin gene] and estrogen biosynthesis and metabolism (such as *CYP17*, *CYP19*, *CYP1A1*, and *CYP1B1*) (17), although many of these associations have not been reliably replicated. In 2009, four large genome-wide association studies (GWASs) in women of European ancestry (EA) were published that together identified two novel genetic loci associated with age at menarche, *LIN28B* and the intergenic region 9q13.2 (18–21). Some of these variants are probably involved in general growth rate, as *LIN28B* variants are associated with pubertal timing, height and body mass index (BMI) growth in children (20,22,23) as well as with body size and pubertal traits in animal models (24). Variants near the 9q13.2 SNP are also associated with height in GWAS (25). More recently, a GWAS in over 85 000 EA women identified a further 30 genome-wide significant loci, and 10 suggestive loci, yielding a total of 42 loci associated with menarche timing (26). A number of these had been previously identified as obesity loci, highlighting genetic pleiotropy between female adiposity and timing of menarche, an observation that supports the long-recognized link between these traits from epidemiologic studies (7,27).

There is ethnic variation in the timing of menarche, with African-American (AA) girls currently experiencing menarche ~4–6 months earlier, on average, than EA girls in the USA (6,28–32). In addition, compared with non-Hispanic White women, non-Hispanic Black women in the USA tend to have twice the prevalence of chronic diseases known to be related to early age at menarche, including childhood obesity (33), the Metabolic Syndrome (34) and diabetes (35), as well as higher prevalence and earlier onset of hypertension (36). Despite the significant heritability of age at menarche and the persistent ethnic variation in both age at menarche and its associated diseases, only one recently published study has sought to identify menarche-related genetic variants in AA women (37).

Here, we present a meta-analysis of GWAS from 15 studies including over 18 000 women to test the association of common genetic variants with age at menarche in AA women. We also conducted a targeted investigation of variants within a ±250 kb region around the 42 SNPs recently reported in EA women, in order to test whether these loci contain variants associated with menarche in AA women and to potentially identify stronger markers of the associations. The study demonstrates (i) suggestive evidence for association of age at menarche in AA with a number of variants in loci involved in growth and insulin signaling, (ii) multiple independent SNP associations in or near *RORA*, previously identified as a possible menarche locus in EA women and (iii) cross-ethnic generalization of the majority of menarche loci identified to date in EA women.

RESULTS

A total of 18 089 AA women with self-reported age at menarche were included in the Stage 1 meta-analysis. Participants were drawn from seven population-based cohort studies and eight breast-cancer case–control studies, in which association analyses were conducted in cases and controls separately. All Stage 1 studies used agnostic, genome-wide SNP genotyping arrays that were not enriched for SNPs in any particular molecular pathways or candidate regions (see Supplementary Material, Table S1, and Methods). A total of 2850 AA women in the Black Women's Health Study (BWHS) were genotyped *de novo* as the Stage 2 replication sample. Descriptive characteristics of each study are presented in Table 1 and in Supplementary Material, Text S1. Mean age at menarche in the studies was 12.6 years (range 8–21 years). Not all studies reported the year of birth; for those that did, the year of birth at the individual level ranged from 1908 to 1978, and studies that were born later, on average (e.g. CARDIA) had lower mean age at menarche than studies that were born earlier (e.g. ARIC), which is consistent with the downward secular trend in age at menarche during the 20th century (38).

An overview of the flow of experiments/analyses performed in this study and a summary of their results is provided in Figure 1. The following paragraphs provide details on the results from these two primary experiments: (i) a meta-analysis of GWAS of age at menarche in AA women and (ii) a targeted interrogation of 42 loci previously reported to be associated with age at menarche in EA women.

Meta-analysis of GWASs of age at menarche in AA women

All Stage 1 studies performed regression analyses to test the linear association of each SNP genotype with age at menarche using an additive genetic model. Covariates included study center (if appropriate), year of birth (or age at study enrollment if birth year not available) to account for known secular trends and the first 10 principal components scores from EIGENSTRAT to adjust for population stratification. Further details of the genotyping, quality control (QC) and analysis methods used for each study are provided in Supplementary Material, Table S1. A quantile-quantile plot of the meta-analysis *P*-values shows that the test statistics follow the null expectations, with no excess of small *P*-values beyond that expected by chance (Supplementary Material, Fig. S1; lambda = 1.03). No SNP passed the pre-specified $P < 5 \times 10^{-8}$ threshold for genome-wide significance in Stage 1 (Supplementary Material, Fig. S2).

Table 2 displays SNPs with the lowest *P*-values from the Stage 1 meta-analysis, using the threshold of $P < 1 \times 10^{-5}$, and other criteria that are described in the Materials and Methods section. Regional association plots for all 20 of these top regions are provided in Supplementary Material, Figure S3. There was little evidence of heterogeneity of SNP effects by study as indicated by *P* for heterogeneity generally >0.05 and never <0.02. There was no indication of systematic deviation of results in breast cancer cases compared with controls or population-based cohort samples (data not shown).

The most statistically significant association was with SNP rs4557202, near *B3GALNT3* ($P = 3.51 \times 10^{-7}$), a gene involved in lipid synthesis and metabolic pathways. An intronic enhancer SNP on chromosome 11q23 (rs11216435) near *DSCAML1* (Down-syndrome cell-adhesion molecule-like 1) and an SNP on chromosome 15q22 (rs339978) near *RORA* (the nuclear hormone receptor, RAR-like orphan receptor-alpha) were associated at $P < 1 \times 10^{-6}$. A second SNP (also an intronic enhancer) near *RORA* (rs980000) was associated at $P = 4.86 \times 10^{-6}$. These latter findings provide evidence of cross-ethnic validation for *RORA*, which had suggestive (but not confirmed) association with age at menarche in the EA ReproGen analysis (26). The two intronic variants we identified at *RORA* are common in AA women [minor allele frequency (MAF) of 0.20 for rs339978 and 0.26 for rs980000] but not in EA women (MAF of 0.02 and 0.05 in 1KGP EUR). These SNPs are in low linkage disequilibrium (LD) with one another in HapMap 1000 Genomes Project (1KGP) African samples (AFR in 1KGP, $r^2 = 0$) and are only modestly correlated in EA populations (CEU in 1KGP, $r^2 = 0.34$). Neither of these SNPs were in LD in either population with the index signal (rs3743266) reported previously at *RORA* in women of EA ($r^2 = 0$).

We also replicated the *ZNF483* locus previously reported in EA women (26) in AA women. The two most significant associations in Stage 1 were intronic enhancer variants rs7873730 and rs10441737 near *ZNF483* on chromosome 9q31. SNP rs10441737 is a near-perfect proxy for the known menarche variant rs10980926 at this locus in EA women (EUR in 1KGP, $r^2 = 0.98$), with LD also observed in African samples (AFR in 1KGP, $r^2 = 0.44$). The SNP rs7873730 was imputed in all studies (MACH r^2 was between 0.72 and 0.86 across studies),

Table 1. Description of participating cohorts

Consortium name/cohort name		Cohort acronym	Age at menarche			Birth year	
			n	Mean (SD)	Range	Mean	Range
AABC (African American Breast Cancer Cohorts)	The Women's Contraceptive and Reproductive Experience Study	CARE (cases)	357	12.4 (1.7)	8–18	NA	NA
		CARE (controls)	215	12.3 (1.74)	9–18	NA	NA
	The Carolina Breast Cancer Study	CBCS (cases)	634	12.6 (1.8)	8–21	NA	NA
		CBCS (controls)	586	12.6 (1.75)	8–18	NA	NA
	The Multiethnic Cohort	MEC (cases)	532	12.9 (1.66)	10–17	NA	NA
		MEC (controls)	972	13.2 (1.6)	10–17	NA	NA
	The Nashville Breast Health Study	NBHS (cases)	304	12.6 (1.99)	8–21	NA	NA
		NBHS (controls)	182	12.4 (1.9)	8–21	NA	NA
	Northern California Breast Cancer Family Registry/San Francisco Breast Cancer Study	NC-BCFR/SFBCS (cases)	575	12.6 (1.8)	8–20	NA	NA
		NC-BCFR/SFBCS (controls)	269	12.6 (1.8)	8–20	NA	NA
	The Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial	PLCO (Cases)	56	13.3 (1.3)	9–16	NA	NA
		PLCO (controls)	116	13.1 (1.76)	9–16	NA	NA
	The Women's Circle of Health Study	WCHS (cases)	260	12.7 (1.9)	9–19	NA	NA
		WCHS (controls)	238	12.7 (1.7)	9–18	NA	NA
	The Wake Forest University Breast Cancer Study	WFBC (cases)	112	12.5 (1.6)	8–16	NA	NA
		WFBC (controls)	116	12.7 (1.7)	9–18	NA	NA
CARe (Candidate Gene Association Resource)	Atherosclerosis Risk in Communities	ARIC	1,690	12.87 (1.66)	9–17	1934	1921–1945
	Coronary Artery Disease in Young Adults	CARDIA	630	12.48 (1.47)	9–17	1959	1957–1969
	Cleveland Family Study	CFS	169	12.22 (1.37)	10–16	1964	1908–1997
	Jackson Heart Study	JHS	1,228	12.77 (1.70)	9–17	1952	1910–1982
Women's Health Initiative		WHI	8,086	12.6 (1.64)	9–17	1935	1913–1948
Healthy Aging in Neighborhoods of Diversity across the Life Span		HANDLS	617	12.6 (1.80)	9–17	1962	1946–1980
Bogalusa Heart Study		BHS	145	12.5 (1.37)	9–17	1966	1959–1978
		TOTAL Stage 1	18,089				
Black Women's Health Study (Stage 2 Replication Cohort)		BWHS	2,850	12.4 (1.6)	9–17	1947	1925–1974

was less common in each population than the index SNP (MAF of 0.06 in EUR and 0.10 in AFR) and was only weakly correlated with either of the two SNPs in both EUR and AFR (r^2 of 0.08–0.18).

Conditional analysis

To further explore the evidence of multiple independent signals within the *RORA* and *ZNF483* loci, we performed conditional analyses in which both of our top SNPs and the index SNP reported for EA were included as covariates in each of the two independent regression models (Table 3; Fig. 2). For the *RORA* locus, we found evidence of two independent signals; both rs980000 ($P = 6.8 \times 10^{-5}$) and rs339978 ($P = 2.5 \times 10^{-3}$) remained significant when considered in the same model with the EA index SNP. This finding suggests that there may be multiple functional variants for menarche at this locus in AA women. For *ZNF483*, we found evidence of a single signal, as only one of the three SNPs (rs10441737) remained nominally significant ($P = 0.025$) when rs7873730 and rs10980926 (the EA index SNP) were included in the regression model.

Stage 2 analysis in the black women's health study

We examined the 20 top SNPs from the Stage 1 meta-analysis in AA women from the BWHS. For 16 of the 20 SNPs that underwent testing in Stage 2, the direction of effect in the replication sample was consistent with Stage 1 (Table 2). Two SNPs also replicated at a nominal P value (<0.05): rs8014131 near *FLRT2* (BWHS $P = 0.021$; combined $P = 3.4 \times 10^{-7}$) and rs10940138 near *PIK3R1* (BWHS $P = 0.018$; combined $P = 4.1 \times 10^{-7}$), which is involved in the metabolic functions of insulin. Suggestive evidence for replication (consistent direction and nominally significant P value) was also noted for rs12907866 near the aromatase gene *CYP19A1* (BWHS $P = 0.065$; combined $P = 4.4 \times 10^{-7}$) centrally involved in estrogen synthesis, and rs17669535 in *DLGAP2* (BWHS $P = 0.057$; combined $P = 6.4 \times 10^{-7}$). No SNPs passed a Bonferroni-corrected P-value threshold for significance in Stage 2.

Examination of the Stage 1 findings in EA women

We also evaluated potential associations between the 20 top SNPs from our Stage 1 meta-analysis in AA women and age at menarche in women of EA using data from the ReproGen con-

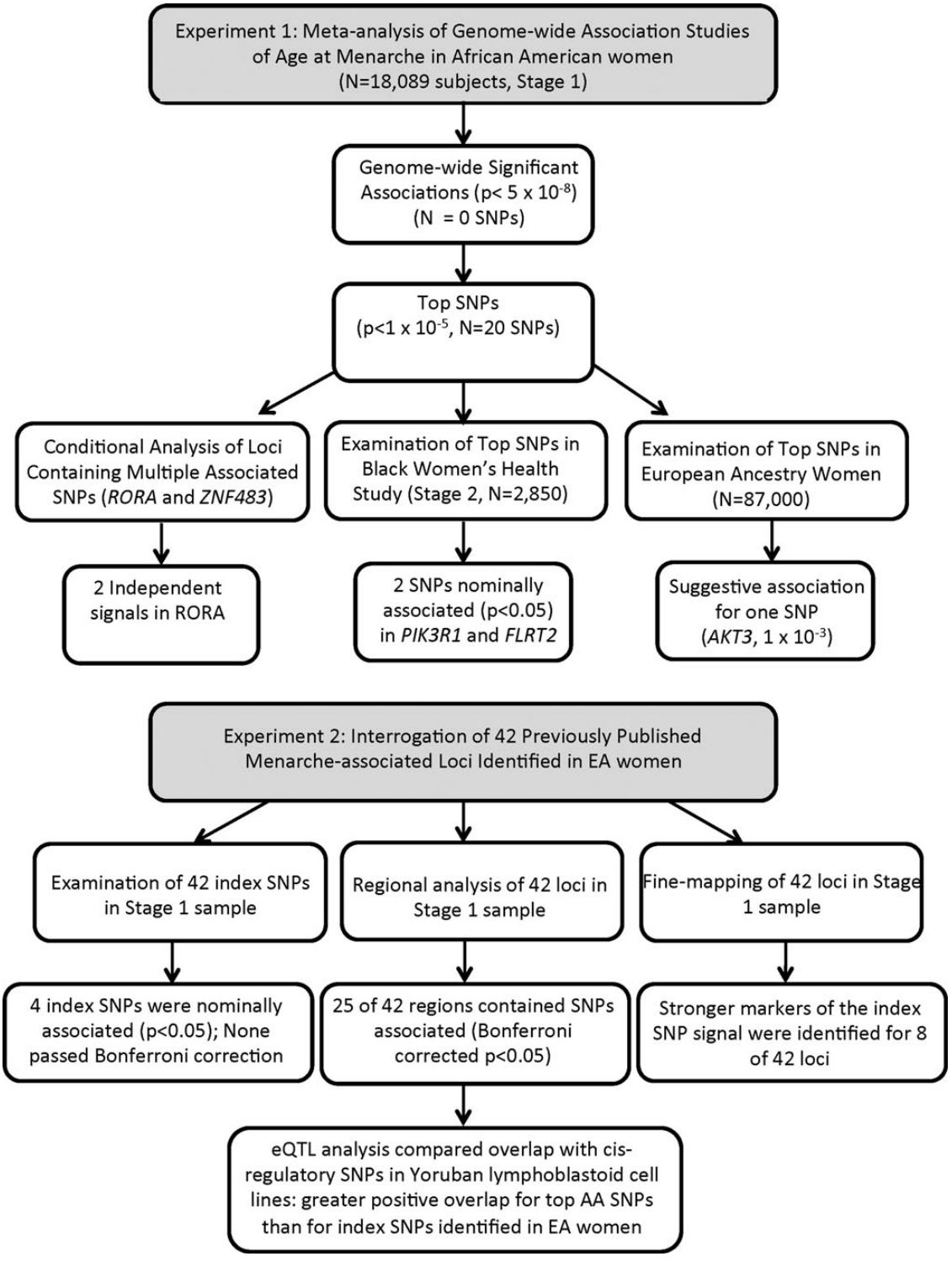

Figure 1. Overview of analysis and results. Flow of analyses and brief summary of results from each analysis are presented for the genome-wide meta-analysis of age at menarche in AA women and for the targeted interrogation of 42 menarche loci previously reported in EA women.

sortium (Supplementary Material, Table S2). AA associations were directionally consistent with EA associations for 14 of the 20 variants, 3 of which were associated with age at menarche (Bonferoni-corrected $P < 0.05$). We found suggestive evidence of association near *AKT3*. The SNP rs320320 was associated in ReproGen at $P = 1.06 \times 10^{-3}$, and the combined Stage 1 AA + ReproGen EA meta-analysis yielded $P = 1.18 \times 10^{-7}$). *AKT3* is one of the AKT kinases, which encodes RAC-gamma serine/threonine-protein kinase, and regulates cell signaling in response to insulin and growth factors. In addition to *AKT3*,

Table 2. Meta-analysis of age at menarche in AA women: Stage 1 and 2 results[a]

SNP	Chr.	Nearest gene	Position (Build 36)	Alleles[b]	EAF[c]	Stage 1 (maximum $n = 18\,089$)			Stage 2 (maximum $n = 2850$)		Stage 1 and Stage 2 (maximum $n = 20\,939$)	
						Effect β (SE)[d]	P	P_{het}	Effect β (SE)	P	Effect β (SE)	P
rs4557202[e]	3	*B3GALNT1*	162303218	C/G	0.43	−4.90 (0.96)	3.51E − 07	0.238	−0.50 (2.24)	0.822	−4.21 (0.88)	1.86E − 06
rs11216435	11	*DSCAML1*	116894142	T/C	0.32	5.11 (1.02)	6.33E − 07	0.966	0.30 (2.49)	0.904	4.41 (0.95)	3.31E − 06
rs339978	15	*RORA*	58724694	T/C	0.20	5.90 (1.21)	9.95E − 07	0.193	2.00 (2.85)	0.484	5.31 (1.11)	1.76E − 06
rs1476150	2	*NAP5*	133592865	C/G	0.65	5.69 (1.18)	1.23E − 06	0.683	−1.36 (2.35)	0.056	4.28 (1.05)	4.51E − 05
rs7754121	6	*HDGFL1*	23281592	A/G	0.10	7.69 (1.59)	1.36E − 06	0.434	−0.28 (3.79)	0.942	6.49 (1.47)	9.61E − 06
rs320320	1	*AKT3*	241901809	A/G	0.48	4.55 (0.95)	1.84E − 06	0.117	1.03 (2.29)	0.652	4.03 (0.88)	4.68E − 06
rs12907866	15	*CYP19A1*	49332746	A/G	0.84	6.15 (1.31)	2.53E − 06	0.587	5.63 (3.05)	0.065	6.06 (1.20)	4.36E − 07
rs6468994	8	*ZFPM2*	106365896	T/C	0.64	4.67 (1.00)	2.81E − 06	0.309	−2.69 (2.34)	0.251	3.54 (0.92)	1.14E − 04
rs11071033	15	*UNC13C*	52167492	T/C	0.71	4.80 (1.03)	3.54E − 06	0.749	2.67 (2.50)	0.286	4.49 (0.96)	2.70E − 06
rs7807441	7	*FLJ13195*	66826223	T/C	0.55	−4.32 (0.94)	4.14E − 06	0.618	−2.28 (2.25)	0.311	−4.02 (0.87)	3.48E − 06
rs17669535	8	*DLGAP2*	1231631	C/G	0.97	14.50 (3.15)	4.20E − 06	0.966	14.49 (7.61)	0.057	14.50 (2.91)	6.37E − 07
rs6947406	7	*C7orf10*	41013150	A/G	0.87	−6.43 (1.40)	4.78E − 06	0.963	−0.16 (3.31)	0.961	−5.48 (1.29)	2.34E − 05
rs980000	15	*RORA*	58688255	T/C	0.26	4.88 (1.07)	4.86E − 06	0.048	3.55 (2.57)	0.168	4.69 (0.99)	2.03E − 06
rs8014131	14	*FLRT2*	85033609	A/C	0.42	−4.48 (0.98)	5.24E − 06	0.157	−5.29 (2.29)	0.021	−4.61 (0.90)	3.44E − 07
rs7819115	8	*DLGAP2*	1549163	A/C	0.36	−4.52 (0.99)	5.52E − 06	0.471	−1.19 (2.36)	0.614	−4.01 (0.92)	1.18E − 05
rs7873730	9	*ZNF483*	113343500	A/T	0.88	−7.43 (1.65)	6.35E − 06	0.026	−4.84 (2.92)	0.104	−6.82 (1.44)	2.17E − 06
rs10441737	9	*ZNF483*	113341406	T/C	0.58	−4.37 (0.97)	6.55E − 06	0.084	−0.11 (2.24)	0.961	−3.70 (0.89)	3.22E − 05
rs10940138	5	*PIK3R1*	67230225	T/C	0.19	5.43 (1.21)	6.78E − 06	0.526	6.77 (2.87)	0.018	5.64 (1.11)	4.09E − 07
rs7911165	10	*EBF3*	131516640	T/C	0.54	4.72 (1.05)	7.09E − 06	0.985	1.80 (2.21)	0.415	4.18 (0.95)	1.05E − 05
rs2796200	1	*ZRANB2*	71431476	A/G	0.66	4.46 (0.99)	7.10E − 06	0.745	−2.81 (2.35)	0.232	3.35 (0.92)	2.45E − 04

[a]Top independent (pairwise $r^2 < 0.3$) SNPs, all with $n > 10\,000$ out of 18 089, MAF > 0.03, and $P < 10^{-5}$ in meta-analysis.
[b]Effect/non-effect allele.
[c]Effect allele frequency.
[d]Effect β is in weeks.
[e]Stage 2 was conducted using proxy snp rs7651087 (effect allele = C/non-effect allele = T, EAF = 0.4353, $r^2 = 1.0$, 1000 Genomes Project—YRI).

Table 3. Conditional analysis of multiple SNPs near ZNF483 and RORA

Chr., gene	SNP	Position (Build 36)	Coded allele, frequency in AFR	Marginal beta[a], *P*	Conditional beta[a], *P*
9, *ZNF483*	rs10980926 (index)	113333455	A, 0.61	2.34, 0.019	−0.12, 0.93
9, *ZNF483*	rs7873730	113343500	A, 0.88	$-7.43, 6.4 \times 10^{-6}$	−0.56, 0.77
9, *ZNF483*	rs10441737	113341406	T, 0.58	$-4.37, 6.6 \times 10^{-6}$	−3.22, 0.025
15, *RORA*	rs3743266 (index)	58568805	C, 0.33	−0.69, 0.49	−0.016, 0.43
15, *RORA*	rs339978	58724694	T, 0.20	$5.90, 1.0 \times 10^{-6}$	$3.68, 2.54 \times 10^{-3}$
15, *RORA*	rs980000	58688255	T, 0.26	$4.88, 4.9 \times 10^{-6}$	$4.31, 6.78 \times 10^{-5}$

[a]Beta values are for effect of SNP on menarche age, in weeks. Conditional analyses were conducted in the largest cohort/studies (WHI, CARe and AABC) using individual-level genotype data in two linear regression models (one for each locus), each of which included the three SNPs, birth year (or enrollment age), study center (if applicable) and the top 10 PCs as covariates. The results were then meta-analyzed using METAL. Bonferroni-corrected *P* values were used to identify independent signals in the conditional analyses, with $P < 0.05$ as the criterion for independence.

both of the SNPs near *ZNF483 (*rs7873730 and rs10441737) also replicated in EA women, which was expected given that *ZNF483* was originally identified as a menarche locus in the ReproGen cohorts (described above) and because these SNPs were in high LD with the index SNP in this locus.

Interrogation of previously published menarche-associated Loci

The second goal of our study was to systematically interrogate the set of 42 menarche loci discovered in EA populations in AA women to confirm cross-ethnic replication of these loci and to identify possible stronger markers of the signals. First we examined the 42 EA index SNPs in these loci and found 26 had the same direction of effect on age at menarche in AA women, although only four of these were also nominally associated with age at menarche in our AA samples ($P < 0.05$). Other than *ZNF483* (discussed above), these included SNPs near *CCDC85A*, *C6orf173*, and *RXRG* (Supplementary Material, Table S3). None of these passed the Bonferroni-adjusted *P*-value threshold for significance ($P < 0.0011$).

We next investigated SNPs in a 250 kb region surrounding the 42 index SNPs that were associated with age at menarche in EA (see Materials and Methods) (Table 4), using a Bonferroni correction for the number of effective SNPs queried in each region. Generally, the SNP with the lowest *P*-value in African populations was in low LD with the index EA SNP in AA women ($r^2 < 0.2$) but was often in high LD with the index SNP in European populations ($r^2 > 0.8$), reflecting the same signal but better localizing it than was possible in the EA studies. Supplementary Material, Figure S4 shows regional association plots of age at menarche in AA women with SNPs within each 42 of the interrogated loci. The main finding from this analysis was that we found cross-population validation for a large proportion of the 42 loci (25 of 42, or 60%). The strongest evidence of association was found for SNPs in *RORA* and *ZNF483* (as discussed above in relation to the top Stage 1 results), but there was also some evidence for cross-population locus replication of seven obesity-associated loci (26,39), including *FTO* (rs12149832, $P = 1.6 \times 10^{-3}$), *SEC16B* (rs543874; $P = 1.47 \times 10^{-2}$), *STK33*/*TRIM66* (rs12575252; $P = 3.27 \times 10^{-2}$) and *RXRG* (rs3767342; $P = 4.49 \times 10^{-2}$) where SNPs were in high LD with the index SNP in EUR ($r^2 > 0.8$), as well as *BSX* ($P = 4.39 \times 10^{-3}$), *TMEM18* (rs2685252; $P = 2.06 \times 10^{-2}$) and *LRP1B* (rs7607295; $P = 4.74 \times 10^{-2}$), which were not in high LD with the index SNP in EUR ($r^2 < 0.1$). In addition, as in EA women, age at menarche was associated with genetic variants near *LIN28B*, *PLCL1*, *NR4A2*, *MKL2* (corrected *P* value $< 5 \times 10^{-3}$ for all), and with variants in *INHBA* ($P = 1.3 \times 10^{-2}$). Inhibin A is secreted by the granulosa cells of the ovarian follicles in the ovaries to provide negative feedback on follicle-stimulating hormone and is a strong candidate gene for pubertal timing.

Next, we sought to identify stronger markers of the index signals in AAs through additional fine-mapping (see details of the approach in Materials and Methods). We found SNPs in 8 of the 42 EA regions that were more strongly associated with age at menarche in AA women when compared with the index signal in EA women (i.e. had a *P*-value for association with menarche <0.004, and a *P* value at least 1 degree of magnitude lower than the *P* for the corresponding index association in EA women), and also were in moderate to strong LD with the EA index SNP with $r^2 > 0.4$ with the index SNP in EA (i.e. represented the same genetic 'signal') (Table 5). These included *SEC16B, CCDC85A, EEFSEC, LIN28B, BSX, NARS2, STK33, and FTO*. For instance, at the obesity-related locus *SEC16B*, we detected a variant (rs543874) approximately 50 kb upstream of the index signal previously identified in EAs (rs633715) that was more strongly associated with menarche in AA women ($P = 4.9 \times 10^{-4}$) than was the index signal ($P = 0.12$). SNP rs543874 is more strongly correlated with rs633715 in EA populations ($r^2 = 0.91$ in 1KGP EUR) than in AA populations ($r^2 = 0.18$ in 1KGP AFR), which suggests rs543874 may be a better marker of the putatively functional variant in AAs. These relationships are illustrated for the index SNP and the stronger marker in *SEC16B* in a regional association plot (Fig. 3**)**. Similarly, in the widely replicated obesity locus *FTO*, rs12149832 may be a stronger marker of the functional variant due to its stronger evidence of association in AA women ($P = 2.0 \times 10^{-4}$) when compared with the index SNP identified in EA women (rs9939609, $P = 0.83$), and because it again represents the same signal as the index SNP in EUR populations ($r^2 = 0.88$ in 1KGP EUR) but not in AFR populations ($r^2 = 0.05$ in 1KGP AFR). Of interest, five of the eight stronger marker SNPs in this analysis were found in/near loci previously implicated in adiposity via GWA (*SEC16B*, *FTO*) (39) were

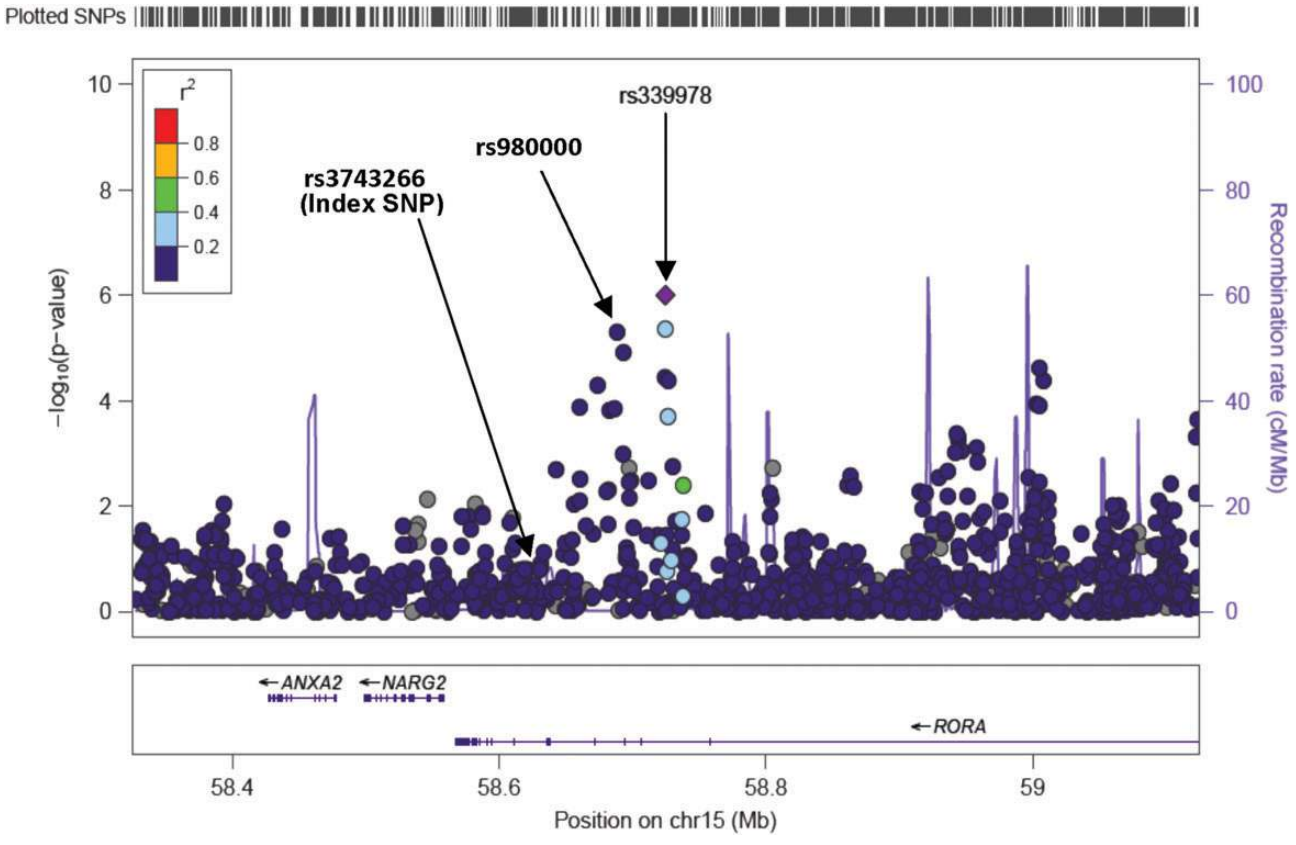

Figure 2. Conditional analysis identifies two independent signals for age at menarche near *RORA*, both of which are different from the previously identified index SNP in EA women. SNPs are plotted using Locus Zoom by position on the chromosome against association with age at menarche ($-\log_{10} P$). Estimated recombination rates are plotted in blue to reflect local LD structure using the 1KGP AFR reference panel, and the SNPs surrounding the top SNP from the Stage 1 meta-analysis (rs339978, purple diamond) are color coded to reflect their LD with this SNP. Both the first (represented by rs339978, $P = 1 \times 10^{-6}$) and the second signal (represented by rs980000, $P = 2 \times 10^{-6}$) were independent of the index SNP (rs3743266) previously reported for EA women (see Table 3).

associated with BMI in the GIANT consortium (*STK33/TRIM66*, *NARS2/GAB2*) (26), or are involved in neuronal feeding-control circuits (*BSX*) (40).

eQTL analysis of lead AA SNPs

For the 42 EA loci, there were 29 SNPs with at least one UCSC, Vega or RefSeq transcript showing expression in Yoruban African lymphoblastoid cell lines (LCLs). A total of 109 *cis*-regulatory SNPs were compared for the analysis of the index SNPs, while a total of 111 *cis*-regulatory SNPs were compared for the AA top SNP analysis. While 11.4% of the queried index SNPs positively overlapped with *cis*-regulatory SNPs observed in YRI LCLs, twice as many (23.4%) of the top AA SNPs from the regional analysis had positive overlap with *cis*-regulatory SNPs [nominal P value for $\chi^2 = 0.026$; P (Permutation) < 0.05]. This provides some evidence for the greater functional relevance of the SNPs identified in AA women when compared with the EA index SNPs. When limiting the comparison to SNPs that were significantly stronger markers of the index signal in the fine-mapping experiment, their overlap with *cis*-regulatory SNPs was not found to be significantly greater than for the index SNPs.

DISCUSSION

Identification of genetic variation controlling the development of chronic disease risk factors in childhood, such as early menarche, is important because it may point to effective targets for environmental and behavioral interventions in early life, before disease processes are fully entrenched. AA women now experience significantly earlier sexual development (28) and carry a much higher burden of obesity and diabetes than EA women (33,35); therefore, the search for genetic determinants of menarche timing may be of particular value in this population. Nonetheless, virtually all GWAS to date have been conducted in individuals of EA (41), and this is true of menarche GWAS as well; a recent systematic review on the genetics of menarche (42) found only one existing study that provided any estimates of measured genotype effects on age at menarche for AA women. Subsequently, there has been one study published using a targeted genome-wide approach [i.e. using the Metabochip (43) in ∼4000 AA women (37)]. In that study, no SNP association passed correction for multiple testing, and relatively poor coverage by the Metabochip of the previously reported menarche loci in EA women meant that cross-ethnic replication and generalization study was hampered (37).

Table 4. Twenty-five of 42 menarche loci identified in European Americans generalize to AA women[a]

Nearest genes	Chr	Index SNP identified in EA women[b]	Best SNP in the region in AA women[c]	LD (index SNP—AA best SNP), YRI Reference panel [R^2]	LD (index SNP—AA best SNP), CEU reference panel [R^2]	Best SNP coded allele (frequency)	Best SNP β (weeks)	Adjusted P-value[d]
RXRG	1	rs466639	rs3767342	0.839	0.877	T (0.85)	4.26	**0.045**
SEC16B	1	rs633715	rs543874	0.144	0.917	A (0.75)	3.77	**0.015**
LRP1B	2	rs12472911	rs7607295	0.206	NA	T (0.90)	−8.83	**0.047**
PLCL1	2	rs12617311	rs7557664	0.037	0.11	A (0.48)	−3.62	**0.003**
NR4A2	2	rs17188434	rs1113060	NA	0.002	T (0.36)	−4.25	**0.0004**
CCDC85A	2	rs17268785	rs17047854	0.318	0.943	A (0.34)	3.41	**0.010**
TMEM18	2	rs2947411	rs2685252	0.004	0.031	T (0.75)	3.41	**0.021**
SFRS10	3	rs2002675	rs4686718	0.002	0.073	T (0.35)	−3.23	0.05
EEFSEC	3	rs2687729	rs9819578	0.543	0.075	T (0.23)	5.47	**0.024**
ECE2	3	rs3914188	rs6770142	0.044	0	A (0.30)	2.87	0.093
3q13.32	3	rs6438424	rs16827902	0.012	NA	T (0.94)	12.58	**0.036**
TMEM108	3	rs6439371	rs7613434	0.129	0.005	A (0.13)	−4.65	**0.029**
RBM6;RBM5	3	rs6762477	rs12629572	0.003	0.071	T (0.76)	3.68	0.075
CCDC71	3	rs7617480	rs1464567	0.113	0.243	C (0.81)	−2.22	0.49
VGLL3	3	rs7642134	rs2879790	0.017	0.046	A (0.21)	−8.18	**0.012**
PHF15	5	rs13187289	rs12655967	0.023	0.017	A (0.53)	−7.71	0.029
JMJD1B	5	rs757647	rs11750854	0.013	0.082	A (0.67)	−4.27	0.46
C6orf173	6	rs1361108	rs9401888	0.404	0.806	A (0.78)	3.62	**0.018**
PRDM13	6	rs4840086	rs7740247	0.000	0.009	C (0.02)	−15.27	0.085
LIN28B	6	rs7759938	rs9386427	0.229	0.075	T (0.29)	4.10	**0.0025**
INHBA	7	rs1079866	rs17171859	0.097	NA	C (0.95)	11.89	**0.010**
PXMP3	8	rs7821178	rs6473010	0.042	0.006	A (0.99)	−47.64	0.097
ZNF483	9	rs10980926	rs7873730	0.093	0.118	A (0.88)	−7.43	**0.0021**
TMEM38B	9	rs2090409	rs7041138	0.002	0.129	T (0.51)	3.31	**0.015**
NARS2	11	rs10899489	rs1006441	0.105	0.841	C (0.15)	3.90	0.10
PHF21A	11	rs16938437	rs11600515	0.014	0.007	C (0.04)	8.61	**0.01**
STK33	11	rs4929923	rs12575252	0.439	0.959	C (0.51)	3.10	**0.033**
BSX	11	rs6589964	rs17126930	0.128)	0	T (0.91)	−6.42	**0.0044**
ARNTL	11	rs900145	rs7925241	0.025	NA	A (0.87)	−6.22	0.094
C13orf16	13	rs9555810	rs1163630	0.024	0.028	C (0.66)	−3.11	0.16
BEGAIN	14	rs6575793	rs941930	0.003	0.015	A (0.16)	4.73	**0.011**
RORA	15	rs3743266	rs339978	0.012	0.008	T (0.20)	5.90	**0.00004**
IQCH	15	rs7359257	rs7174933	0.00	NA	A (0.02)	−12.76	0.11
NFAT5	16	rs1364063	rs8054051	0.015	NA	A (0.97)	−14.49	0.74
MKL2	16	rs1659127	rs39826	0.035	0.019	A (0.26)	−3.84	**0.0082**
FTO	16	rs9939609	rs12149832	0.057	0.934	A (0.12)	−5.54	**0.0016**
CA10	17	rs9635759	rs12452390	0.001	0.001	T (0.86)	4.98	**0.011**
FUSSEL18	18	rs1398217	rs1036349	0.026	0.133	T (0.08)	−4.22	0.32
SLC14A2	18	rs2243803	rs9973059	0.004	0.001	C (0.84)	3.88	0.092
CRTC1	19	rs10423674	rs875396	0.003	0.067	A (0.27)	−2.55	0.54
PIN1	19	rs1862471	rs10425175	0.032	0	T (0.27)	3.30	0.079
PCSK2	20	rs852069	rs4814606	0.006	0.011	A (0.13)	4.44	**0.034**

[a]Locus generalization defined as Bonferonni-corrected P value for best SNP in the region <0.05.
[b]Index SNPs in EA women are from Elks *et al.* (26).
[c]All SNPs in a 250 kb region in either direction of the index SNP were interrogated for association with age at menarche and the SNP with the lowest Bonferonni-correct P value was considered the best.
[d]Bonferonni-corrected P value ($0.05/n$), based upon number of n independent tests (SNPs) within each region; corrected P values < 0.05 are shown in bold.

Meta-analysis of GWASs of age at menarche

The present study remains the largest and most comprehensive genetic examination of menarche timing in AA women to date, including all known available data (from 15 observational cohort and case–control studies) in AA women having both age at menarche information and genome-wide genotype data. Nonetheless, it included far fewer samples than are now available for EA women ($\geq$100 000 in ReproGen). Effect sizes of the variants found via GWA in EA women (26) were fairly small (e.g. accounting for between 1 and 6 weeks variation in age at menarche per copy of the risk allele); therefore, our lack of genome-wide significant associations could stem from a lack of statistical power. Our power calculations show (Supplementary Material, Fig. S5) that for SNPs with MAF $>$ 0.2, we had $\geq$80% power to detect a relatively small effect size [e.g. 6.5 weeks (0.12 years) earlier age at menarche per copy of the risk allele]. This is an effect size at the upper end of the range observed previously in EA populations [e.g. in *LIN28B*, a ~6.9-week reduction in age at menarche per allele copy has been seen (18,20,21,26)]. As many of the SNPs identified in EAs were novel, it is also possible that the reported effect sizes in EA women were overestimated (i.e. the phenomenon of the 'winner's curse'), which would indicate that while our study was adequately powered to test variants having effects in the range of previously reported SNPs, it was in reality substantially underpowered.

Table 5. Fine-mapping of 42 putative menarche loci localized in EA women: identification of stronger markers of association in AA women[a]

Chr., Nearest Gene	Index SNP identified in EA women	Coded allele, frequency in AA	Index SNP beta (weeks), *P* in AA	Stronger marker	Coded allele, frequency in AA	Stronger marker beta (weeks), *P* in AA	r^2 EUR[b]	r^2 AFR[b]
1, *SEC16B*	rs633715	C, 0.10	−2.5, 0.12	rs543874	A/G, 0.75	$3.8, 4.9 \times 10^{-4}$	0.91	0.18
2, *CCDC85A*	rs17268785	A, 0.74	−3.1, 0.017	rs17047854	A/G, 0.34	$3.4, 5.8 \times 10^{-4}$	0.99	0.52
3, *EEFSEC*	rs2687729	A, 0.66	−1.8, 0.075	rs2075402	T/C, 0.27	$-3.2, 2.2 \times 10^{-3}$	0.56	0.14
6, *LIN28B*	rs7759938	C, 0.53	2.5, 0.15	rs314266	T/C, 0.33	$-3.8, 2.9 \times 10^{-4}$	0.65	0.53
11, *BSX*	rs6589964	A, 0.38	0.3, 0.72	rs1461499	A/C, 0.63	$3.5, 3.8 \times 10^{-4}$	0.41	0.06
11, *NARS2*	rs10899489	A, 0.31	0.7, 0.48	rs1006441	C/G, 0.15	$3.9, 3.8 \times 10^{-3}$	na	na
11, *STK33*	rs4929923	C, 0.55	−1.5, 0.11	rs12575252	C/G, 0.51	$3.1, 9.9 \times 10^{-4}$	0.92	0.55
16, *FTO*	rs9939609	A, 0.47	−0.2, 0.83	rs12149832	A/G, 0.12	$-5.5, 2.0 \times 10^{-4}$	0.88	0.05

[a]SNPs selected were those within ±250 kb of index signal, with $r^2 > 0.4$ with index SNP in EUR, *P* value for marker association <0.004 and at least 1 degree of magnitude lower than p for index SNP.
[b]LD (r^2) between Index SNP and Stronger Marker SNP is based on 1000 Genome Project.

The primary outcome of our GWA experiment was to provide the first cross-ethnic validation of *RORA*, strengthening the evidence for its role in menarche. Genetic variants near *RORA* were previously reported to influence age at menarche in EA women, but at *P*-values below genome-wide significant thresholds (26). *RORA* encodes one of the ROR nuclear receptors that regulate the transcription of numerous other genes and is expressed in human endometrium (44). Recently, *RORA* expression has been found to regulate aromatase (*CYP19A1*), which converts testosterone to estrogen (45). A SNP in *CYP19A1* was among our top Stage 1 results and was marginally associated in Stage 2 ($P = 0.065$). Variants in genes in the *CYP19* gene family have been found to be associated with age at menarche (46,47) as well as other reproductive traits in women (17,18,48,49). Furthermore, a conditional analysis identified two independent signals in *RORA* in AA women, both of which were independent of the previously reported index SNP identified in EA women. Localization of multiple independent variants that are statistically associated with disease traits is an important first step toward identifying causal variants. Our results suggest two independent signals in/near *RORA*, refining this putative menarche locus.

In addition, results of the GWA meta-analysis highlight biological pathways that may be important in AA women. A number of the most strongly associated variants from this meta-analysis implicate growth factor and insulin signaling in menarche timing. SNPs in Stage 1 that were also associated with menarche age in Stage 2 included rs10940138 near *PIK3R1* (phosphatidylinositol 3-kinase receptor 1, alias p13k in mice), which is part of the *PI3K/AKT/mTOR* inflammatory pathway. Enhanced activity of this pathway is strongly implicated in ER-positive breast cancer, ovarian cancer and endometrial cancer (50), is involved in over 30 insulin-signaling networks (51) and contains variants associated with body fatness and leptin levels (52). The second SNP from Stage 1 that was associated with menarche in the Stage 2 sample (rs8014131) is located near *FLRT2*, encoding the fibronectin leucine-rich transmembrane protein 2. *FLRT2* acts as a cell-adhesion or signaling molecule and interacts with numerous growth factors including *FGFR1*, *GnRH* and *GnRHR* to control diverse developmental processes. Lastly, a suggestive association was noted with SNP rs320320 near *AKT3* in Stage 1 and was also associated with menarche in our sample of EA women in the ReproGen study ($P = 1 \times 10^{-3}$, combined *P*-value $= 1 \times 10^{-7}$). *AKT3* (also known as protein kinase B) is a member of the serine/threonine-protein kinase family, and functions to regulate extracellular signals including platelet-derived growth factor, insulin and insulin-like growth factor 1 (53,54).

Interrogation of menarche loci reported in EA women

While there are numerous pitfalls in the use of diverse populations for GWA at present, including poorer genomic coverage with existing SNP panels and lower imputation quality (55) and complex admixture patterns across regions of the genome (56), diverse populations are very important in building on the findings in individuals of EA (41,55,57). Owing to wide population variation in allele frequencies, sampling of diverse populations is critical, and African ancestry populations in particular should theoretically yield greater resolution on the location of causal variants influencing a trait, given their lower average LD (56). We undertook two investigations to leverage these properties of AA populations to expand the information on menarche variants already identified in EA women. First, we hypothesized that we would find locus replication (association of SNPs in the same region), but not necessarily SNP replication in AA women. Therefore, we examined SNPs in a 250 kb region of the previously reported menarche loci in EAs. Secondly, we hypothesized that by taking advantage of the lower LD structure in AAs, we could gain insight into the fine structure of these loci and localize potentially causal variants.

As recently shown for lipid traits, significant inter-population differences exist in the contributions of individual SNPs within a given locus as well as their magnitude of effect on a given trait (57,58). Similarly, in the present analysis, none of the 42 index SNPs identified in EA women was associated with age at menarche in AA women after Bonferroni correction for multiple testing. In contrast, 60% of the 42 loci contained SNPs (within ±250 kb of the index SNP) were associated with menarche after region-based Bonferroni correction, showing significant overlap in the genes involved in menarcheal timing across race/ethnicity. This finding is important, first, because it strengthens the evidence for these particular loci being involved in menarcheal timing generally. We found, further, that the SNP with the lowest *P*-value in AA women in each region generally represented the same signal as in EA women (was in high LD

with the index SNP in EA populations), although it was in low LD with the index EA SNP in AA populations. These findings point to the value of examining African Ancestry populations to better localize associations identified in EA populations. We also showed modest evidence in our eQTL analysis that particular SNPs in the 42 loci that were associated with menarche in AA women were more likely to influence local gene expression than were the index SNPs in EA women. A limitation of eQTL analysis is that expression is tissue and cell-type specific, and the cell-type (LCL) used here, while from Yoruban (African) ancestry samples, may not be as informative for investigation of gene variants regulating reproductive timing as hypothalamic or ovarian tissues would be, if available.

In a second analysis to identify specific SNPs in the 42 loci that may better capture the association of the EA signal in AAs, we targeted only SNPs in LD ($r^2 > 0.4$) with the index signal in EAs. Through this fine-mapping work, we identified SNPs in at least eight regions that better captured the association with age at menarche in AAs than the SNPs identified in EAs, most of which were in obesity-related loci such as *FTO* and *SEC16B*. In the case of *SEC16B*, the LD structure of AA women was particularly helpful in localizing the signal to a smaller region. The results suggest a close link between female adiposity and the timing of pubertal development in AA women as was found for EA women (26) and again showcase the value of examining AA populations to narrow the subset of potentially functional alleles in loci identified via GWA in EA populations. In future, this work may be enhanced through trans-ethnic meta-analysis (59), which takes into account the expected similarity in allelic effects in more closely related populations while allowing for heterogeneity between more diverse ethnic groups. This approach has already been shown to both increase power to detect association and improve localization of causal variants by combining diverse population data in a single meta-analysis (60,61).

Interpretation

The lack of strong genome-wide significant associations, in combination with significant overlap in loci associated with age at menarche in both AA and EA women, should not be interpreted to mean that ethnic differences in menarche timing are driven solely by environmental factors. First, African ancestry populations have greater haplotype diversity than European and Asian populations, which yields lower sensitivity for GWA because lower genome-wide LD makes the identification of loci less sensitive given the same degree of genomic coverage on a given genotyping array. Better imputation strategies are needed using population-specific sequencing data to detect low-frequency variants and provide better coverage of genomic regions for African ancestry populations (62,63). However, greater haplotypic diversity also allows greater refinement of loci of interest (64), and may have contributed to our greater success in refining multiple RORA signals than in the European cohort studies of menarche (26). Secondly, there are other classes of genetic variants (e.g. less common alleles, copy number variants, other structural variants) that were not assayed here that may be shown to play a role in age at menarche. Thirdly, gene-by-environment interactions may have masked the effect of genetic variants; the presence of such interactions on a genome-wide basis requires much larger sample size than is available at this time for AA women. Therefore, expansion to additional cohorts to increase the sample size to reach the sizes now available for EA individuals (>100 000) will also be necessary before a full assessment of genetic and environmental contributors to ethnic variation in menarche timing can be made.

Limitations

In addition to the issue of sample size and the focus on common variants discussed above, there are a number of limitations of the study. The participating studies used SNP arrays that were designed to capture common variants in populations of EA and thus, a substantial fraction of common variation in AA populations is likely to have been missed or imprecisely tagged following imputation to a reference source such as HapMap (64). The trait under investigation was self-reported; except for subjects in the Bogalusa Health Study, the subjects included in this meta-analysis were adults when detailed reproductive history data were collected. However, recalled age at menarche is highly correlated with observed age at menarche (65), even 30 years later (66). We did not detect significant heterogeneity across cohorts in our genetic meta-analysis, but heterogeneity in data collection methods may have nonetheless contributed to lower precision of our estimates.

Finally, the timing of menarche is sensitive to early nutritional status (including body fatness), economic disadvantage and, more recently, exposure to endocrine-disrupting chemicals (67), but data on these factors prior to or at the time of menarche were not available for our study cohorts. Environmental heterogeneity between AA and EA women may explain the lack of replication of some loci, as we did not have adequate data to adjust for such factors in our analysis. The rate of decline in age at menarche over the 20th century was more rapid in AA than EA women (68), highlighting the potential effects of the changing nutritional environment for our study population. This is important because environmental variation between and within populations may mask genetic effects when those differences are not accounted for (69,70). Birth year is a potentially useful proxy for numerous nutritional (e.g. protein intake) and non-nutritional (e.g. endocrine disruptors) exposures that have changed over time and may influence developmental timing. It is possible that one reason for the lack of genome-wide significant findings in the present analysis is that birth year heterogeneity (and the environmental variation it may index) could have masked genetic associations and contributed to our lack of genome-wide significant SNP discovery. In this regard, we recently showed a menarche genetic risk score-by-birth year interaction effect on childhood BMI, in which the aggregate effect of 42 menarche-related SNPs was greater in those born recently when compared with those born earlier in the 20th century in the same cohort (71). However, in the present study, we had insufficient statistical power to conduct an SNP × birth year interaction analysis at the genome-wide level, and it was furthermore unlikely that this would have significantly altered our meta-analysis results, as we found little evidence for effect heterogeneity across cohorts that varied widely in mean birth year. Nonetheless, this is a limitation of the present analysis, and an interesting avenue for future investigation.

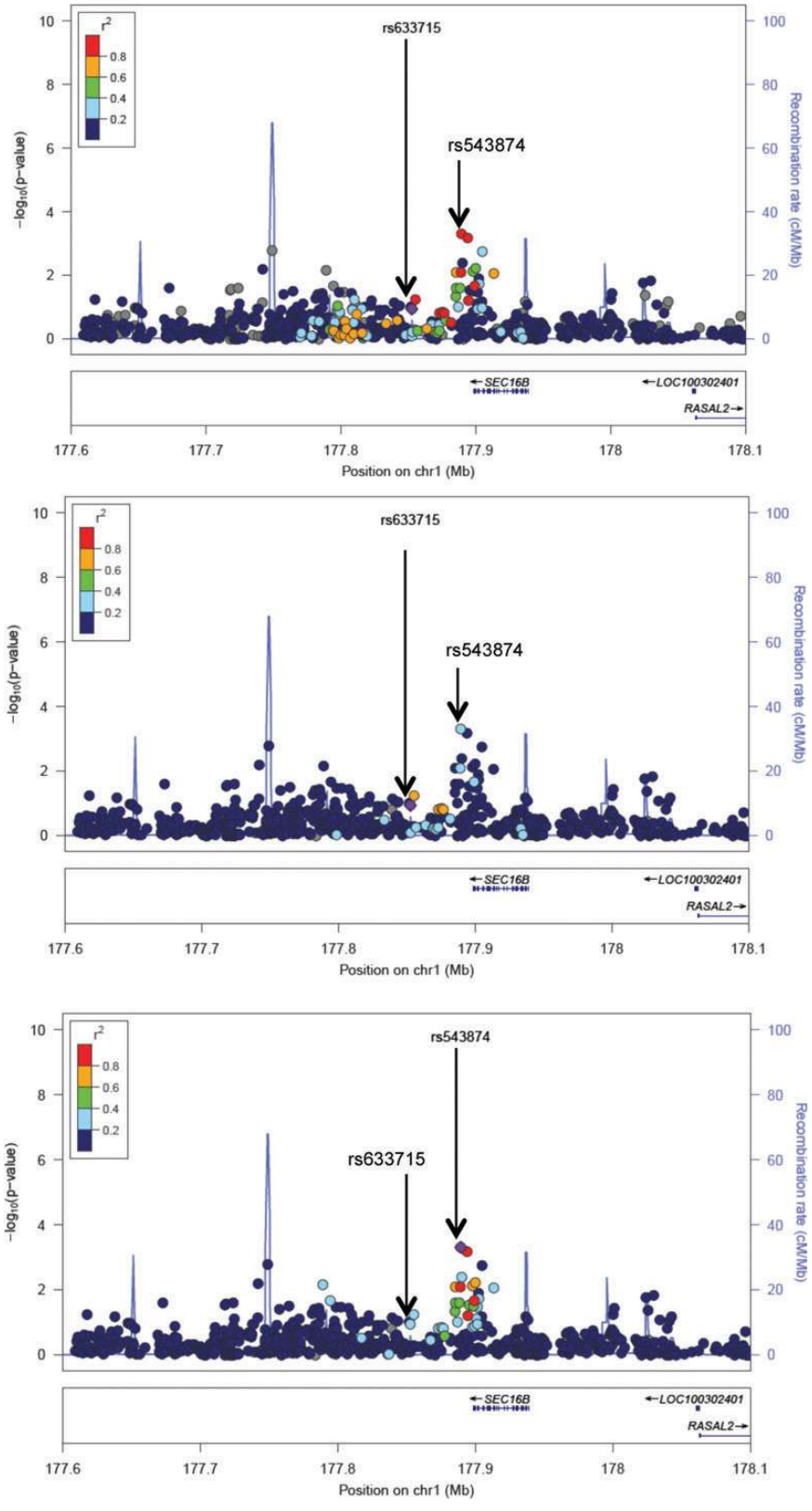

Figure 3. Fine-mapping of the *SEC16B* locus in AA women reveals a stronger genetic marker for age at menarche than the index SNP identified in European American women. (**A**) SNP associations with age at menarche near *SEC16B* in AAs, using LD from the 1KGP EUR reference panel, in relation to the index SNP rs633715 identified in EA women (purple diamond). The SNP with the lowest *P* value in the AA meta-analysis (rs543874) is 0.5 Mb upstream from the index SNP and has significantly lower *P* value (4.9×10^{-4} versus 0.12). The two SNPs are in strong LD in EUR populations (red color indicates $r^2 > 0.8$), showing that it would be considered the same signal in EA women. (**B**) SNP associations with age at menarche near *SEC16B* in AAs, using LD from the 1KGP AFR reference panel, in relation to the index

Conclusions

In summary, we confirmed that many menarche loci identified in EA women generalize to AA women, and for some of these loci, examination of AA samples allowed resolution of multiple signals, better localization of their respective signals and stronger associations with menarche than the originally reported SNPs. We present findings from the largest genome-wide association meta-analysis of age at menarche in AA women to date and, although no single SNP reached genome-wide significance, we identified a number of suggestive associations that may help define novel biological pathways involved in this important early life risk factor.

MATERIALS AND METHODS

Subjects

A total of 18 089 AA women with self-reported age at menarche collected at the baseline visit for each study were included in the Stage 1 meta-analysis. Participants were drawn from seven population-based cohort studies including the Women's Health Initiative (WHI, $n = 8086$), four cohorts within the Candidate Gene Association Resource (CARe): Atherosclerosis Risk in Communities (ARIC; $n = 1690$), Coronary Artery Risk Development in young Adults (CARDIA; $n = 630$), Cleveland Family Study (CFS; $n = 169$) and Jackson Heart Study (JHS; $n = 1228$); the Bogalusa Heart Study (BHS; $n = 145$), the Healthy Aging in Neighborhoods of Diversity across the Life Span study (HANDLS; $n = 617$) and eight breast cancer case–control studies in the African American Breast Cancer Consortium (AABC) (72)(73), including the Carolina Breast Cancer Study (CBCS; $n = 634$ cases/586 controls), the Los Angeles component of the Women's Contraceptive and Reproductive Experience Study (CARE; $n = 357$ cases/215 controls), the Multiethnic Cohort (MEC; $n = 532$ cases/972 controls), the Nashville Breast Health Study (NBHS; $n = 304$ cases/182 controls), the Northern California Breast Cancer Family Registry/San Francisco Breast Cancer Study (NC-BCFR/SFBCS; $n = 575$ cases/269 controls), the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (PLCO; $n = 56$ cases/116 controls), the Wake Forest University Breast Cancer study (WFBC; $n = 112$ cases/116 controls), and the Women's Circle of Health Study (WCHS; $n = 260$ cases/238 controls). A further 2850 AA women in the Black Women's Health Study (BWHS) were included in the Stage 2. Detailed descriptions of all studies are provided in Supplementary Material, Text S1 and Table 1.

Phenotypes

Age at menarche was reported to the whole year, and ranged from 8 to 21 years of age, except in the case of the MEC, in which age at menarche was reported within 2-year age groups, the mid-point of which was used in the analysis. Self-reported age at menarche in adult women has been shown to be a valid proxy for prospectively collected age at menarche (66,74,75). Age at menarche is a normally distributed trait and therefore was not transformed prior to analysis.

Genotyping and QCs

The Affymetrix Genome-Wide Human SNP array 6.0 (for ARIC, CARDIA, CFS, JHS, and WHI), the Illumina Human1M-Duo BeadChip array (for HANDLS, WFBC, WCHS, NBHS, PLCO, MEC, CBCS, CARE, NC-BCFR, SFBCS) or the Illumina 610K/Illumina CVD SNP array (BHS) is used according to the manufacturer's protocol for genome-wide genotyping. *De novo* genotyping was conducted at the Broad Institute for the replication samples using a custom-designed Sequenom chip and Taqman assays for SNPs that could not be multiplexed. Several QC filters were applied to the genome-wide genotype data: DNA concordance checks; sample and SNP genotyping success rate [$>95\%$, MAF $> 1\%$, minor allele count > 3]; sample heterozygosity rate, identity-by-descent analysis to identify population outliers, problematic samples and cryptic relatedness. A detailed description of the QC checks applied to the genotypes in each study and consortium is provided in Supplementary Material, Table S1.

SNP imputation

To increase coverage and facilitate comparison with other datasets, imputed genotype data were obtained using MACH (76,77), using all SNPs that passed the QC steps described above, and employing a 1:1 mixture of HapMap phase II CEU and YRI data as the reference panel for imputation.

Ancestry estimation

In all cohorts, SNPs on the GWA arrays were subjected to principal components analysis using EIGENSTRAT (78) to infer genetic ancestry. The top 10 principal components were included in the study-specific genetic association models as covariates to correct for population stratification.

Association analysis

Within each cohort (and within the breast cancer cases and controls separately in AABC), we tested associations between the imputed and genotyped SNPs with age at menarche using an additive genetic model. Linear regression analysis in PLINK (version 1.07) (79) or ProAbel (80) was used for cohorts of unrelated individuals (ARIC, CARDIA, JHS, WHI, BHS, HANDLS

SNP rs633715 identified in EA women (purple diamond). The SNP with the lowest *P* value in the AA meta-analysis (rs543874) was in relatively weak LD (light blue color indicates r^2 between 0.2 and 0.4) with the index SNP in AFR, suggesting they lie on different haplotypes. (**C**) SNP associations with age at menarche near *SEC16B* in AAs, using LD from the 1KGP AFR reference panel, in relation to the putatively stronger marker SNP rs543874 (purple diamond). Strong LD in AFR populations between rs543874 and a cluster of SNPs surrounding it, but low LD with SNPs near the index SNP rs633715 is seen. This suggests rs543874 may better localize the causal variant giving rise to the association of menarche age with SNPs near *SEC16B* previously reported in EA women. Note: SNPs are plotted using Locus Zoom by position on the chromosome against association with age at menarche ($-\log_{10} P$). Estimated recombination rates are plotted in blue to reflect local LD structure, and the SNPs surrounding the index SNP (or most significant SNP) in each case are color coded to reflect their LD with this SNP, marked as a purple diamond.

and all AABC studies) and linear mixed-effect models in R were used to model family structure for cohorts including related individuals (CFS). Covariates included the woman's year of birth (or age at diagnosis or recruitment for AABC and WHI), if available, to account for the known secular trends in age at menarche, and the first ten principal components from EIGENSTRAT to account for global population stratification.

Meta-analysis

Cohort-specific association results were combined using an inverse variance-weighted meta-analysis approach as implemented in METAL (81). A genome-wide significance threshold was set at $P \leq 5 \times 10^{-8}$.

Stage 2 analysis

AA women in the Black Women's Health Study (BWHS) were included in the replication stage (description provided in Supplementary Material, Text S1). Given the lack of genome-wide significant findings, we used the following serial inclusion criteria to select top SNPs from the meta-analysis for presentation (Table 2) and for replication testing: SNPs with $P < 1 \times 10^{-5}$ (43 SNPs), SNPs tested in >10 000 women in Stage 1 (yielding 35 SNPs), SNPs with an MAF >0.03 (yielding 34 SNPs) and including only SNPs in relatively low LD with other top SNPs within a 500 kb region ($r^2 < 0.3$) (yielding 20 SNPs). Genotyping of these 20 SNPs was carried out at the Broad Institute Center for Genotyping and Analysis using the Sequenom MassArray iPLEX technology. An average reproducibility of 99.6% was obtained among the blinded duplicates. The call rate was 98.6% or higher for each SNP. A total of 2850 samples were included in the final analyses. The top 30 ancestral informative markers (AIMs) from the Phase 3 admixture panel (82) were genotyped to estimate and control for population stratification due to European admixture; these 30 AIMs are highly correlated with estimates from the whole admixture panel and thus provide efficient and valid adjustment for stratification (83). An additive linear model for associations of age at menarche with genotype was used, adjusting for year of birth and percent EA as continuous variables. All regression models were run using the SAS statistical software version 9.1.3 (SAS Institute, Inc., Cary, NC, USA). One SNP could not be accommodated on the panel (rs4557202) and therefore a proxy SNP in high LD with it ($r^2 = 0.967$ in 1KG-AFR) was chosen (rs7651087).

Replication of SNP associations in EA women

The 20 SNPs genotyped in the AA replication sample were also examined for association with age at menarche in the 32-study Stage 1 meta-analysis results of EA women (26) in over 87 000 women. A Bonferroni-corrected significance threshold of $P < 0.05/20$ was applied.

Analysis of secondary signals at known loci

Multiple signals within a single locus in the top GWA results were evaluated through conditional analyses in the largest cohort/studies (WHI, CARe and AABC) using individual-level genotype data. Linear regression analyses were conducted that included all such SNPs, birth year, study center (if applicable) and the top 10 PCs as covariates. The results were then meta-analyzed using METAL and the resulting beta coefficients and *P*-values were compared to assess whether there were multiple independent risk variants within the regions. We applied a Bonferroni-corrected *P*-value, correcting for the number of comparisons to determine the significance of independent signals.

Interrogation of 42 menarche loci identified in GWAS of EA women

Our second aim was to interrogate the 42 loci previously reported to be associated with age at menarche in EA women in our AA sample (26); 32 of which were genome-wide significant and 10 demonstrated suggestive associations in the previous study. First, we developed a set of criteria to validate the EA index SNPs and interrogate regions around each of these 42 loci. For each index SNP in EA, we looked-up the respective association result with age at menarche in AA. To accommodate the difference of LD structure and possible allelic heterogeneity across different ethnicities, we then interrogated the 250 kb flanking region around each lead SNP for locus replication to determine whether there exist other SNPs in the locus with stronger associations in AA with the outcome. We used the following criteria to identify the top AA SNP: (i) the SNP with the smallest association *P*-value within the region; (ii) MAF > 0.01; (iii) location of the AA lead SNP within the same recombination block of the lead EA SNP, where the recombination block was defined as a 20% recombination rate. The statistical significance of each identified SNP was evaluated using a region-specific Bonferroni correction for the multiple comparisons. We determined the number of independent SNPs based on the variance inflation factor, which was calculated recursively within a sliding window with size 50 SNPs and pairwise r^2 value of 0.2 using PLINK. If a SNP was identified with Bonferroni-adjusted *P*-value <0.05 within a locus, then this served as evidence of locus replication in AAs.

Secondly, we interrogated all common genotyped and imputed SNPs (MAF > 0.01) within the ±250 kb flanking region of the index SNPs to identify, specifically (a) variants that capture the association in the region in AA women *significantly better* than the index SNP and (b) variants that may represent secondary signals. We have previously estimated (84) a threshold of significance for (a) as $P < 0.004$, which is a correction based on the number of tag SNPs in the HapMap YRI population needed to capture ($r^2 \geq 0.8$) all SNPs that are correlated with the index signal in the HapMap CEU ($r^2 \geq 0.2$). In an attempt to eliminate minor fluctuations in *P*-values for correlated SNPs, we took a more conservative approach than for the conditional analyses and further required the *P*-value to decrease by more than one order of magnitude compared with the association of the EA index signal in AAs. We also required an $r^2 > 0.4$ between the index marker and the more associated marker in AAs and we assessed phase to ensure that the more associated marker is on the same haplotype as the GWAS-reported risk allele in the HapMap CEU population.

For all of the remaining markers that were weakly correlated ($r^2 < 0.20$) with the index signal (in Europeans), and thus may define secondary signals, we applied a more stringent α level

for defining statistical significance. Here we set the threshold as 5.6×10^{-6}, which is a correction for the number of tag SNPs needed to capture all common alleles (MAF > 0.05, with $r^2 > 0.8$) in the YRI HapMap population. Both (a) and (b) were estimated empirically based on ~30 regions of 500 kb in size in a previous study of the prostate cancer risk loci (84).

Expression database analysis of menarche SNPs

We queried existing human lymphocyte gene expression databases to determine whether the top SNPs that we identified in each of the 42 loci (Table 4) were more likely to be associated with the expression of nearby genes than the originally identified SNPs in these regions from studies in women of EA. To do so, we applied a sensitive technique for mapping cis-regulatory SNPs (85) in 56 unrelated Yoruban African LCLs (YRI LCLs) used by the HapMap consortium (86).

Statistical power

The Stage 1 meta-analysis of GWA results had ≥80% power to detect relatively small effect sizes (e.g. 0.12 years, or 6.5 weeks earlier menarche per copy of the risk allele) for SNPs with MAF > 0.2 at $P < 5 \times 10^{-8}$ (Supplementary Material, Fig. S5).

The Stage 2 replication sample in 2850 women in the BWHS provided >80% power to detect an SNP having an effect of 7 weeks earlier menarche per copy of the risk allele for alleles with MAF > 0.25 (which included 13 of the 20 queried variants) at $P < 0.05$ corrected for 20 comparisons (Supplementary Material, Fig. S6).

Ethics statement

All participants gave informed written consent for the use of their genomic material in studies of cardiovascular disease, cancer and aging risk factors, and the project was approved by the institutional review boards at all participating institutions.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at *HMG* online.

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CARDIA: NHLBI had input into the overall design and conduct of the CARDIA study.

HANDLS: Data analyses for the HANDLS study utilized the high-performance computational capabilities of the Biowulf Linux cluster at the National Institutes of Health, Bethesda, MD (http://biowulf.nih.gov).

WHI: This manuscript was prepared in collaboration with investigators of the WHI, and has been reviewed and/or approved by the Women's Health Initiative (WHI). WHI investigators are listed at https://cleo/researchers/Documents%20%20Write%20a%20Paper/WHI%20Investigator%20Long%20List.pdf.

Conflict of Interest statement. None declared.

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Supplemental Figure Legends

SFigure 1. Quantile-Quantile Plot. Distribution of observed versus predicted p values under the null hypothesis of no association, from Stage 1 meta-analysis of genome-wide association studies of age at menarche in 18,089 African-American women.

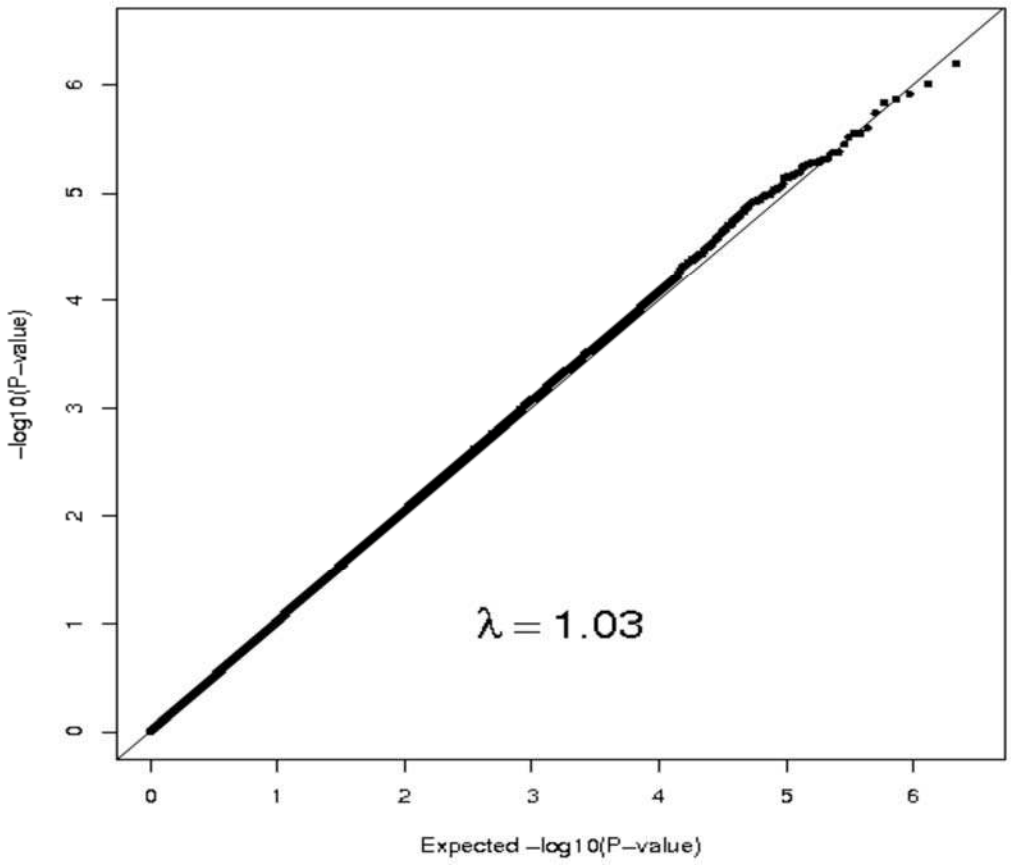
SFigure 2. Manhattan Plot. –log10 p values from meta-analysis of genome-wide association studies of age at menarche in 18,089 African-American women, by chromosome. Each point indicates the p value for the additive association of each of ~2.5 million imputed SNPs in the analysis.

SFigure 3. Regional association of meta-analysis results. Regional association plots of the 20 top SNPs from the Stage 1 meta-analysis of age at menarche genome-wide association studies are presented showing linkage disequilibrium with the top SNP (named and indicated by purple diamond) using the 1KGP AFR reference panel.

SFigure 4. Regional association in African American women of 42 previously reported menarche loci. Regional association plots of 42 previously reported menarche loci with age at menarche in African American (AA) women showing linkage disequilibrium with the index SNP (named and indicated by purple diamond) using the 1KGP EUR reference panel.

SFigure 5. Statistical Power of the Meta-Analysis (Stage 1). Power to detect an additive association between a SNP (varying in MAF from 0.05 to 0.50) and age at menarche (continuous), over a range of expected effect sizes (in weeks), in a sample of 18,089 unrelated individuals, assuming two-sided $p = 5 \times 10^{-8}$.

SFigure 6. Statistical Power of the Replication Study (Stage 2). Power to detect an additive association between a SNP (varying in MAF from 0.05 to 0.50) and age at menarche (continuous), over a range of expected effect sizes (in weeks), in a sample of 2,850 unrelated individuals, assuming two-sided p = 0.05/20.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Expected −log10(P−value)
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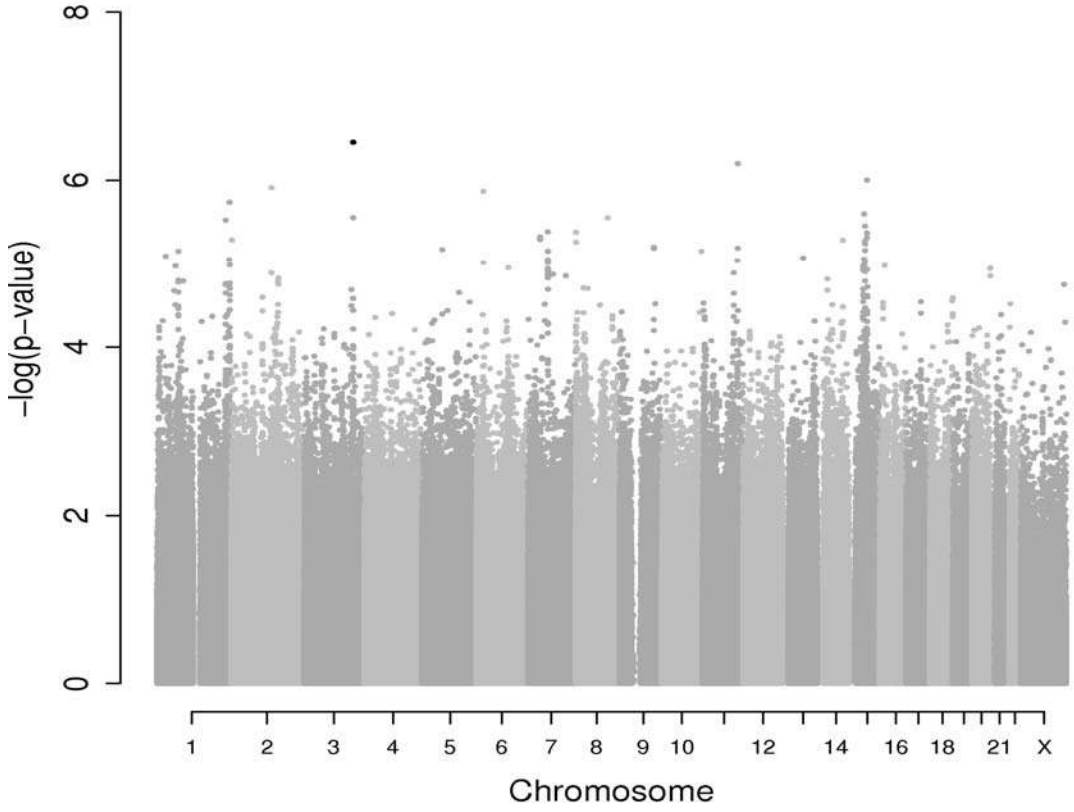
2
3
4
5
6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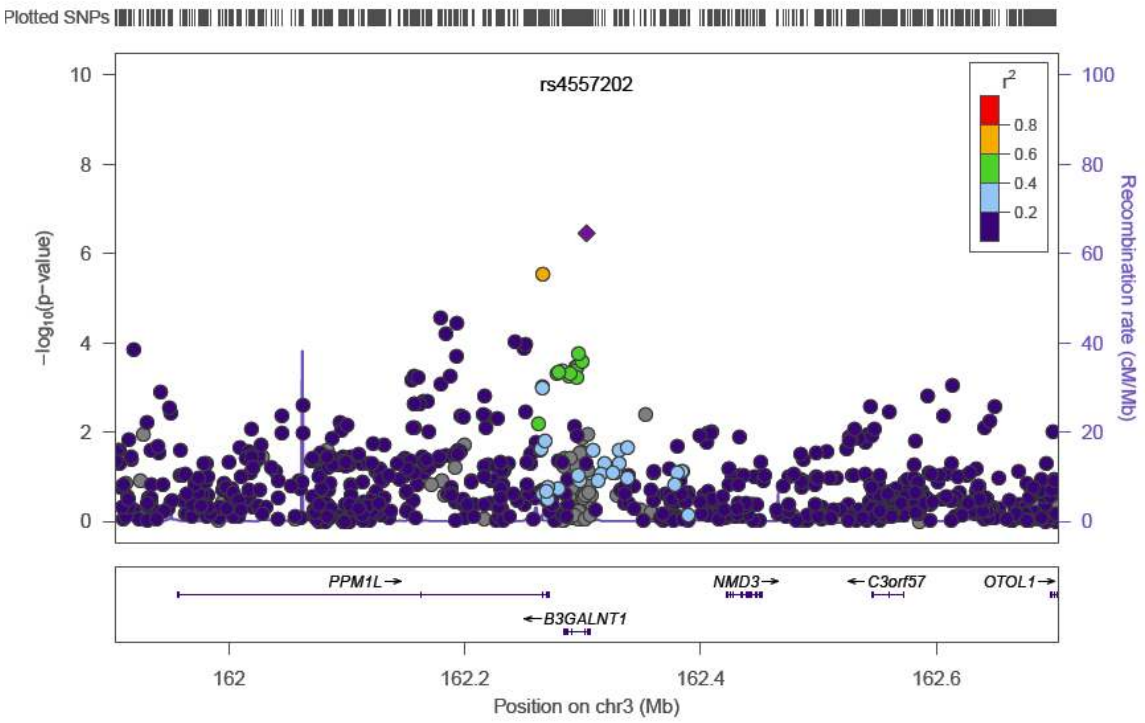
X
Chromosome

B3GALNT1



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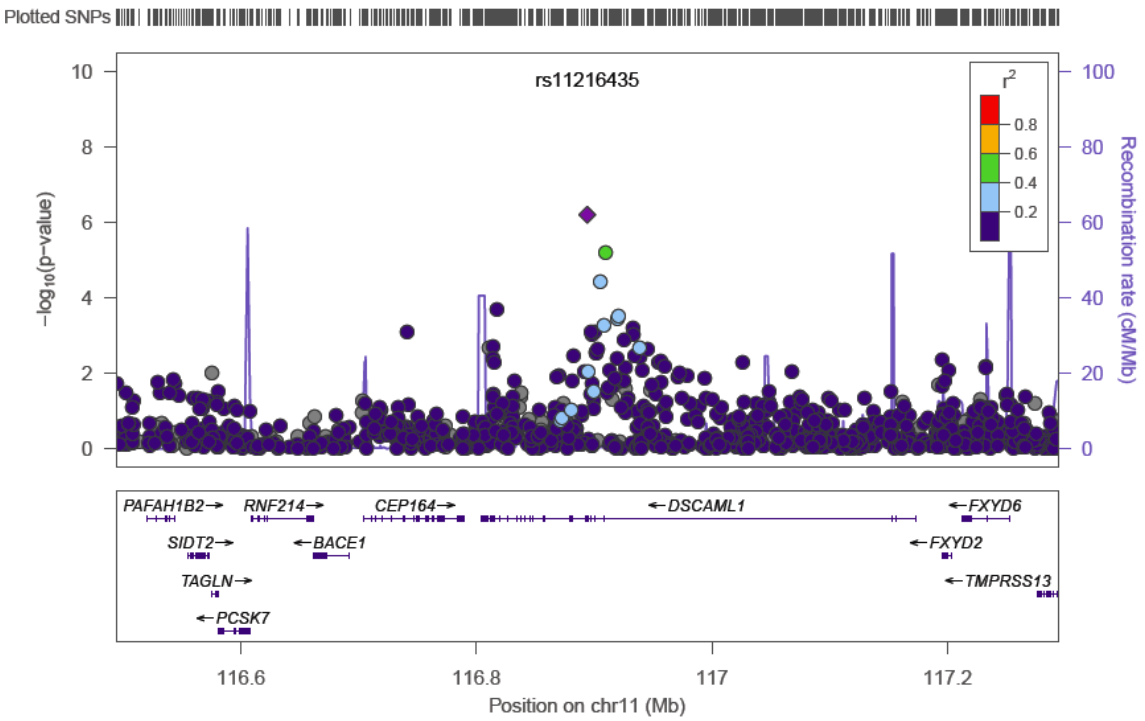

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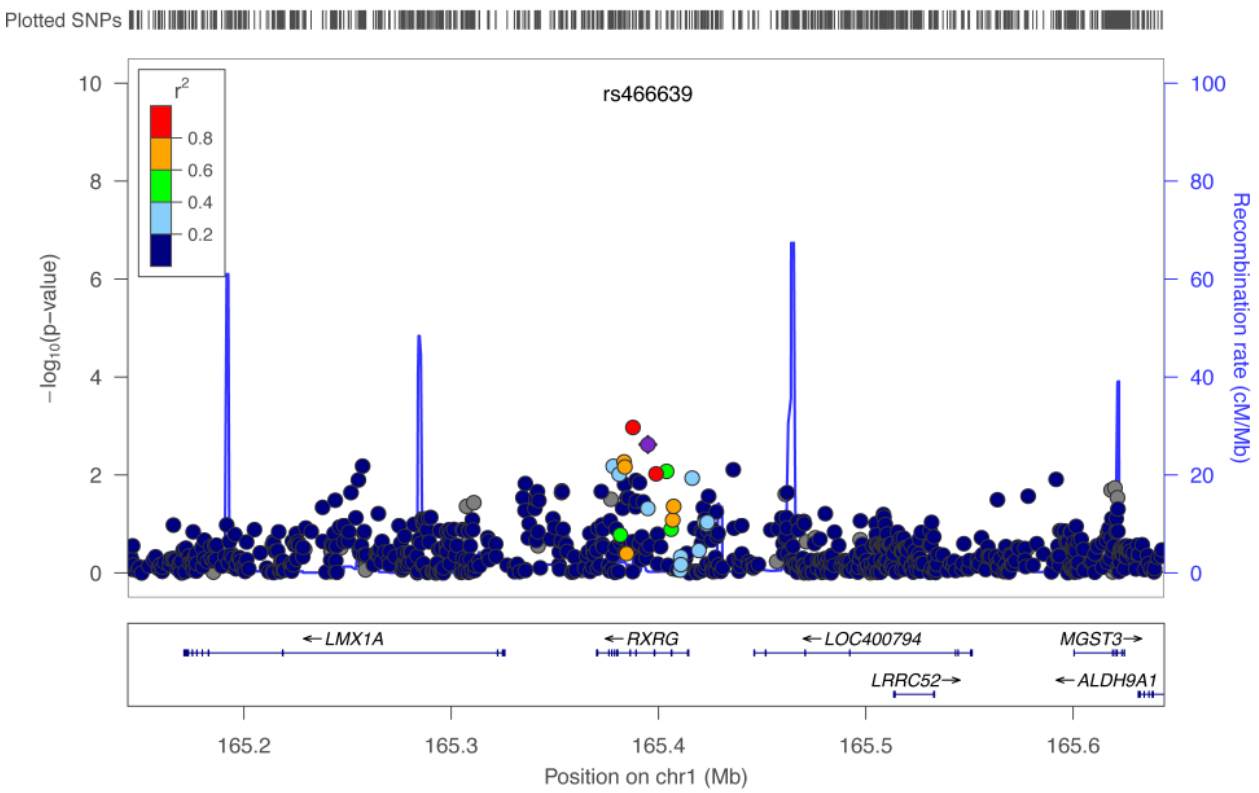
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r²
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0.6
0.4
0.2
−log10(p−value)
Recombination rate (cM/Mb)
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RXRG
LOC400794
MGST3
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ALDH9A1
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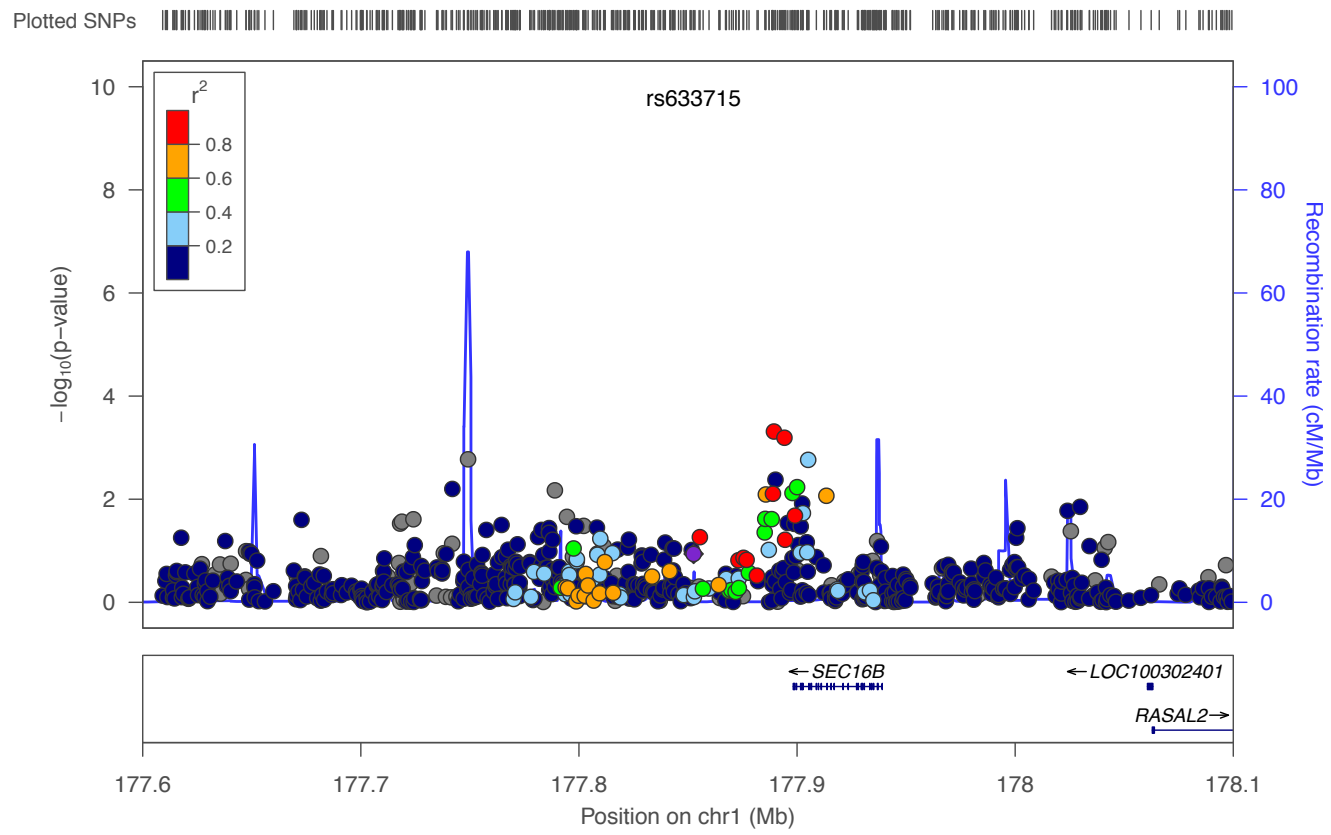
165.3
165.4
165.5
165.6
Position on chr1 (Mb)

SEC16B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r²
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0.6
0.4
0.2
−log10(p−value)
Recombination rate (cM/Mb)
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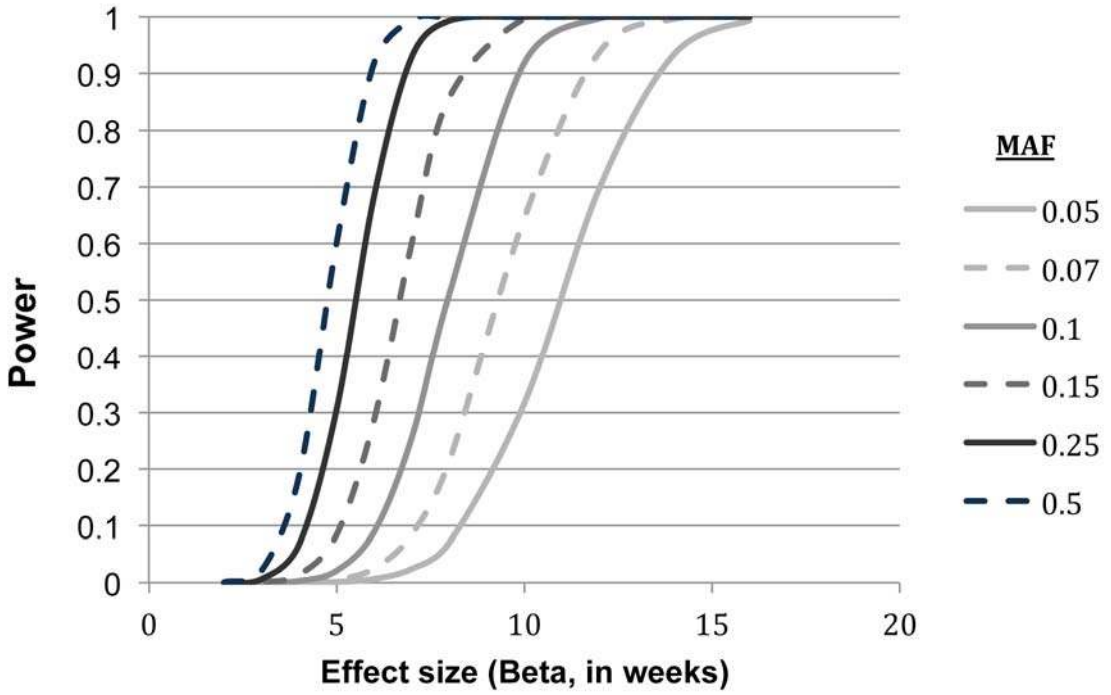
178
178.1
Position on chr1 (Mb)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0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
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0
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10
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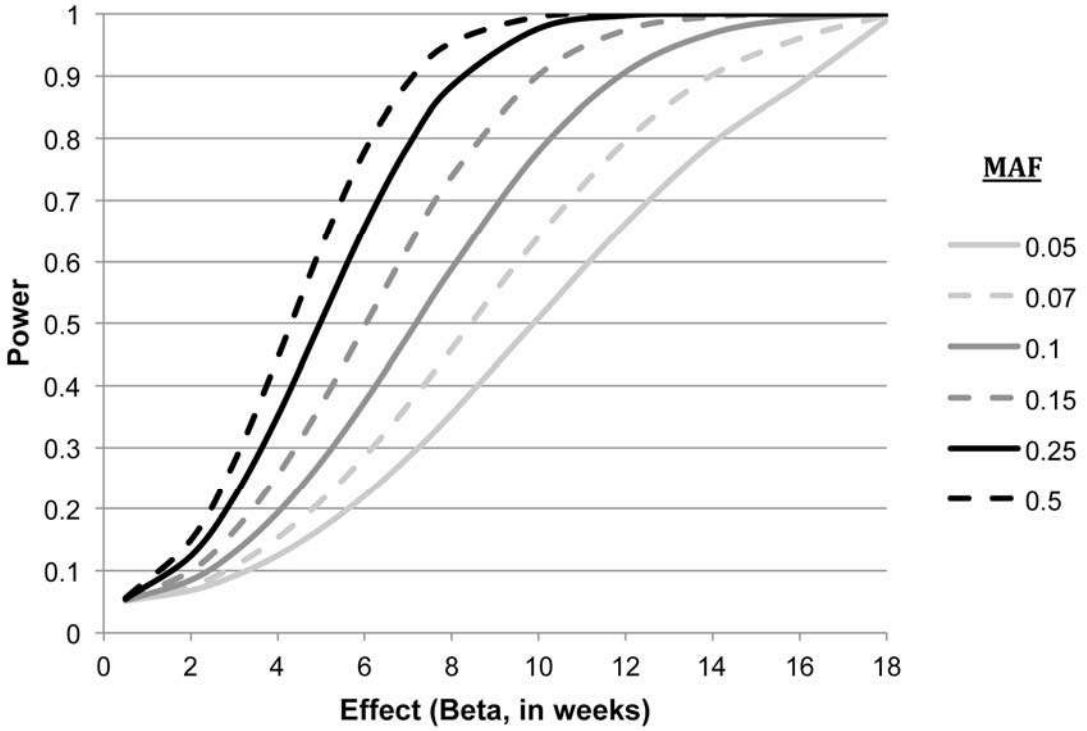
20
Effect size (Beta, in weeks)
MAF
0.05
0.07
0.1
0.15
0.25
0.5


Power
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0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
2
4
6
8
10
12
14
16
18
Effect (Beta, in weeks)
MAF
0.05
0.07
0.1
0.15
0.25
0.5

Supplemental Text 1: Cohort Descriptions

AABC STUDIES

The Carolina Breast Cancer Study (CBCS)
The CBCS is a population-based case-control study conducted between 1993 and 2001 in 24 counties of central and eastern North Carolina [1]. Cases were identified by rapid case ascertainment system in cooperation with the North Carolina Central Cancer Registry and controls were selected from the North Carolina Division of Motor Vehicle and United States Health Care Financing Administration beneficiary lists. Participants' ages ranged from 20 to 74 years. For stage 1, DNA samples were provided from 656 African American cases with invasive breast cancer and 608 African American controls.

The Los Angeles component of The Women's Contraceptive and Reproductive Experiences (CARE) Study
The NICHD Women's CARE Study is a large multi-center population-based case-control study that was designed to examine the effects of oral contraceptive (OC) use on invasive breast cancer risk among African American women and white women ages 35-64 years in five U.S. locations [2]. Cases in Los Angeles County were diagnosed from July 1, 1994 through April 30, 1998, and controls were sampled by random-digit dialing (RDD) from the same population and time period; 380 African American cases and 224 African American controls were included in stage 1 of the scan.

The Multiethnic Cohort Study (MEC)
The MEC is a prospective cohort study of 215,000 men and women in Hawaii and Los Angeles [3] between the ages of 45 and 75 years at baseline (1993-1996). Through December, 31 2007, a nested breast cancer case-control study in the MEC included 556 African American cases (544 invasive and 12 in situ) and 1,003 African American controls. An additional 178 African American breast cancer cases (ages: 50-84) diagnosed between June 1, 2006 and December 31, 2007 in Los Angeles County (but outside of the MEC) were combined with the MEC samples in the analysis.

The Nashville Breast Health Study (NBHS)
The NBHS is a population-based case-control study of incident breast cancer conducted in Tennessee [4]. The study was initiated in 2001 to recruit patients with invasive breast cancer or ductal carcinoma in situ, and controls, recruited through RDD between the ages of 25 and 75 years. NBHS contributed 310 African American cases (57 in situ), and 186 African American controls to stage 1 of the GWAS.

The Northern California Breast Cancer Family Registry/San Francisco Breast Cancer Study (NC-BCFR/SFBCS):
The NC-BCFR is a population-based family study conducted in the Greater San Francisco Bay Area, and is one of 6 international sites collaborating in the NCI-funded Breast Cancer Family Registry (BCFR) [5]. African American breast cancer cases in NC-BCFR were diagnosed after January 1, 1995 and between the ages of 18 and 64 years; population controls were identified through RDD. Stage 1 genotyping was conducted for 440 invasive African American cases and 53 African American controls.
The SFBCS is a population-based case-control study of invasive breast cancer in Hispanic, African American and non-Hispanic White women conducted between 1995

and 2003 in the San Francisco Bay Area [6]. African American cases, ages 35-79 years, were diagnosed between April 1, 1995 and April 30, 1999, with controls identified through RDD. Stage 1 included 172 invasive African American cases and 231 African American controls from SFBCS.

The Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO) Cohort
PLCO, coordinated by the U.S. National Cancer Institute (NCI) in 10 U.S. centers, enrolled during 1993 - 2001 approximately 155,000 men and women, aged 55-74 years, in a randomized, two-arm trial to evaluate the efficacy of screening for these four cancers [7]. A total of 64 African American invasive breast cancer cases and 133 African American controls contributed to stage 1 of the GWAS.

Wake Forest University Breast Cancer Study (WFBC)
African American breast cancer cases and controls in WFBC were recruited at Wake Forest University Health Sciences from November 1998 through December 2008 [8]. Controls were recruited from the patient population receiving routine mammography at the Breast Screening and Diagnostic Center. Age range of participants was 30-86 years. WFBC contributed 125 cases (116 invasive and 9 in situ) and 153 controls to the stage 1 analysis.

The Women's Circle of Health Study (WCHS)
The WCHS is an ongoing case-control study of breast cancer among European, American and African American women in the New York City boroughs (Manhattan, the Bronx, Brooklyn and Queens) and in seven counties in New Jersey (Bergen, Essex, Hudson, Mercer, Middlesex, Passaic, and Union) [9]. Eligible cases included women with invasive breast cancer between 20 and 74 years of age; controls were identified through RDD. The WCHS contributed 272 invasive African American cases and 240 African American controls to stage 1 of the GWAS.

BOGALUSA HEART STUDY (BHS)

Between 1973 and 2010, 9 cross-sectional surveys of children aged 4-17 years and 10 cross-sectional surveys of adults aged 18-48 years, who had been previously examined as children, were conducted for CVD risk factor examinations in Bogalusa, Louisiana. Collection of age at menarche information in the BHS has been previously described [10]. Briefly, girls in the 3rd grade and up were interviewed individually about menstrual history by a registered nurse during the collection of anthropometric measures, health habits, and cardiovascular risk factors. In the ongoing Longitudinal Aging Study funded by NIH and NIA since 2000, there are 1,202 subjects who have been examined 4-14 times from childhood to adulthood and have DNA available for GWA genotyping. Based on the analysis of identity-by-state (IBS) sharing from whole genome genotyping data, we focus on a subset of 145 genotyped women who are of African-American ancestry, unrelated, and have information about the onset of menarche.

BLACK WOMEN'S HEALTH STUDY (BWHS)

Replication genotyping was conducted in samples that were part of a nested case-control study of breast cancer in the ongoing prospective Black Women's Health Study (BWHS) [11]. A description of the BWHS is as follows. In 1995, 59,000 African-American women 21-69 years of age from across the U.S. enrolled in the BWHS by completing a 14-page postal health questionnaire. The median age at entry was 38, and

participants were residents of 17 states in mainland U.S. The baseline questionnaire elicited information on a wide range of variables including age at menarche, and biennial follow-up questionnaires are used to identify new cases of disease outcomes and to update covariate information. DNA samples were obtained from BWHS participants by the mouthwash-swish method and were stored in freezers at -80^0 C. Approximately 50% of participants, 27,800 women, provided a sample. Women who provided samples were slightly older than women who did not, but the two groups were closely similar with regard to educational level, geographic region, body mass index, and reproductive factors. Breast cancer cases and controls matched to the cases on year of birth, geographic region of residence, and country of birth (U.S. or other) were included in the current analysis.

CARe COHORTS

ARIC

The ARIC study is a multi-center prospective investigation of atherosclerotic disease in a bi-racial population [12]. Men and women aged 45-64 years at baseline were recruited from 4 communities: Forsyth County, North Carolina; Jackson, Mississippi; suburban areas of Minneapolis, Minnesota; and Washington County, Maryland. A total of 15,792 individuals participated in the baseline examination in 1987-1989, with four follow-up examinations in approximate 3-year intervals, during 1990-1992, 1993-1995, and 1996-1998. Only African-American women with genotype data and age at menarche between 9 and 17 years of age were included in this analysis (N=1,817). This study was approved by the institutional review board at each field center, and this analysis was approved by the University of North Carolina at Chapel Hill School of Public Health Institutional Review Board on research involving human subjects. All subjects provided written informed consent.

CARDIA

The CARDIA study is a prospective, multi-center investigation of the natural history and etiology of cardiovascular disease in African Americans and whites 18-30 years of age at the time of initial examination. The initial examination included 5,115 participants selectively recruited to represent proportionate racial, gender, age, and education groups from four communities: Birmingham, AL; Chicago, IL; Minneapolis, MN; and Oakland, CA. Participants from the Birmingham, Chicago, and Minneapolis centers were recruited from the total community or from selected census tracts. Participants from the Oakland center were randomly recruited from the Kaiser-Permanente health plan membership. Details of the study design have been published [13]. From the time of initiation of the study in 1985-1986, five follow-up examinations have been conducted at years 2, 5, 7, 10, 15, and 20. DNA extraction for genetic studies was performed at the Y10 examination. After taking into account availability of adequate amounts of high quality DNA, appropriate informed consent and genotyping quality control and assurance procedures, genotype data were available on 955 African-American individuals.

CFS

The Cleveland Family Study (CFS) is a family-based study of sleep apnea comprising 2534 individuals (46% AA) from 352 families, which were evaluated on up to 4 different occasions, at exam cycles each occurring approximately every 4 years over a 16 year period (1990-2006). A description of the study and data collection procedures have been described previously [14] [15]. Briefly, the initial aim of the CFS study was to

quantify the familial aggregation of sleep apnea by recruiting index probands (n=275) from 3 area sleep centers if the proband had a confirmed diagnosis of sleep apnea and there were at least 2 first-degree relatives available to be studied. In the first 5 years, neighborhood control probands (n=87) with 2 or more living relatives available were also recruited. All available first-degree relatives and spouses of the case and control probands were recruited. Second-degree relatives, including half-sibs, aunts, uncles and grandparents, were also included if they lived near the first degree relatives, or if the family had been found to have 2 or more relatives with sleep apnea. In the first 3 exams, participants underwent in-home sleep studies, anthropometry (weight, height, waist, hip, and neck circumferences), blood pressure and spirometry tests, completed a, standardized questionnaire evaluation of symptoms and other health and habits, and venipuncture. DNA was isolated for subjects participating in the last 2 exam cycles (n=1447). The fourth exam was designed to include only 700 subjects, with oversampling of minorities, and those in whom a microsatellite genome scan had been conducted. In this sub-study, we include all African-American women with genotyping and menarche data (n=213). This study was approved by the Institutional Review Board of University Hospitals and all other centers involved. All subjects provided informed consent.

JHS

The Jackson Heart Study is a community-based observational study whose primary objective is to investigate causes of cardiovascular disease in an African American population. The cohort (N=5,301) was sampled from non-institutionalized African Americans 35-84 years old in urban and rural areas of three counties in Mississippi (Hinds, Madison, and Rankin) and involved four components: African Americans from Jackson, MS who were in the ARIC cohort (31%), a Family Cohort of relatives of index participants (22%; all ages ≥ 21included), a component recruited by sampling randomly selected households (17%), and a constrained volunteer sample designed to reflect the demographics of the overall population (30%). [16]. Only women with data for genotype and age at menarche who did not overlap with the ARIC cohort were included in the JHS analysis (N=185). This study was approved by the institutional review board, and all subjects provided written informed consent.

HEALTHY AGING IN NEIGHBORHOODS OF DIVERSITY ACROSS THE LIFESPAN (HANDLS)

The Healthy Aging in Neighborhoods of Diversity across the Life Span study (HANDLS) is an interdisciplinary, community-based, prospective longitudinal epidemiologic study examining the influences of race and socioeconomic status (SES) on the development of age-related health disparities among socioeconomically diverse African Americans and whites in Baltimore [17]. This study investigates whether health disparities develop or persist due to differences in SES, differences in race, or their interaction. The HANDLS design is an area probability sample of Baltimore based on the 2000 Census. The study protocol facilitated our ability to recruit 3722 participants from Baltimore, MD with mean age 47.7 (range 30-64) years, % males/female, 2200 African Americans (59%) and 1522 whites (41%); 41% reported household incomes below the 125% poverty delimiter. Genotyping was focused on a subset of participants self-reporting as African American was undertaken at the Laboratory of Neurogenetics, National Institute on Aging, National Institutes of Health. In the larger genotyping effort, a small set of self-reported European

ancestry samples were included. This research was supported by the Intramural Research Program of the NIH, National Institute on Aging and the National Center on Minority Health and Health Disparities.

WOMEN'S HEALTH INITIATIVE (WHI)

WHI is a prospective population-based cohort study investigating post-menopausal women's health in the U.S [18]. A total of 161, 838 women aged 50–79 years old were recruited from 40 US clinical centers between 1993 and 1998 to participate in the observational study (OS) and in clinical trials (CT): postmenopausal hormone therapy (estrogen alone or estrogen plus progestin), a calcium and vitamin D supplement trial, and a dietary modification trial. Demographics and medical history were assessed using a questionnaire and body height, weight, systolic and diastolic blood pressures were measured as described previously [18]. Baseline medication use was ascertained using a computer-driven inventory system at the first screening visit. Study protocols and consent forms were approved by the institutional review boards at all participating institutions. Age of menopause was accessed using a questionnaire using the following question: " How old were you when you had your first menstrual period (menses)?" Categories of age were: 9 or less, 10, 11, 12, 13, 14, 15, 16 and 17 or more.

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Supplemental Table 1. Genotyping and imputation platforms used by participating studies

	Array type	Genotype calling	QC filters (removed) for genotyped SNPs used for imputation	No of SNPs used for imputation	Imputation	Imputation Backbone for phased haplotypes (NCBI build)	Filtering of imputed genotypes	Data management and statistical analysis
AABC Consortium	Illumina 1M	Illumina GenomeStudio	call rate < 95%	1043036	MACH (1.0.16)	combined CEU+YRI reference panel	MAF<1%, $r^{2<}$ 0.4	C++
CARE Consortium	Affymetrix 6.0	Birdseed v1.33	all chip QC + pi_hat 0.05 for rate step	763537 to 846628	MACH, 2 rounds	combined CEU+YRI reference panel	MAF<1%, $r^{2<}$ 0.3	PLINK – dosage
WHI	Affymetrix 6.0	Birdseed 2	genome-wide genotyping success rate <95%, duplicate discordance or sex mismatch, SNPs with genotyping success rate <90%, monomorphic SNPs, SNPs with minor allele frequency (MAF) <1%, and SNPs that mapped to several genomic locations were removed	798,230	MACH	combined CEU+YRI reference panel	MAF < 1% (m = 14,014), HWE p-value < 1e-6 (m = 16,327), or Genotype completeness < 90% (m = 1,633)	ProbAbel
HANDLS	Illumina 1M SNP coverage*	Illumina GenomeStudio	HWE p-value < 1e-7, missing by haplotype p-values < 1e-7, minor allele frequency < 1%, call rate < 95%, related individuals	907763	MACH, 2 rounds	combined CEU+YRI reference panel	None	R, MACH2qtl V1.08
BHS	Illumina Human610 Genotyping BeadChip and HumanCVD BeadChip	Illumina BeadStudio	call rate < 95%	N/A	MACH v.1.0.16	combined CEU+YRI reference panel	None	PLINK – dosage

*(The majority of samples used Illumina 1M and 1Mduo arrays, the remainder used a combination of Illumina 550K, 370K, 510S and 240S to reach the 1 million SNP level of coverage)

Supplementary Table 2. Replication of African American GWA findings in women of European ancestry[1]

						ReproGen 32-study meta-analysis results					Combined
SNP	**Chr.**	**Nearest gene**	**Effect Allele**	**AA**	**EAF**	**ReproGen EAF**	**Effect β[2]**	**SE**	**Same direction as AA?**	**p[3]**	**p[4]**
rs4557202	3	*B3GALNT1*	C	0.43		0.48	0.296	0.364	N	4.52E-01	2.94E-01
rs11216435	11	*DSCAML1*	T	0.32		0.22	-0.926	0.468	N	6.78E-02	7.71E-01
rs339978	15	*RORA*	T	0.20		0.02	0.624	1.612	Y	7.22E-01	3.60E-05
rs1476150	2	*NAP5*	C	0.65		0.40	0.083	0.520	Y	8.76E-01	3.66E-05
rs7754121	6	*HDGFL1*	A	0.10		0.02	-0.026	1.716	N	9.89E-01	3.66E-02
rs320320	1	*AKT3*	A	0.48		0.80	1.659	0.468	Y	**1.06E-03**	**1.18E-07**
rs12907866	15	*CYP19A1*	A	0.84		0.53	-0.432	0.364	N	2.74E-01	9.10E-01
rs6468994	8	*ZFPM2*	T	0.64		0.55	0.359	0.364	Y	3.63E-01	1.16E-02
rs11071033	15	*UNC13C*	T	0.71		0.41	1.134	0.364	Y	4.58E-03	7.12E-06
rs7807441	7	*FLJ13195*	T	0.55		0.57	-0.281	0.364	Y	4.83E-01	1.73E-02
rs17669535	8	*DLGAP2*	C	0.97		0.88	0.426	0.624	Y	5.14E-01	1.18E-01
rs6947406	7	*C7orf10*	A	0.87		0.98	0.385	1.352	N	7.94E-01	2.83E-03
rs980000	15	*RORA*	T	0.26		0.03	1.274	1.300	Y	3.67E-01	3.41E-05
rs8014131	14	*FLRT2*	A	0.42		0.44	-0.156	0.364	Y	6.92E-01	4.62E-02
rs7819115	8	*DLGAP2*	A	0.36		0.78	-0.728	0.468	Y	1.37E-01	7.86E-04
rs7873730	9	*ZNF483*	A	0.88		0.93	-5.122	0.780	Y	**3.50E-09**	**3.80E-15**
rs10441737	9	*ZNF483*	T	0.58		0.64	-2.777	0.416	Y	**4.88E-11**	**2.56E-15**
rs10940138	5	*PIK3R1*	T	0.19		0.21	0.296	0.520	Y	5.80E-01	2.17E-02
rs7911165	10	*EBF3*	T	0.54		0.63	-0.198	0.364	N	6.31E-01	3.38E-01
rs2796200	1	*ZRANB2*	A	0.66		0.92	0.806	0.676	Y	2.86E-01	4.23E-04

From Elks et al., 2010

Effect is in weeks

P-value from stage 1 meta analysis (up to 87,802 women from 32 studies); values meeting Bonferonni-correction threshold are bolded

p-value from Stage 1 + ReproGen

plemental Table 3. Association of index SNPs in 42 previously reported menarche loci with age at menarche in African American (AA) women

r	index SNP identified in EA women	Nearest Genes	Coded/Non-coded alleles for the index SNP	Coded allele freq for index SNP in EA (from Elks 2010)	Index SNP β in EA (weeks)	Coded allele frequency for the index SNPs in AA	Index SNP β in AA (weeks)	Same direction of effect	p-value for index snp in AA[1]
	rs466639	*RXRG*	C/T	0.87	4.2	0.86	4.1	Y	0.002
	rs633715	*SEC16B*	C/T	0.20	-2.6	0.10	-2.5	Y	0.115
	rs12472911	*LRP1B*	C/T	0.20	2.5	0.51	-1.4	N	0.144
	rs12617311	*PLCL1*	A/G	0.32	-3.0	0.17	-0.4	Y	0.738
	rs17188434	*NR4A2*	C/T	0.07	-4.5	0.02	1.6	N	0.871
	rs17268785	*CCDC85A*	A/G	0.83	-3.2	0.74	-3.1	Y	0.017
	rs2947411	*TMEM18*	A/G	0.17	2.8	0.23	-0.1	N	0.942
	rs2002675	*SFRS10*	A/G	0.58	-2.2	0.69	-2.0	Y	0.051
	rs2687729	*RUVBL1;EEFSEC*	A/G	0.73	-2.3	0.66	-1.8	Y	0.075
	rs3914188	*PSMD2;ALG3;ECE2;CAMK2 N2;EIF4G1;FAM131A;CLCN2*	C/G	0.73	2.2	0.75	1.2	Y	0.326
	rs6438424	*3q13.32*	A/C	0.50	-2.7	0.73	0.0	N	0.999
	rs6439371	*TMEM108;NPHP3*	A/G	0.66	-2.3	0.58	0.7	N	0.487
	rs6762477	*RBM6;RBM5*	A/G	0.56	-2.5	0.73	-1.7	Y	0.111
	rs7617480	*LOC646498;USP19;KLHDC8 B;CCDC71;CCDC36;LAMB2*	A/C	0.22	2.4	0.32	-0.2	N	0.815
	rs7642134	*VGLL3*	A/G	0.38	-2.4	0.54	-0.4	Y	0.696
	rs13187289	*PHF15*	C/G	0.80	-3.0	0.77	-2.2	Y	0.062
	rs757647	*JMJD1B;CDC25C;FAM53C; KDM3B*	A/G	0.22	-2.4	0.41	-1.1	Y	0.261
	rs1361108	*C6orf173;TRMT11*	C/T	0.54	2.1	0.69	3.0	Y	0.003
	rs4840086	*PRDM13;MCHR2*	A/G	0.58	2.1	0.87	2.3	Y	0.125
	rs7759938	*LIN28B*	C/T	0.32	6.4	0.53	2.5	Y	0.152
	rs1079866	*INHBA*	C/G	0.85	-3.9	0.91	-0.8	Y	0.660
	rs7821178	*PXMP3*	A/C	0.34	-2.4	0.47	-0.3	Y	0.727
	rs10980926	*ZNF483;LTB4DH;KIAA0368*	A/G	0.36	2.5	0.61	2.3	Y	0.019
	rs2090409	*TMEM38B*	A/C	0.31	-4.7	0.38	-1.5	Y	0.391
	rs10899489	*NARS2;GAB2*	A/C	0.15	3.1	0.31	0.7	Y	0.482
	rs16938437	*PHF21A*	C/T	0.91	3.7	0.77	-2.6	N	0.022
	rs4929923	*STK33; TRIM66*	C/T	0.64	-2.3	0.55	-1.5	Y	0.109
	rs6589964	*BSX;HSPA8;C11orf63*	A/C	0.48	-2.7	0.38	0.3	N	0.722
	rs900145	*ARNTL*	C/T	0.30	2.3	0.53	0.6	Y	0.524
	rs9555810	*C13orf16, ARHGEF7*	C/G	0.72	-2.3	0.81	0.1	N	0.922
	rs6575793	*BEGAIN;WDR25*	C/T	0.42	2.3	0.78	-2.6	N	0.057

rs3743266	*RORA;NARG2*	C/T	0.32	-2.0	0.33	-0.7	Y	0.489
rs7359257	*IQCH*	A/C	0.45	1.7	0.65	1.4	Y	0.162
rs1364063	*NFAT5*	C/T	0.43	2.1	0.23	-0.7	N	0.553
rs1659127	*MKL2*	A/G	0.34	2.4	0.30	1.8	Y	0.116
rs9939609	*FTO*	A/T	0.40	-2.1	0.47	-0.2	Y	0.833
rs9635759	*CA10*	A/G	0.32	3.0	0.14	-0.5	N	0.754
rs1398217	*IER3IP1; FUSSEL18*	C/G	0.57	2.7	0.74	-0.7	N	0.514
rs2243803	*SLC14A2*	A/T	0.40	2.0	0.84	-0.3	N	0.804
rs10423674	*CRTC1;KLHL26*	A/C	0.35	2.3	0.54	-0.8	N	0.412
rs1862471	*PIN1;OLFM2;UBL5*	C/G	0.53	-2.0	0.81	0.3	N	0.842
rs852069	*PCSK2*	A/G	0.37	-2.1	0.50	-1.8	Y	0.058

*p*value unadjusted for multiple comparisons