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Title

Integrating Genome-Wide Association and eQTLs Studies Identifies the Genes Associated with Age at Menarche and Age at Natural Menopause

Short title

Identifying the Genes Associated with Age at Menarche and Age at Natural Menopause

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Abstract

Objective: An early onset of menarche and, later, menopause are well-established risk factors for the development of breast cancer and endometrial cancer. Although the largest GWASs have identified 389 independent signals for age at menarche (AAM) and 44 regions for age at menopause (ANM), GWAS can only identify the associations between variants and traits. The aim of this study was to identify genes whose expression levels were associated with AAM or ANM due to pleiotropy or causality by integrating GWAS data with genome-wide expression quantitative trait loci (eQTLs) data. We also aimed to identify the pleiotropic genes that influenced two phenotypes.
Method: We employed GWAS data of AAM and ANM and Genome-wide eQTL data from whole blood. The summary data-based Mendelian randomization (SMR) method was used to prioritize the associated genes for further study. The colocalization analysis was used to identify the pleiotropic genes.
Results: We identified 31 genes whose expression was associated with AAM and 24 genes whose expression was associated with ANM due to pleiotropy or causality. Two pleiotropic genes were identified to be associated with two phenotypes.
Conclusion: The results point out the most possible genes which were responsible for the association. Our study prioritizes the associated genes for further functional mechanistic study of AAM and ANM and illustrates the benefit of integrating different omics of data into the study of complex traits.

Introduction

Menarche is the first menstrual cycle and signals the possibility of fertility. An early onset of menarche is associated with risks for obesity, type 2 diabetes, cardiovascular disease, breast cancer and all-cause mortality [1]. Menopause is defined as the permanent cessation of menses due to the loss of ovarian follicular activity. Younger age at natural menopause (ANM) is associated with low risk of breast cancer and ovarian cancer, but higher risks of osteoporosis, cardiovascular disease and type 2 diabetes [1]. A Mendelian randomization study have found that later ANM causally increased the risk of breast cancer [2]. These two traits also mark the beginning and the end of a woman's reproductive life [3].

Genome-wide association studies (GWAS) are capable to identify the association between target phenotypes and million genetic variants. GWAS of age at menarche (AAM) identified 106 loci containing 389 independent signals [4]. GWAS of ANM has successfully identified dozens of loci [2, 5, 6]. Most of these loci encode factors that appear to be involved in DNA repair, immune response and breast cancer processes [2, 5]. However, GWAS can only identify those SNPs strongly associated with target phenotypes, without pinpointing the target genes and the underlying biological mechanism. For example, the largest GWAS of ANM identified 44 loci containing at least one common variant significantly associated with ANM [2]. However, the significant SNPs in 21 loci were annotated to more than one gene in each locus. It suggested that the specific causal genes remain mostly unidentified.

A large part of genetic variants influence the target phenotypes by causal regulatory effect rather than directly influencing the structure of protein [7]. Expression quantitative trait loci (eQTL), which is a genetic variant influencing a target gene's expression, is often used to explain the underlying biological mechanism of significant SNPs identified by GWAS. Previous studies have suggested that in the significant loci, those SNPs which were also eQTLs were more likely to be functional SNPs [8]. Zhu et al. proposed a summary-based Mendelian randomization (SMR) analysis to combine GWAS and eQTL data into a single analysis [7]. SMR integrates GWAS data and eQTL identified from whole blood tissue to identify potential functionally relevant genes at the significant loci identified in GWAS. Previous studies have shown that whole blood can be a proxy of relevant tissues for various of phenotypes and disease [7, 9].

In this study, we identified genes whose expression levels were associated with AAM or ANM due to pleiotropy or causality, by integrating ANM GWAS data with eQTL data. We conducted a colocalization analysis to identify significant SNPs causally associated with both phenotypes.

Materials and Methods

AAM GWAS summary dataset

Using 1000 Genomes Project–imputed genotype data in up to ~370,000 women, 389 independent signals ($P < 5 \times 10^{-8}$) were identified for age at menarche [4]. The summary data were downloaded from the following website (http://www.reprogen.org).

ANM GWAS summary dataset

The largest-scale GWAS meta-analysis summary data of ANM was used in this study [2]. The GWAS meta-analysis was conducted with a total sample of 69,360 individuals of European descent. SNPs with the minor allele frequency (MAF) no less than 0.01 and the imputation quality larger than 0.4 were included in the meta-analysis. The summary data were downloaded from the following website (http://www.reprogen.org).

eQTL dataset

Because the Westra eQTL data [9] had a low coverage of human genes, in this study we used the genetic architecture of gene expression (GAGE) eQTL data to do the SMR test [10]. The GAGE study was performed to investigate the genetic architecture of gene expression in peripheral blood in 2,765 European individuals [10]. In the GAGE data, there were 11,829 unique probes. We set the p-value threshold to select the top associated eQTL for the SMR test to be 5×10^{-8}. After removing those probes where the p value of the top eQTL was less than 5×10^{-8}, there were 8,144 probes left in the eQTL summary data. The binary summary data can be download from http://cnsgenomics.com/software/smr/#DataResource.

SMR analysis

The method of SMR was fully described in previous paper [7]. In brief, there were three models including causality (Z -> X -> Y), pleiotropy (Z -> X and Z -> Y) and linkage (Z_1 -> X, Z_2 -> Y, and Z_1 and Z_2 are two variants in linkage disequilibrium (LD) in the cis-eQTL region). In this study, we tried to identify those genes with pleiotropy effect on ANM. To distinguish the causality and pleiotropy model from the linkage model, we conducted the heterogeneity in dependent instruments (HEIDI) test. The null hypothesis of HEIDI test was that there was no heterogeneity in the b_{XY} (the effect of gene's expression on the target phenotype) values. So we used $P_{HEIDI} > 0.05$ to exclude those genes belonging to linkage model. The SMR software was downloaded from http://cnsgenomics.com/software/smr/#Download.

Colocalization analysis

Colocalization analysis was used to identify the genetic variants affecting both phenotypes. The method was detailed in previous paper [11]. In brief, the method based on a hierarchical Bayesian model can be used to find the region containing a variant that influences both phenotypes. It estimates the probability that a given genomic region either 1) contains a genetic variant that influences the first trait, 2) contains a genetic variant that influences the second trait, 3) contains a genetic variant that influences both

traits, or 4) contains both a genetic variant that influences the first trait and a separate genetic variant that influences the second trait [11]. The threshold of posterior probability equal to 0.9 was used to control the false discovery rate at level 0.1 [11].

Results

After removing those probes with the top eQTL at P_{eQTL} smaller than 5×10^{-8}, there were 8,144 probes left in the eQTL summary data. The genome-wide significant level for SMR analysis was $P_{smr} < 6.14\times10^{-6}$ (0.05/8,144, Bonferroni test). We identified 98 gene-trait associations with $P_{smr} < 6.14\times10^{-6}$. After application of the HEIDI test, this reduced to 54 gene-trait associations ($P_{HEIDI} > 0.05$). Those genes which did not pass the HEIDI test may be associated with AAM or ANM due to linkage.

We identified 31 genes which associated with AAM (Table 1) and 24 genes which associated with ANM due to pleiotropy or causality (Table 2). Three of the 31 genes can be considered as novel, i.e. no previously identified SNP, reported as genome-wide significant in the primary GWAS paper in the cis-eQTL region of the probes. Among the 24 genes, seven genes (MSH6, TLK1, SYCP2L, BRCA1, PGAP3, DIDO1 and DDX17) were previously annotated to be responsible for the association based on distance, biological function, eQTL effect and non-synonymous SNP in high LD. We also identified 5 new genes (AK125462, MSL2, CLSTN3, TRAPPC2L, DDX5 and CPNE1) where there was no significant SNP ($p < 5\times10^{-8}$) in the cis-eQTL region of the probes. C17orf46 was the only gene identified to be associated with both phenotypes

Table 1. Genes identified by SMR analysis for AAM.

probeID	Chr	Gene	topSNP	topSNP_bp	p_GWAS	p_eQTL	b_SMR	p_SMR	p_HEIDI
ILMN_1869109	1	NUCKS1	rs823094	205689807	4.83E-08	3.39E-38	-0.0637368	8.24E-07	0.24
ILMN_1765061	2	OXER1	rs12617390	42985395	1.25E-07	4.61E-31	0.0725266	2.45E-06	0.46
ILMN_1659854	2	PRPF40A	rs7592669	153550668	7.80E-12	2.08E-10	0.163474	3.75E-06	0.98
ILMN_1783304	3	ATP1B3	rs2115935	141616198	0.5	3.82E-23	-0.0101008	3.82E-23	1.00
ILMN_1732452	3	MAPKAPK3	rs13096264	50678280	3.26E-10	5.31E-47	-0.0699323	1.37E-08	1.00
ILMN_1752631	3	CGGBP1	rs9814057	88214472	4.67E-09	1.86E-107	-0.0427618	2.75E-08	0.98
ILMN_1744471	3	ZNF654	rs7653652	88189341	3.56E-09	8.55E-79	-0.0499757	3.17E-08	0.95
ILMN_2285568	4	NAAA	rs4859572	76857388	0.5	1.90E-146	0.00402107	1.90E-146	0.51
ILMN_1749409	6	HLA-F	rs3870968	29647149	1.16E-09	4.51E-37	0.0726109	6.40E-08	0.09
ILMN_1697309	7	NCF1	rs2267812	74138121	1.87E-13	8.72E-42	-0.0884765	1.54E-10	0.13
ILMN_2112988	7	NCF1C	rs2267812	74138121	1.87E-13	2.41E-31	-0.102885	7.10E-10	0.16
ILMN_2083333	7	PMS2L5	rs3846966	74113141	3.23E-12	3.35E-22	-0.111675	2.10E-08	0.09
ILMN_1788384	9	C9orf5	rs12686736	111888739	1.45E-14	1.59E-74	-0.0619751	2.24E-12	0.13
ILMN_2191929	9	C9orf6	rs874864	111728718	5.66E-13	6.02E-24	0.10741	6.09E-09	0.11
ILMN_2163306	9	FAM120A	rs1055710	96214928	1.35E-08	2.83E-15	0.116356	5.46E-06	0.86
ILMN_2377829	10	NANOS1	rs671736	120811073	4.38E-08	3.49E-88	-0.0425769	2.37E-07	0.16
ILMN_1665964	11	GAB2	rs901105	77924607	3.51E-13	8.11E-13	0.15873	3.99E-07	0.78
ILMN_1767642	11	C11orf46	rs7926666	30363101	6.58E-08	8.15E-34	-0.066431	1.31E-06	0.24

ILMN_2094106	11	HSD17B12	rs7118906	43817320	3.67E-07	1.07E-82	0.0398518	1.62E-06	0.05
ILMN_1695585	12	RPS26	rs1131017	56435929	3.25E-07	0	0.0186654	7.70E-07	0.36
ILMN_2142353	13	GRTP1	rs4907616	114008744	0.5	1.52E-12	0.0159608	1.52E-12	0.87
ILMN_1727271	14	WARS	rs1570305	100808155	2.63E-09	1.08E-226	0.0289293	9.21E-09	0.11
ILMN_2080760	15	SNX22	rs12102207	64607472	2.15E-10	9.29E-17	-0.118627	5.98E-07	0.49
ILMN_1724406	16	INO80E	rs4787491	30015337	6.98E-13	4.48E-18	0.132111	4.14E-08	0.06
ILMN_1717565	16	CLEC18A	rs3748388	69974448	3.75E-14	3.89E-09	0.197866	3.71E-06	0.10
ILMN_2056687	17	C17orf56	rs1048775	79202329	5.78E-08	4.60E-38	0.066233	9.46E-07	0.19
ILMN_1707391	17	STXBP4	rs244293	53230722	2.26E-13	4.87E-09	0.198429	5.35E-06	0.93
ILMN_1715968	19	MLL4	rs17638853	36234652	1.89E-09	1.41E-12	-0.118845	5.89E-06	0.23
ILMN_1776188	20	MAP1LC3A	rs4564863	33179367	1.94E-08	8.12E-110	-0.037662	9.50E-08	0.25
ILMN_1781225	22	C22orf27	rs5753373	31283719	1.42E-11	1.76E-78	0.0569386	3.57E-10	0.95
ILMN_2103591	22	MORC2	rs7284474	31390187	6.47E-11	7.08E-24	-0.101685	5.99E-08	0.10

topSNP was the most significant SNP in the cis-region of the probe.
topSNP_bp was the position of the most significant SNP.
p_GWAS was p value from GWAS.
p_eQTL was p value from eQTL study.
b_smr was effect size from SMR test.
p_smr was p value from SMR test.
p_HEIDI was p value from HEIDI test.

Table 2. Genes identified by SMR analysis for ANM.

probeID	Chr	Gene	topSNP_bp	topSNP	p_GWAS	p_eQTL	b_SMR	p_SMR	p_HEIDI
ILMN_1810915	1	FAAH	46747301	rs12142240	6.60E-09	5.31E-28	0.381001	2.24E-08	5.54E-02
ILMN_1716004	1	NSUN4	46806703	rs10489769	8.20E-08	9.38E-77	0.209601	1.14E-08	3.25E-01
ILMN_1793461	1	AK125462	149848885	rs1260246	1.60E-06	3.92E-79	0.207352	5.91E-06	9.53E-01
ILMN_1732810	2	SNX17	27644464	rs1728922	1.20E-14	5.12E-23	0.596554	5.06E-10	6.58E-02
ILMN_1670096	2	NRBP1	27584666	rs7586601	2.30E-14	1.59E-10	0.931532	5.85E-07	1.82E-01
ILMN_1729051	2	MSH6	48018081	rs1800932	3.20E-11	4.44E-47	0.30321	1.34E-07	3.86E-01
ILMN_1811029	2	TLK1	171871997	rs13004273	5.90E-17	1.67E-10	1.01194	1.89E-07	5.00E-01
ILMN_1788053	2	SLC25A12	172704291	rs4668414	2.30E-07	8.11E-21	-0.418	4.40E-07	2.27E-01
ILMN_1766859	3	MSL2	136518670	rs13433683	1.10E-05	2.00E-44	0.234233	3.08E-07	9.18E-01
ILMN_1779743	6	SYCP2L	10895260	rs6899676	2.20E-19	3.03E-10	1.07936	1.14E-06	6.02E-01
ILMN_1798804	6	SRPK1	35809776	rs17705020	1.70E-06	7.09E-34	0.280719	3.79E-06	5.41E-02
ILMN_1767642	11	C11orf46	30363101	rs7926666	1.90E-11	8.15E-34	-0.39443	1.99E-08	6.12E-02
ILMN_1734021	12	CLSTN3	7284301	rs2167285	1.20E-06	9.69E-20	-0.44306	2.53E-06	5.06E-02
ILMN_1654421	12	MPHOSPH9	123634122	rs884548	6.80E-07	2.39E-18	-0.43820	7.57E-07	5.77E-01
ILMN_1859908	16	TRAPPC2L	88927221	rs3826061	2.80E-06	3.93E-198	-0.10996	8.13E-07	7.80E-02
ILMN_1805636	17	PGAP3	37833035	rs2941506	2.00E-09	2.98E-57	-0.27618	1.75E-09	6.23E-01
ILMN_2311089	17	BRCA1	41215825	rs3092994	2.80E-10	1.18E-66	0.275418	8.81E-11	1.58E-01
ILMN_1700690	17	VAT1	41215825	rs3092994	2.80E-10	4.47E-15	-0.60833	1.77E-07	3.21E-01
ILMN_1805344	17	DDX5	62502435	rs1991401	9.60E-07	2.73E-84	0.216125	9.84E-09	8.01E-02
ILMN_1802053	19	ZNF91	23545004	rs296092	1.50E-06	5.82E-118	0.151764	8.78E-08	6.96E-01
ILMN_2307025	20	CPNE1	34221155	rs6060524	1.40E-05	5.28E-244	0.100523	7.62E-07	1.00E+00

ILMN_1812934	20	DIDO1	61558775	rs910831	7.10E-10	2.65E-16	0.554822	3.19E-08	4.00E-01
ILMN_2371590	22	DDX17	39021522	rs5757187	1.30E-12	1.88E-28	-0.49116	9.01E-11	1.00E-01
ILMN_1668535	22	JOSD1	39065172	rs3788545	3.60E-12	3.06E-12	0.782725	1.46E-07	7.48E-01

topSNP was the most significant SNP in the cis-region of the probe.
topSNP_bp was the position of the most significant SNP.
p_GWAS was p value from GWAS.
p_eQTL was p value from eQTL study.
b_smr was effect size from SMR test.
p_smr was p value from SMR test.
p_HEIDI was p value from HEIDI test.

To identify more pleiotropic SNPs and genes associated with both phenotypes, we conducted a colocalization analysis. One region was identified to contain a variant influencing two phenotypes with the posterior probability of 0.92 (Table 3). Thirteen regions were considered to influence two phenotypes through different variants (Table 3). rs3136249, with the largest probability, was considered to be the causal SNP influencing two phenotypes.

Table 3. colocalization analysis results of AAM and ANM.

chunk	chr	st	sp	PPA_3	PPA_4
162	chr2	47318990	48212562	0.92	0.064
1419	chr15	88370262	90473690	2.5E-17	1.00
1579	chr19	614967	2098015	1.1E-08	1.00
360	chr3	135458294	137370076	4.1E-06	1.00
653	chr6	28918936	29737846	3.3E-05	1.00
799	chr7	92500845	93966036	5.4E-05	1.00
1637	chr20	32819871	34960201	6.7E-05	0.99
656	chr6	31572333	32682429	3.5E-03	0.97
655	chr6	30798697	31568469	6.1E-04	0.94
1682	chr22	19913726	22355640	4.0E-04	0.94
128	chr1	241582668	242070731	2.5E-04	0.93
1644	chr20	42680811	44838112	1.9E-03	0.93
1693	chr22	37570784	39306630	3.0E-04	0.92
1213	chr12	55665948	57543572	3.7E-04	0.91

Chunk was the internal numerical identifier for the segment.
chr: chromosome
st: star position
sp: end position
PPA_3 was the posterior probability of model 3.
PPA_4 was the posterior probability of model 4.

Discussion

In this study, we identified 31 genes whose expression was associated with AAM and 24 genes whose expression was associated with ANM due to pleiotropy or causality. In total, we identified 7 new genes where there was no significant SNP in the gene region. Many of these genes participated in DNA repair, immune response, and breast cancer process [2, 5]. C17orf46 was identified to be associated with both phenotypes by integrating GWAS and eQTLs data. We also found one region influencing two phenotypes though the colocalization analysis.

SMR demonstrated that it was useful to prioritize novel genes associated with AAM or ANM. In the significant loci, we redefined the functional genes which were more likely to play important roles in the process of natural menopause. For example, a previous study that combined modification quantitative trait locus (mQTL) and eQTL identified that NRBP1 may be a functional gene associated with menopause in the significant locus [12]. Our study found that the expression of NRBP1 was associated with age at natural menopause due to pleiotropy or causality. SRPK1, encoding the splicing factor kinase SRSF protein kinase 1, was highly expressed in basal breast cancer cells [13]. The knockdown of SRPK1 significantly suppressed metastasis of breast cancer cells [14].

SMR tests reduced the multiple hypothesis burdens by testing tens of thousands of genes instead of millions of SNPs [15]. So, SMR was useful in identifying novel genes associated with AAM or ANM. DDX5, which is also known as p68, is a prototypic member of the DEAD box family of RNA helicases that encompasses multiple functions. DDX5 was highly expressed in a high proportion of breast cancers. Patients with a detectable levels of both DDX5 and polo-like kinase-1 (pLK1) often had a poor prognosis [16]. Fatty acid amide hydrolase (FAAH), the enzyme that breaks down the endocannabinoid anandamide and controls its levels, is regulated by estrogen [17].

Despite the common belief that multiple genes are responsible for controlling the timing of menarche and natural menopause, very few genes have been identified that contain common genetic variants associated with AAM and ANM. In this study, we identified two genes rs3136249 is located in the intronic region of MSH6. MSH6, which is a mismatch repair gene, was found to be associated with ANM by previous study [18]. Although the function of C11orf46 is unknown, further studies are needed to prove this result.

The present study may have some limitations that should be considered. Although we redefined the functional genes in the significant loci, these genes may be associated with age at natural menopause due to pleiotropy which meant that some of these genes may be not the causal genes. Due to the limitation of method, we did not distinguish those pleiotropic genes from causal genes. So, further works are warranted to confirm the functional genes and explore the underlying mechanism.

In conclusion, we highlighted the putative functional genes in the significant loci for

AAM and ANM. Our study prioritizes the associated genes for further functional mechanistic study of AAM and ANM and illustrates the benefit of integrating different omics of data into the study of complex traits.

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