Genome-wide association study in East Asians identifies two novel breast cancer susceptibility loci

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Abstract
Breast cancer is one of the most common malignancies among women worldwide. Genetic factors have been shown to play an important role in breast cancer aetiology. We conducted a two-stage genome-wide association study (GWAS) including 14 224 cases and 14 829 controls of East Asian women to search for novel genetic susceptibility loci for breast cancer. Single nucleotide polymorphisms (SNPs) in two loci were found to be associated with breast cancer risk at the genome-wide significance level. The first locus, represented by rs12118297 at 1p22.3 (near the LMO4 gene), was associated with breast cancer risk with odds ratio (OR) and (95% confidence interval (CI)) of 0.91 (0.88–0.94) and a P-value of $4.48 \times 10^{-8}$. This association was replicated in another study, DRIVE GAME-ON Consortium, including 16 003 cases and 41 335 controls of European ancestry (OR = 0.95, 95% CI = 0.91–0.99, P-value = 0.19). The second locus, rs16992204 at 21q22.12 (near the LINC00160 gene), was associated with breast cancer risk with OR (95% CI) of 1.13 (1.07–1.18) and a P-value of $4.63 \times 10^{-8}$. The risk allele frequency for this SNP is zero in European-ancestry populations in 1000 Genomes Project and thus its association with breast cancer risk cannot be assessed in DRIVE GAME-ON Consortium. Functional annotation using the ENCODE data indicates that rs12118297 might be located in a repressed element and locus 21q22.12 may affect breast cancer risk through regulating oestrogen receptor signalling. Our findings provide additional insights into the genetics of breast cancer.

Introduction
Breast cancer is the most common malignancy among women in the United States and many other countries around the world (1). Genetic factors have been shown to play an important role in breast cancer aetiology (2,3). Since 2007, genome-wide association studies (GWAS) have identified approximately 100 common genetic susceptibility loci for breast cancer risk (3–32). To date, most GWAS have been conducted primarily among women of European ancestry, and genetic risk variants identified in these studies explain approximately 16% of familial breast cancer risk in European descendants (5). Many variants discovered in European ancestry populations showed a weak or no association with breast cancer risk in other ethnic groups (14,33–36). Therefore, it is necessary to conduct GWAS in non-European populations to discover additional genetic risk variants for breast cancer. In 2008, we initiated the Asia Breast Cancer Consortium (ABCC), a GWAS in East Asians to search for novel genetic susceptibility loci for breast cancer risk. Over the years, this consortium has grown into a large collaboration involving cases and controls recruited in studies conducted in multiple Asian countries (13). We have identified 10 novel susceptibility loci for breast cancer risk (13,15,19–22,29,37), and many of these loci were subsequently replicated in studies of European descendants (13,15,20,38). Studies from African and Latino-ancestry populations also have identified novel susceptibility variants associated with breast cancer risk (30,32). In this paper, we report novel findings from an expanded ABCC that included additional samples in the discovery stage and imputed the genome-wide scan data using data from the 1000 Genomes Project as reference (39).

Results
Association analyses among East Asian women
The current study included data from 29 053 women (14 224 cases and 14 829 controls) as part of the ABCC. All study participants were of East Asian ancestry and recruited from eight studies conducted in multiple countries (Table 1, Supplementary Material, Text S1). Our discovery stage (stage I) included three studies with genome-wide scan data comprising a total of 7619 cases and 6286 controls, including 4866 Chinese women (SBCGS) (13,29), 4298 Korean women (SeBCS1) (40), and 4741 Japanese women (BBJ1) (41,42). Imputation was performed within each study using Minimac2 (43). SBCGS and BBJ1 were imputed with the 1000 Genomes Project Phase 3 as reference and SeBCS1 was imputed with the 1000 Genomes Project Phase 1 as reference. Only single nucleotide polymorphisms (SNPs) imputed with high imputation quality (RSQR $> 0.5$) and minor allele frequency (MAF) $> 0.01$ were included in the discovery stage analyses. A meta-analysis of imputed data from SBCGS, SeBCS1 and BBJ1 was conducted using fixed-effects, inverse variance meta-analysis using the METAL software (44). In the discovery stage, we have evaluated the association of risk variants in 106 loci identified previously for breast cancer risk via GWAS. Among those 106 SNPs, 80 SNPs were available among all three breast cancer datasets in our current GWAS. We found that the 35 SNPs were associated with breast cancer risk at $P$-value $< 0.05$ with the same direction as observed in previous reports (Supplementary Material, Table S1).

In order to select SNPs for fast-track replication (stage II), we used the following criteria: (i) an imputation score of RSQR $> 0.8$ in at least two studies with genome-wide scan data; (ii) an MAF of $> 0.05$ in all stage I studies with available data; (iii) $P < 1.0 \times 10^{-4}$ in the stage I meta-analysis; (iv) the same direction of association in all stage I studies; and (v) no strong linkage disequilibrium (LD) ($r^2 < 0.3$ in Asians) with any of the known breast cancer susceptibility loci (5,13–15,19,20,29). The top 32 SNPs were selected for replication in an independent set of 6605 cases and 8543 controls from five studies participating in the ABCC. After filtering out SNPs with low quality among designable SNPs, 28 SNPs were evaluated in stage II, in which 5 SNPs were associated with breast cancer risk at $P < 0.05$ (Supplementary Material, Table S2).
In the combined data from both stages, the association for two SNPs reached the genome-wide significance level (P < 5.0 x 10^{-8}): rs12118297 at 1p22.3 with OR (95% CI) of 0.91 (0.88–0.94) and P = 4.48 x 10^{-8}, and rs16992204 at 21q22.12 with OR (95% CI) of 1.13 (1.07–1.18) and P = 4.63 x 10^{-8} (Table 2).

With the exception of the Nagoya study for rs12118297, the direction of the association between breast cancer risk and each of the two SNPs was consistent across all studies included in the present analysis (P for heterogeneity > 0.05) (Fig. 1).

In analyses stratified by study population, although the associations of both SNPs rs12118297 and rs16992204 were stronger for Chinese than for Korean and Japanese participants, heterogeneity tests were not statistically significant (Supplementary Material, Table S3). Both SNPs showed a stronger association for ER-positive breast cancer than ER-negative breast cancer, and the difference was statistically significant for rs16992204 (P = 0.05) (Supplementary Material, Table S4).

Evaluation of the two SNPs in European-ancestry women

To investigate the association of these two SNPs with breast cancer risk in women of European ancestry, we accessed data from the DRIVE GAME-ON Consortium (45), consisting of 16 003 cases and 41 335 controls. SNP rs12118297 showed a significant association with breast cancer risk in women of European ancestry at P = 0.019. The OR for the association was 0.95 (95% CI = 0.91–0.99), consistent with the association observed in the East Asian population. The MAF of this SNP was much lower in European descendants (0.18) than in East Asians (0.38), and the strength of the association was weaker in European than in East Asian women (P for heterogeneity = 0.03). We were not able to evaluate rs16992204 in the DRIVE GAME-ON Consortium since this SNP showed an allele frequency of 0 in European ancestry in the 1000 Genomes Project. Therefore, it is likely that this SNP cannot be imputed to 1000 Genomes project for GWAS of European ancestry, like the DRIVE GAME-ON Consortium.

Expression quantitative trait loci (eQTL) analyses and functional annotation

To explore potentially regulated target genes for the newly identified loci, we conducted eQTL analysis to evaluate the association of rs12118297 and rs16992204 with the expression levels of genes within 1Mb region in breast tumour tissue using data from The Cancer Genome Atlas (TCGA) (46) and the Molecular Taxonomy of Breast Cancer International Consortium (METABRIC) (47) (see Methods). Additionally, two publicly available databases, Gene Expression Omnibus (GEO) (48) and the 1000 Genomes Project (49), were used to identify additional eQTL and functional annotations for these loci.
available eQTL databases, GTEx database (48) and HaploReg V4 (49) were also examined. To investigate whether nearby genes may be involved in breast carcinogenesis, we performed differential gene expression analysis between breast tumour tissue and adjacent normal tissue using data from 87 patients included in TCGA. The functional significance of both newly identified loci was evaluated using the Encyclopedia of DNA Elements (ENCODE) Project (50), HaploReg V4 (49), and RegulomeDB (51).

For locus 1p22.3, SNP rs12118297 is located 14 934 bp upstream of the LMO4 gene (LIM-only protein 4) (Fig. 2A). No significant associations were found between this SNP and expression of genes within 1 Mb region based on the eQTL analysis in GTEx, TCGA, or METABRIC data. However, a search of eQTL results from both HaploReg V4 and RegulomeDB showed that this SNP was correlated with LMO4 gene expression in human monocytes (52) and brain tissue (cerebellum and temporal cortex) (53). The expression level of the LMO4 gene was significantly lower in tumour tissue than in adjacent normal tissue ($P = 5.71 \times 10^{-5}$) among breast cancer cases included in TCGA (Supplementary Material, Table S5). We also found some evidence of eQTLs for nearby genes. They were associated with expression levels of CLCA2 (chloride channel accessory 2) gene and SH3GLB1 (SH3-Domain GRB2-Like Endophilin B1) gene ($P < 0.05$), located $\sim 857$ kb and $\sim 565$ kb upstream of rs12118297, respectively (Supplementary Material, Table S7). The expression levels of both genes were significantly lower in tumour tissues than in adjacent normal tissues (CLCA2 gene, $P = 1.10 \times 10^{-4}$ and SH3GLB1 gene, $P = 1.25 \times 10^{-2}$) (Supplementary Material, Table S5). These findings support possible roles of CLCA2 and SH3GLB1 as potential tumour suppressors in breast carcinogenesis (54,55). ChromHMM annotation using ENCODE data suggests that rs12118297 might be located in a polycomb-repressed element. In RegulomeDB, this SNP has been annotated as a potentially functional SNP with a score of 1f, indicating that it may lie within a region containing a transcription factor (TF) binding site, matched TF motif and DNase I hypersensitive site. Consistently, the annotation using HaploReg indicated that it might be located in a predicted LRF motif (Supplementary Material, Table S6).

For locus 21q22.12, SNP rs16992204 is located 1722 bp upstream of the LINCO0160 gene (Long Intergenic Non-Protein Coding RNA 160) (Fig. 2A). We could not evaluate whether this SNP was an eQTL in GTEx, TCGA, and METABRIC data because the MAF of this SNP is less than 0.01 in European populations. The expression level of the LINCO0160 gene was significantly higher in tumour tissue than in adjacent normal tissue ($P = 1.13 \times 10^{-4}$) among breast cancer cases included in TCGA (Supplementary Material, Table S5). At the 21q22.12, we examined nearby genes and found some evidence of eQTLs with MAF $> 0.01$ (Supplementary Material, Table S7). SNP rs16992204 was associated with expression of a nearby gene, KCNE1 ($P = 0.03$), which is located $\sim 283$ kb downstream of this SNP at 21q22.12 (Supplementary Material, Table S7). The expression level of the KCNE1 gene was significantly lower in tumour tissues than in adjacent normal tissues ($P = 4.97 \times 10^{-24}$) (Supplementary Material, Table S5). Our analysis showed some evidence of eQTLs for nearby genes, including RUNX1 (Runt-Related Transcription Factor 1) gene and RCAN1 (Regulator Of Calcineurin 1) gene ($P < 0.05$), located $\sim 49$ kb upstream and $\sim 124$ kb downstream of rs16992204, respectively (Supplementary Material, Table S7). Expression levels of RUNX1 gene were significantly higher in tumour tissues than in adjacent normal tissues ($P = 1.92 \times 10^{-8}$) (Supplementary Material, Table S5). This result is consistent with recent studies showing that RUNX1 expression was correlated with breast cancer progression and metastasis (56,57). Furthermore, we found that the expression level of the RCAN1 gene was significantly lower in tumour tissues than in adjacent normal tissues ($P = 5.18 \times 10^{-27}$) which supports the role of RCAN1 as a potential tumour suppressor.

Figure 1. Forest plots for risk variants in the two newly identified breast cancer risk loci by study site and stage. Per-allele OR estimates and fixed-effect summary OR estimates are presented. The size of the square box is proportional to the number of cases and controls in each study site.
breast cancer suppressor reported from previous studies (58,59). The function of the SNP rs16992204 is still not known.

Discussion

In this two-stage GWAS based on 14,224 cases and 14,829 controls of East Asian women, we identified two new breast cancer susceptibility loci at 1p22.3 (rs12118297) and 21q22.12 (rs16992204). The association of SNP rs12118297 with breast cancer risk was replicated in 16,003 cases and 41,335 controls of European ancestry from the DRIVE GAME-ON Consortium. We also found that SNP rs16992204 showed stronger association for ER-positive breast cancer than ER-negative breast cancer with statistically significant difference. These SNPs were not in LD with any of the previously reported GWAS loci for breast cancer.

Our first SNP, rs12118297 in the 1p22.3 region, is located 14,934 bp upstream of the LMO4 gene. The LMO4 gene belongs to a family of LIM-only transcriptional regulators that function as molecular adaptors for protein-protein interactions. The mechanism of LMO4 function is not yet fully known. Several studies indicated a role for the LMO4 gene as an oncogene (60,61) which is inconsistent with our differential gene expression results using TCGA data: decreased expression among breast tumour tissues with Log2 fold changes = -0.53 (Supplementary Material, Table S5). However, the expression level of LMO4 is largely affected by the change in the stoichiometry of LMO4-containing complexes, such as those comprising CtIP, BRCA1, DEAF1, and/or Ldb1 (62,63). Thus, given the primary function of LMO4, which is an adaptor for protein-protein interactions, it remains to be established whether LMO4 is amplified or deregulated by other means in breast cancer. Our finding about SNP rs12118297
identified in the present study for breast cancer risk was not associated with LMO4 expression level in breast tissues using data from GTEx, TCGA or METABRIC, however, it has been shown to be an eQTL for LMO4 in human monocytes (52) and brain tissue (53). Therefore, even though the underlying biology is still not known, our study suggests that it is possible that SNP rs12118297 affects breast cancer risk through genetic mechanisms associated with the LMO4 gene.

Our second SNP, rs16992204 in the 21q22.12 region, is located 1722 bp upstream of the LINC00160 gene. The LINC00160 gene has numerous classes of non-protein coding transcripts longer than 200 nucleotides. Recently, Jonsson et al. (64) reported that LINC00160 is a direct target of ERα, and 17β-estradiol treatment up-regulated LINC00160 expressions in breast cancer MCF-7 and T47D cells. The ChiP-qPCR experiments confirmed that ERα binds to LINC00160 in both MCF-7 and T47D cells (64). The LINC00160 was expressed at higher levels in ERα-positive tumours, compared with both ERα-negative tumours and normal tissue among TCGA samples (64). Furthermore, silencing of LINC00160 using siRNA reduces MCF-7 cell proliferation (64). We also found that the association of SNP rs16992204 with breast cancer risk was mainly observed in ER-positive breast cancer. Taken together, genetic variation at the locus 21q22.12 may affect breast cancer risk through regulating LINC00160 expressions and interaction with ER signalling. Our analysis showed no evidence for rs16992204 as cis-eQTL for this gene due to the very low MAF in European populations. However, we found some evidence of eQTLs for nearby genes with MAF ≥ 0.01 at 21q22.12 (Supplementary Material, Table S7). In particular, many studies have demonstrated a possible link of the RUNX1 gene with breast cancer development (46,65), and we found evidence of eQTLs for nearby genes.

In summary, we report common variants at two genomic loci as new genetic risk factors for breast cancer in East Asian populations, providing additional insights into the genetics and biology of breast cancer. We have explored possible biological mechanisms for the observed associations. In particular, in silico analyses support a functional significance of one of these common SNPs at 1p22.3/LMO4. However, the other biological mechanism may also be involved. Future studies, including fine-mapping and functional experimental investigations, are needed to gain additional insights into the biological basis for the genetic associations with breast cancer risk in these two loci identified in our study.

Materials and Methods

Study populations

All study participants provided written informed consent, and the protocols for all participating studies were approved by the relevant institutional review boards. Detailed descriptions of participating studies are included in the S1 File. Briefly, as part of the ABCG study, this study includes 14 224 cases and 14 829 controls from eight studies (Table 1), including 4866 Chinese, 17 356 Korean, and 6831 Japanese women. Data for Chinese women came from four studies based in Shanghai (n = 4866; the Shanghai Breast Cancer Study (SBCC), the Shanghai Breast Cancer Survival Study (SBCSS), the Shanghai Endometrial Cancer Study (SECS; controls only) and the Shanghai Women’s Health Study (SWHS) (13,66–68). Data for Korean women came from four studies: the Seoul Breast Cancer Study (SeBCS; n = 6177) (40), the Korea Genome Epidemiology Study (KoGES; n = 3209) (69), the Korean Hereditary Breast Cancer study (KOHBRA; n = 1397) (70), and the Hwasun Cancer Epidemiology Study-Breast (HCES-Br; n = 6573) (71–73). Data for Japanese women came from three studies: the Biobank Japan Project (BBJ1; n = 4741) (41), the Nagoya Study (n = 1288) (74), and the Nagano Breast Cancer Study (n = 802) (75) (Table 1).

Genotyping and quality control

Three GWAS were included in stage I, in which 4866 Chinese women, 4928 Korean women, and 4741 Japanese women were genotyped. Genotyping protocols for stage I have been described elsewhere (13,15,19,20,29,40–42). In the Chinese GWAS (SBCCS), samples were scanned primarily using Affymetrix Genome-Wide Human SNP Array 6.0, and the initial 300 samples were scanned using the Affymetrix GeneChip Mapping 500K Array Set. In the present study, only data from Affymetrix SNP Array 6.0 were used to perform imputation. After quality control exclusions, the final data set included 2731 cases and 2135 controls for 668 499 markers. For the Korean GWAS (SeBCS1), Affymetrix Genome-Wide Human SNP Array 6.0 was used. After quality control exclusions, the final data set included 2246 cases and 2052 controls for 555 117 markers. For the Japanese GWAS (BBJ1), Illumina OmniExpress BeadChip was used. A total of 550 026 SNPs from 2642 cases and 2099 controls were included after quality control exclusions.

Genotyping in stage II was completed at the Vanderbilt Molecular Epidemiology Laboratory using the iPLEX MassArray platform for 15 148 samples from the KOHBRA/KoGES, HCES-Br, SeBCS2, Nagoya, and Nagano studies. QC samples were used in the Sequenom assay, including one negative control (water), two blinded duplicates and two samples from the HapMap project in each 96-well plate. We excluded samples or SNPs that had a genotyping call rate of < 95%. We also excluded SNPs that had a concordance with the QC samples of < 95% or an unclear genotype call.

Statistical analysis

Imputation and haplotype estimation (phasing) were carried out for autosomal SNPs using Minimac2 and SHAPEIT (76) with the 1000 Genomes Project Phase 3 as the reference data for the Chinese and Japanese GWAS. The Korean GWAS was imputed using the 1000 Genomes Project Phase 1 as the reference. We only included SNPs with an MAF > 0.01 and high imputation quality (RSQ > 0.5) in three GWAS in the analyses. Association analyses of dosage data for imputed SNPs in each stage I study were analysed using the Mach2dat for SeBCS1 and Rtests for SBCCS and BBJ1 (see URLs). The first five principal components estimated through EIGENSTRAT software (see URLs) (77) were included in the logistic regression models for adjustment of population structures. ORs associated with each SNP and 95% CIs were estimated under a log-additive model. To analyze genotype data, we used SAS version 9.3, which provides results identical to those generated with dosage data using Mach2dat and Rtests. Summary ORs and 95% CIs for SNPs were obtained using fixed-effect inverse variance meta-analysis using METAL software (see URLs). Stratified analyses by ancestry and ER status were carried out. Heterogeneity across studies, among ancestry groups, and according to ER status was assessed with a Cochran’s Q test. In the combined analysis, a significant threshold P-value of < 5 × 10–8 was used to determine GWAS SNPs.

In collaboration with the DRIVE GAME-ON Consortium, data from 16 003 cases and 41 335 controls were assessed to conduct
in silico replication of the SNPs included in stage II analyses (Supplementary Material, Text S1). Forest plots were generated using STATA version 23 and regional association plots were generated using LocusZoom (see URLs). To identify proxy SNPs, pairwise LD \( r^2 \) was calculated based on the 1000 Genomes Project Phase 3 Asian populations. All genomic references are based on NCBI Human Genome Build 37, and P-values presented are based on 2-sided tests.

**Imputation accuracy**

In the current study, 714 individuals from stage I SBCGS data were genotyped for 28 SNPs using the iPLEX Sequenom MassArray platform. These genotypes were used to evaluate the imputation accuracy by examining the correlation between array genotypes and imputed dosages. Accuracy was calculated using Pearson correlation coefficient. The imputed data were highly consistent with the genotype data from Sequenom for the two SNPs identified in this study (Supplementary Material, Table S8, squared correlation coefficient \( r^2 = 0.99 \) for both loci). Similarly, imputed data and genotype data were consistent for the remaining 26 SNPs (squared correlation coefficient \( r^2 > 0.8 \)).

**eQTL analysis**

We extracted the RNA-Seq V2 data (level 3) of 1006 breast cancer tumour tissues and 94 adjacent normal tissues from the TCGA data portal (see URLs). We also downloaded DNA methylation data which were measured by the Illumina HumanMethylation450 BeadChip from TCGA level 3 data. SNP data genotyped using the Affymetrix SNP 6.0 array were also retrieved. Genotype data within the 1 Mb regions flanking the two loci were extracted and then imputed using Minimac2 and SHAPEIT with the 1000 Genomes Project Phase 3 as the reference data. Copy number variation (CNV) data for genotypes within a 1Mb region of the two loci for TCGA samples were collected from the cBioPortal (see URLs) for tumour tissues. We analyzed a total of 621 breast tumour tissues in the European population and 55 breast tumour tissues for the Asian population separately, including matched CNV, genotype, methylation and expression data. The eQTL analysis was performed in tumour tissue as previously described (78,79). Briefly, we transformed the RNA-Seq by the Expectation Maximization (RSEM) value of each gene, and performed principal component correction in gene expression data to remove potential batch effects. Residual linear regression analysis was then used to detect eQTLs while adjusting for methylation and CNV, according to the approach proposed by Li et al. (78,79).

In addition to TCGA, we conducted eQTL analyses using the GTEx database (see URLs), and data from the METABRIC project (47). We extracted matched genotypes and gene expression levels in a total of 1981 breast cancer tumour tissues from the METABRIC project. Gene expression profiling was generated on the Illumina HT12 arrays and downloaded from the Synapse (syn1757063, see URLs). A total of 49 576 transcripts are included in gene expression profiling and have been normalized as described previously (47). Genotype data using the Affymetrix SNP 6.0 array were downloaded from EBI (EGAD000100000164, see URLs). We used R package CRLMM (see URLs) to process genotype calls from the original image array-based data for METABRIC (80,81). Only probes of high qualities with intensities more than 3000 at 95% calling rate were included. Imputation was performed on the genotypes for the 1Mb regions flanking the two loci using Minimac2 and SHAPEIT with the 1000 Genomes Project Phase 3 as the reference data. The eQTL analysis was performed using Matrix eQTL (82) to evaluate the association between genotypes and gene expression levels. In the current study, we focused only on the SNPs imputed with high imputation quality (RSQR > 0.5) and an MAF > 0.01 within the 1Mb regions flanking the two newly identified risk loci to identify cis-eQTLs.

**Differential gene expression analysis**

To perform differential expression analysis on genes near the newly identified loci, we extracted their expression values from a total of 87 patients, consisting of tumour tissue sample and the corresponding adjacent normal tissue sample from TCGA. We first performed surrogate variable analysis on gene expression between tumour and normal tissues to reduce potential batch effects and other artefacts (83). The full model includes the tumour-normal comparison of interest adjusted for the paired design, and a null model was adjusted only for the paired design. The total number of latent factors and the values of the surrogate variables were identified and estimated using the two models. After adjusting for the surrogate variables, limma software package from Bioconductor was used to extract differential expression of genes (84). False discovery rate-adjusted (Benjamini and Hochberg method) P-values are presented (85).

**Assessment of regulatory functions**

We investigated the potential function of the two newly identified loci using epigenomic data from ENCODE (see URLs). First, we investigated whether they are located in regulatory elements (i.e. promoter and enhancer) using ChromHMM annotation tracks in ENCODE from the UCSC Genome Browser (see URLs) including nine cell lines: HMEC (breast normal cell line), GM12878, H1-hESC, K562, HepG2, HUVEC, NHEK, and NHLF (86). We also evaluated DNase I hypersensitive and TF binding sites in all cell lines analyzed by ENCODE, including breast normal cell line, HMEC, and breast cancer cell lines, T-47D and MCF-7. We assessed the histone modification markers H3K4Me1, H3K4Me3, and H3K27Ac in all cell lines analyzed by ENCODE using the layered histone tracks from the UCSC Genome Browser. Two publicly-available tools, RegulomeDB (see URLs) (51) and HaploReg V4 (see URLs) (49), were also used to evaluate candidate functional variants.

**URLs**

UCSC Genome Browser, http://genome.ucsc.edu/ last accessed on March, 2016


Supplementary Material
Supplementary Material is available at HMG online.

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