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ARTICLE

Examination of the associations between m^{6}A-associated single-nucleotide polymorphisms and blood pressure

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Abstract

N^6-methyladenosine (m^{6}A) has been shown to play critical roles in many biological processes and a variety of diseases. The aim of this study was to investigate the association between m^{6}A-associated single-nucleotide polymorphisms (m^{6}A-SNPs) and blood pressure (BP) in large-scale genome-wide association studies and to test whether m^{6}A-SNPs are enriched among the SNPs that were associated with BP. Furthermore, gene expression analysis was performed to obtain additional evidence for the identified m^{6}A-SNPs. We found 1236 m^{6}A-SNPs that were nominally associated with BP, and 33 of them were significant genome wide. The proportion of m^{6}A-SNPs with a $P < 0.05$ was significantly higher than that of non-m^{6}A-SNPs. Using fgwas, we found that SNPs associated with diastolic BP ($P < 5 \times 10^{-8}$) were significantly enriched with m^{6}A-SNPs (log 2 enrichment of 2.67, 95% confidence interval: [0.42, 3.68]). Approximately 10% of the BP-associated m^{6}A SNPs were associated with coronary artery disease or stroke. Most of these m^{6}A-SNPs were strongly associated with gene expression. We showed that rs56001051, rs9847953, rs197922, and rs740406 were associated with *C1orf167* ($P = 0.019$), *ZNF589* ($P = 0.013$), *GOSR2* ($P = 0.001$), and *DOT1L* ($P = 0.032$) expression levels in peripheral blood mononuclear cells of 40 Chinese individuals, respectively. The present study identified many BP-associated m^{6}A-SNPs and demonstrated their potential functionality. The results suggested that m^{6}A might play important roles in BP regulation.

Keywords Blood pressure · m^{6}A · Methylation · Genome-wide association study · Gene expression

Introduction

Hypertension is one of the most important risk factors for cardiovascular diseases, which is the leading cause of death worldwide [1]. As with other complex traits, evidence from familial studies suggests that hypertension is caused by a combination of genetic and environmental factors. The heritability of blood pressure (BP) has been estimated at approximately 40–60% [2]. Large-scale meta-analysis studies of genome-wide association studies (GWAS) have identified numerous single-nucleotide polymorphisms (SNPs) for BP [3–5]. In addition, BP-associated missense variants have been identified by exome-wide studies of large samples [6–8].

Although GWAS have revolutionized the understanding of the genetic architecture of BP, the interpretation of the GWAS results is still a major challenge. Delineation of GWAS variants by distinguishing the functional variants from the rest should be helpful for this. Nonsynonymous variants, as well as genetic variants that have the capacity to alter protein binding [9] and affect RNA splicing [10] and editing [11], are potential BP functional variants.

N^6-methyladenosine (m^{6}A) is a pervasive RNA modification in eukaryotes. It has become a hot research topic because of its critical roles not only in the regulation of gene expression [12], messenger RNA (mRNA) stability [13], and homeostasis [14] but also in the pathogenesis of various diseases [15]. Recent studies have shown that genetic variants influence m^{6}A by changing the RNA sequences of the

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target sites or key flanking nucleotides [16]. This kind of putative functional SNP is called an m^{6}A-associated SNP (m^{6}A-SNP). If m^{6}A modification was interrupted by an m^{6}A-SNP, the biological process by which m^{6}A functions would be disturbed [16]. Thus, m^{6}A-SNPs may have regulatory potential to affect gene expression and mRNA stability and homeostasis, which may consequently affect diseases such as hypertension.

Evaluation of the effect of genetic variants on m^{6}A modification will increase our understanding of the variants' pathogenic molecular mechanisms and uncover new causal variants. However, although GWAS have identified >400 loci that harbor DNA sequence variants that influence BP [5, 17], identification of m^{6}A-SNPs is very rare, and thus far, the relationship between m^{6}A-SNPs and BP is still unclear. In addition, determining the association between m^{6}A and BP in large samples on a genome-wide scale is difficult to achieve. Using the GWAS identified BP-associated m^{6}A-SNPs as a bridge, we can indirectly assess the relationship between m^{6}A and hypertension. Thus, in this study, we investigated the association between m^{6}A-SNPs and BP in large-scale GWAS and aimed to identify the enrichment of m^{6}A-SNPs among the SNPs that were associated with BP.

Methods

Determination of m^{6}A-SNPs for BP

In this study, we first investigated the effect of a new functional variant, m^{6}A-SNPs, on systolic BP (SBP) and diastolic BP (DBP) in the published summary data of three large-scale GWAS [3–5]. The first is the 2011 GWAS, which comprised 69,395 individuals. The raw data used in the present analysis were the downloaded summary results from the initial GWAS, which included association *P* values of almost 2.5 million SNPs. The second is the 2016 GWAS, which examined the association between 196,725 variants of the custom genotyping microarray Cardio-Metabochip and SBP and DBP in 201,529 individuals of European ancestry. The available summary data contained *P* values for 128,272 variants. We also evaluated the effect of the m^{6}A-SNPs on BP in the summary data from the 2018 GWAS, the third study [5]. This GWAS dataset comprised the summary results for the association between more than 10 million SNPs and SBP and DBP, which were evaluated in 317,756 individuals from the UK Biobank [18].

To screen out the m^{6}A-SNPs from these millions of SNPs, we annotated them using a list of m^{6}A-SNPs that were downloaded from the m6AVar database (http://m6avar.renlab.org/). The list contains 13,703 high (miCLIP/PA-m^{6}A-seq experiments), 54,222 medium (MeRIP-Seq experiments), and 284,089 low (genome-wide prediction based on Random Forest algorithm) confidence level m^{6}A-SNPs for humans [16]. After annotation of the SNPs in the GWAS summary dataset by the list of m^{6}A-SNPs (merged the entire set of m^{6}A-SNPs with each of the BP GWAS datasets), we identified the m^{6}A-SNPs that were associated with BP. Those m^{6}A-SNPs with *P* values <0.05 were considered in the following analyses.

Enrichment analysis

Among BP-associated SNPs, we determined if m^{6}A-SNPs were overrepresented compared to what would be expected by chance. We randomly sampled 1000 sets of non-m^{6}A-SNPs (the same number of m^{6}A-SNPs) from the GWAS datasets for SBP and DBP as the matched background and then determined if the proportion of m^{6}A-SNPs with *P* value <0.05 was significantly higher than the proportion of non-m^{6}A-SNPs with *P* value <0.05 in the 1000 sets for each trait.

In addition, fgwas was used to assess the functional enrichment of m^{6}A-SNPs as a functional annotation in SBP and DBP. The fgwas, which was a practical tool for genetic and developmental analysis of complex traits or diseases, takes advantage of summary statistics of genome-wide association and incorporates functional annotation information into a GWAS to estimate the enrichment of GWAS findings in the annotation type (e.g., m^{6}A-SNPs) [19]. The software (version 0.3.6) can be downloaded at https://github.com/joepickrell/fgwas.

Association in East Asian populations

Because of substantial ethnic differences in allele frequencies and disease susceptibility, BP association has not been necessarily replicated in East Asians [20]. We looked for evidence on associations between the identified m^{6}A-SNPs and BP traits in three GWAS from East Asian populations. The first study analyzed genome-wide (475,157) SNP data of 400 matched pairs of young-onset hypertensive patients and normotensive controls genotyped with the Illumina HumanHap550-Duo BeadChip [21]. The second study was a GWAS meta-analysis of mean arterial pressure and pulse pressure among 26,600 East Asian participants [22]. Genome-wide data for approximately 2.4 million SNPs were available. Third, the supplementary data from a meta-analysis of GWASs of BP and hypertension in 11,816 Chinese individuals followed by replication studies, including 69,146 additional individuals were also searched [23]. The m^{6}A-SNPs with *P* values <0.05 were considered.

Association with coronary artery disease and stroke

We evaluated the associations between the identified BP-associated m^{6}A-SNPs and coronary artery disease (CAD) using the summary data from a large-scale 1000 Genomes-based GWAS meta-analysis of CAD carried out by the CARDIoGRAMplusC4D consortium [24]. This study comprised approximately 185,000 individuals who were mainly (77%) of European descent. This dataset is publicly available at http://www.cardiogramplusc4d.org/data-downloads/.

We also evaluated the associations between the identified m^{6}A-SNPs and ischemic stroke using the summary data from previous GWAS, which comprised 60,341 ischemic stroke patients and approximately 400,000 controls [25]. The raw data used in the present analysis were the downloaded summary results from the initial GWAS, which included association *P* values of almost eight million SNPs and indels for ischemic stroke. The dataset is publicly available at the MEGASTROKE website (http://megastroke.org/). The m^{6}A-SNPs with CAD or stroke association *P* values <0.05 were considered in this analysis.

Gene expression analysis

The m^{6}A-SNPs may take part in gene expression regulation through exerting influence on RNA modification; thus, they may be associated with gene expression levels. We carried out expression quantitative locus (eQTL) analysis to obtain evidence on associations between the identified BP-associated m^{6}A-SNPs and gene expression using the HaploReg browser (https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php). HaploReg is a database that manually collates and updates data from large sample eQTL studies and contains data from the ENCODE project [26]. Furthermore, we tested genotypes and mRNA expression levels in peripheral blood mononuclear cells (PBMCs) of 40 unrelated Chinese Han individuals (age range from 27 to 67 years) using reverse transcription-polymerase chain reaction to obtain additional evidence to support the top BP-associated m^{6}A-SNPs.

Results

BP-associated m^{6}A-SNPs

The first step of this study was to select m^{6}A-SNPs from the BP GWAS dataset according to the annotation information of the 352,014 m^{6}A-SNPs in the m6AVar database. We found 1712 unique m^{6}A-SNPs in the 2011 GWAS dataset. Among these SNPs, 97 (5.7%) and 129 (7.5%) (166 unique) were nominally ($P < 0.05$) associated with SBP and DBP, respectively. Two of the 1712 tested m^{6}A-SNPs reached a significance level of 1.5×10^{-5}. The most significant m^{6}A-SNP for SBP ($P = 2.68 \times 10^{-6}$) and DBP ($P = 9.58 \times 10^{-8}$) was rs7398833 in the *CUX2* gene. The second m^{6}A-SNP was rs13096477 in the *SLC4A7* gene ($P = 2.85 \times 10^{-6}$ for DBP).

We found 94 unique m^{6}A-SNPs in the 2016 GWAS dataset, and a total of 32 m^{6}A-SNPs were associated with SBP and/or DBP among the 128,272 variants ($P < 0.05$). Rs9847953 (*ZNF589*), rs1801253 (*ADRB1*), and rs7398833 (*CUX2*) reached the genome-wide significance level ($P < 5 \times 10^{-8}$) (Table 1). The association between rs197922 (*GOSR2*) and SBP was also significant ($P = 9.58 \times 10^{-8}$).

In the UK Biobank data of GWAS-2018, 761 (11.1%) and 799 (12.1%) (1236 unique) m^{6}A-SNPs were nominally ($P < 0.05$) associated with SBP and DBP (Supplementary Tables 1 and 2), respectively. Among these SNPs, 13 and 26 (33 unique) were associated with SBP and DBP at the genome-wide significance level (Tables 2 and 3), respectively. These genome-wide significant m^{6}A-SNPs were in linkage disequilibrium ($r^2 \geq 0.2$ in Europeans) with the sentinel SNPs of the corresponding BP-associated loci (Supplementary Table 3). The four significant m^{6}A-SNPs found in GWAS-2016 were all significant in GWAS-2018.

Table 1 The significant m^{6}A -SNPs for SBP and DBP in GWAS-2016

SNP rsID	Gene	CHR	Position[a]	MA	MAF	*P* value				m^{6}A Function[b]
						SBP 2011	DBP 2011	SBP 2016	DBP 2016	
rs9847953	*ZNF589*	3	48,282,695	G	0.23	2.30E − 04	9.18E − 04	7.36E −08	1.39E −09	Loss
rs1801253	*ADRB1*	10	115,805,056	C	0.34	4.11E − 04	3.41E − 04	7.08E − 13	1.17E − 13	Loss
rs7398833	*CUX2*	12	111,786,892	C	0.24	2.68E − 06	9.58E −08	2.07E − 07	3.50E −09	Gain
rs197922	*GOSR2*	17	45,008,570	A	0.35	5.62E − 03	6.24E − 02	9.58E − 08	3.91E − 03	Loss

m^{6}A-SNP N^6-methyladenosine-associated single-nucleotide polymorphisms, *GWAS* genome-wide association studies, *CHR* chromosome, *DBP* diastolic blood pressure, *MA* minor allele, *MAF* minor allele frequency, *SBP* systolic blood pressure, *SNP* single-nucleotide polymorphism

[a]Assembly: GRCh37.p13

[b]The minor alleles may result in loss or gain of m^{6}A sites

Table 2 The significant m^{6}A-SNPs for SBP in GWAS-2018

SNP rsID	Gene	CHR	Position[a]	EA	EAF	GWAS							eQTL effect	Confidence level	m^{6}A function[c]
						β SBP	*P* SBP	*β* CAD	*P* CAD	*β* stroke	*P* stroke	Asian[b]			
rs56001051	*C1orf167*	1	11,838,841	G	0.10	−0.0378	1.49E − 21	–	–	–	–	Yes	*cis*	Low	Gain
rs715	*CPS1*	2	211,543,055	C	0.29	−0.0155	5.67E − 09	–	–	–	–	No	*trans*	Low	Loss
rs1733860	*PODXL*	7	131,185,995	A	0.36	0.0160	1.70E − 10	–	–	–	–	No	*trans*	Low	Gain
rs9657518	*RP1L1*	8	10,467,589	C	0.34	0.0160	2.89E − 08	–	–	–	–	Yes	*trans*	Low	Gain
rs4639	*NEIL2*	8	11,644,751	G	0.41	0.0160	1.03E − 10	–	–	–	–	No	*cis*	Low	Gain
rs1801253	*ADRB1*	10	115,805,056	C	0.34	0.0182	9.24E − 11	–	–	–	–	No	*cis*	Low	Loss
rs3758911	*CWF19L2*	11	107,197,640	C	0.32	0.0142	4.28E − 08	–	–	–	–	No	*cis*	Low	Gain
rs7398833	*CUX2*	12	111,786,892	C	0.24	−0.0201	7.33E −12	−0.0344	1.22E−03	−0.0431	1.78E−05	No	*trans*	Low	Gain
rs1885986	*SMG6*	17	2,203,175	G	0.34	−0.0173	2.07E − 11	–	–	–	–	No	*cis*	Medium	Loss
rs17650901	*MAPT*	17	44,039,691	G	0.23	−0.0168	9.87E − 09	–	–	–	–	No	*cis*	Low	Loss
rs1881193	*KANSL1*	17	44,248,769	C	0.23	−0.0169	8.73E − 09	–	–	–	–	No	*trans*	Medium	Loss
rs197922	*GOSR2*	17	45,008,570	A	0.35	0.0166	1.30E − 10	0.0313	9.70E − 04	–	–	Yes	*cis*	Low	Loss
rs740406	*DOT1L*	19	2,232,221	G	0.07	0.0295	2.79E − 08	−0.0381	3.03E − 02	–	–	Yes	*cis*	Low	Gain

m^{6}A-SNP N^6-methyladenosine-associated single-nucleotide polymorphisms, *GWAS* genome-wide association studies, *CAD* coronary artery disease, *CHR* chromosome, *DBP* diastolic blood pressure, *EA* effect allele, *EAF* effect allele frequency, *eQTL* expression quantitative locus, *SBP* systolic blood pressure, SE standard error, *SNP* single-nucleotide polymorphism

[a]Assembly: GRCh37.p13

[b]Whether the BP association has been replicated in East Asians

[c]The effect alleles may result in loss or gain of m^{6}A sites

The 1236 m^{6}A-SNPs were associated with 1241 m^{6}A sites, 4.2% and 18.0% of which belong to the high and medium confidence levels, respectively. Among these high and medium confidence level SNPs, 2 and 8 (9 unique) were associated with SBP and DBP at the genome-wide significance level (Tables 2 and 3), respectively.

Enrichment of m^{6}A-SNPs

By applying the genome-wide association data of GWAS-2011 and GWAS-2018, we carried out the enrichment analyses. The proportion of non-m^{6}A-SNPs with GWAS *P* values <0.05 for DBP (95% confidence interval [CI]: [4.4%, 6.5%], $P = 6.68 \times 10^{-5}$) was significantly lower than that of the m^{6}A-SNPs in the GWAS-2011 data. However, it was not significant for SBP (95% CI: [4.3%, 6.5%], $P = 0.31$). In the GWAS-2018 data, the proportion of non-m^{6}A-SNPs with GWAS *P* values <0.05 for SBP (95% CI: [9.5%, 10.9%], $P = 3.47 \times 10^{-3}$) and DBP (95% CI: [9.8%, 11.2%], $P = 5.81 \times 10^{-6}$) was significantly lower than the m^{6}A-SNPs. Using fgwas, we found that SNPs associated with DBP ($P < 5 \times 10^{-8}$) were significantly enriched with m^{6}A-SNPs (log 2 enrichment of 2.67, 95% CI: [0.42, 3.68]).

Association in East Asian populations

In the three GWAS from East Asian populations, we found that 57 of the 1236 identified m^{6}A-SNPs were nominally associated with BP (Supplementary Tables 1 and 2). Among the m^{6}A-SNPs that were significantly associated with BP at a genome-wide significance level in Europeans, rs56001051 (*C1orf167*, $P = 0.046$) and rs197922 (*GOSR2*, $P = 0.041$) were associated with hypertension. rs853678 (*ZSCAN31*, $P = 0.021$), rs9657518 (*RP1L1*, $P = 0.024$), rs197922 (*GOSR2*, $P = 0.031$), and rs740406 (*DOT1L*, $P = 0.018$) were associated with mean arterial pressure. rs740406 (*DOT1L*, $P = 5.0 \times 10^{-4}$) was associated with pulse pressure in East Asian populations.

Association with CAD and stroke

To further evaluate whether the 1236 BP-associated m^{6}A-SNPs were associated with CAD, we tested for association in approximately 185,000 individuals by using the CARDIoGRAMplusC4D GWAS dataset [24]. We found that 78 of the 761 SBP-associated m^{6}A-SNPs and 72 of the 799 DBP-associated m^{6}A-SNPs seemed to be associated with CAD ($P < 0.05$) (Supplementary Tables 1 and 2). The most significant ($P = 4.50 \times 10^{-9}$) m^{6}A-SNP for CAD was the nonsense SNP rs12286 of the *ADAMTS7* gene (Supplementary Table 2). We found that 56 of the 761 SBP-associated m^{6}A-SNPs and 61 of the 799 DBP-associated m^{6}A-SNPs seemed to be associated with ischemic stroke (*P*

Table 3 The significant m^{6}A -SNPs for DBP in GWAS-2018

SNP rsID	Gene	CHR	Position[a]	EA	EAF	GWAS							eQTL effect	Confidence level	m6A function[c]
						β DBP	*P* DBP	β CAD	*P* CAD	β stroke	*P* stroke	Asian[b]			
rs56001051	*C1orf167*	1	11,838,841	G	0.10	−0.0354	4.50E − 19	–	–	–	–	Yes	*cis*	Low	Gain
rs8024	*IPO9*	1	201,845,575	A	0.32	0.0166	1.62E − 10	0.0416	3.60E − 05	0.0258	7.03E − 03	No	*cis*	High	Loss
rs2275155	*SDCCAG8*	1	243,493,907	T	0.33	0.0257	1.06E − 21	–	–	–	–	No	*cis*	Low	Loss
rs9847953	*ZNF589*	3	48,282,695	G	0.23	−0.0152	7.59E −09	−0.0317	1.99E −03	–	–	No	*cis*	Low	Loss
rs1353776	*AMOTL2*	3	134,077,470	G	0.39	−0.0142	3.14E − 08	–	–	−0.0267	7.95E − 03	No	*trans*	Low	Gain
rs3757138	*BTN3A2*	6	26,376,103	G	0.11	−0.0199	3.49E − 08	–	–	–	–	No	*cis*	High	Loss
rs1978	*BTN3A2*	6	26,377,573	A	0.11	−0.0200	3.10E − 08	–	–	–	–	No	*cis*	Low	Gain
rs853678	*ZSCAN31*	6	28,297,313	A	0.11	−0.0188	1.48E − 08	–	–	0.0258	3.04E − 02	Yes	*cis*	Medium	Loss
rs2232423	*ZSCAN12*	6	28,366,151	G	0.08	−0.0243	2.26E − 10	–	–	–	–	No	*trans*	Medium	Loss
rs450630	*SCAND3*	6	28,542,424	A	0.42	−0.0151	9.56E − 10	–	–	–	–	No	*cis*	Medium	Loss
rs9262143	*PPP1R18*	6	30,652,781	T	0.09	−0.0207	2.63E − 09	–	–	–	–	No	*cis*	Low	Gain
rs1049633	*DDR1*	6	30,867,527	A	0.09	−0.0211	8.35E − 10	–	–	–	–	No	*trans*	Medium	Loss
rs2233974	*C6orf15*	6	31,080,016	G	0.15	−0.0195	2.96E − 10	–	–	–	–	No	*trans*	Low	Gain
rs707908	*HLA-C*	6	31,238,053	C	0.39	0.0209	3.80E − 16	–	–	–	–	No	*cis*	Low	Loss
rs3115672	*MSH5*	6	31,727,897	T	0.08	−0.0254	1.94E −12	–	–	–	–	No	*trans*	Low	Gain
rs4993986	*HLA-DQB1*	6	32,627,652	G	0.50	0.0152	3.45E −09	–	–	–	–	No	*trans*	High	Loss
rs564449	*EPO*	7	100,321,138	G	0.11	−0.0240	3.61E − 10	−0.0387	9.45E − 03	–	–	No	*trans*	Low	Gain
rs1733860	*PODXL*	7	131,185,995	A	0.36	0.0141	1.94E − 08	–	–	–	–	No	*trans*	Low	Gain
rs2979247	*ERI1*	8	8,888,948	G	0.41	0.0173	4.41E − 12	–	–	–	–	No	*cis*	Low	Loss
rs4639	*NEIL2*	8	11,644,751	G	0.41	0.0177	9.98E − 13	–	–	–	–	No	*cis*	Low	Gain
rs1801253	*ADRB1*	10	115,805,056	C	0.34	0.0242	6.04E − 18	–	–	–	–	No	*cis*	Low	Loss
rs7398833	*CUX2*	12	111,786,892	C	0.24	−0.0257	2.00E −18	−0.0344	1.22E −03	−0.0431	1.78E − 05	No	*trans*	Low	Gain
rs12828755	*PITPNM2*	12	123,470,586	T	0.22	−0.0201	7.00E − 11	–	–	−0.0299	1.14E − 02	No	*cis*	Low	Gain
rs72681869	*SOS2*	14	50,655,357	C	0.01	−0.0779	4.97E − 11	–	–	–	–-	No	*trans*	Low	Gain
rs1885986	*SMG6*	17	2,203,175	G	0.34	−0.0155	2.06E −09	–	–	–	–	No	*cis*	Medium	Gain
rs6066802	*PREX1*	20	47,261,017	C	0.26	−0.0157	2.04E − 08	0.0293	3.61E − 03	–	–	No	*cis*	Low	Gain

m^{6}A-SNP *N^6*-methyladenosine-associated single-nucleotide polymorphisms, *GWAS* genome-wide association studies, *CAD* coronary artery disease, *CHR* chromosome, *DBP* diastolic blood pressure, *EA* effect allele, *EAF* effect allele frequency, *eQTL* expression quantitative locus, *SBP* systolic blood pressure, SE standard error, *SNP* single-nucleotide polymorphism

[a]Assembly: GRCh37.p13

[b]Whether the BP association has been replicated in East Asians

[c]The effect alleles may result in loss or gain of m^{6}A sites

< 0.05) (Supplementary Tables 1 and 2). The most significant ($P = 1.78 \times 10^{-5}$) m^{6}A-SNP for ischemic stroke was rs7398833 in the 3′-untranslated region (UTR) of the *CUX2* gene. For the 33 BP-associated m^{6}A-SNPs that passed the genome-wide significance level, rs7398833, rs197922, rs740406, rs8024, rs9847953, rs564449, and rs6066802 were associated with CAD, and rs7398833, rs8024, rs1353776, rs853678, and rs12828755 were associated with ischemic stroke ($P < 0.05$) (Tables 2 and 3). Among them, rs7398833 in *CUX2* and rs8024 in *IPO9* were associated with both CAD ($P = 1.22 \times 10^{-3}$ and 1.78×10^{-5}) and ischemic stroke ($P = 3.60 \times 10^{-5}$ and 7.03×10^{-3}) with consistent effects, respectively.

Gene expression analysis

To further clarify the possible functional mechanisms underlying the identified m^{6}A-SNPs in association with BP, we investigated whether they were associated with the expression levels of local genes. Most of these m^{6}A-SNPs were strongly associated with gene expression in *cis* or *trans* effects. In total, 359 (217 for SBP and 246 for DBP) of the 1236 BP-associated m^{6}A-SNPs ($P < 0.05$) may have a *cis* effect on the 339 corresponding genes in different cells or tissues (Supplementary Tables 1 and 2). Some of these 359 m^{6}A-SNPs were found in multiple tissues or cells, and 20 of them could be considered *cis*-eQTLs ($P < 5 \times 10^{-8}$) (Tables 2 and 3). These *cis*-eQTLs are proxies of the sentinel variants in the BP-associated loci (Supplementary Table 3). Fifty-eight of the 359 *cis*-effect m^{6}A-SNPs showed associations with CAD, and 33 showed associations with ischemic stroke. The most significant *cis*-eQTLs for SBP, DBP, CAD, and ischemic stroke were rs56001051 in *C1orf167* ($P = 1.49 \times 10^{-21}$), rs2275155 in *SDCCAG8* ($P = 1.06 \times 10^{-21}$), rs12286 in *ADAMTS7* ($P = 4.50 \times 10^{-9}$), and rs5213 in *KCNJ11* ($P = 2.46 \times 10^{-5}$), respectively.

We noticed that rs56001051 (*C1orf167*), rs197922 (*GOSR2*), rs740406 (*DOT1L*), rs8024 (*IPO9*), rs9847953 (*ZNF589*), rs853678 (*ZSCAN31*), rs12828755 (*PITPNM2*), and rs6066802 (*PREX1*) were significantly associated with BP ($P < 5 \times 10^{-8}$) and *cis*-eQTLs in Europeans and showed evidence of association with BP traits in the East Asian populations or association with CAD or stroke. We tested the relationship between these eight BP-associated m^{6}A-SNPs and mRNA expression levels of the corresponding genes in PBMCs from Chinese individuals. We found that rs56001051, rs9847953, rs197922, and rs740406 were associated with *C1orf167* ($P = 0.019$), *ZNF589* ($P = 0.013$), *GOSR2* ($P = 0.001$), and *DOT1L* ($P = 0.032$) expression levels, respectively (Fig. 1).

Discussion

This study represents the first effort to identify m^{6}A-SNPs that influence BP by excavating data from large-scale GWAS. We found many m^{6}A-SNPs that were associated with BP. These m^{6}A-SNPs (e.g., rs9847953 and rs197922) might be functional variants that have the potential to affect gene expression (e.g., *ZNF589*, *GOSR2*), by which they affect BP.

Delineation of m^{6}A-SNPs for BP has the potential to pinpoint this kind of multifunctional SNP as a causal variant. Studies have shown that m^{6}A modification plays a pivotal role in the regulation of downstream molecular events, such as nuclear export, stability, translatability,

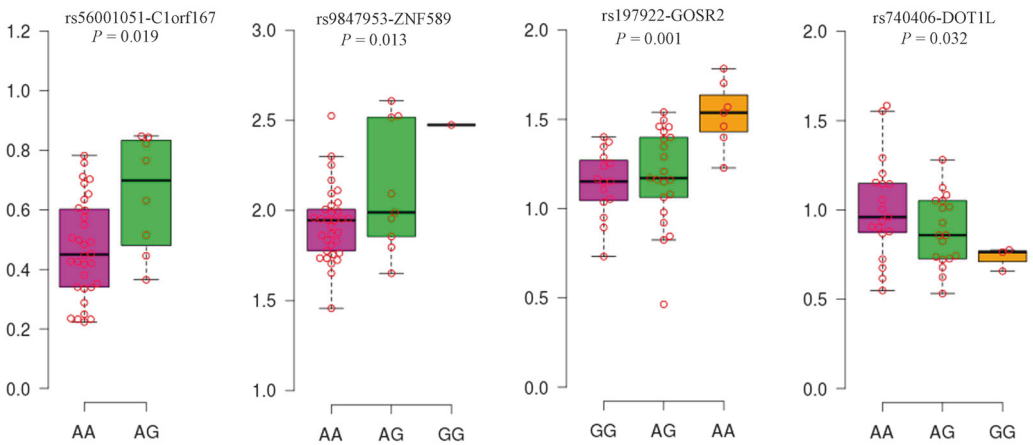

Fig. 1 Association between top blood pressure (BP)-associated N^6-methyladenosine-associated single-nucleotide polymorphisms (m^{6}A-SNPs) and messenger RNA (mRNA) expression levels. The association between m^{6}A-SNPs and mRNA expression levels was evaluated in peripheral blood mononuclear cells (PBMCs) of 40 Chinese individuals. The minor allele G carriers of rs56001051 tended to have higher *C1orf167* gene expression levels. The minor allele G carriers of rs9847953 tended to have higher *ZNF589* gene expression levels. The minor allele A carriers of rs197922 tended to have higher *GOSR2* gene expression levels. The minor allele G carriers of rs740406 tended to have higher *DOT1L* gene expression levels

splicing, and miRNA processing [27]. The m^{6}A-SNPs, which were quite close to the methylation sites or were the exact methylation site, would lead to gain or loss of m^{6}A methylation sites [16]. Therefore, one of the functional interpretations for the association between m^{6}A-SNPs and BP is that the SNPs could influence m^{6}A methylation. On the other hand, we noticed that many of the identified m^{6}A-SNPs were missense mutations or variants that could influence transcription. Indeed, most of the identified m^{6}A-SNPs have the potential to alter the binding of regulatory elements, which may in turn regulate gene expression. The nonsynonymous variants could not only change the encoded amino acids but also influence promoter activity, mRNA stability, and structure, and subcellular localization of proteins [28]. SNPs in UTRs are known to have the ability to alter microRNA or transcription factor binding. Thus, the functional interpretations for the missense and regulatory m^{6}A-SNPs should include these well-known inherent attributes. However, although we have identified many BP-associated m^{6}A-SNPs and first demonstrated that m^{6}A-SNPs may play important roles in BP regulation, to date, how m^{6}A affects BP is unclear. The underlying mechanisms still need to be elucidated.

Some of the identified m^{6}A-SNPs and genes showed strong associations with BP. For example, the nonsynonymous m^{6}A-SNP rs9847953 in *ZNF589* has the potential to alter the binding of 32 proteins and a regulatory motif LBP-1 and is located in CpG islands and DNase I hypersensitive sites (Supplementary Fig. 1). ZNF589 protein, also known as stem cell zinc-finger 1 (SZF1), belongs to the large family of Krüppel-associated box domain zinc-finger transcription factors. A recent study has established the role of SZF1/ZNF589 as a new functional regulator of the hematopoietic system [29]. Data from the Atherosclerosis Risk in Communities study and the Women's Genome Health Study have shown that the missense (Lys67Arg) m^{6}A-SNP rs197922 in *GOSR2* is associated with hypertension in white individuals [30]. As our analyses showed that rs9847953 and rs197922 were strongly associated with gene expression levels, the identified m^{6}A-SNPs may have the potential to regulate BP by altering gene expression. The nonsynonymous m^{6}A-SNP rs1801253 in *ADRB1* has the ability to alter the binding of proteins ELF1 and MAX and a regulatory motif STAT and is located in CpG islands and DNase I hypersensitive sites (Supplementary Fig. 1). The β_1-adrenergic receptor is crucial in regulating cardiac output and agents by preventing lower blood pressure [31]. The association between rs1801253 (known as Arg389Gly, which was identified in 1999) and hypertension has been well studied [31–33]. This polymorphism and β-blocker treatment duration are independent factors associated with β-blocker treatment outcome [34]. The detected BP-associated m^{6}A-SNP in 12q24.11 was rs7398833 in the *CUX2* gene, while the reported SNP was rs3184504 in *SH2B3* [3]. The *CUX2* gene has been shown to directly regulate the expression of NeuroD and function at multiple levels during spinal cord neurogenesis [35]. The association between rs7398833 and BP has not been reported before, but SNPs in this gene have been shown to be associated with type 1 diabetes [36] and CAD [37]. The present study showed that the rs7398833 C allele may result in the gain of an m^{6}A site and may lead to lower SBP and DBP levels and lower risk of CAD and ischemic stroke. The associations between these m^{6}A-SNPs and BP were not significant in GWAS-2011. However, as the sample size increased in GWAS-2016 and GWAS-2018, the associations were confirmed. These m^{6}A-SNPs and genes should be important candidates for further genetic association and functional studies.

This study has several limitations. First, the sample sizes of BP GWAS from East Asian populations were small, so very few associations between m^{6}A-SNPs and BP have been identified. Second, only a very small proportion of m^{6}A SNPs were examined in this study (1712 of 313,000). To fully recognize the impact of m^{6}A-SNPs on BP regulation, the effects of a large number of m^{6}A-SNPs (especially the rare variants) on BP should be evaluated in larger datasets (e.g., sequencing data). Finally, the functionalities of the detected SNPs, especially the effects on m^{6}A modification, have not been validated by technical and biological experiments. Further experimental studies are needed to determine their functions, for example, by performing gene editing at the single nucleotide of the SNP and observing the effect on gene expression in cell lines or the effect on BP in animals.

In summary, the present study found many BP-associated m^{6}A-SNPs and demonstrated their potential functionalities. This study increased our understanding of the regulation patterns of SNPs and may provide new clues for further detection of the functional mechanism underlying the associations between SNPs and hypertension. Although we found supplementary functional information to support the significant findings, further studies are needed to elucidate the mechanisms.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK, He J. Global burden of hypertension: analysis of worldwide data. Lancet. 2005;9455:217–23.
2. Kupper N, Willemsen G, Riese H, Posthuma D, Boomsma DI, de Geus EJ. Heritability of daytime ambulatory blood pressure in an extended twin design. Hypertension. 2005;1:80–5.
3. Ehret GB, Ferreira T, Chasman DI, Jackson AU, Schmidt EM, Johnson T, et al. The genetics of blood pressure regulation and its target organs from association studies in 342,415 individuals. Nat Genet. 2016;10:1171–84.
4. Ehret GB, Munroe PB, Rice KM, Bochud M, Johnson AD, Chasman DI, et al. Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. Nature. 2011;7367:103–9.
5. Evangelou E, Warren HR, Mosen-Ansorena D, Mifsud B, Pazoki R, Gao H, et al. Genetic analysis of over 1 million people identifies 535 new loci associated with blood pressure traits. Nat Genet. 2018;10:1412–25.
6. Liu C, Kraja AT, Smith JA, Brody JA, Franceschini N, Bis JC, et al. Meta-analysis identifies common and rare variants influencing blood pressure and overlapping with metabolic trait loci. Nat Genet. 2016;10:1162–70.
7. Surendran P, Drenos F, Young R, Warren H, Cook JP, Manning AK, et al. Trans-ancestry meta-analyses identify rare and common variants associated with blood pressure and hypertension. Nat Genet. 2016;10:1151–61.
8. Yu B, Pulit SL, Hwang SJ, Brody JA, Amin N, Auer PL, et al. Rare exome sequence variants in CLCN6 reduce blood pressure levels and hypertension risk. Circ Cardiovasc Genet. 2016;1:64–70.
9. Mao F, Xiao L, Li X, Liang J, Teng H, Cai W, et al. RBP-Var: a database of functional variants involved in regulation mediated by RNA-binding proteins. Nucleic Acids Res. 2016;D1:D154–63.
10. Wu X, Hurst LD. Determinants of the usage of splice-associated *cis*-motifs predict the distribution of human pathogenic SNPs. Mol Biol Evol. 2016;2:518–29.
11. Ramaswami G, Deng P, Zhang R, Anna Carbone M, Mackay TF, Li JB. Genetic mapping uncovers *cis*-regulatory landscape of RNA editing. Nat Commun. 2015;6:8194.
12. Meyer KD, Jaffrey SR. The dynamic epitranscriptome: N^6-methyladenosine and gene expression control. Nat Rev Mol Cell Biol. 2014;5:313–26.
13. Wang X, Lu Z, Gomez A, Hon GC, Yue Y, Han D, et al. N^6-methyladenosine-dependent regulation of messenger RNA stability. Nature. 2014;7481:117–20.
14. Edupuganti RR, Geiger S, Lindeboom RGH, Shi H, Hsu PJ, Lu Z, et al. *N*(6)-methyladenosine (m(6)A) recruits and repels proteins to regulate mRNA homeostasis. Nat Struct Mol Biol. 2017;10:870–8.
15. Visvanathan A, Somasundaram K. mRNA traffic control reviewed: N^6-methyladenosine (m(6) A) takes the driver's seat. BioEssays. 2017;1:1700093.
16. Zheng Y, Nie P, Peng D, He Z, Liu M, Xie Y. et al. m6AVar: a database of functional variants involved in m6A modification. Nucleic Acids Res. 2018;D1:D139–45.
17. Kraja AT, Cook JP, Warren HR, Surendran P, Liu C, Evangelou E, et al. New blood pressure-associated loci identified in meta-analyses of 475 000 individuals. Circ Cardiovasc Genet. 2017;10: e001778.
18. Sudlow C, Gallacher J, Allen N, Beral V, Burton P, Danesh J, et al. UK biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. PLoS Med. 2015;3:e1001779.
19. Pickrell JK. Joint analysis of functional genomic data and genome-wide association studies of 18 human traits. Am J Hum Genet. 2014;4:559–73.
20. Liu Z, Qi H, Liu B, Liu K, Wu J, Cao H, et al. Genetic susceptibility to salt-sensitive hypertension in a Han Chinese population: a validation study of candidate genes. Hypertens Res. 2017;10:876–84.
21. Yang HC, Liang YJ, Chen JW, Chiang KM, Chung CM, Ho HY, et al. Identification of IGF1, SLC4A4, WWOX, and SFMBT1 as hypertension susceptibility genes in Han Chinese with a genome-wide gene-based association study. PLoS ONE. 2012;3:e32907.
22. Kelly TN, Takeuchi F, Tabara Y, Edwards TL, Kim YJ, Chen P, et al. Genome-wide association study meta-analysis reveals transethnic replication of mean arterial and pulse pressure loci. Hypertension. 2013;5:853–9.
23. Lu X, Wang L, Lin X, Huang J, Charles Gu C, He M, et al. Genome-wide association study in Chinese identifies novel loci for blood pressure and hypertension. Hum Mol Genet. 2015;24:865–74.
24. Nikpay M, Goel A, Won HH, Hall LM, Willenborg C, Kanoni S, et al. A comprehensive 1,000 Genomes-based genome-wide association meta-analysis of coronary artery disease. Nat Genet. 2015;10:1121–30.
25. Malik R, Chauhan G, Traylor M, Sargurupremraj M, Okada Y, Mishra A, et al. Multiancestry genome-wide association study of 520,000 subjects identifies 32 loci associated with stroke and stroke subtypes. Nat Genet. 2018;4:524–37.
26. Ward LD, Kellis M. HaploReg: a resource for exploring chromatin states, conservation, and regulatory motif alterations within sets of genetically linked variants. Nucleic Acids Res. 2012;40: D930–34.
27. Visvanathan A, Somasundaram K. mRNA traffic control reviewed: N^6-methyladenosine (m(6) A) takes the driver's seat. BioEssays . 2018;1:1700093.
28. Shastry BS. SNPs: impact on gene function and phenotype. Methods Mol Biol. 2009;578:3–22.
29. Venturini L, Stadler M, Manukjan G, Scherr M, Schlegelberger B, Steinemann D, et al. The stem cell zinc finger 1 (SZF1)/ZNF589 protein has a human-specific evolutionary nucleotide DNA change and acts as a regulator of cell viability in the hematopoietic system. Exp Hematol. 2016;4:257–68.
30. Meyer TE, Shiffman D, Morrison AC, Rowland CM, Louie JZ, Bare LA, et al. GOSR2 Lys67Arg is associated with hypertension in whites. Am J Hypertens. 2009;2:163–8.
31. Bengtsson K, Melander O, Orho-Melander M, Lindblad U, Ranstam J, Rastam L, et al. Polymorphism in the beta(1)-adrenergic receptor gene and hypertension. Circulation. 2001;2:187–90.
32. Kong H, Li X, Zhang S, Guo S, Niu W. The beta1-adrenoreceptor gene Arg389Gly and Ser49Gly polymorphisms and hypertension: a meta-analysis. Mol Biol Rep. 2013;6:4047–53.
33. Wang H, Liu J, Liu K, Liu Y, Wang Z, Lou Y, et al. beta1-adrenoceptor gene Arg389Gly polymorphism and essential hypertension risk in general population: a meta-analysis. Mol Biol Rep. 2013;6:4055–63.
34. Wu D, Li G, Deng M, Song W, Huang X, Guo X, et al. Associations between ADRB1 and CYP2D6 gene polymorphisms and the response to beta-blocker therapy in hypertension. J Int Med Res. 2015;3:424–34.
35. Iulianella A, Sharma M, Durnin M, Vanden Heuvel GB, Trainor PA. Cux2 (Cutl2) integrates neural progenitor development with cell-cycle progression during spinal cord neurogenesis. Development. 2008;4:729–41.
36. Huang J, Ellinghaus D, Franke A, Howie B, Li Y. 1000 Genomes-based imputation identifies novel and refined associations for the Wellcome Trust Case Control Consortium Phase 1 Data. Eur J Hum Genet. 2012;7:801–5.
37. Lee JY, Lee BS, Shin DJ, Woo Park K, Shin YA, Joong Kim K, et al. A genome-wide association study of a coronary artery disease risk variant. J Hum Genet. 2013;3:120–6.