Genome-wide association study on blood pressure traits in the Iranian population suggests ZBED9 as a new locus for hypertension

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High blood pressure is the heritable risk factor for cardiovascular and kidney diseases. Genome-wide association studies (GWAS) on blood pressure traits increase our understanding of its underlying genetic basis. However, a large proportion of GWAS was conducted in Europeans, and some roadblocks deprive other populations to benefit from their results. Iranians population with a high degree of genomic specificity has not been represented in international databases to date, so to fill the gap, we explored the effects of 652,919 genomic variants on Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP), and Hypertension (HTN) in 7694 Iranian adults aged 18 and over from Tehran Cardiometabolic Genetic Study (TCGS). We identified consistent signals on ZBED9 associated with HTN in the genome-wide borderline threshold after adjusting for different sets of environmental predictors. Moreover, strong signals on ABHD17C and suggestive signals on FBN1 were detected for DBP and SBP, respectively, while these signals were not consistent in different GWA analysis. Our finding on ZBED9 was confirmed for all BP traits by linkage analysis in an independent sample. We found significant associations with similar direction of effects and allele frequency of genetic variants on ZBED9 with DBP (genome-wide threshold) and HTN (nominal threshold) in GWAS summary data of UK Biobank. Although there is no strong evidence to support the function of ZBED9 in blood pressure regulation, it provides new insight into the pleiotropic effects of hypertension and other cardiovascular diseases.

High blood pressure as a heritable risk factor with direct and indirect effects on the incidence of cardiovascular diseases (CVD), stroke, and chronic renal failure is the leading cause of morbidity and mortality worldwide1,2. In the analysis of world data, one in three adults is expected to be affected by high blood pressure up to 2025, while macro or microvascular complications are not limited to extreme ranges of systolic and diastolic blood pressures (SBP and DBP)3. Elevated BP is categorized into primary and secondary hypertension based on disease etiology. While the latter is secondary due to other diseases, some medications, and related side effects, the former is known as essential hypertension and is prevalent in all populations4,5.

Genetic and environmental risk factors such as age, obesity, and lifestyle are attributed to the heterogeneity of essential hypertension risk in the general population6,7. Emerging new genotyping technologies has facilitated studies with Genome-Wide Association (GWA) design in non-communicable and infectious diseases to detect

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Material and methods

Study subjects. The Tehran Cardiometabolic Genetic Study (TCGS) is a family-based genetic analysis of the Tehran Lipid and Glucose Study (TLGS) as the oldest Iranian cohort. In total, more than 20,000 participants are followed in 7 phases since 1999 and underwent a clinical examination on more than 230 metabolic-related traits in each phase of the study. The Medical Ethics Committee of Shahid Beheshti University of Medical Sciences approved this study and all methods were performed in accordance with the relevant guidelines and regulations. All participants gave written informed consent to participate in the original cohort and TCGS. In the case of younger participants, written formal consent was obtained from the parents or guardians. Principles for clinical investigations could be found in the original TCGS and TLGS papers.

In the present study, 17,462 participants from the five phases of TCGS (1999–2014) were recruited to investigate hypertension. More details on DNA sample collection, genotype quality control process, phenotype measurements, covariate imputation, case definition, and selection criteria are explained in the supplementary information 1.

Study design. Based on critical points of BP changes in the age trajectory, all study subjects aged 18 or above were included in the present study, and average values of SBP and DBP during follow up visits were considered in GWA analysis. For binary trait analysis, HTN incident cases and a random sample of healthy individuals with two or more follow-up records were included in the analysis (Supplementary file, Figure S1, S2). Age, sex, Body Mass Index (BMI), Waist Circumference (WC), insulin resistance and top 5 PCs were included in both GWA analysis on quantitative and binary traits after imputing their missing values, using the Expectation-Maximization method with Bootstrapping (EMB) approach by Amelia package in R (Supplementary file, Figure S3).

Quality control of genotypes. To maximize power against the removal of individuals and markers, quality control (QC) of genotyping data was implemented on a per-individual basis before per-marker using PLINK version 1.9 and R 2,23. Accordingly, a standard QC pipeline on 652,919 SNP, with an average mean distance of 4 kilobases, were performed in 7694 adults after excluding Individuals with missing phenotype, discordance of 2 or more follow-up records were included in the analysis (Supplementary file, Figure S1). genotype calls ≤ 10%, and high heterozygosity (Fstatistics ± 3 standard deviation). Moreover, related subjects with Identity By Decent (IBD) ≥ 18.5% were excluded from the study. In the genotype level, variants with minor allele frequency (MAF) < 1%, missing genotype calls > 5%, and Hardy Weinberg Equilibrium (HWE) P value < 1e-6 for quantitative traits, P value < 1e-10 in the HTN cases and P value < 1e-6 in controls were filtered out (Supplementary file, Figure S4).

Statistical analysis. After checking collinearity for all covariates (r² > 0.8), linear and logistic regression tests were performed. To explore any associations between BP traits and genetic variants, the covariates and top 5 Principal Components (PC) were adjusted and additive and overdominant inheritance models on autosomal chromosomes were checked for GWA on the quantitative and binary traits in PLINK v1.9, respectively. A conventional genome-wide threshold of 5 × 10⁻⁸ was considered for a significant P value. The genomic inflation factor was computed for each analysis, and observed versus expected P values were highlighted in the Q-Q plots to check for population stratification. Finally, four regression-based multivariate analyses evaluated the predictive accuracy of initial GWA outputs for quantitative traits on discovery dataset and also an independent sample of TCGS (N = 2799). Accordingly, Polygenic Risk Score (PRS) was calculated after adjusting for the covariates and top 5 PCs, to evaluate proportion of variance, which is explained by genomic variants (R²). We used a standard pipeline to evaluate R² through calculating PRS on our GWA summary data. Moreover, discrimination of best...
fit PRS was assessed for each analysis according to sex. The PRS was computed in the following steps by PLINK and R:

i. Pruning: A window size of 200 variants, sliding across the genome with step size of 50 variants at a time were considered, and any SNPs with $r^2 > 0.25$ were filter out.

ii. Clumping: SNPs within 250 k of the index SNP were considered for clumping. Then, all SNPs are correlated with each other were removed ($r^2 > 0.2$) with $P$ value ≤ 1 and only index SNP captured.

iii. Seven $P$ value thresholds (0.001, 0.05, 0.1, 0.2, 0.3, 0.4, and 0.5) for inclusion of SNPs in the PRS was extracted.

iv. PRS generated in different ranges of $P$ values for SBP and DBP.

v. To find “best-fit” score for the quantitative traits, PRS calculated in different ranges of $P$ values thresholds.

vi. $R^2$ as an index of trait variance explained by genomic variants calculated in different ranges of $P$ values thresholds for two sets of covariates, (including age, WC and Ins. resistance and top 5 PCs, and also age, BMI and Ins. resistance and top 5 PCs).

vii. Calculated $R^2$ was plotted by different ranges of $P$ values using “ggplot2”.

viii. The best-fit PRS was plotted against quantitative traits based on best result of $R^2$ by sex using “ggplot2”.

**Confirmation study.** The confirmation study was conducted on 1618 participants in 210 selected TCGS families with an age range of 1 to 93 and familial aggregation of two or more affected (HTN) cases. Similar to initial GWAS, the QC processes were applied in the confirmation study. After removing Mendelian errors and pruning out SNPs in linkage disequilibrium (LD) with a $r^2 > 0.2$, the effects of significant independent SNPs in the initial GWAS were tested in the presence of the same covariate sets using two-level Haseman-Elston regression model by SAGE version 6.4. This model-free linkage analysis is mathematically equivalent to the likelihood-based score test in variance component linkage analysis, which compute a random effect for genetic variants with $T$ score test.

**Post GWAS.** Four consecutive steps were followed to explain probable functions and pathophysiologic pathway(s) of discovered variants’ effects on BP with $P$ values less than $1 \times 10^{-4}$. In the first step, chromosomal coordinates, genes, transcripts, and variants on protein sequence were annotated in Ensemble Variant Effect Predictor. In the second step, GWAS catalog information was retrieved to identify the association of specified loci on BP traits. Functional analysis was performed for those variants with consistent results and at least a $P$ value less than $5 \times 10^{-7}$ after adjusting for different covariate sets. In the case of a similar locus, ldlink browser by National Cancer Institute and LinDA (LINkage Disequilibrium-based Annotation) browser were checked for LD of detected and previously reported variant(s) in all populations using the website http://analysistools.nci.nih.gov/ldlink/ and http://linda.irgb.cnr.it/, respectively. In the third step, a list of detailed information on all loci was retrieved separately in Open Targets Post GWAS, including disease associations, protein interactions, pathways, similar targets based on diseases in common, RNA, and protein baseline expression by the anatomical system and organ. In the final step, the overall association score was retrieved for each locus and other genes whose protein products interact with new loci through protein interaction networks. Open Target Platform provides the score from 20 data sources, is ranged from 0 to 1, that the former implies no evidence and later corresponds to the most reliable evidence supporting evidence based on frequency, severity, and significance of association.

**Results**

**GWAS datasets.** Table 1 describes the phenotypes and covariates characteristics in three datasets of discovery and confirmation studies. After applying DNA samples and genomic markers metrics, 4657 individuals with 616,263 SNPs on the quantitative traits and 4214 individuals with 616,308 SNPs on the HTN trait passed...
Table 2. GWA findings on BP traits in discovery and confirmation studies. EAF effect allele frequency in TCGS, St. β standardized effect estimate, CI confidence interval. NA not applicable due to removing variant in LD pruning process (r^2 > 0.2). *Reach the genome-wide borderline P value threshold after adjustment for WC. † Reach genome-wide threshold after adjustment for age and sex. ‡ Reach the genome-wide borderline P value threshold after adjustment for BMI/WC. *Odds ratio.

GWAS result. Table 2 describes GWAS findings in the discovery and confirmation studies. In the genome-wide analysis of quantitative traits, two signals on ABHD17C detected for DBP corresponding to the genome-wide significance threshold. Moreover, suggestive signals within 6p22.1 (ZBED9) and FBN1 with borderline P values detected with the HTN and SBP traits, respectively (Fig. 1). The detected variants on ZBED9 and ABHD17C associated with HTN and DBP by two different sets of covariates, respectively. Moreover, P values for detected SNPs on ZBED9 were slightly attenuated after adjusting for age, sex and top 5 PCs (Supplementary file, Table S1). The test statistic of genomic inflation was low for both analyses on quantitative and binary traits (λ: 1.001–1.01), and there was no population stratification (Fig. 2).

In the confirmation analysis for detected SNPs in initial GWAS, the association of rs450630 on ZBED9 was replicated for SBP, DBP, and HTN traits after LD pruning on other variants and adjusting for three covariate sets (P value < 0.05). The associations of other genetic variants on ZBED9 with QRS amplitude and interval in Europeans and also ABHD17C and FBN1 with BP traits or cardiovascular diseases in Europeans and East Asians were previously reported in GWAS literature. However, there is no correlation (LD) between detected and previously reported genetic variants in the same locus (Supplementary file, Figure S7).

In an inquiry of GWAS summary data on blood pressure related phenotypes in UK Biobank and also Japanese population, we found out similar associations (direction of effect and allele frequency) of detected variants on ZBED9 with DBP (P value ≤ 5 × 10^{-4}) and HTN (P value ≤ 1 × 10^{-4}) in UK Biobank, while the associations were not significant (even in nominal threshold of 5 × 10^{-2}) in Japanese population (Table 3). We checked out for LD of associated variants in 6p22.1 by LinDA. However, we could not find any reports in previous studies for association between correlated variants (r^2 > 0.4) and BP traits in the region. Except for Africans, allele frequencies of detected variants on ZBED9 are similar in Europeans, South Asians, Middle East (Qatari) and Iranian population, while the allele frequencies of detected variants on FBN1 and ABHD17C is less than 1% in Europeans (Supplementary file, Table S2).

Polycgenic risk score estimation. Multivariate analysis of PRS on initial GWAS outputs in the discovery dataset showed evidence of correlations between calculated PRS with SBP and DBP in three out of four models. The genomic variants account for 4.5–12.7% of the total variance of quantitative traits. Moreover, a similar distribution of PRS-trait correlation was seen by sex for each statistical model in discrimination analysis (Fig. 3).

In the discovery and confirmation datasets, while high collinearity between BMI and WC was highlighted; hence, these effects were adjusted in three different sets of covariates including, (1) age, BMI, Ins. resistance and top 5 PCs, (2) age, WC, Ins. resistance and top 5 PCs, and (3) age, sex and top 5 PCs (Supplementary file, Figures S5, S6).

Variant effects on protein-coding sequence. In the locus-based regulatory annotation, detected signals on ABHD17C and ZBED9 were annotated as an intronic variant. All detected SNPs on the FBN1 gene were in relatively high LD, while rs363830 and rs363838 were missense and splice variants. Also, two variants on ABHD17C were in moderate LD (r^2: 0.4–0.6). In further investigation via sentinel variants, we found rs853684
on ZSCAN31 as a missense consequence in moderate LD ($r^2 > 0.60$) and five missense variants in perfect LD ($r^2 = 1.0$) with detected variants on ZBED9 (Fig. 5).

**Prioritization of targets by scoring target-disease association.** The cumulative evidence for association with BP traits for three detected loci was retrieved in the Open Targets POST GWAS. The overall association scores were summarized in Fig. 6, with varying blue shades: the darker the blue, the stronger the association. Two previously reported loci of FBN1 and ABHD17C to show evidence for a strong association with BP and cardiovascular disease traits, so they were prioritized as biological targets (overall association score = 1). Further, the protein interaction network suggests gene subnetworks for ZBED9 with relatively low association with BP and cardiovascular diseases (overall association score ≤ 0.40), respectively.

**Discussion**

To our knowledge, this is the first GWAS to examine genetic associations with BP traits in the Iranian population. This study identified suggestive and strong signals on ZBED9, FBN1, and ABHD17C, associated with HTN, SBP, and DBP, respectively. We found significant associations with similar direction of effect and allele frequency of detected variants on ZBED9 with SBP (genome-wide threshold) and HTN (nominal threshold) in UK Biobank. However, there is no LD between detected and previously reported genetic variants on three loci in Europeans and other populations including, South Asian, Hispanic or Latin American, East Asian, African American, and Afro-Caribbean populations. The detected signals were not consistent after adjusting for two different sets of covariates on FBN1 and ABHD17C. On the other hand, the association of genetic variants on ZBED9 and BP traits has not been previously reported in other populations, while their effects were consistent after controlling for environmental factors by two different GWA analysis. Further, the confirmed association of genetic variants on ZBED9 in discovery study with SBP, DBP, and HTN in an independent sample of TCGS families substantially increase our knowledge of its effects on HTN in the Iranian population.

In an effort to explain the function of new loci by RNA and protein baseline expression, the rate of expression for all detected loci was in the range of low to moderate in the circulatory system, including blood, endothelial cells of the umbilical vein, aorta, coronary artery, left heart ventricle, tibial artery, atrium auricular region, and heart muscle, provided by Human Protein Atlas and Expression Atlas.

There is no evidence to support the functional effects of ZBED9 on BP regulation in the literature. However, GeneHancer-gene association results indicate some known BP loci, including EBF1, NR2F2, SOX5, and PRDM6, are likely acting as transcription factor binding sites or gene targets for ZBED9. In this way, significant...
Figure 2. Q–Q plots of observed versus expected $P$ values for BP quantitative and binary traits.
association with QRS interval and amplitude as a surrogate of myocardial mass in European population\(^41\) and replication with SBP, DBP and also HTN using different criteria based on reference adjusted curves in the Iranian children and adolescents\(^47\), who was included in TCGS confirmation study is a signal for further investigations on the functional role of \textit{ZBED9} in BP regulation pathway in other populations.

High blood pressure is directly associated with vascular mortality due to stroke, cardiomyopathy, aneurysm, cardiac hypertrophy, aorta stenosis, sudden cardiac arrest, and myocardial infarction\(^48\). Accordingly, gene-set-based analysis by Open Target Post GWAS highlighted the probable pleiotropic effects of detected loci, which are likely acting as a common genetic etiology for BP and cardiovascular diseases. Accordingly, the lethal cardiovascular diseases may affect allele frequency of effect variants with advancing age in our study due to survival reduction, so overlook the effect of functional variants in older groups as a competing risk\(^49,50\).

Table 3. Association of GWA findings on BP traits in UKbiobank and Japanese population. \textit{EAF} effect allele frequency, \textit{\(\beta\)} effect estimate, se standard error.

| Locus nearby | Position | rs ID    | Effect/other allele | UKbiobank |        |        | Japanese pop |        |        |
|--------------|----------|----------|---------------------|-----------|--------|--------|--------------|--------|--------|
|              |          |          |                     | HTN       | DBP    | SBP    | HTN          | DBP    | SBP    |
|              |          |          | \(\text{\textit{\(\beta\)} (se)}\) | \(P\) value | \(\text{\textit{\(\beta\)} (se)}\) | \(P\) value | \(\text{\textit{\(\beta\)} (se)}\) | \(P\) value | \(\text{\textit{\(\beta\)} (se)}\) | \(P\) value |
| \textit{FBN1} | 15:48420560 | T/G      | 0.008               | 7.2E−03   | 0.49   | 3.6E−03 | 2.1E−02      | 2.3E−03 | 8.4E−01 |
|              | 15:48428455 | T/C      | 0.017               | 8.8E−03   | 0.47   | 3.7E−03 | 2.3E−03      | 1.8E−04 | 9.8E−01 |
|              | 15:48420679 | G/T      | 0.02                | 7.0E−03   | 0.47   | 3.9E−03 | 2.3E−03      | 1.8E−04 | 9.8E−01 |
| \textit{ABHD17C} | 15:80717774 | C/T      | 0.003               | 1.3E−02   | 0.46   | 3.6E−03 | 2.6E−02      | 1.9E−02 | 5.1E−01 |
|              | 15:80689205 | C/T      | 0.003               | 1.2E−02   | 0.46   | 3.6E−03 | 2.6E−02      | 1.9E−02 | 5.1E−01 |
| \textit{ZBED9} | 6:28574647 | A/G      | 0.46                | 3.6E−03   | 0.47   | 3.6E−03 | 2.3E−03      | 1.3E−04 | 2.1E−01 |
|              | 6:28611694 | T/C      | 0.47                | 3.9E−03   | 0.47   | 3.6E−03 | 2.3E−03      | 1.3E−04 | 2.1E−01 |
|              | 6:28602172 | T/C      | 0.47                | 3.9E−03   | 0.47   | 3.6E−03 | 2.3E−03      | 1.3E−04 | 2.1E−01 |
|              | 6:28554918 | G/A      | 0.46                | 3.6E−03   | 0.47   | 3.6E−03 | 2.3E−03      | 1.3E−04 | 2.1E−01 |
|              | 6:28682576 | A/G      | 0.49                | 3.6E−03   | 0.47   | 3.6E−03 | 2.3E−03      | 1.3E−04 | 2.1E−01 |

We acknowledge that some limitations are evident in our study. First, there is evidence for significant differences in the magnitude of genetic variants effects on high blood pressure between men and women\(^31\). However, there was inadequate statistical power to conduct GWAS by sex after removing related individuals in our study. Moreover, the calculated PRS for the quantitative traits showed similar patterns by sex. Second, despite the high predictive accuracy of PRS by genomic variants and confirm significant SNPs on \textit{ZBED9} with family-based regression analysis, it was a case for overfitting due to selecting individuals with similar genetic background from the TCGS cohort. Accordingly, our findings validated in an independent sample of the Iranian population. However, our results still needs to be cross-validated in a different sample of the Iranian population as well\(^32,36\). Finally, we found signals which were not in LD with previously reported genetic variants on BP traits. Further investigation is necessary to fine mapping of these regions when imputed variants on the Iranian genome become available\(^37\).
Figure 3. Predictive accuracy corresponding to a range of $P$ value thresholds in regression models and the scatter plot of best fit PRS by sex in the discovery dataset.
Figure 4. Predictive accuracy corresponding to a range of P value thresholds in regression models and the scatter plot of best fit PRS by sex in the independent sample of TCGS.
Figure 5. Locus zoom plot of GWAS findings in detected loci.
Conclusion
We identified three loci, of which two loci were previously reported in individuals with European and Non-European ancestries. The association of genetic variants on ZBED9 and BP traits has not been previously reported in other populations. Their effects were consistent after controlling environmental factors by two different GW A analyses and confirmed in a family-based linkage study. Although there is no strong evidence to support the function of ZBED9 in blood pressure regulation by Open Target Post GW AS, its association with QRS interval and QRS amplitude as surrogates of myocardial mass may provide new insight into pleiotropic effects of hypertension and other cardiovascular diseases.

Data availability
Summary-level data for BP traits in UK Biobank and Japanese population are publicly available in (http://www.nealelab.is/uk-biobank/) and (http://jenger.riken.jp/en/result), respectively.

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**Author contributions**
M.S.D. and G.K. had full access to all the data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis; G.K., M.S.D., S.S., M.A., and F.H. contributed to study concept, design, and supervision; M.S.D., B.S.K., K.G., M.A., and G.K. contributed in acquisition of data; G.K., M.S.D., S.S., M.A., F.H., and B.S.K. contributed in analysis and interpretation of data; G.K., M.S.D., M.A., S.S., and S.R. contributed in statistical analysis; F.A., M.S.D., and F.H. contributed in administrative, technical, or material support; G.K., M.S.D., B.S.K., M.A., S.S., F.H., K.G., M.A.R. and F.A.: drafting of the manuscript and critical revision of the manuscript for important intellectual content. All authors read and approved the final manuscript.

**Competing interests**
The authors declare no competing interests.

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