Published in final edited form as:
Hypertension. 2012 February ; 59(2): 248–255. doi:10.1161/HYPERTENSIONAHA.111.181990.

Genome-wide association study using a high-density SNP-array and case-control design identifies a novel essential hypertension susceptibility locus in the promoter region of *eNOS*

Erika Salvi, **Zoltán Kutalik**, **Nicola Glorioso**, **Paola Benaglio**, **Francesca Frau**, **Tatiana Kuznetsova**, **Hisatomi Arima**, **Clive Hoggart**, **Jean Tichet**, **Yury P. Nikitin**, **Costanza Conti**, **Jitka Seidlerova**, **Valérie Tikhonoff**, **Katarzyna Stolarz-Skrzypek**, **Toby Johnson**, **Nabila Devos**, **Laura Zagato**, **Simonetta Guarrera**, **Roberta Zaninello**, **Andrea Calabria**, **Benedetta Stancanelli**, **Chiara Troffa**, **Lutgarde Thijs**, **Federica Rizzi**, **Galina Simonova**, **Sara Lupoli**, **Giuseppe Argiolas**, **Daniele Braga**, **Maria C. D'Alessio**, **Maria F. Ortu**, **Fulvio Ricceri**, **Maurizio Mercurio**, **Patrick Descombes**, **Maurizio Marconi**, **John Chalmers**, **Stephen Harrap**, **Jan Filipovsky**, **Murielle Bochud**, **Licia Iacoviello**, **Justine Ellis**, **Alice V. Stanton**, **Maris Laan**, **Sandosh Padmanabhan**, **Anna F. Dominiczak**, **Nilesh J. Samani**, **Olle Melander**, **Xavier Jeunemaitre**, **Paolo Manunta**, **Amnon Shabo**, **Paolo Vineis**, **Francesco P. Cappuccio**, **Mark J. Caulfield**, **Giuseppe Matullo**, **Carlo Rivolta**, **Patricia B. Munroe**, **Cristina Barlassina**, **Jan A Staessen**, **Jacques S. Beckmann**, and **Daniele Cusi**
Dept. of Medicine, Surgery and Dentistry, University of Milano. Graduate School of Nephrology, University of Milano, Division of Nephrology, San Paolo Hospital, Milano, Italy (E.S., F.F., A.C., S.L., C.B., D.C.); Filarete Foundation, Genomic and Bioinformatics Unit, Milano (E.S., F.F, A.C., S.L., C.B., D.C.); Department of Medical Genetics, University of Lausanne, Switzerland (Z.K. , P.B., C.R, J.S.B).; Swiss Institute of Bioinformatics, Lausanne, Switzerland (Z.K.); Hypertension and Related Diseases Centre-AOU, University of Sassari, Italy (N.G., R.Z., C.T., G.A., M.F.O.); Studies Coordinating Centre, Division of Hypertension and Cardiovascular Rehabilitation, Department of Cardiovascular Diseases, University of Leuven, Leuven, Belgium (T.K., L.T., J.A.S.); The George Institute for Global Health, University of Sydney and the Royal Prince Alfred Hospital (H.A., J.C.); Department of Epidemiology and Biostatistics, School of Public Health, Imperial College of London, London, United Kingdom (C.H., P.V.); IRSA, Institut inter Régional pour la Santé, Tours, France (J.T.); Institute of Internal Medicine, Siberian Branch of the Russian Academy of Medical Sciences – Novosibirsk – Russian Federation (Y.P.N., G.S.); I.M.S., Milano, Italy (C.C., M.C.D.); 2nd Department of Internal Medicine, Charles University, Medical, Faculty, Pilsen, Czech Republic (J.S.); University of Padova, Department of Clinical and Experimental Medicine, Padova, Italy (V.T.); First Department of Cardiology and Hypertension, Jagiellonian University Medical College, Krakow, Poland (K.S-S.); Clinical Pharmacology and The Genome Centre, William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, UK (T.J., M.J.C., P.B.M.); INSERM, UMRS-970, PARCC, Paris France (N.D., X.J.); Chair of Nephrology, Università Vita Salute San Raffaele; Nephrology, Dialysis and Hypertension Unit, San Raffaele Scientific Institute, Milan, Italy (L.Z., P.M.); Human Genetics Foundation (HUGEF), Turin, Italy (S.G., P.V.); Department of

Corresponding Author: Prof. Daniele Cusi MD Coordinator of HYPERGENES Project Dept. of Medicine, Surgery and Dentistry, University of Milano Chairman Division of Nephrology, San Paolo Hospital, Milano Viale Ortles 22/4, 20139 Milano Italy daniele.cusi@unimi.it Tel: +39 /02 56660130 Fax: + 39 / 02 537250.

Europe PMC Funders Author Manuscripts

Medicine, University of Catania, Italy (B.S.); KOS Genetic, Milano, Italy (F.R., D.G., M.M.); Department of Genetics, Biology and Biochemistry, University of Torino and Human Genetics Foundation (HUGEF) (F.R., G.M.); Genomics Platform, NCCR "Frontiers in Genetics" CMU University of Geneva Switzerland (P.D.); Center of Transfusion Medicine and Immunohematology, Department of Regenerative Medicine, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano (M.M.), Department of Physiology, University of Melbourne, Melbourne (S.H.); 2nd Medical Department, Cardiology and Angiology, 1st Medical Faculty, Charles University, Prague, Czech Republic (J.F.); Institute of Social and Preventive Medicine, Centre Hospitalier Universitaire Vaudois (CHUV) and University of Lausanne, 1005 Lausanne, Switzerland (M.B.); Research Laboratories "John Paul II" Centre for High Technology Research and Education in Biomedical Sciences, Catholic University, Campobasso, Italy (L.I.); Murdoch Childrens Research Institute, Melbourne, Australia. Department of Physiology, University of Melbourne, Melbourne, Australia (J.E.); Molecular & Cellular Therapeutics, Royal College of Surgeons in Ireland, Dublin 2, Ireland (A.V.S.); Institute of Molecular and Cell Biology, University of Tartu, Tartu, Estonia (M.L.); BHF Glasgow Cardiovascular Research Centre, University of Glasgow, Glasgow, G12 8TA, UK (A.F.D., S.P.); BHF Glasgow Cardiovascular Research Centre, University of Glasgow, Glasgow, G12 8TA, UK (N.J.S.); Department of Cardiovascular Sciences, University of Leicester, Leicester, UK LE3 9QP (N.J.S.); Hypertension and Cardiovascular Disease, Department of Clinical Sciences, Lund University, Malmö Sweden (O.M.); Centre of Emergency Medicine, Skåne University Hospital, Malmö, Sweden (O.M.); University Paris Descartes, Paris France (X.J.); APHP, Department of Genetics, Hopital Europeen Georges Pompidou, Paris France (X.J.); IBM Haifa Research Lab, Haifa Univ. Mount Carmel Haifa, Israel (A.S.); University of Warwick, Warwick Medical School, Coventry, United Kingdom (F.P.C.); Genetic Epidemiology Unit, Department of Epidemiology, Maastricht University, 6200 MD Maastricht, Netherlands (J.A.S.); Service of Medical Genetics, Centre Hospitalier Universitaire Vaudois(CHUV) University Hospital, Lausanne, Switzerland (J.S.B.)

Abstract

Essential hypertension is a multi-factorial disorder and is the main risk factor for renal and cardiovascular complications. The research on the genetics of hypertension has been frustrated by the small predictive value of the discovered genetic variants. The HYPERGENES Project investigated associations between genetic variants and essential hypertension pursuing a two-stage study by recruiting cases and controls from extensively characterized cohorts recruited over many years in different European regions.

The discovery phase consisted of 1,865 cases and 1,750 controls genotyped with 1M Illumina array. Best hits were followed up in a validation panel of 1,385 cases and 1,246 controls that were genotyped with a custom array of 14,055 markers. We identified a new hypertension susceptibility locus (rs3918226) in the promoter region of the endothelial nitric oxide synthase (eNOS) gene (odds ratio 1.54; 95% CI 1.37-1.73; combined $p=2.58\cdot10^{-13}$). A meta-analysis, using other *in-silico*/*de novo* genotyping data for a total of 21714 subjects, resulted in an overall odds ratio of 1.34 (95% CI 1.25-1.44, $p=1.032\cdot10^{-14}$). The quantitative analysis on a population-based sample revealed an effect size of 1.91 (95% CI 0.16-3.66) for systolic and 1.40 (95% CI 0.25-2.55) for diastolic blood pressure. We identified *in-silico* a potential binding site for ETS transcription-factors directly next to *rs3918226,* suggesting a potential modulation of eNOS expression. Biological evidence links eNOS with hypertension, as it is a critical mediator of cardiovascular homeostasis and blood pressure control via vascular tone regulation. This finding supports the hypothesis that there may be a causal genetic variation at this locus.

Keywords

genetic epidemiology; risk factors; genetics-association studies; nitric oxide; Essential Hypertension

Background

Essential hypertension (EH) is a clinical condition affecting a large proportion (25-30%) of the adult population and is a major risk factor for cardiovascular and renal diseases.[1,2] It is a complex trait influenced by multiple susceptibility genes, environmental and lifestyle factors and their interactions. [3] In the last years, huge efforts have been performed in recruiting and genotyping tens of thousands of individuals and meta-analysing dozens of cross-sectional population-based studies. In spite of this, the research on the genetics of EH has been frustrated by the small predictive value of the discovered genetic variants and by the fact that these variants explain a small proportion of the phenotypic variation. [4-13] EH is a late-onset disease and therefore the small discovered effect sizes could in part be due to the effect of misclassification, sample selection bias and inappropriate phenotyping of cases and controls.[9,14,15] The selection of cases and controls may have important effects on the results as misclassification bias can lead to loss of power. For common traits, such as EH, this bias can be remedied by defining more stringent selection criteria, by recruiting hyper-normal controls and adopting a more stringent case definition.[14,15]

The HYPERGENES Project pursued a two-stage study to investigate novel genetic determinants of essential hypertension. Cases and controls were recruited from extensively characterized cohorts over many years in different European regions using standardized clinical ascertainment. Particular care was devoted to control selection. A large proportion of the sample has been followed for 5–10 years after DNA collection, allowing for the exclusion of controls that developed hypertension at a later age, thereby defining the hyper-normal controls.

Methods

Study Population

Cases and controls were recruited from extensively characterized cohorts using standardized clinical ascertainment, collected over many years in different European regions (balanced within North Europe, Continental Italy and Sardinia). The inclusion criteria are described in Methods S1 (available online at http://hyper.ahajournals.org). In order to perform a genetic association with continuous BP phenotypes, we considered two additional cohorts (FLEMENGHO-EPOGH, n=1514 and WHSS, n=306, see methods S2 (please see http://hyper.ahajournals.org) that provided population-based data. Description of the different samples is reported in the methods S2 (http://hyper.ahajournals.org).

Genotyping and Imputation

Genotyping details are shown in methods S3-S6 (please see http://hyper.ahajournals.org). Briefly, in the Discovery phase, the samples were genotyped using the Illumina 1M-duo array and the imputation was performed with MACH [16] using as reference the 1000 Genomes haplotypes (release June 2010) (method S3, please see http://hyper.ahajournals.org). To validate and fine map the genes found associated with EH in discovery phase an Illumina custom chip of 14,055 markers was created starting from the list of best-associated and of candidate SNPs based on *a priori* biological knowledge (methods S4-S5, please see http://hyper.ahajournals.org). For the replication stage, we used

the in-silico data of rs3918226 from ASCOT/AIBIII/NBS, BRIGHT, EPIC Turin, HYPEST and NORDIL/MDC studies (methods S6, please see http://hyper.ahajournals.org).

Statistical analysis

All quality controls and statistical analyses were performed in accordance with the protocols written by C.A Anderson [17] and G.M. Clarke [18] (methods S7-S9, http://hyper.ahajournals.org). We tested each SNP for association with hypertension using a logistic regression under an additive model with adjustment for sex and for the first 10 principal components. Combined analysis for discovery, validation and replication results was conducted using METAL. [19] The quantitative effect of rs3918226 on SBP and DBP was tested on two additional population-based cohorts (methods S2**,** please see http://hyper.ahajournals.org). Moreover, we tested for multiplicative interaction between rs3918226 and the most plausible interactive partners of eNOS gene: actin genes and Heat Shock Protein-90 genes (methods S9**,** please see http://hyper.ahajournals.org). The quantitative effect of rs3918226 on SBP and DBP has been tested on two additional population-based cohorts (EPOGH-FLEMENGO and WHSS, see methods S2 http://hyper.ahajournals.org).

The recognition sequences for transcription factors (TFs) in eNOS region were searched using TRANSFAC [20,21] and TFSEARCH database [22] (methods S10, please see http://hyper.ahajournals.org).

Results

A classical two-stage case-control strategy was employed with a discovery phase of 1,865 cases and 1,750 controls (2,294 males, 1,321 females), all genotyped on the Illumina 1M Duo chip. The sample consisted of an ethnically diverse population (25.06% North Europeans, 38.70 % Sardinians and 36.24% Continental Italy subjects). The discovery phase was followed by a validation phase of additional 1,385 cases and 1246 controls (1,417 males and 1,214 females). According to ethnicity, the validation sample was comprised of 1262 North Europeans (47.97%), 788 Sardinians (29.95 %) and 581 Continental Italians (22.08%). Tables S1 and S2 (please see http://hyper.ahajournals.org) show the demographic characteristics and baseline measures.

Principal Component Analysis (PCA) of the genotype data was carried out to find the major axes of variation used as covariates to correct for population stratification. [23] The discovery samples in the principal component map showed three (roughly) equal-sized distinct clusters corresponding to the three main ethnic groups, as expected from the study design (Figure S1, please see http://hyper.ahajournals.org). All association analyses were adjusted for the ancestry principal components and sex by including them as covariates in the logistic regression model. In addition genomic control (GC) correction was applied (since genomic inflation factor was 1.04). In the discovery phase, 90 SNPs (57% intragenic) with p-value < $1{\cdot}10^{-4}$ were identified after GC (Figure S2, Table S4, please see http://hyper.ahajournals.org). The most promising SNPs were genotyped in the validation samples using an Illumina Infinium Custom chip. The meta analysis of the discovery and validation data revealed SNP rs3918226 to be associated with EH in Caucasians, reaching a $P_{combined}$ of $2.58{\cdot}10^{-13}$ and OR of 1.54 per T allele (95% CI, 1.37-1.73) under an additive model (Figure 1, Table 1 and Figure S4 at http://hyper.ahajournals.org**).** Estimated odds ratios in the Discovery and Validation samples were consistent across the different Caucasian populations of the HYPERGENES sample (Figure S5, please see http://hyper.ahajournals.org).

The polymorphism rs3918226 maps to the promoter region of the eNOS gene (−665 C>T, NOS3). [24,25] The T allele frequencies in the present study are 13.8% in cases and 8.9% in controls. SNP rs3918226 is monomorphic in the non-Caucasian HYPERGENES samples (Wandsworth Heart & Stroke Study cohort, WHSS) and African and Asian HapMap samples. The second best hit chr7:150,314,954 (G/A SNP, MAF of A allele= 3%) with P value $2.46 \cdot 10^{-6}$ and OR 2.25 was imputed based on the 1000 Genomes haplotypes (release June 2010), its imputation quality was very high (r2-hat = 0.94). Unfortunately we couldn't replicate the observation in validation due to low imputation quality. Further 7 SNPs within eNOS gene showed significant p-values ($1 \cdot 10^{-3}$ < p-values < $1 \cdot 10^{-5}$): *rs2853792* (intronic, $P_{combined} = 7.76 \cdot 10^{-5}$), *rs1549758* (coding, $P_{combined} = 3.32 \cdot 10^{-4}$), *rs1800779* (intronic, $P_{combined} = 1.16 \cdot 10^{-3}$), *rs6951150* (intergenic, $P_{combined} = 1.64 \cdot 10^{-3}$), *rs743507* (intronic, $P_{combined} = 1.76 \cdot 10^{-3}$), *rs1800780* (intronic, $P_{combined} = 1.96 \cdot 10^{-3}$), *rs1800783* (intronic, $P_{combined} = 2.89 \cdot 10^{-3}$) (Figure 1).

Table 1 shows also other significant SNPs with p-values between $1 \cdot 10^{-3}$ and $1 \cdot 10^{-5}$ mapping different genes as calcium-activated potassium channel subunit alpha-1 (*KCNMA1*), plasminogen (*PLG*), retinoid-related orphan receptor alpha (*RORA*) and WW domain-containing protein 1 (*WWC1*).

Moreover, the signals of SNPs previously presented in literature are in our study in the same direction as the original studies [5,6,8] showing evidence of a marginally significant association in HYPERGENES (Table S5**,** please see http://hyper.ahajournals.org).

We meta-analyzed rs3918226 using in silico data from ASCOT/AIBIII/NBS, BRIGHT, EPIC-Turin, HYPEST and NORDIL/MDC samples (methods S2, S6, please see http://hyper.ahajournals.org) resulting in an overall OR of 1.34 per T allele (95% CI 1.25-1.44, $P_{combined} = 1.032 \cdot 10^{-14}$) (Table 2 and Figure 2) for a total of 21,714 subjects. Since case and control definitions differed between HYPERGENES and the *in-silico* replication samples, the ORs are not directly comparable. In our study, the p value of heterogeneity calculated for HYPERGENES samples is 0.13. It decreased slightly, but remained non-significant, as expected, when also EPIC-Turin was considered together in the meta-analysis (p=0.092) since the recruitment criteria for cases and controls were identical. Conversely, the heterogeneity increased significantly (p=0.005) when HYPERGENES samples were meta-analyzed with all the other samples (ASCOT/AIBIII/NBS, BRIGHT, HYPEST and NORDIL/MDC).

Moreover we tested for epistatic multiplicative interactions between *eNOS rs3918226* and all available polymorphisms in genes known to be involved in targeting and regulating the overall availability of eNOS at the cell membrane [26,27,28] actin genes (ACTA1, ACTA2, ACTB, ACTG1, ACTG2) [29,30] and HSP90 genes (HSP90AA1, HSP90AA2, HSP90AB1).[26] Nominally significant interactions were observed between *rs3918226* and *rs13447427* (p-value $=1.34 \cdot 10^{-3}$) in actin beta gene (*ACTB)*, *rs7503750* (p-value $=1.57 \cdot 10^{-3}$) in actin gamma 1 (*ACTG1)* and *rs4922796* and *rs17309979* (p-value $=3.47 \cdot 10^{-3}$, p-value $=4.88 \cdot 10^{-3}$) in heat shock protein alpha 2 (*HSP90AA2*) (Table S6, please see http://hyper.ahajournals.org). When controlling for multiple testing these interactions remained significant at a False Discovery Rate of 20%.

The quantitative analysis confirmed the qualitative observation. In fact, the β coefficient of the regression between SBP or DBP with rs3918226 is respectively 1.91 (95% CI 0.16-3.66) and 1.40 (95% CI 0.25-2.55) per T allele. The coefficient is the effect size on blood pressure in mm Hg per coded allele based on an additive genetic model. The BP distribution according to rs3918226 genotype is shown in Table S7 (please see http://hyper.ahajournals.org).

Since *rs3918226* maps to the promoter region of *eNOS*, we tested whether it may fall into a regulatory binding site. Using the PATCH algorithm of TRANSFAC database [21] we characterized a putative binding site for transcription-factors of ETS family directly next to *rs3918226*. The ETS family members are present in endothelial cells and participated in activation of the eNOS promoter. [31] Using the TFSEARCH tool [22] we confirmed this finding with a score of 87.3.

We also tested the degree of evolutionary conservation of rs3918226 locus in primates and placental mammals using the conservation track of UCSC genome browser. Figure S6 (please see http://hyper.ahajournals.org) shows that the region in which rs3918226 lies is conserved from placental mammals to primates.

Discussion

Essential hypertension (EH) is a complex clinical condition representing the main risk factor responsible for renal and cardiovascular complications. The HYPERGENES Project investigated undiscovered associations between genetic variants and EH pursuing a two-stage study by recruiting cases and controls from extensively characterized cohorts recruited in different European regions.

We discovered rs3918226 in the promoter region of the eNOS gene (endothelial nitric oxide synthase) to be significantly associated with hypertension (OR, 1.54; 95% CI, 1.37 to 1.73; p-value = $2.58 \cdot 10^{-13}$). The result was confirmed by meta-analyzing *in-silico data* for a total of 21714 subjects (OR, 1.34; 95% CI 1.25-1.44; p-value = $1.032 \cdot 10^{-14}$). We observed heterogeneity in the findings of meta-analysis (p=0.005 for Q-test of heterogeneity) that could be due to both different sample sizes and recruitment criteria not directly comparable between HYPERGENES and the i*n-silico* replication samples (Figure 2).

The quantitative effect of rs3918226 was also estimated in continuous BP phenotypes, resulting in a β coefficient of 1.91 for SBP and 1.40 for DBP, despite the low p-values of the regression probably due to the low sample size. This finding reinforces the observation on the qualitative phenotype.

We identified a potential transcription-factor binding site for the ETS-family domain directly next to rs3918226. The members of ETS family, as ETS-1 and ELF-1, are essential factors for the activation of eNOS promoter.[31] This suggests that, by affecting transcription factor-binding affinity, *rs3918226* might modulate the transcription of *eNOS gene*.

It is also worth noting that the region in which *rs3918226* lies is conserved from placental mammals to primates.

We propose rs3918226 as a novel susceptibility SNP since among the GWASs so far published this is the first that points to eNOS: the novelty of the rs39118226 finding is that the association between eNOS and Hypertension has been found in Caucasians using a GWAS approach.

The use of the Illumina 1M array and Human CVD BeadArray was crucial in detecting the association since *rs3918226* is not present on other commercial arrays.[32] Besides being poorly covered by other genotyping platforms, the region has a relatively high recombination rate towards the coding region (Figure 1). This has resulted in low linkage disequilibrium (LD) with markers present on older platforms (e.g. rsq-hat < 0.2 for Affy500K platform). These facts largely limited the potential to replicate our finding using data from other GWAS samples, almost all of which relied on older platforms.

Indeed eNos has been found inconsistently associated to hypertension with several underpowered candidate gene studies, many of which only focused on a few variants with relatively small numbers of cases and controls, compared to the large sample sizes of GWAS. Positive studies were substantially on Asian cohorts [33,34,35], whereas the majority were negative in Caucasians, as summarised in a recent meta-analysis.[36] The polymorphisms studied in our Caucasian sample, G894T (rs1799983) and T-786C (rs2070744) did not reach genome-wide significant association with hypertension. If looked with candidate gene threshold, the p-value and the sample size of the present study by far outnumber all the other published so far. rs1799983 was associated with EH with a p value = 2.63×10^{-3} (OR =1.038) and rs2070744 with a p value = 6.42×10^{-4} (OR = 1.04), as shown in Table S8 (please see http://hyper.ahajournals.org). To summarize, the ORs are clinically irrelevant. We would like to underline the low LD between rs3918226 and rs1799983 (R^2=0.16) and rs2070744 (R^2=0.17) suggesting that these two SNPs are independent from rs3918226 and do not have any additional effect on the phenotype.

There is considerable biological evidence linking eNOS with hypertension and hypertension-associated cardiovascular target organ damage. [37] eNOS, which catalyses the synthesis of nitric oxide (NO) by vascular endothelium, is responsible for the vasodilator tone that is fundamental for the regulation of blood pressure. Furthermore, eNOS is a critical mediator of cardiovascular homeostasis through regulation of blood vessels diameter and of the maintenance of an anti-proliferative and anti-apoptotic environment.

As NO is highly active, it cannot be stored inside producing cells. Indeed, eNOS signalling capacity must be controlled, at least in part, by regulating its targeting from Golgi apparatus to plasma membrane, by its compartmentalization within the plasma membrane and by its later internalization from the plasma membrane to the cytoplasm. eNOS is a dually acylated peripheral membrane protein that is targeted to endothelial plasmalemmal caveolae through an interaction with the caveolae structural protein, Caveolin-1 (*Cav1*). [26,27] Cav1 inhibition of eNOS is lessened by Calmodulin (*Calm*) causing dissociation of eNOS from Caveolin. This regulatory mechanism is further altered by Heat Shock Protein-90 (HSP90) [27] which binds to eNOS and facilitates displacement of Cav1 by Calm. Moreover, eNOS directly interacts with actin cytoskeleton. [29] Recently, Kondrikov added that beta-actin is associated with eNOS oxygenase domain increasing eNOS activity and NO production.[30] To explore such pathway we tested the interaction between the discovered eNOS SNP and its most plausible interactive partners. We observed nominally significant interactions between *rs3918226* and *rs13447427* in actin beta (*ACTB)*, *rs7503750* in actin gamma 1 (*ACTG1)* and *rs4922796* and *rs17309979* in Heat Shock Protein-90 alpha 2 (*HSP90AA2)* gene.

In conclusion, with a stringent case-control design and a population based study, we identified a novel hypertension susceptibility locus in the promoter region of *eNOS* with a relatively high effect size. Our finding could provide new insights into the mechanism of vascular regulation and could help in better understanding the genetics of EH. Furthermore, we believe that this indication can be useful to guide fine-mapping or sequencing efforts to single out causal variants.

Perspectives

Further investigations and high-throughput sequencing of region of interest will help to identify the real causal variant and to clarify the functional role of eNOS in essential hypertension.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The complete list is reported in supplemental material.

Sources of Funding This work was supported by the HYPERGENES project (European Network for Genetic-Epidemiological Studies: building a method to dissect complex genetic traits, using essential hypertension as a disease model), grant HEALTH-2007-201550, funded by EU within the FP7.

Disclosures Toby Johnson was supported by the Wellcome Trust (grant number 093078/Z/10/Z). John Chalmers has received research grants from Servier International and from the NHMRC (Australia), administered through the University of Sydney, for the PROGRESS and ADVANCE Trials and for the ADVANCE-Post-trial follow-up study. He has also received honoraria for speaking about these studies at scientific meetings.

Mark Caulfield was supported by British Heart Foundation grant for developing CVD BeadArray and KASPAr assay.

References

1. Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK, He J. Global burden of hypertension: analysis of worldwide data. Lancet. 2005; 365:217–223. [PubMed: 15652604]
2. Lawes CM, Vander Hoorn S, Rodgers A, International Society of Hypertension. Global burden of blood-pressure-related disease, 2001. Lancet. 2008; 371:1513–1518. [PubMed: 18456100]
3. Kunes J, Zicha J. The interaction of genetic and environmental factors in the etiology of hypertension. Physiol Res. 2009; 58:S33–41. [PubMed: 20131935]
4. Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature. 2007; 447:661–678. [PubMed: 17554300]
5. Newton-Cheh C, Johnson T, Gateva V, Tobin MD, Bochud M, Coin L, Najjar SS, Zhao JH, Heath SC, Eyheramendy S, Papadakis K, Voight BF, Scott LJ, Zhang F, Farrall M, Tanaka T, Wallace C, Chambers JC, Khaw KT, Nilsson P, van der Harst P, Polidoro S, Grobbee DE, Onland-Moret NC, Bots ML, Wain LV, Elliott KS, Teumer A, Luan J, Lucas G, Kuusisto J, Burton PR, Hadley D, McArdle WL, Wellcome Trust Case Control Consortium. Brown M, Dominiczak A, Newhouse SJ, Samani NJ, Webster J, Zeggini E, Beckmann JS, Bergmann S, Lim N, Song K, Vollenweider P, Waeber G, Waterworth DM, Yuan X, Groop L, Orho-Melander M, Allione A, Di Gregorio A, Guarrera S, Panico S, Ricceri F, Romanazzi V, Sacerdote C, Vineis P, Barroso I, Sandhu MS, Luben RN, Crawford GJ, Jousilahti P, Perola M, Boehnke M, Bonnycastle LL, Collins FS, Jackson AU, Mohlke KL, Stringham HM, Valle TT, Willer CJ, Bergman RN, Morken MA, Döring A, Gieger C, Illig T, Meitinger T, Org E, Pfeufer A, Wichmann HE, Kathiresan S, Marrugat J, O'Donnell CJ, Schwartz SM, Siscovick DS, Subirana I, Freimer NB, Hartikainen AL, McCarthy MI, O'Reilly PF, Peltonen L, Pouta A, de Jong PE, Snieder H, van Gilst WH, Clarke R, Goel A, Hamsten A, Peden JF, Seedorf U, Syvänen AC, Tognoni G, Lakatta EG, Sanna S, Scheet P, Schlessinger D, Scuteri A, Dörr M, Ernst F, Felix SB, Homuth G, Lorbeer R, Reffelmann T, Rettig R, Völker U, Galan P, Gut IG, Hercberg S, Lathrop GM, Zelenika D, Deloukas P, Soranzo N, Williams FM, Zhai G, Salomaa V, Laakso M, Elosua R, Forouhi NG, Völzke H, Uiterwaal CS, van der Schouw YT, Numans ME, Matullo G, Navis G, Berglund G, Bingham SA, Kooner JS, Connell JM, Bandinelli S, Ferrucci L, Watkins H, Spector TD, Tuomilehto J, Altshuler D, Strachan DP, Laan M, Meneton P, Wareham NJ, Uda M, Jarvelin MR, Mooser V, Melander O, Loos RJ, Elliott P, Abecasis GR, Caulfield M, Munroe PB. Genome-wide association study identifies eight loci associated with blood pressure. Nat Genet. 2009; 41:666–676. [PubMed: 19430483]
6. Levy D, Ehret GB, Rice K, Verwoert GC, Launer LJ, Dehghan A, Glazer NL, Morrison AC, Johnson AD, Aspelund T, Aulchenko Y, Lumley T, Köttgen A, Vasan RS, Rivadeneira F, Eiriksdottir G, Guo X, Arking DE, Mitchell GF, Mattace-Raso FU, Smith AV, Taylor K, Scharpf RB, Hwang SJ, Sijbrands EJ, Bis J, Harris TB, Ganesh SK, O'Donnell CJ, Hofman A, Rotter JI, Coresh J, Benjamin EJ, Uitterlinden AG, Heiss G, Fox CS, Witteman JC, Boerwinkle E, Wang TJ, Gudnason V, Larson MG, Chakravarti A, Psaty BM, van Duijn CM. Genome-wide association study of blood pressure and hypertension. Nat Genet. 2009; 41:677–687. [PubMed: 19430479]
7. Munroe PB, Johnson T, Caulfield M. The Genetic Architecture of Blood Pressure Variation. Current Cardiovascular Risk Reports. 2009; 3:418–425.

8. Padmanabhan S, Melander O, Johnson T, Di Blasio AM, Lee WK, Gentilini D, Hastie CE, Menni C, Monti MC, Delles C, Laing S, Corso B, Navis G, Kwakernaak AJ, van der Harst P, Bochud M, Maillard M, Burnier M, Hedner T, Kjeldsen S, Wahlstrand B, Sjögren M, Fava C, Montagnana M, Danese E, Torffvit O, Hedblad B, Snieder H, Connell JM, Brown M, Samani NJ, Farrall M, Cesana G, Mancia G, Signorini S, Grassi G, Eyheramendy S, Wichmann HE, Laan M, Strachan DP, Sever P, Shields DC, Stanton A, Vollenweider P, Teumer A, Völzke H, Rettig R, Newton-Cheh C, Arora P, Zhang F, Soranzo N, Spector TD, Lucas G, Kathiresan S, Siscovick DS, Luan J, Loos RJ, Wareham NJ, Penninx BW, Nolte IM, McBride M, Miller WH, Nicklin SA, Baker AH, Graham D, McDonald RA, Pell JP, Sattar N, Welsh P, Global BPgen Consortium. Munroe P, Caulfield MJ, Zanchetti A, Dominiczak AF. Genome-Wide Association Study of Blood Pressure Extremes Identifies Variant near UMOD Associated with Hypertension. PLoS Genet. 2010; 6:e1001177. [PubMed: 21082022]

9. Ehret GB. Genome-wide association studies: contribution of genomics to understanding blood pressure and essential hypertension. Curr Hypertens Rep. 2010; 12:17–25. [PubMed: 20425154]

10. Hong KW, Jin HS, Lim JE, Kim S, Go MJ, Oh B. Recapitulation of two genome-wide association studies on blood pressure and essential hypertension in the Korean population. J Hum Genet. 2010; 55:336–341. [PubMed: 20414254]

11. Fox ER, Young JH, Li Y, Dreisbach AW, Keating BJ, Musani SK, Liu K, Morrison AC, Ganesh S, Kutlar A, Ramachandran VS, Polak JF, Fabsitz RR, Dries DL, Farlow DN, Redline S, Adeyemo A, Hirschorn JN, Sun YV, Wyatt SB, Penman AD, Palmas W, Rotter JI, Townsend RR, Doumatey AP, Tayo BO, Mosley TH Jr, Lyon HN, Kang SJ, Rotimi CN, Cooper RS, Franceschini N, Curb JD, Martin LW, Eaton CB, Kardia SL, Taylor HA, Caulfield MJ, Ehret GB, Johnson T, International Consortium for Blood Pressure Genome-wide Association Studies (ICBP-GWAS); Chakravarti A, Zhu X, Levy D, Munroe PB, Rice KM, Bochud M, Johnson AD, Chasman DI, Smith AV, Tobin MD, Verwoert GC, Hwang SJ, Pihur V, Vollenweider P, O'Reilly PF, Amin N, Bragg-Gresham JL, Teumer A, Glazer NL, Launer L, Zhao JH, Aulchenko Y, Heath S, Sõber S, Parsa A, Luan J, Arora P, Dehghan A, Zhang F, Lucas G, Hicks AA, Jackson AU, Peden JF, Tanaka T, Wild SH, Rudan I, Igl W, Milaneschi Y, Parker AN, Fava C, Chambers JC, Kumari M, Go MJ, van der Harst P, Kao WH, Sjögren M, Vinay DG, Alexander M, Tabara Y, Shaw-Hawkins S, Whincup PH, Liu Y, Shi G, Kuusisto J, Seielstad M, Sim X, Nguyen KD, Lehtimäki T, Matullo G, Wu Y, Gaunt TR, Onland-Moret NC, Cooper MN, Platou CG, Org E, Hardy R, Dahgam S, Palmen J, Vitart V, Braund PS, Kuznetsova T, Uiterwaal CS, Campbell H, Ludwig B, Tomaszewski M, Tzoulaki I, Palmer ND, CARDIoGRAM consortium; CKDGen consortium; KidneyGen consortium; EchoGen consortium; CHARGE-HF consortium. Aspelund T, Garcia M, Chang YP, O'Connell JR, Steinle NI, Grobbee DE, Arking DE, Hernandez D, Najjar S, McArdle WL, Hadley D, Brown MJ, Connell JM, Hingorani AD, Day IN, Lawlor DA, Beilby JP, Lawrence RW, Clarke R, Collins R, Hopewell JC, Ongen H, Bis JC, Kähönen M, Viikari J, Adair LS, Lee NR, Chen MH, Olden M, Pattaro C, Bolton JA Hoffman, Köttgen A, Bergmann S, Mooser V, Chaturvedi N, Frayling TM, Islam M, Jafar TH, Erdmann J, Kulkarni SR, Bornstein SR, Grässler J, Groop L, Voight BF, Kettunen J, Howard P, Taylor A, Guarrera S, Ricceri F, Emilsson V, Plump A, Barroso I, Khaw KT, Weder AB, Hunt SC, Bergman RN, Collins FS, Bonnycastle LL, Scott LJ, Stringham HM, Peltonen L, Perola M, Vartiainen E, Brand SM, Staessen JA, Wang TJ, Burton PR, Artigas M Soler, Dong Y, Snieder H, Wang X, Zhu H, Lohman KK, Rudock ME, Heckbert SR, Smith NL, Wiggins KL, Shriner D, Veldre G, Viigimaa M, Kinra S, Prabhakaran D, Tripathy V, Langefeld CD, Rosengren A, Thelle DS, Corsi AM, Singleton A, Forrester T, Hilton G, McKenzie CA, Salako T, Iwai N, Kita Y, Ogihara T, Ohkubo T, Okamura T, Ueshima H, Umemura S, Eyheramendy S, Meitinger T, Wichmann HE, Cho YS, Kim HL, Lee JY, Scott J, Sehmi JS, Zhang W, Hedblad B, Nilsson P, Smith GD, Wong A, Narisu N, Stanáková A, Raffel LJ, Yao J, Kathiresan S, O'Donnell C, Schwartz SM, Ikram MA, Longstreth WT Jr, Seshadri S, Shrine NR, Wain LV, Morken MA, Swift AJ, Laitinen J, Prokopenko I, Zitting P, Cooper JA, Humphries SE, Danesh J, Rasheed A, Goel A, Hamsten A, Watkins H, Bakker SJ, van Gilst WH, Janipalli C, Mani KR, Yajnik CS, Hofman A, Mattace-Raso FU, Oostra BA, Demirkan A, Isaacs A, Rivadeneira F, Lakatta EG, Orru M, Scuteri A, Ala-Korpela M, Kangas AJ, Lyytikäinen LP, Soininen P, Tukiainen T, Würz P, Ong RT, Dörr M, Kroemer HK, Völker U, Völzke H, Galan P, Hercberg S, Lathrop M, Zelenika D, Deloukas P, Mangino M, Spector TD, Zhai G, Meschia JF, Nalls MA, Sharma P, Terzic J, Kumar MJ, Denniff M, Zukowska-Szczechowska E, Wagenknecht LE, Fowkes FG, Charchar FJ, Schwarz PE, Hayward C, Guo X, Bots ML, Brand E, Samani N,

Polasek O, Talmud PJ, Nyberg F, Kuh D, Laan M, Hveem K, Palmer LJ, van der Schouw YT, Casas JP, Mohlke KL, Vineis P, Raitakari O, Wong TY, Tai ES, Laakso M, Rao DC, Harris TB, Morris RW, Dominiczak AF, Kivimaki M, Marmot MG, Miki T, Saleheen D, Chandak GR, Coresh J, Navis G, Salomaa V, Han BG, Kooner JS, Melander O, Ridker PM, Bandinelli S, Gyllensten UB, Wright AF, Wilson JF, Ferrucci L, Farrall M, Tuomilehto J, Pramstaller PP, Elosua R, Soranzo N, Sijbrands EJ, Altshuler D, Loos RJ, Shuldiner AR, Gieger C, Meneton P, Uitterlinden AG, Wareham NJ, Gudnason V, Rettig R, Uda M, Strachan DP, Witteman JC, Hartikainen AL, Beckmann JS, Boerwinkle E, Boehnke M, Larson MG, Järvelin MR, Psaty BM, Abecasis GR, Elliott P, van Duijn CM, Newton-Cheh C. Association of genetic variation with systolic and diastolic blood pressure among African Americans: the Candidate Gene Association Resource study. Hum Mol Genet. 2011; 20:2273–2284. [PubMed: 21378095]

12. Lettre G, Palmer CD, Young T, Ejebe KG, Allayee H. Genome-wide association study of coronary heart disease and its risk factors in 8,090 African Americans: the NHLBI CARe Project. PLoS Genet. 2011; 7:e1001300. [PubMed: 21347282]
13. International Consortium for Blood Pressure Genome-Wide Association Studies; Ehret GB, Munroe PB, Rice KM, Bochud M, Johnson AD, Chasman DI, Smith AV, Tobin MD, Verwoert GC, Hwang SJ, Pihur V, Vollenweider P, O'Reilly PF, Amin N, Bragg-Gresham JL, Teumer A, Glazer NL, Launer L, Zhao JH, Aulchenko Y, Heath S, Sõber S, Parsa A, Luan J, Arora P, Dehghan A, Zhang F, Lucas G, Hicks AA, Jackson AU, Peden JF, Tanaka T, Wild SH, Rudan I, Igl W, Milaneschi Y, Parker AN, Fava C, Chambers JC, Fox ER, Kumari M, Go MJ, van der Harst P, Kao WH, Sjögren M, Vinay DG, Alexander M, Tabara Y, Shaw-Hawkins S, Whincup PH, Liu Y, Shi G, Kuusisto J, Tayo B, Seielstad M, Sim X, Nguyen KD, Lehtimäki T, Matullo G, Wu Y, Gaunt TR, Onland-Moret NC, Cooper MN, Platou CG, Org E, Hardy R, Dahgam S, Palmen J, Vitart V, Braund PS, Kuznetsova T, Uiterwaal CS, Adeyemo A, Palmas W, Campbell H, Ludwig B, Tomaszewski M, Tzoulaki I, Palmer ND, CARDIoGRAM consortium; CKDGen Consortium; KidneyGen Consortium; EchoGen consortium; CHARGE-HF consortium. Aspelund T, Garcia M, Chang YP, O'Connell JR, Steinle NI, Grobbee DE, Arking DE, Kardia SL, Morrison AC, Hernandez D, Najjar S, McArdle WL, Hadley D, Brown MJ, Connell JM, Hingorani AD, Day IN, Lawlor DA, Beilby JP, Lawrence RW, Clarke R, Hopewell JC, Ongen H, Dreisbach AW, Li Y, Young JH, Bis JC, Kähönen M, Viikari J, Adair LS, Lee NR, Chen MH, Olden M, Pattaro C, Bolton JA, Köttgen A, Bergmann S, Mooser V, Chaturvedi N, Frayling TM, Islam M, Jafar TH, Erdmann J, Kulkarni SR, Bornstein SR, Grässler J, Groop L, Voight BF, Kettunen J, Howard P, Taylor A, Guarrera S, Ricceri F, Emilsson V, Plump A, Barroso I, Khaw KT, Weder AB, Hunt SC, Sun YV, Bergman RN, Collins FS, Bonnycastle LL, Scott LJ, Stringham HM, Peltonen L, Perola M, Vartiainen E, Brand SM, Staessen JA, Wang TJ, Burton PR, Artigas MS, Dong Y, Snieder H, Wang X, Zhu H, Lohman KK, Rudock ME, Heckbert SR, Smith NL, Wiggins KL, Doumatey A, Shriner D, Veldre G, Viigimaa M, Kinra S, Prabhakaran D, Tripathy V, Langefeld CD, Rosengren A, Thelle DS, Corsi AM, Singleton A, Forrester T, Hilton G, McKenzie CA, Salako T, Iwai N, Kita Y, Ogihara T, Ohkubo T, Okamura T, Ueshima H, Umemura S, Eyheramendy S, Meitinger T, Wichmann HE, Cho YS, Kim HL, Lee JY, Scott J, Sehmi JS, Zhang W, Hedblad B, Nilsson P, Smith GD, Wong A, Narisu N, Stancáková A, Raffel LJ, Yao J, Kathiresan S, O'Donnell CJ, Schwartz SM, Ikram MA, Longstreth WT Jr, Mosley TH, Seshadri S, Shrine NR, Wain LV, Morken MA, Swift AJ, Laitinen J, Prokopenko I, Zitting P, Cooper JA, Humphries SE, Danesh J, Rasheed A, Goel A, Hamsten A, Watkins H, Bakker SJ, van Gilst WH, Janipalli CS, Mani KR, Yajnik CS, Hofman A, Mattace-Raso FU, Oostra BA, Demirkan A, Isaacs A, Rivadeneira F, Lakatta EG, Orru M, Scuteri A, Ala-Korpela M, Kangas AJ, Lyytikäinen LP, Soininen P, Tukiainen T, Würtz P, Ong RT, Dörr M, Kroemer HK, Völker U, Völzke H, Galan P, Hercberg S, Lathrop M, Zelenika D, Deloukas P, Mangino M, Spector TD, Zhai G, Meschia JF, Nalls MA, Sharma P, Terzic J, Kumar MV, Denniff M, Zukowska-Szczechowska E, Wagenknecht LE, Fowkes FG, Charchar FJ, Schwarz PE, Hayward C, Guo X, Rotimi C, Bots ML, Brand E, Samani NJ, Polasek O, Talmud PJ, Nyberg F, Kuh D, Laan M, Hveem K, Palmer LJ, van der Schouw YT, Casas JP, Mohlke KL, Vineis P, Raitakari O, Ganesh SK, Wong TY, Tai ES, Cooper RS, Laakso M, Rao DC, Harris TB, Morris RW, Dominiczak AF, Kivimaki M, Marmot MG, Miki T, Saleheen D, Chandak GR, Coresh J, Navis G, Salomaa V, Han BG, Zhu X, Kooner JS, Melander O, Ridker PM, Bandinelli S, Gyllensten UB, Wright AF, Wilson JF, Ferrucci L, Farrall M, Tuomilehto J, Pramstaller PP, Elosua R, Soranzo N, Sijbrands EJ, Altshuler D, Loos RJ, Shuldiner AR, Gieger C, Meneton P, Uitterlinden AG, Wareham NJ, Gudnason V, Rotter JI,

Rettig R, Uda M, Strachan DP, Witteman JC, Hartikainen AL, Beckmann JS, Boerwinkle E, Vasan RS, Boehnke M, Larson MG, Järvelin MR, Psaty BM, Abecasis GR, Chakravarti A, Elliott P, van Duijn CM, Newton-Cheh C, Levy D, Caulfield MJ, Johnson T, Tang H, Knowles J, Hlatky M, Fortmann S, Assimes TL, Quertermous T, Go A, Iribarren C, Absher D, Risch N, Myers R, Sidney S, Ziegler A, Schillert A, Bickel C, Sinning C, Rupprecht HJ, Lackner K, Wild P, Schnabel R, Blankenberg S, Zeller T, Münzel T, Perret C, Cambien F, Tiret L, Nicaud V, Proust C, Dehghan A, Hofman A, Uitterlinden A, van Duijn C, Levy D, Whitteman J, Cupples LA, Demissie-Banjaw S, Ramachandran V, Smith A, Gudnason V, Boerwinkle E, Folsom A, Morrison A, Psaty BM, Chen IY, Rotter JI, Bis J, Volcik K, Rice K, Taylor KD, Marciante K, Smith N, Glazer N, Heckbert S, Harris T, Lumley T, Kong A, Thorleifsson G, Thorgeirsson G, Holm H, Gulcher JR, Stefansson K, Andersen K, Gretarsdottir S, Thorsteinsdottir U, Preuss M, Schreiber S, Meitinger T, König IR, Lieb W, Hengstenberg C, Schunkert H, Erdmann J, Fischer M, Grosshennig A, Medack A, Stark K, Linsel-Nitschke P, Bruse P, Aherrahrou Z, Peters A, Loley C, Willenborg C, Nahrstedt J, Freyer J, Gulde S, Doering A, Meisinger C, Wichmann HE, Klopp N, Illig T, Meinitzer A, Tomaschitz A, Halperin E, Dobnig H, Scharnagl H, Kleber M, Laaksonen R, Pilz S, Grammer TB, Stojakovic T, Renner W, März W, Böhm BO, Winkelmann BR, Winkler K, Hoffmann MM, O'Donnell CJ, Voight BF, Altshuler D, Siscovick DS, Musunuru K, Peltonen L, Barbalic M, Melander O, Elosua R, Kathiresan S, Schwartz SM, Salomaa V, Guiducci C, Burtt N, Gabriel SB, Stewart AF, Wells GA, Chen L, Jarinova O, Roberts R, McPherson R, Dandona S, Pichard AD, Rader DJ, Devaney J, Lindsay JM, Kent KM, Qu L, Satler L, Burnett MS, Li M, Reilly MP, Wilensky R, Waksman R, Epstein S, Matthai W, Knouff CW, Waterworth DM, Hakonarson HH, Walker MC, Mooser V, Hall AS, Balmforth AJ, Wright BJ, Nelson C, Thompson JR, Samani NJ, Braund PS, Ball SG, Smith NL, Felix JF, Morrison AC, Demissie S, Glazer NL, Loehr LR, Cupples LA, Dehghan A, Lumley T, Rosamond WD, Lieb W, Rivadeneira F, Bis JC, Folsom AR, Benjamin E, Aulchenko YS, Haritunians T, Couper D, Murabito J, Wang YA, Stricker BH, Gottdiener JS, Chang PP, Wang TJ, Rice KM, Hofman A, Heckbert SR, Fox ER, O'Donnell CJ, Uitterlinden AG, Rotter JI, Willerson JT, Levy D, van Duijn CM, Psaty BM, Witteman JC, Boerwinkle E, Vasan RS, Köttgen A, Pattaro C, Böger CA, Fuchsberger C, Olden M, Glazer NL, Parsa A, Gao X, Yang Q, Smith AV, O'Connell JR, Li M, Schmidt H, Tanaka T, Isaacs A, Ketkar S, Hwang SJ, Johnson AD, Dehghan A, Teumer A, Paré G, Atkinson EJ, Zeller T, Lohman K, Cornelis MC, Probst-Hensch NM, Kronenberg F, Tönjes A, Hayward C, Aspelund T, Eiriksdottir G, Launer LJ, Harris TB, Rampersaud E, Mitchell BD, Arking DE, Boerwinkle E, Struchalin M, Cavalieri M, Singleton A, Giallauria F, Metter J, de Boer J, Haritunians T, Lumley T, Siscovick D, Psaty BM, Zillikens MC, Oostra BA, Feitosa M, Province M, de Andrade M, Turner ST, Schillert A, Ziegler A, Wild PS, Schnabel RB, Wilde S, Munzel TF, Leak TS, Illig T, Klopp N, Meisinger C, Wichmann HE, Koenig W, Zgaga L, Zemunik T, Kolcic I, Minelli C, Hu FB, Johansson A, Igl W, Zaboli G, Wild SH, Wright AF, Campbell H, Ellinghaus D, Schreiber S, Aulchenko YS, Felix JF, Rivadeneira F, Uitterlinden AG, Hofman A, Imboden M, Nitsch D, Brandstätter A, Kollerits B, Kedenko L, Mägi R, Stumvoll M, Kovacs P, Boban M, Campbell S, Endlich K, Völzke H, Kroemer HK, Nauck M, Völker U, Polasek O, Vitart V, Badola S, Parker AN, Ridker PM, Kardia SL, Blankenberg S, Liu Y, Curhan GC, Franke A, Rochat T, Paulweber B, Prokopenko I, Wang W, Gudnason V, Shuldiner AR, Coresh J, Schmidt R, Ferrucci L, Shlipak MG, van Duijn CM, Borecki I, Krämer BK, Rudan I, Gyllensten U, Wilson JF, Witteman JC, Pramstaller PP, Rettig R, Hastie N, Chasman DI, Kao WH, Heid IM, Fox CS, Vasan RS, Glazer NL, Felix JF, Lieb W, Wild PS, Felix SB, Watzinger N, Larson MG, Smith NL, Dehghan A, Grosshennig A, Schillert A, Teumer A, Schmidt R, Kathiresan S, Lumley T, Aulchenko YS, König IR, Zeller T, Homuth G, Struchalin M, Aragam J, Bis JC, Rivadeneira F, Erdmann J, Schnabel RB, Dörr M, Zweiker R, Lind L, Rodeheffer RJ, Greiser KH, Levy D, Haritunians T, Deckers JW, Stritzke J, Lackner KJ, Völker U, Ingelsson E, Kullo I, Haerting J, O'Donnell CJ, Heckbert SR, Stricker BH, Ziegler A, Reffelmann T, Redfield MM, Werdan K, Mitchell GF, Rice K, Arnett DK, Hofman A, Gottdiener JS, Uitterlinden AG, Meitinger T, Blettner M, Friedrich N, Wang TJ, Psaty BM, van Duijn CM, Wichmann HE, Munzel TF, Kroemer HK, Benjamin EJ, Rotter JI, Witteman JC, Schunkert H, Schmidt H, Völzke H, Blankenberg S, Chambers JC, Zhang W, Lord GM, van der Harst P, Lawlor DA, Sehmi JS, Gale DP, Wass MN, Ahmadi KR, Bakker SJ, Beckmann J, Bilo HJ, Bochud M, Brown MJ, Caulfield MJ, Connell JM, Cook HT, Cotlarciuc I, Davey Smith G, de Silva R, Deng G, Devuyst O, Dikkeschei LD, Dimkovic N, Dockrell M, Dominiczak A, Ebrahim S, Eggermann T, Farrall M, Ferrucci L, Floege J, Forouhi NG,

Gansevoort RT, Han X, Hedblad B, van der Heide JJ Homan, Hepkema BG, Hernandez-Fuentes M, Hypponen E, Johnson T, de Jong PE, Kleefstra N, Lagou V, Lapsley M, Li Y, Loos RJ, Luan J, Luttropp K, Maréchal C, Melander O, Munroe PB, Nordfors L, Parsa A, Peltonen L, Penninx BW, Perucha E, Pouta A, Prokopenko I, Roderick PJ, Ruokonen A, Samani NJ, Sanna S, Schalling M, Schlessinger D, Schlieper G, Seelen MA, Shuldiner AR, Sjögren M, Smit JH, Snieder H, Soranzo N, Spector TD, Stenvinkel P, Sternberg MJ, Swaminathan R, Tanaka T, Ubink-Veltmaat LJ, Uda M, Vollenweider P, Wallace C, Waterworth D, Zerres K, Waeber G, Wareham NJ, Maxwell PH, McCarthy MI, Jarvelin MR, Mooser V, Abecasis GR, Lightstone L, Scott J, Navis G, Elliott P, Kooner JS. Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. Nature. 2011; 478:103–109. [PubMed: 21909115]

14. McCarthy MI, Abecasis GR, Cardon LR, Goldstein DB, Little J, Ioannidis JP, Hirschhorn JN. Genome-Wide association studies for complex traits: consensus, uncertainty and challenges. Nature Review Genetics. 2008; 9:356–369. [PubMed: 18398418]
15. Padmanabhan S, Melander O, Hastie C, Menni C, Delles C, Connell JM, Dominiczak AF. Hypertension and genome-wide association studies: combining high fidelity phenotyping and hypercontrols. J Hypertens. 2008; 26:1275–1281. [PubMed: 18550997]
16. Li Y, Willer CJ, Ding J, Scheet P, Abecasis GR. MaCH: using sequence and genotype data to estimate haplotypes and unobserved genotypes. Genet Epidemiol. 2010; 34:816–834. [PubMed: 21058334]
17. Anderson CA, Pettersson FH, Clarke GM, Cardon LR, Morris AP, Zondervan KT. Data quality control in genetic case-control association studies. Nat Protoc. 2010; 5:1564–1573. [PubMed: 21085122]
18. Clarke GM, Anderson CA, Pettersson FH, Cardon LR, Morris AP, Zondervan KT. Basic statistical analysis in genetic case-control studies. Nat Protoc. 2011; 6:121–133. [PubMed: 21293453]
19. Willer CJ, Li Y, Abecasis GR. METAL: fast and efficient meta-analysis of genomewide association scans. Bioinformatics. 2010; 26:2190–2191. [PubMed: 20616382]
20. Heinemeyer T, Wingender E, Reuter I, Hermjakob H, Kel AE, Kel OV, Ignatieva EV, Ananko EA, Podkolodnaya OA, Kolpakov FA, Podkolodny NL, Kolchanov NA. Databases on Transcriptional Regulation: TRANSFAC, TRRD, and COMPEL. Nucleic Acids Res. 1998; 26:364–370.
21. Matys V, Fricke E, Geffers R, Gössling E, Haubrock M, Hehl R, Hornischer K, Karas D, Kel AE, Kel-Margoulis OV, Kloos DU, Land S, Lewicki-Potapov B, Michael H, Münch R, Reuter I, Rotert S, Saxel H, Scheer M, Thiele S, Wingender E. TRANSFAC: transcriptional regulation, from patterns to profiles. Nucleic Acids Res. 2003; 31:374–378. [PubMed: 12520026]
22. http://www.cbrc.jp/research/db/TFSEARCH.html
23. Novembre J, Johnson T, Bryc K, Kutalik Z, Boyko AR, Auton A, Indap A, King KS, Bergmann S, Nelson MR, Stephens M, Bustamante CD. Genes mirror geography within Europe. Nature. 2008; 456:98–101. [PubMed: 18758442]
24. Marsden PA, Heng HH, Scherer SW, Stewart RJ, Hall AV, Shi XM, Tsui LC, Schappert KT. Structure and chromosomal localization of the human constitutive endothelial nitric oxide synthase gene. J Biol Chem. 1993; 268:17478–17488. [PubMed: 7688726]
25. Zhang R, Min W, Sessa WC. Functional analysis of the human endothelial nitric oxide synthase promoter. Sp1 and GATA factors are necessary for basal transcription in endothelial cells. J Biol Chem. 1995; 270:15320–15326. [PubMed: 7541039]
26. Oess S, Icking A, Fulton D, Govers R, Müller-Esterl W. Subcellular targeting and trafficking of nitric oxide synthases. Biochem J. 2006; 396:401–409. [PubMed: 16722822]
27. Fleming I. Molecular mechanisms underlying the activation of eNOS. *Pflugers* Arch. 2010; 459(6): 793–806. [PubMed: 20012875]
28. Moncada S, Higgs A. The L-arginine-nitric oxide pathway. N Engl J Med. 1993; 329:2002–2012. [PubMed: 7504210]
29. Su Y, Edwards-Bennett S, Bubb MR, Block ER. Regulation of endothelial nitric oxide synthase by the actin cytoskeleton. Am J Physiol Cell Physiol. 2003; 284:C1542–C1549. [PubMed: 12734108]
30. Kondrikov D, Fonseca FV, Elms S, Fulton D, Black SM, Block ER, Su Y. Beta-actin association with endothelial nitric-oxide synthase modulates nitric oxide and superoxide generation from the enzyme. J Biol Chem. 2010; 285:4319–4327. [PubMed: 19946124]

31. Karantzoulis-Fegaras F, Antoniou H, Lai SL, Kulkarni G, D'Abreo C, Wong GK, Miller TL, Chan Y, Atkins J, Wang Y, Marsden PA. Characterization of the human endothelial nitric-oxide synthase promoter. J Biol Chem. 1999; 274:3076–3093. [PubMed: 9915847]

32. Sõber S, Org E, Kepp K, Juhanson P, Eyheramendy S, Gieger C, Lichtner P, Klopp N, Veldre G, Viigimaa M, Döring A, Kooperative Gesundheitsforschung in der Region Augsburg Study; Putku M, Kelgo P, HYPertension in ESTonia Study; Shaw-Hawkins S, Howard P, Onipinla A, Dobson RJ, Newhouse SJ, Brown M, Dominiczak A, Connell J, Samani N, Farrall M, MRC British Genetics of Hypertension Study. Caulfield MJ, Munroe PB, Illig T, Wichmann HE, Meitinger T, Laan M. Targeting 160 candidate genes for blood pressure regulation with a genome-wide genotyping array. PLoS ONE. 2009; 4:1–13.

33. Li J, Cun Y, Tang WR, Wang Y, Li SN, Ouyang HR, Wu YR, Yu HJ, Xiao CJ. Association of eNOS gene polymorphisms with essential hypertension in the Han population in southwestern China. Genet Mol Res. 2011; 10:2202–2212. [PubMed: 21968727]

34. Yan-Yan L. Endothelial Nitric Oxide Synthase G894T Gene Polymorphism and Essential Hypertension in the Chinese Population: a Meta-Analysis Involving 11,248 Subjects. Intern Med. 2011; 50:2099–2106. [PubMed: 21963726]

35. Men C, Tang K, Lin G, Li J, Zhan Y. ENOS-G894T polymorphism is a risk factor for essential hypertension in China. Indian J Biochem Biophys. 2011; 48:154–157. [PubMed: 21793305]

36. Niu W, Qi Y. An updated meta-analysis of endothelial nitric oxide synthase gene: three well-characterized polymorphisms with hypertension. PLoS One. 2011; 6:e24266. [PubMed: 21912683]

37. Huang PL, Huang Z, Mashimo H, Bloch KD, Moskowitz MA, Bevan JA, Fishman MC. Hypertension in mice lacking the gene for endothelial nitric oxide synthase. Nature. 1995; 377:239–242. [PubMed: 7545787]

Europe PMC Funders Author Manuscripts

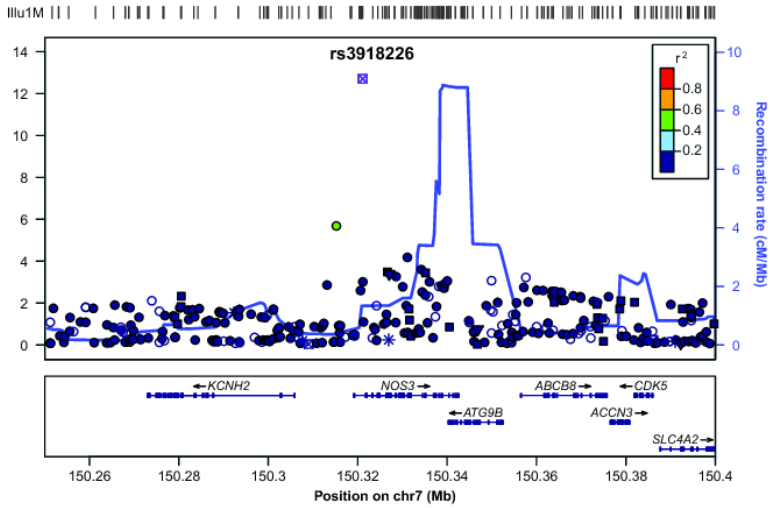

Figure 1. Local Manhattan plot for the NOS3 (endothelial NOS) region

Each circle represents a SNP, its y-coordinate is the −log10 association P value for hypertension, the x-coordinate represents the physical position on the chromosome (on build 36, hg18). When replication data was available the combined P value was used, otherwise the discovery P value. Circles are filled with colours according to the LD (r^2) between the given SNP and the lead SNP (rs3918266, violet square). Blue line indicates the recombination rate. The second best hit with P value 2.46E-6 in discovery stage (named chr7:150,314,954 according to 1000 genome project) was imputed based on the 1000 Genomes haplotypes (release June 2010), its imputation quality was very high (r^2-hat = 0.94). In validation stage the imputation quality was very low (r^2-hat = 0.17).

Europe PMC Funders Author Manuscripts

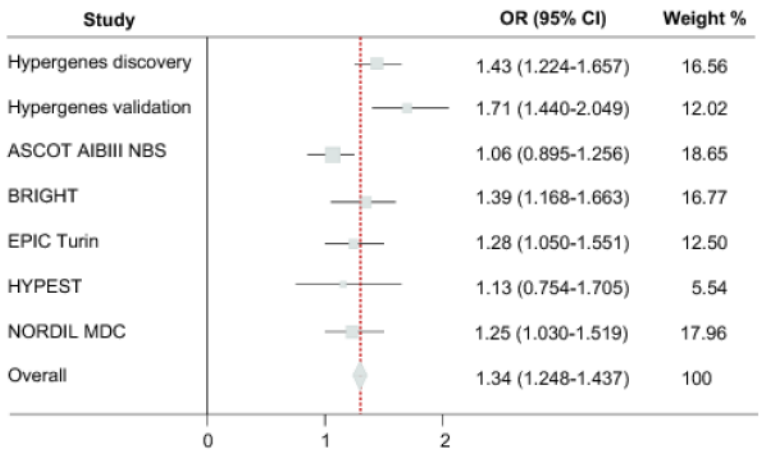

Figure 2. Forest plot of meta-analysis between Hypergenes Discovery, Hypergenes Validation, ASCOT/AIBIII/NBS, BRIGHT, EPIC Turin, HYPEST and NORDIL/MDC studies

The squares and the horizontal lines correspond to the OR and 95% CI of each study, the size of squares is proportional to weights (also shown as percentage), the dotted red line and the diamond represent the overall combined OR and 95% CI.

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

Table 1

Meta-analysis results for the top SNPS in the HYPERGENES study

The table shows association results (OR and p-values) for Discovery and for Validation samples, and for the combined analysis (both inverse variance weighting and Z-score meta-analysis). P values and ORs with the associated 95% CI have been calculated under an additive model using logistic regression adjusted for gender and PCs. To retrieve information about SNPs and their genomic context (the nearest gene) we used the hg18 (NCBI 36) assembly.

Marker Name	Chr	Position	Effect/ other allele	Gene	OR Discovery	P Discovery	OR Validation	P Validation	OR combined	CI combined	Inverse variance weighted P combined	Z-score P combined
rs3918226	7	150321109	T/C	NOS3	1.425	4.81E-06	1.71	2.55E-09	1.538	1.372-1.726	1.98E-13	2.58E-13
rs341408	15	58928982	G/A	RORA	0.786	1.74E-06	0.956	4.29E-01	0.856	0.79-0.92	3.98E-05	2.79E-05
rs4976593	5	167710021	G/A	WWC1	1.27	3.75E-06	1.045	4.60E-01	1.169	1.08-1.26	6.64E-05	5.29E-05
rs631208	16	9307225	G/A	RP11-473I1.1	0.798	8.09E-06	0.951	3.84E-01	0.862	0.80-0.93	8.89E-05	6.36E-05
rs7907270	10	78550949	G/A	KCNMA1	1.27	2.35E-06	0.989	8.53E-01	1.141	1.06-1.23	5.75E-04	4.25E-04
rs10519080	15	58925751	G/A	RORA	1.369	5.79E-06	0.979	7.95E-01	1.187	1.07-1.31	1.09E-03	8.49E-04
rs1406891	6	161107070	G/A	PLG	1.251	3.99E-06	0.949	3.50E-01	1.112	1.03-1.19	3.87E-03	2.97E-03
rs783182	6	161088538	G/A	PLG	0.797	2.95E-06	1.068	2.42E-01	0.902	0.84-0.97	5.31E-03	4.15E-03
rs1084656	6	161101282	C/A	PLG	1.243	6.67E-06	0.936	2.39E-01	1.103	1.03-1.18	7.66E-03	6.35E-03
rs783145	6	161072439	G/A	PLG	0.788	8.53E-07	1.102	8.45E-02	0.909	0.84-0.98	9.27E-03	6.85E-03
rs1247558	6	161110189	G/A	PLG	1.24	8.30E-06	0.932	2.14E-01	1.100	1.02-1.18	9.42E-03	7.93E-03

Europe PMC Funders Author Manuscripts

Table 2

In silico meta-analysis results for rs3918226 (T/C, effect allele/other allele)

Top: association results (Odds Ratios, Standard Errors, Confidence intervals and p-values) for Discovery, Validation and combined analysis of the HYPERGENES samples. Middle: results for ASCOT/AIBIII/NBS, BRIGHT, Epic Turin, HYPEST and NORDIL/MDC studies and combined analysis of replication _in silico_ samples. Bottom: Meta-analysis results for all samples using both the z-score and inverse variance weighted p-value methods.

	Study	Sample Size	OR	SE	95% CI	P-value
Hypergenes Samples	HYPERGENES_DISCOVERY	3596	1.43	0.11	1.224-1.657	4.81E-06
	HYPERGENES_VALIDATION	2610	1.71	0.155	1.440-2.049	2.55E-09
	Combined Analysis HYPERGENES	6206	1.54	0.038	1.372-1.726	2.58E-13
Replication Samples	ASCOT_AIBIII_NBS	4049	1.06	0.092	0.895-1.256	4.97E-01
	BRIGHT	3641	1.39	0.126	1.168-1.663	2.32E-04
	EPIC Turin	2714	1.28	0.126	1.050-1.551	1.44E-02
	HYPEST	1204	1.13	0.236	0.754-1.705	5.45E-01
	NORDIL_MDC	3900	1.25	0.124	1.030-1.519	2.40E-02
	Combined Analysis of Replication Samples	15508	1.23	0.056	1.125-1.344	6.50E-06
	META-ANALYSIS	**Sample Size**	**OR (combined)**	**95% CI (combined)**	**Combined P (Z-score)**	**Combined P (Inverse variance weighted)**
		21714	1.34	1.248-1.437	1.032E-14	6.198E-16