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Abstract—Essential hypertension is a multifactorial 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 2-stage study by recruiting cases and controls from extensively characterized cohorts recruited over many years

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in different European regions. The discovery phase consisted of 1865 cases and 1750 controls genotyped with 1M Illumina array. Best hits were followed up in a validation panel of 1385 cases and 1246 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 NO synthase gene (odds ratio: 1.54 [95% CI: 1.37–1.73]; combined $P=2.58 \cdot 10^{-13}$). A meta-analysis, using other in silico/de novo genotyping data for a total of 21 714 subjects, resulted in an overall odds ratio of 1.34 (95% CI: 1.25–1.44; $P=1.032 \cdot 10^{-14}$). 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 endothelial NO synthase expression. Biological evidence links endothelial NO synthase with hypertension, because 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. (***Hypertension*. 2012;59:00-00.) • Online Data Supplement**

Key Words: genetic epidemiology ■ risk factors ■ genetics association studies ■ NO ■ essential hypertension

Essential hypertension (EH) is a clinical condition affecting a large proportion (25% to 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-analyzing 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 because of 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, because 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 hypernormal controls and adopting a more stringent case definition.[14,15]

The HYPERGENES Project pursued a 2-stage study to investigate novel genetic determinants of EH. 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 to 10 years after DNA collection, allowing for the exclusion of controls that developed hypertension at a later age, thereby defining the hypernormal 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 the Methods (S1) section of the online Data Supplement (available at http://hyper.ahajournals.org). To perform a genetic association with continuous blood pressure (BP) phenotypes, we considered 2 additional cohorts (FLEMENGHO-EPOGH, n=1514, and Wandsworth Heart & Stroke Study, n=306, see Methods [S2] of the online Data Supplement) that provided population-based data. Description of the different samples is reported in the Methods S2 section.

Genotyping and Imputation

Genotyping details are shown in Methods S3 through S6 of the online Data Supplement. 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). 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 single nucleotide polymorphisms (SNPs) based on a priori biological knowledge (Methods S4 and S5). For the replication stage, we used the in silico data of rs3918226 from Anglo-Scandinavian Cardiac Outcomes Trial/AIBIII/NBS, BRIGHT, EPIC Turin, HYPEST, and NORDIL/MDC studies (Methods S6).

Statistical Analysis

All of the quality controls and statistical analyses were performed in accordance with the protocols written by Anderson et al[17] and Clarke et al[18] (Methods S7 through S9). 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 systolic BP and diastolic BP was tested on 2 additional population-based cohorts (Methods S2). Moreover, we tested for multiplicative interaction between rs3918226 and the most plausible interactive partners of the endothelial NO synthase (eNOS) gene, actin genes and heat shock protein (HSP) 90 genes (Methods S9). The quantitative effect of rs3918226 on systolic BP and diastolic BP has been tested on 2 additional population-based cohorts (FLEMENGHO-EPOGH and Wandsworth Heart & Stroke Study, see Methods S2). The recognition sequences for transcription factors in the eNOS region were searched using TRANSFAC[20,21] and the TFSEARCH database[22] (Methods S10).

Results

A classic 2-stage case-control strategy was used with a discovery phase of 1865 cases and 1750 controls (2294 males and 1321 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 an additional 1385 cases and 1246 controls (1417 males and 1214 females). According to ethnicity, the validation sample was composed of 1262 North Europeans (47.97%), 788 Sardinians (29.95%), and 581 continental Italians (22.08%). Tables S1 and S2 (available in the online Data Supplement) show the demographic characteristics and baseline measures.

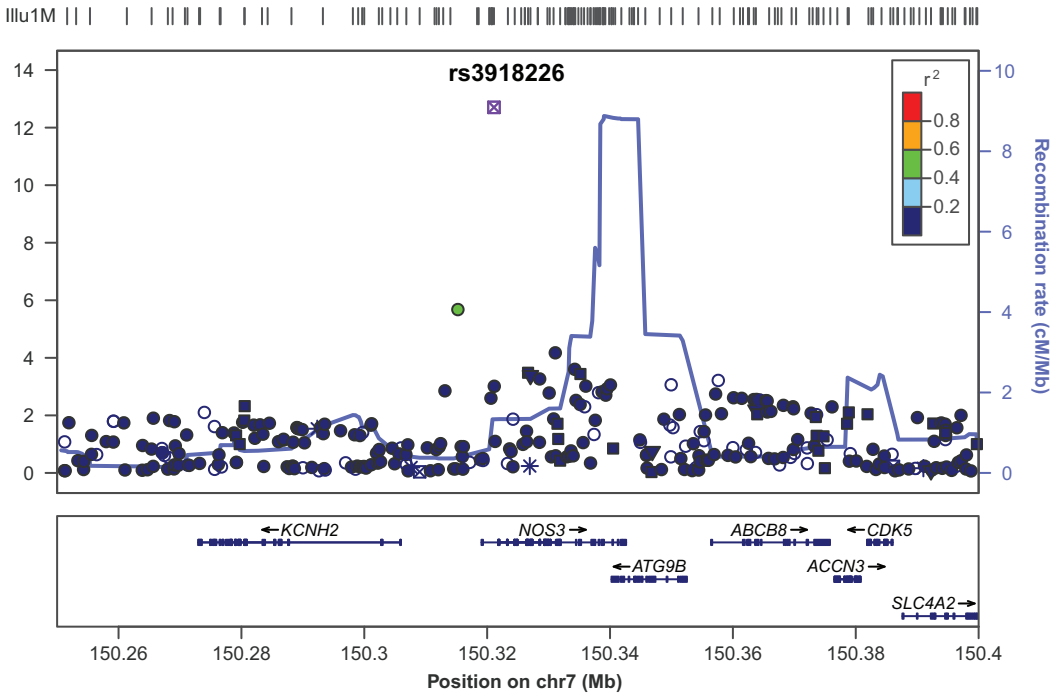

Figure 1. Local Manhattan plot for the NOS3 (endothelial NO synthase) region. Each circle represents a single nucleotide polymorphism (SNP), its *y* coordinate is the −log10 association *P* value for hypertension, and the *x* coordinate represents the physical position on the chromosome (on build 36, hg18). When replication data were available, the combined *P* value was used, otherwise the discovery *P* value was used. Circles are filled with colors according to the linkage disequilibrium (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 the discovery stage (named chr7:150 314 954 according to the 1000 Genome Project) was imputed based on the 1000 Genomes haplotypes (release June 2010), and 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).

Principal component analysis of the genotype data were 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 3 (roughly) equal-sized distinct clusters corresponding with the 3 main ethnic groups, as expected from the study design (Figure S1). All of the 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 correction was applied (because genomic inflation factor was 1.04). In the discovery phase, 90 SNPs (57% intragenic) with *P* value $<1 \cdot 10^{-4}$ were identified after genomic control (Figure S2 and Table S4). 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 whites, reaching a $P_{combined}$ of $2.58 \cdot 10^{-13}$ and odds ratio (OR) of 1.54 per T allele (95% CI: 1.37–1.73) under an additive model (Figure 1 and Table 1 and Figure S4). Estimated ORs in the discovery and validation

Table 1. Meta-Analysis Results for the Top SNPs in the HYPERGENES Study

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

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% CIs have been calculated under an additive model using logistic regression adjusted for sex and principal components. To retrieve information about single nucleotide polymorphisms and their genomic context (the nearest gene) we used the hg18 (National Center for Biotechnology Information 36) assembly. OR indicates odds ratio; *P*, *P* values; CI, confidence interval; Chr, chromosome; SNP, single nucleotide polymorphism.

Table 2. In Silico Meta-Analysis Results for rs3918226 (T/C, Effect Allele/Other Allele)

Variable	Study	Sample Size	OR	SE	95% CI	*P*
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	15 508	1.23	0.056	1.125–1.344	6.50E-06
	Meta-analysis	21 714	1.34*	1.248–1.437†	1.032E-14‡	6.198E-16§

Top section shows association results (odds ratios, SEs, CIs, and *P* values) for discovery, validation, and combined analysis of the HYPERGENES samples. Middle section shows results for ASCOT/AIBIII/NBS, BRIGHT, Epic Turin, HYPEST, and NORDIL/MDC studies and combined analysis of replication in silico samples. Bottom section shows meta-analysis results for all of the samples using both the *z* score and inverse variance-weighted *P* value methods.

*Data are OR combined.

†Data are 95% CI combined.

‡Data are combined *P* (*z* score).

§Data are combined *P* (inverse variance weighted).

samples were consistent across the different white populations of the HYPERGENES sample (Figure S5).

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 nonwhite HYPERGENES samples (Wandsworth Heart & Stroke Study cohort) and African and Asian HapMap samples. The second best hit chr7:150,314,954 (G/A SNP, minor allele frequency 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 could not replicate the observation in validation because of low imputation quality. An additional 7 SNPs within eNOS gene showed significant *P* values ($1 \cdot 10^{-3} < P < 1 \cdot 10^{-5}$): *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, $P_{combined}=1.96 \cdot 10^{-3}$), and *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 α-1 (*KCNMA1*), plasminogen (*PLG*), retinoid-related orphan receptor-α (*RORA*), and WW domain-containing protein 1 (*WWC1*). Moreover, the signals of SNPs presented previously 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).

We meta-analyzed rs3918226 using in silico data from Anglo-Scandinavian Cardiac Outcomes Trial/AIBIII/NBS, BRIGHT, EPIC-Turin, HYPEST, and NORDIL/MDC samples (Methods S2 and S6), 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. Because 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 nonsignificant, as expected, when EPIC-Turin was also considered together in the meta-analysis

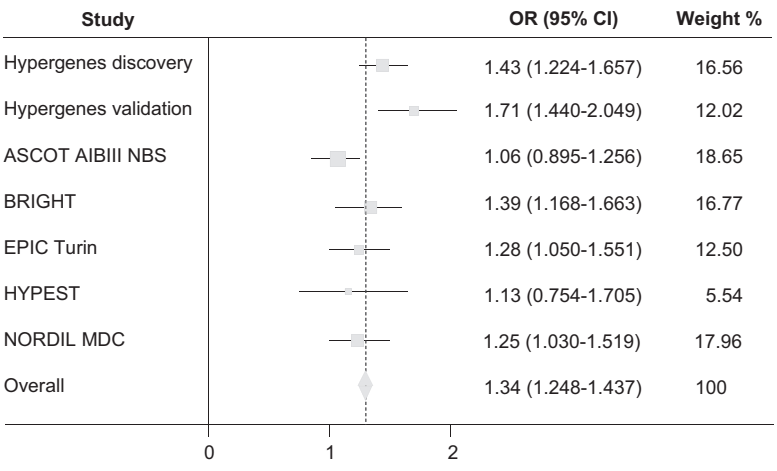

Figure 2. Forest plot of meta-analysis between HYPERGENES Discovery, HYPERGENES Validation, ASCOT/AIBIII/NBS, BRIGHT, EPIC Turin, HYPEST, and NORDIL/MDC studies. The squares and the horizontal lines correspond with the odds ratio (OR) and 95% CI of each study; the size of squares is proportional to weights (also shown as percentage); and the dotted line and the diamond represent the overall combined OR and 95% CI.

($P=0.092$), because 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 of the other samples (Anglo-Scandinavian Cardiac Outcomes Trial/AIBIII/NBS, BRIGHT, HYPEST, and NORDIL/MDC).

Moreover, we tested for epistatic multiplicative interactions between *eNOS rs3918226* and all of the available polymorphisms in genes known to be involved in targeting and regulating the overall availability of eNOS at the cell membrane[26–28]: actin genes (ACTA1, ACTA2, ACTB, ACTG1, and ACTG2)[29,30] and HSP90 genes (HSP90AA1, HSP90AA2, and HSP90AB1).[26] Nominally significant interactions were observed between *rs3918226* and *rs13447427* ($P=1.34\cdot10^{-3}$) in actin-β gene (*ACTB*), *rs7503750* ($P=1.57\cdot10^{-3}$) in actin-γ1 (*ACTG1*), and *rs4922796* and *rs17309979* ($P=3.47\cdot10^{-3}$, $P=4.88\cdot10^{-3}$) in HSP-α2 (*HSP90AA2*; Table S6). 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 systolic BP or diastolic BP 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 BP in millimeter of mercury per coded allele based on an additive genetic model. The BP distribution according to rs3918226 genotype is shown in Table S7.

Because *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 the 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 the University of California, Santa Cruz genome browser. Figure S6 shows that the region in which rs3918226 lies is conserved from placental mammals to primates.

Discussion

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 2-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 to be significantly associated with hypertension (OR: 1.54 [95% CI: 1.37–1.73]; $P=2.58\cdot10^{-13}$). 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]; $P=1.032\cdot10^{-14}$). We observed heterogeneity in the findings of meta-analysis ($P=0.005$ for Q test of heterogeneity) that could be attributed to both different sample sizes and recruitment criteria not directly comparable between HYPERGENES and the in 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 systolic BP and 1.40 for diastolic BP, despite the low P values of the regression probably because of 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 the eNOS promoter.[31] This suggests that, by affecting transcription factor–binding affinity, *rs3918226* might modulate the transcription of the *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, because among the genomewide association studies 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 whites using a genomewide association study approach.

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

Indeed, eNOS has been found inconsistently associated with 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 with the large sample sizes of genomewide association studies. Positive studies were substantially on Asian cohorts,[33–35] whereas the majority were negative in whites, as summarized in a recent meta-analysis.[36] The polymorphisms studied in our white sample G894T (rs1799983) and T-786C (rs2070744) did not reach genomewide 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 of the other published so far. rs1799983 was associated with EH with a P value of 2.63×10^{-3} (OR=1.038) and rs2070744 with a P value of 6.42×10^{-4} (OR=1.04), as shown in Table S8. To summarize, the ORs are clinically irrelevant. We underline the low linkage disequilibrium between rs3918226 and rs1799983 ($R^2=0.16$) and rs2070744 ($R^2=0.17$), suggesting that these 2 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 catalyzes the synthesis of NO by vascular endothelium, is responsible for the vasodilator tone that is fundamental for the regulation of BP. Furthermore, eNOS is a critical mediator of cardiovascular homeostasis through regulation of blood vessels diameter and of the maintenance of an antiproliferative and antiapoptotic environment.

Because NO is highly active, it cannot be stored inside producing cells. Indeed, eNOS signaling 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 HSP90,[27] which binds to eNOS and facilitates displacement of Cav1 by Calm. Moreover, eNOS directly interacts with actin cytoskeleton.[29] Recently, Kondrikov et al[30] added that β-actin is associated with the eNOS oxygenase domain increasing eNOS activity and NO production. To explore such a 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 *ACTB*, *rs7503750* in *ACTG1*, and *rs4922796* and *rs17309979* in the *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 EH.

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ONLINE SUPPLEMENT

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*

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Running title: eNOS gene as hypertension susceptibility locus

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Supplemental methods

Supplemental Methods S1: Inclusion Criteria

Definition of case:
A participant could be included as hypertensive case if he/she could self report to be of Caucasian Origin, was unrelated with other participants, had diastolic blood pressure (DBP) > 90 mmHg and systolic blood pressure (SPB) > 140 mmHg or under antihypertensive treatment before the age of 50.
Definition of control:
A participant could be included as normotensive if he/she could self report to be of Caucasian Origin, was unrelated with other participants, had DBP <85 mmHg and SBP <135 at least until 55 years of age and had never been treated for hypertension.
All hypertensive and normotensive subjects were otherwise healthy, non obese (body mass index < 30), non dyslipidemic (serum cholesterol <250; serum triglycerides <200 mg/dl - values obtained at screening prior inclusion in the study) and had no abnormal findings on physical examination.
A large proportion of the sample has been followed for many years after DNA collection, allowing for the exclusion of controls that developed hypertension at a later age. In particular the FLEMENGHO-EPOGH cohorts have been followed from 2 to 10 years and the Milan Cohorts from 5 to 15 years.

Supplemental Methods S2: HYPERGENES Consortium

Data presented here are part of data generated within the HYPERGENES Project, an EU supported IP, under FP7 (http://www.hypergenes.eu), aimed at the definition of a comprehensive genetic-epidemiological model of essential hypertension and of the intermediate phenotypes of hypertension, specifically associated Target Organ Damage (TOD).
Institutional review boards at each collection site approved the study and all individuals gave their informed consent. A further Ethical Revision of the University of Milano and of the HYPERGENES Internal Ethical Steering Board approved the entire process.

Cohorts contributing to the discovery sample:

- The FLEMENGHO (Flemish Study on Environment, Genes and Health Outcomes) - EPOGH (European Project on Genes in Hypertension) cohort has been the first large-scale study on genetic epidemiology of blood pressure and associated phenotypes in Europe and has already produced 35 scientific papers. It recruited family-based random samples in 5 eastern and 2 western European countries.[1,2] Overall the cohort available for HYPERGENES totals 4889 individuals (~13% hypertensive). According to our "macro-region concept, the FLEMENGHO/EPOGH cohort is considered a "North-European" cohort. From FLEMENGO-EPOGH cohort, 248 cases and 275 controls were genotyped in the discovery sample.
- The IMMIDIET study is a population-based cross-sectional study, funded by the European Union (FP5).[3] It compares healthy couples from regions of England, Belgium and Italy in order to evaluate the present dietary habits and the risk profile of the three communities at different risk of myocardial infarction. A large body of clinical (mostly cardiovascular) and environmental data are available. In the discovery phase, 106 controls and 163 cases were recruited and genotyped.
- Milano and Sassari cohorts (Italian and Sardinian cohorts) have been collected with a different target, since the focus was more directed on the pathophysiology on EH and TOD. For this reason, in most hypertensives, the phenotypes were collected before any pharmacological treatment was started.[4] The specificity of the cohorts is in the possibility of providing results for 2 important secondary outcome analyses. In fact, while today there is no substantial difference in shared environment between North Sardinia and Milano, there is no doubt that substantial genetic difference exists [5] (figure S1). Overall the Italian cohort available for HYPERGENES totals 1544 individuals (~52% hypertensives) while Sardinian cohort totals 1599 (~53% hypertensives).
- The cohort of the Wandsworth Heart & Stroke Study (WHSS) [6] has the characteristic of being a multiethnic community-based study, drawn from the same geographical area of South London. The sample totals 1577 individuals (~40% hypertensives), evenly distributed for ethnic origin; 33% Caucasians, 33% of African descent, 33% of South Asian descent. The population has been extensively phenotyped. Mortality follow-up through death certificates and cancer registrations are available. It is a unique multiethnic cohort sharing the same urban environment. Seventy eight cases and forty six controls with Caucasian origin were included in the discovery phase.

Cohorts contributing to the Validation sample

Additional samples were received from the major contributing units:

- Milano (Continental Italy). In the validation phase, 318 controls and 250 cases were recruited.
- Paris (France): The French hypertensive patients were selected from the HYPERGENES dataset of hypertensive families consecutively recruited in Paris, since 1990.[7] The dataset comprises more than 1000 index cases and more than 500 hypertensive siblings. In this study, only the 958 subjects (604 hypertensives and 354 normotensives) filling the criteria for inclusion in the HYPERGENES study with DNA available were included. Three hundred fifty four French normotensive patients were selected by the Institute Regional pour la Santé (IRSA, Pr J Tichet) during an annual medical visit of preventive medicine. All of the them were Caucasians, normotensive after 50 years of age and had no history of diabetes mellitus and were recruited in three main centers of the regions Centre and Picardie which are located about 200 kms South and North of Paris, respectively. These controls were previously used in other case control studies made by the Paris group.[8]
- PROGRESS cohort. The "Perindopril protection against recurrent stroke study" (PROGRESS) was designed to determine the effects of a blood-pressure-lowering regimen in hypertensive and non-hypertensive patients with a history of stroke or transient ischemic attack.[9] In the validation phase, we genotyped 281 subjects divided between 119 affected and 162 normotensives.
- Sassari (Sardinia). Eight hundred and thirty one samples (47% hypertensives) were included as validation samples.
- VFHS (Victorian Family Heart Study), a general population study of 2959 individuals.[10] Only 213 unrelated subjects satisfying the criteria for inclusion as controls in the HYPERGENES study were included.

Cohorts contributing to the replication stage

- ASCOT/AIBIII/NBS: The cases (BP of >160/100mmHg untreated or >140/90mmHg treated and >40 years + other risk factors) were derived from the UK/Irish participants of the Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT).[11] The normotensives were derived from two resources: NBS study and AIBIII study (Irish controls). The total sample is composed of 4049 subjects.
- BRIGHT study (The BRItish Genetics of HyperTension)[12] : the inclusion criteria for the hypertensives were a diagnosis of hypertension prior to 50 years, and BP ≥ 150/100mmHg for a single reading or ≥ 145/95mmHg for 3 consecutive readings. Exclusion criteria included BMI>35, diabetes, secondary hypertension or a co-existing illness. Normotensives had SBP ≤140mmHg and DBP≤ 90mmHg and were not taking any anti-hypertensive medications, the controls were recruited from similar geographical regions to the cases. A total of 3641 individuals were included in these analyses.
- EPIC Turin study is a longitudinal cohort of 10,603 volunteers, aged 35-64 years at baseline, from the Turin area, Italy. Blood pressure was measured using a mercury sphygmomanometer, in seated position, on the left arm. Full details of the cohort are reported elsewhere.[13] Only 2714 individuals were considered here, to apply HYPERGENES selection criteria.
- HYPEST (HYPertension in ESTonia)[14]: cases were selected based on the clinical diagnosis and profile of blood pressure specialists during the patients' ambulatory visits or hospitalization at the North Estonia Medical Center, Centre of Cardiology, or at the

Cardiology Clinic, Tartu University Hospital, Estonia. The controls were recruited from long-term blood donors. The total sample used in the current study is comprised of 1204 individuals.

- NORDIL/MDC: The cases were selected from the Nordic Diltiazem (NORDIL) study.[15] Hypertensives were defined as having at least two consecutive BP measurements greater than 160mmHg SBP and 100mmHg DBP, with the diagnosis made before age 63 years. The controls were identified from the Malmö Diet and Cancer study (MDC).[16] These individuals had to have a SBP < 120mmHg and DBP < 80mmHg and were at least 50 years of age and free from cardiovascular events (coronary events and stroke) during 10 years of follow up and not on any antihypertensive medication. The total sample is made up of 3900 cases and controls.

Population-based cohorts contributing to the quantitative analysis on SBP and DBP

In order to estimate the effect of continuous BP phenotypes, we considered two cohorts of the HYPERGENES Consortium that had also collected a population-based sample. We then assumed a normal linear regression model for SBP and DBP.
For individuals taking antihypertensive medication, we added 15 and 10 mmHg to the measured SBP and DBP, respectively, to account for treatment.[17,33] SBP and DBP were adjusted for sex, age, age^2 and body mass index.

- EPOGH-FLEMENGO sample: we considered a random sample of 1514 subjects with an age between 40 and 60 years (table S3).
- WHSS random population sample: we considered a random sample of 306 Caucasians subjects with an age between 40 and 60 years for the random population study. Besides Caucasians, we also genotyped 254 black Caribbean, 141 black Africans and 407 South Asians for a total of 1132 subjects. The eNOS SNP is very rare in non-Caucasians; in this dataset the T allele of rs3918226 had frequency of 0.75% (table S3).

Supplemental Methods S3: Genotyping and data filtering in the discovery

DNA was extracted from peripheral blood with standard procedures.
In the discovery phase all samples were genotyped using the Illumina 1M-duo arrays (Illumina Inc, San Diego, CA, USA). The chip captures 1199187 SNPs. In addition to markers necessary for broad genome coverage the chip contains 21877 non- synonymous SNPs, 51207 SNPs in sex chromosomes, 138 in mitochondrial DNA, 35969 SNPs covering recently reported copy number variant regions, 30908 SNPs in MHC and ADME regions.
Genotyping was performed in two different genotyping centres: Milan University (UNIMI) and Lausanne University (UNIL). UNIMI genotyped 2064 subjects (1270 controls and 794 cases) whereas UNIL performed the genotyping of 1995 subjects (665 controls and 1330 cases).
All raw intensity data were collected in UNIMI genotyping centre and analysed with the Illumina Software Genome Studio for genotype calling, using the Illumina reference cluster file. A DNA call rate threshold was set at 0.95 and DNAs with call rate ≤ 0.95 were excluded from the final data set. For each DNA, data from X chromosome were used to check for discordance with ascertained sex.
After association analysis, a final assessment of genotype quality was performed for the significantly associated SNPs (p-value<$1 \cdot 10^{-4}$) with visual inspection of cluster plots.
As genotyping was performed in two Laboratories, replications were designed in order to estimate the genotyping error rate between the two Genotyping Centres. DNAs from 20 individuals were genotyped with the 1M-duo Illumina's BeadChips.
Within samples with Call Rates >0.95, 99.624% of the genotype calls were concordant and 0.376% were discordant.
A cross-check between the scan performances of the two Illumina iScan platforms was also performed. Ten chips (20 samples) were processed and scanned in UNIMI (average DNA call rate 0.994). The same chips were re-scanned in UNIL. We could demonstrate that 99.189% of the calls were concordant and 0.0053% was discordant between the two platforms. For 0.805% of the calls we could not do the comparison since this percentage accounts for calls available in the chip scanned in one Lab but not in the other and vice-versa.

Imputation was performed using MACH [18] using as reference the 1000 Genomes haplotypes (release June 2010). Measured SNPs with >90% call rate, minor allele frequency >1%, and Hardy–Weinberg p-value >$1 \cdot 10^{-7}$ were included as input set. In subsequent analysis imputed SNPs with low imputation quality (r2-hat<0.3 or MAF<1%) were ignored. Whenever the measured genotype was available, it replaced the imputed value.

Supplemental Methods S4: Assembly of the Custom chip

In order to retest and fine map the genes found associated whit EH we selected about 15000 SNPs for a custom iSelect HD Illumina.
The selection was based on:

1. The list of BEST SNPs from case-control analysis at a genome wide level (p-value $<1 \cdot 10^{-4}$). From now onwards these SNPs will be reported as *BEST SNPs*.
2. A list of candidate genes and SNPs historically studied in hypertension or genes selected according to their functional role and involvement in biological pathways relevant in hypertension. These SNPs were included independently of the association results (i.e independently of the p-value for association) and if not already included in the SNP list at point 1. From now onwards these SNPs will be reported as *candidate SNPs*.

The criteria for SNPs selection are illustrated in the following paragraphs:
BEST SNPs selection: We selected a list of SNPs significantly associated with hypertension in the case-control analysis (p-value $<1 \cdot 10^{-4}$). Genotyping clusters of these BEST SNPs were checked by visual inspection using the Illumina software Genome Studio with the aim to exclude any ambiguous SNP. In order to retest and fine map the genes or regions identified in the discovery phase we selected further SNPs, neighboring the BEST SNPs, in order to better describe the genetic variability of the selected region. For this purpose we referred to the Illumina Gene Annotation of 1M duo and 1M quad chips, the densest Illumina chips available, and extracted all the SNPs in the genes mapped by the BEST SNPs. We created an "enriched BEST SNP list"of 106,360 SNPs.
Next steps were:
Step 1
From the "enriched BEST SNP list" we excluded all the SNPs mapping more than 100kb upstream and downstream the gene start and end. We chose this threshold since 100kb is a reasonable distance in order to include SNPs in possible gene regulatory regions. On coding SNPs and on the original BEST SNPs we didn't apply any of the filters described onwards because we decided to keep them on the custom chip.
Step 2
We also performed the gene annotation of the "enriched BEST list". With this annotation we found that:
a) Some SNPs had been merged with a new SNP code (the SNP coordinate doesn't change). We renamed these SNPs with the new SNP-code that is also recognized by Illumina.
b) Some SNPs were annotated as triallelic/quadriallelic. These SNPs were removed, since they should not be supported by the Illumina Infinium genotyping technology.
c) Some SNPs did not univocally map in the genome. These SNPs were removed.
A final "enriched BEST list" was sent to Illumina TechSupport in order to get all validation notes on Illumina assay.
Step 3
We then selected SNPs according to MAF and p-value of association analysis.
We chose SNPs with HapMap MAF ≥0.01 in at least one of the three populations of the confirmation sample: CEU, CHB and YRI (MAF set). SNPs with no MAF data for any of the 3 populations were included anyway. We kept SNPs with a p-value lower than 0.01. SNPs from the 1M quad chip that do not have a p-value of association since they were not on the 1M duo chip used in the discovery phase were not filtered for p-value of association.
Step 4
As a final step we applied a filter on SNPs flanking the original BEST SNP, to reduce the final count. The logic behind the flanking filter was to keep one SNP every five, obtaining a final set of 10349 markers.

Candidate SNPs selection

The following selection steps were followed:

Step 1:

Exclusion of all the SNPs mapping more than 100kb upstream or downstream the of the gene.

Step 2:

Extraction from Illumina annotation of 1M duo and 1M quad chips of all the SNPs assigned to the candidate gene list.

Step 3:

We annotated this list and we found that:

a) Some SNPs had been merged to a new SNP code (the SNP coordinate doesn't change). We renamed these SNPs with the new code.

b) Some SNPs were annotated as triallelic/quadriallelic. These SNPs were removed.

c) Some SNPs did not univocally map in the genome. These SNPs were removed.

A final "enriched candidate list" was sent to Illumina TechSupport in order to get all validation notes on Illumina assay.

Step 4:

We then kept only SNPs with HapMap MAF ≥0.05 in at least one of the three populations of the confirmation sample: CEU, CHB and YRI. SNPs with no MAF data for any of the 3 populations were included.

We applied also a filter based on the p-value of association. We removed SNPs having p-value ≥0.01. Since this list includes SNPs from the 1M quad that do not have a p-value of association (they were not on the chip used in the discovery), we didn't filter out these SNPs.

Step 5:

For each candidate gene we included only 5 SNPs and to reach this number we removed one SNP every two. In this way we reduced the number of candidate SNP to 4651 SNPs.

The list sent to Illumina was comprised of 4651 Candidate SNPs and 10349 BEST SNPs for a total of 15000 SNPs.

Due to the fact that during chip manufacturing not all assays passed the Illumina decoding process, the real number of SNPs on the custom chip was 14055 (4386 candidate SNPs, 9669 Best SNPs)

Supplemental Methods S5: Genotyping and data filtering in the Validation

Genotyping was performed on 2869 samples by Milan University (UNIMI) and Lausanne University (UNIL) who genotyped 1700 and 1169 samples respectively.

All raw intensity data were collected in UNIMI genotyping centre and analysed with the Illumina Software Genome Studio. For custom chips Illumina doesn't supply the reference cluster file for genotype calling we therefore ran training samples to generate a reference cluster file. To create this file all SNPs were visually inspected in order to check the accuracy of the cluster plots. Three hundred and forty ambiguous SNPs were excluded.

Genotype calling for the whole sample was performed using the custom reference file.

In the Validation phase the DNA call rate threshold was set at 0.98 and this led to the exclusion of 173 DNAs from the final data set that comprised of 2696 DNAs.

Supplemental Methods S6: *In-silico* genotype data for replication

We meta-analyzed rs3918226 using *in silico* data from, ASCOT/AIBIII/NBS, BRIGHT, EPIC Turin, HYPEST and NORDIL/MDC studies.

In particular, ASCOT/AIBIII/NBS, BRIGHT and NORDIL/MDC cohorts were genotyped with Illumina Human CVD BeadArray. The HumanCVD BeadChip is the first high-density SNP genotyping standard panel specifically targeted for cardiovascular disease (CVD) studies. The BeadChip features 49,452 markers that capture genetic diversity across approximately 2,100 genes associated with CVD processes such as blood pressure changes, insulin resistance, metabolic disorders, dyslipidemia, and inflammation. HYPEST samples were genotyped using the KASPAr assay at Barts and The London Genome Centre. Support for developing CVD BeadArray and KASPAr assay to MC from British Heart Foundation grant. Only SNPs passing quality control thresholds of Hardy-Weinberg equilibrium ($p>0.001$) and with a call rate >90% were included in the analyses.

The SNP rs3918226 was genotyped in EPIC Turin cohort by the 5'-Nuclease assay (TaqMan) with a fluorogenic Minor Groove Binder probe on ABI-7900 (Applied Biosystems).

Supplemental Methods S7: Quality Control

All QC steps were performed in accordance with the protocol written by C.A Anderson et al [19]

The Discovery sample was comprised of 4059 genotyped individuals who underwent a quality control. 143 Samples having Call Rate <0.95 were excluded. 56 subjects with genotypic sex mismatch (difference between the gender reported in clinical data and the one estimated with sex SNPs genotyped) were identified and removed from the analysis.

Using genome-wide IBD estimation (PLINK version 1.7 [20]) we identified and removed from the analysis 64 duplicated and 156 related subjects (44 family components, 63 siblings, 23 parent/offspring, 26 second degree). Using theEIGENSOFT package version 2.0 [21, 22], we removed 25 outliers defined as individuals that exceed a default number of standard deviations (6.0) from the whole sample. After quality control the final case-control sample comprised of 3615 subjects, 1865 hypertensive cases, 1750 healthy controls (2,294 males, 1,321 females).

According ethnicity the HYPERGENES sample is composed by 906 North European subjects (25.06%), 1399 Sardinians (38.70 %) and 1310 Continental Italy (36.24%). Table S1 shows the sample distribution in different cohorts divided between cases-controls and genotyping centres.

SNPs with a minor allele frequency (MAF) <1%, in significant Hardy-Weinberg disequilibrium (p-value$<1\cdot10^{-8}$) and with call rate <99% were removed leaving 882564 SNPs for analysis.

We assessed population structure within the data using principal components analysis (PCA) as implemented in EIGENSTRAT [21, 22] to infer continuous axes of genetic variation.

After association tests the cluster plots of all SNPs taken forward for validation were manually

inspected to check the fidelity of genotype assignment.

The Validation sample was composed by 2696 genotyped individuals.After data quality control for relatedness/duplicates (32 duplicated and 33 related subjects) the final case-control sample was composed by 2631 subjects, 1385 hypertensives and 1246 controls (1,417 males and 1,214 females).

According to ethnicity, the HYPERGENES sample was comprised of 1262 North Europeans (47.97%), 788 Sardinians (29.95 %) and 581 Continental Italians (22.08%). Table S2 shows the sample distribution in different cohorts divided between cases, controls and genotyping centers.

We filtered out 335 SNPs on the basis of SNP genotype call rates (<99%), 2641 SNPs for Minor Allele Frequency (< 0.01) and 39 markers that failed HWE test in controls (p-value $<1 \cdot 10^{-6}$). After frequency and genotyping pruning, 10684 autosomal SNPs were available for the association analysis.

Supplemental Methods S8: Principal Component Analysis

In the discovery sample, we performed PCA using 1M SNPs using the EIGENSOFT package (version 2.0) [21, 22]. We removed 25 genetic outliers defined as individuals that exceed 6 standard deviations from the whole sample along any of the principal components.

Results for the first 2 PCs are described in figure S1. The plot clearly shows subjects clustering according to their geographical origin. In figure S1A the samples are represented as macro-groups (North Europe, Continental Italy and Sardinia). Figure S1B shows the same clusters with the individuals marked according to the recruitment centres.

We selected as significant the first 10 PCs (P-value < $1 \cdot 10^{-7}$) to include them as covariates in the logistic regression model.

In the Validation sample, PCA was carried out using 10684SNPs. We excluded 21 subjects as outliers (6 standard deviations from the whole sample).

The results, for the first 2 PCs, are shown in figure S3. The distribution of the three macro-groups is very similar in the discovery and validation samples. However, due to limited number of SNPs, clusters are not as well defined as in the discovery sample. To validate the use of the PCs as covariates in logistic regression, we re-run the PCA on the discovery sample using only the common SNPs between the two study phases. We could correctly replicate the distribution of the discovery subjects in the three macro-areas (data not shown). We used as covariates in association analysis the first 10 PCs (P-value < $1 \cdot 10^{-2}$).

Supplemental Methods S9: Statistical Analysis

In the discovery phase, 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 PCs, as implemented in PLINK.[20] Residual inflation (1.04) of the test statistic was corrected using genomic control.

In the Validation phase, logistic regression analysis was carried out using an additive genetic model adjusted for sex and for the first 10 PCs.

The basic statistical analyses were performed in accordance with the protocol written by G.M. Clarke et Al. [23]

Combined analysis for HYPERGENES discovery, HYPERGENES Validation, ASCOT/AIBIII/NBS, BRIGHT, Epic Turin, HYPEST and NORDIL/MDC results was conducted using both Z-score and inverse variance weighting meta-analysis as implemented in METAL.[24]

In the meta-analysis, a genome-wide significance threshold of $5 \cdot 10^{-8}$ was considered as genome-wide significant.

The heterogeneity analysis tests whether observed effect sizes (or test statistics) are homogeneous across samples. A test for heterogeneity examines the null hypothesis that all studies are evaluating the same effect. The usual test statistic (Cochran's *Q*) is computed by summing the squared deviations of each study's estimate from the overall meta-analytic estimate, weighting each study's contribution in the same manner as in the meta-analysis [25,26]. The resulting heterogeneity statistic has n-1 degrees of freedom for n cohorts.

In our study, the p value of heterogeneity calculated for HYPERGENES cohorts is 0.13. It decreased slightly, but remained non-significant, when also EPIC-Turin was considered together in the meta-analysis (p=0.092), as expected, since the recruitment criteria for cases and controls were identical. Conversely, the heterogeneity significantly increased (p=0.005) when HYPERGENES cohort was meta-analyzed with all the other cohorts (ASCOT/AIBIII/NBS, BRIGHT, HYPEST and NORDIL/MDC).

We tested for interaction between the discovered eNOS SNP (rs3918226) and the most plausible interactive partners of the eNOS gene: actin genes (ACTA1, ACTA2, ACTB, ACTG1, ACTG2) and HSP90 genes (HSP90AA1, HSP90AA2, HSP90AB1). In total 159 SNPs were tested for interaction. Usual covariates, sex and ancestry principal components were included in the logistic regression. To assess significance, multiple testing corrections were applied. Since many of the SNPs are in high LD with each other, we estimated the Per Comparison Error Rate (PCER) which provides a weak control of the false discovery rate (FDR) and we permuted the phenotype 500 times. [27] We used a loose 20% false discovery rate threshold providing suggestive evidence for interactions. Since the PCER is estimated via permutations, dependency between the tests is fully accounted for.

The quantitative effect of rs3918226 has been tested on two additional population-based cohorts from the HYPERGENES consortium (EPOGH-FLEMENGO and WHSS, see supplemental methods S2). For individuals taking antihypertensive medication, we added 15 and 10 mmHg to the measured SBP and DBP, respectively, to account for treatment.[28,32] The linear regressions for SBP and DBP were adjusted for sex, age, age^2 and body mass index.

Supplemental Methods S10: Recognition sequences for transcription factors

The recognition sequences for transcription factors (TFs) in eNOS region were searched with the PATCH algorithm of TRANSFAC.[29,30] The TRANSFAC database on eukaryotic transcriptional regulation includes data on transcription factors, their target genes and regulatory binding sites. Potential binding sites for TFs within the region of interest were also searched using the TFSEARCH database with a cutoff score of 85.31.

Authors Contributions

Daniele Cusi designed the study.

Hisatomi Arima, Giuseppe Argiolas, Franco Cappuccio, John Chalmers, Nabila Devos, Justine Ellis, Jan Filipovsky, Nicola Glorioso, Stephen Harrap, Licia Iacoviello, Xavier Jeunemaitre, Tatiana Kuznetsova, Paolo Manunta, Maurizio Marconi, Yury P. Nikitin, Jitka Seidlerova, Galina Simonova, Jan A. Staessen, Benedetta Stancanelli, Katarzyna Stolarz-Skrzypek, Lutgarde Thijs, Jean Tichet, Valérie Tikhonoff, Chiara Troffa and Paolo Vineis collected the samples and provided the clinical data.

Murielle Bochud, Costanza Conti, Maria C. D'Alessio, Federica Rizzi and Amnon Shabo performed ontology and harmonization of phenotypes.

Laura Zagato, Maria Francesca Ortu, Roberta Zaninello managed DNA samples. Cristina Barlassina, Paola Benaglio, Daniele Braga, Patrick Descombes, Carlo Rivolta performed genotyping.

Andrea Calabria developed information system and bioinformatics applications for quality control and SNP selection. Maurizio Mercurio and Amnon Shabo provided database support.

Jacques S. Beckmann, Cristina Barlassina, Daniele Cusi, Francesca Frau, Nicola Glorioso, Clive Hoggart, Zoltán Kutalik, Sara Lupoli, Federica Rizzi, Erika Salvi contributed experimental design, statistical analysis and interpretation.

Mark Caulfield, Anna Dominiczak, Simonetta Guarrera, Toby Johnson, Maris Laan, Giuseppe Matullo, Olle Melander, Patricia Munroe, Fulvio Ricceri, Nilesh Samani, Alice Stanton and Paolo Vineis provided *in silico* replication samples**.**

Daniele Cusi and Erika Salvi wrote the first draft of the manuscript.

Cristina Barlassina, Jacques S. Beckmann, Franco Cappuccio, Mark Caulfield, Daniele Cusi, Francesca Frau, Clive Hoggart, Zoltán Kutalik, Xavier Jeunemaitre, Patricia Munroe, Carlo Rivolta, Federica Rizzi, Erika Salvi, Amnon Shabo and Jan A. Staessen edited and discussed the manuscript.

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Supplemental Figures and tables

Characteristics	North Europe		Continental Italy		Sardinia	
Affection status	**Hypertensives**	**Normotensives**	**Hypertensives**	**Normotensives**	**Hypertensives**	**Normotensives**
number of individuals	563	343	574	736	728	671
Percent women (%)	46.91	53.09	22.3	41.7	32.83	33.08
age (yrs)	48.3 (8.6)	60.0 (5.0)	45.2 (8.8)	59.5 (6.1)	50.9 (12.4)	62.9 (12.0)
body mass index (kg/m^2)	27.9 (5.2)	26.1 (3.8)	26.4 (3.1)	24.6 (2.9)	27.5 (3.9)	26.0 (3.7)
SBP (mm Hg)	143.1 (16.9)	119.2 (9.9)	148.7 (8.9)	125.0 (7.0)	158.5 (14.5)	123.9 (9.8)
SBP after G BP Gen adjustment for treatment* (mm Hg)	150.2 (14.4)	119.2 (9.9)	148.8 (8.9)	125.0 (7.0)	158.5 (14.5)	123.9 (9.8)
DBP (mm Hg)	89.8 (10.5)	74.0 (6.3)	96.4 (6.1)	79.5 (4.6)	102.1 (9.1)	77.1 (6.9)
DBP after G BP Gen adjustment for treatment* (mm Hg)	94.6 (9.0)	74.0 (6.3)	96.5 (6.0)	79.5 (4.6)	102.1 (9.1)	77.1 (6.9)
Treated for hypertension (%)	46.71	-	1.05	-	0	-

Table S1. Demographic and clinical description of discovery samples into the three macro-areas (North Europe, Continental Italy and Sardinia) and divided between cases and controls. The table reports the mean values and, in parenthesis, the standard deviations.

* Tobin MD, et al. Adjusting for treatment effects in studies of quantitative traits: antihypertensive therapy and systolic blood pressure. Stat. Med. 24, 2911–2935 (2005).

Characteristics	North Europe		Continental Italy		Sardinia	
affection status	**Hypertensives**	**Normotensives**	**Hypertensives**	**Normotensives**	**Hypertensives**	**Normotensives**
number of individuals	757	505	256	325	372	416
per cent women (%)	44.78	36.63	19.53	58.46	48.66	64.66
age (yrs)	46.66 (8.46)	62.89 (6.6)	43.06 (8.39)	62.92 (7.22)	50.18 (10.14)	60.59 (6.15)
body mass index (kg/m^2)	26.20 (4.19)	25.68 (3.75)	26.30 (3.22)	25.21 (3.47)	27.42 (4.31)	25.05 (4.46)
SBP (mm Hg)	153.38 (19.80)	119.77 (7.68)	149.78 (14.64)	121.81 (7.20)	158.96 (13.84)	123.49 (9.23)
SBP after G BP Gen adjustment for treatment † (mm Hg)	162.22 (20.84)	119.77 (7.68)	152.25 (14.65)	121.81 (7.20)	158.96 (13.84)	123.49 (9.23)
DBP (mm Hg)	97.20 (12.36)	73.43 (7.15)	96.75 (9.99)	72.47 (6.04)	101.83 (7.96)	75.43 (7.2)
DBP after G BP Gen adjustment for treatment † (mm Hg)	103.10 (12.35)	73.43 (7.15)	98.24 (9.28)	72.47 (6.04)	101.83 (7.96)	75.43 (7.2)
treated for hypertension (%)	60.50	-	14.84	-	0	-

Table S2. Demographic and clinical description of Validation samples into the three macro-areas (North Europe, Continental Italy and Sardinia) and divided between cases and controls. The table reports the mean values and, in parenthesis, the standard deviations.

† Tobin MD, et al. Adjusting for treatment effects in studies of quantitative traits: antihypertensive therapy and systolic blood pressure. Stat. Med. 24, 2911–2935 (2005).

Characteristics	EPOGH-FLEMENGO	Wandsworth Heart & Stroke Study (WHSS)
number of individuals	1514	306
Percent women (%)	52.58	57.19
age (yrs)	48.04 (6.05)	47.83 (4.8)
body mass index (kg/m^2)	26.60 (3.98)	25.36 (4.32)
SBP (mm Hg)	125.30 (15.30)	121.32 (14.99)
SBP after G BP Gen adjustment for treatment ‡ (mm Hg)	126.59 (16.48)	121.32 (14.99)
DBP (mm Hg)	78.34 (10.33)	77.52 (8.56)
DBP after G BP Gen adjustment for treatment ‡ (mm Hg)	79.20 (11.02)	77.52 (8.56)
Treated for hypertension (%)	8.6	0

Table S3. Demographic and clinical description of population based samples (EPOGH-FLEMENGO and WHSS) used for the assessment of the quantitative effect of the rs3918226 on BP.

‡ Tobin MD, et al. Adjusting for treatment effects in studies of quantitative traits: antihypertensive therapy and systolic blood pressure. Stat. Med. 24, 2911–2935 (2005).

Genome wide association results for hypertension-associated SNPs with pvalue < 1e-04 sorted by hypertension analysis

SNP	CHR	Position (bp)	Location	Nearest gene	Coded Allele	Not coded Allele	MAF	OR	Lower limit, CI 95	Upper limit, CI 95	P	P adjusted for GC
rs783145	6	161072439	intronic	PLG	A	G	0.50	1.27	1.15	1.40	8.53E-07	1.41E-06
rs341408	15	58928982	intronic	RORA	A	G	0.38	1.27	1.15	1.40	1.74E-06	2.79E-06
rs7907270	10	78550949	intronic	KCNMA1	G	A	0.40	1.27	1.15	1.40	2.35E-06	3.73E-06
rs783182	6	161088538	intronic	PLG	A	G	0.49	1.25	1.14	1.38	2.95E-06	4.64E-06
rs4976593	5	167710021	intronic	WWC1	G	A	0.33	1.27	1.15	1.41	3.75E-06	5.84E-06
rs1406891	6	161107070	intergenic	PLG	G	A	0.49	1.25	0.73	0.88	3.99E-06	6.20E-06
rs3918226	7	150321109	intronic	NOS3	T	C	0.12	1.42	1.22	1.66	4.81E-06	7.42E-06
rs10519080	15	58925751	intronic	RORA	G	A	0.14	1.37	1.20	1.57	5.79E-06	8.87E-06
rs11686131	2	27362214	intronic	TRIM54	G	A	0.18	1.34	0.66	0.85	5.93E-06	9.08E-06
rs1084656	6	161101282	intergenic	PLG	C	A	0.50	1.24	1.13	1.37	6.67E-06	1.02E-05
rs4665962	2	27368609	intronic	TRIM54	A	G	0.18	1.33	0.66	0.85	7.09E-06	1.08E-05
rs631208	16	9307225	intergenic	LOC653737	A	G	0.41	1.25	0.72	0.88	8.09E-06	1.22E-05
rs1975384	2	27395545	intronic	MPV17	G	A	0.18	1.33	0.66	0.85	8.15E-06	1.23E-05
rs1247558	6	161110189	intergenic	PLG	G	A	0.50	1.24	1.13	1.36	8.30E-06	1.26E-05
rs783165	6	161097832	intergenic	PLG	C	A	0.50	1.24	1.13	1.36	9.73E-06	1.46E-05
rs1621801	6	161116956	intergenic	PLG	C	A	0.49	1.24	0.73	0.89	1.10E-05	1.64E-05
GA006079	6	161094861	intergenic	PLG	C	G	0.50	1.24	1.13	1.36	1.12E-05	1.67E-05
rs6555802	5	167705652	intronic	WWC1	A	C	0.34	1.25	1.13	1.38	1.13E-05	1.69E-05
rs1992291	2	27357816	intergenic	DNAJC5G	A	G	0.20	1.31	0.68	0.86	1.38E-05	2.04E-05
rs2366947	2	85941520	intronic	ST3GAL5	A	G	0.34	1.25	0.73	0.89	1.54E-05	2.27E-05
rs2565721	6	161155088	intergenic	PLG	G	A	0.48	1.23	0.74	0.89	1.71E-05	2.51E-05
rs6721125	2	85958240	intronic	ST3GAL5	G	A	0.35	1.25	0.73	0.89	1.71E-05	2.52E-05
rs6753350	2	85959009	intronic	ST3GAL5	A	G	0.35	1.25	0.73	0.89	1.78E-05	2.61E-05
rs895314	5	13900984	intronic	DNAH5	G	A	0.17	1.32	0.67	0.86	1.83E-05	2.69E-05
rs1511789	15	52442052	intronic	UNC13C	A	G	0.29	1.26	1.13	1.40	1.88E-05	2.76E-05
rs11787445	8	135388710	intergenic	LOC729395	A	G	0.24	1.28	1.14	1.43	1.89E-05	2.77E-05
rs2023843	7	27209746	intergenic	HOXA13	A	G	0.06	1.58	0.51	0.78	2.01E-05	2.94E-05

Genome wide association results for hypertension-associated SNPs with pvalue < 1e-04 sorted by hypertension analysis

SNP	CHR	Position (bp)	Location	Nearest gene	Coded Allele	Not coded Allele	MAF	OR	Lower limit, CI 95	Upper limit, CI 95	P	P adjusted for GC
rs4665960	2	27321102	intergenic	CAD	G	A	0.22	1.29	0.69	0.87	2.03E-05	2.97E-05
rs2930120	12	66600079	intergenic	LOC341333	A	G	0.25	1.27	1.14	1.41	2.03E-05	2.97E-05
rs7733887	5	168551433	intergenic	SLIT3	G	A	0.10	1.42	1.21	1.68	2.13E-05	3.10E-05
rs929250	7	27211626	intergenic	HOXA13	A	C	0.06	1.58	0.51	0.78	2.13E-05	3.11E-05
rs1561535	2	27324529	intergenic	CAD	G	A	0.28	1.26	0.71	0.88	2.18E-05	3.18E-05
rs1122227	2	27397789	intronic	MPV17	G	A	0.18	1.31	0.67	0.86	2.32E-05	3.37E-05
rs783156	6	161151167	intergenic	PLG	G	A	0.49	1.23	0.74	0.90	2.37E-05	3.44E-05
rs7209417	17	72877103	intronic	SEPT9	G	A	0.37	1.24	0.73	0.89	2.42E-05	3.51E-05
rs4553826	2	85948712	intronic	ST3GAL5	G	A	0.35	1.24	0.73	0.89	2.44E-05	3.54E-05
rs2580761	2	27279866	intronic	SLC5A6	G	A	0.22	1.29	0.69	0.87	2.60E-05	3.76E-05
rs2908764	12	66598283	intergenic	LOC341333	A	G	0.25	1.26	1.13	1.40	2.79E-05	4.03E-05
rs4962095	9	135021572	intronic	GBGT1	C	A	0.47	1.23	1.12	1.35	2.89E-05	4.16E-05
rs2073927	9	135021167	intronic	GBGT1	A	G	0.47	1.23	1.12	1.35	2.90E-05	4.18E-05
rs501630	11	65393849	intronic	EFEMP2	A	G	0.43	1.23	1.12	1.35	2.93E-05	4.22E-05
rs2160243	12	66602263	intergenic	LOC341333	G	A	0.25	1.26	1.13	1.40	3.00E-05	4.31E-05
rs4722675	7	27210487	intergenic	HOXA13	G	A	0.06	1.56	0.52	0.79	3.20E-05	4.59E-05
rs9653564	2	27326122	intergenic	SLC30A3	G	A	0.23	1.27	0.70	0.88	3.26E-05	4.67E-05
rs7276718	21	19329160	intergenic	PPIAL3	G	A	0.45	1.23	1.11	1.35	3.36E-05	4.82E-05
rs13404446	2	27372758	intronic	TRIM54	G	A	0.24	1.27	0.70	0.88	3.39E-05	4.86E-05
rs3735533	7	27212418	intergenic	HOXA13	G	A	0.06	1.56	0.52	0.79	3.49E-05	5.00E-05
rs7658173	4	89401542	intergenic	PPM1K	A	G	0.37	1.23	1.12	1.36	3.54E-05	5.06E-05
rs7808249	7	86821651	intronic	CROT	A	G	0.26	1.26	1.13	1.40	3.68E-05	5.25E-05
rs11126918	2	27292214	intronic	C2orf28	G	A	0.22	1.28	0.70	0.88	3.70E-05	5.28E-05
rs7223756	17	74794846	intronic	HRNBP3	G	A	0.47	1.22	1.11	1.35	3.89E-05	5.54E-05
rs1659685	2	27274194	intergenic	SLC5A6	A	G	0.29	1.25	0.72	0.89	4.08E-05	5.80E-05
rs1563447	17	74805025	intronic	HRNBP3	G	A	0.30	1.24	0.73	0.89	4.13E-05	5.88E-05
rs4665376	2	27362536	intronic	TRIM54	A	G	0.24	1.26	0.71	0.88	4.15E-05	5.90E-05

Genome wide association results for hypertension-associated SNPs with pvalue < 1e-04 sorted by hypertension analysis

SNP	CHR	Position (bp)	Location	Nearest gene	Coded Allele	Not coded Allele	MAF	OR	Lower limit, CI 95	Upper limit, CI 95	P	P adjusted for GC
rs1640235	7	5609157	intronic	FSCN1	A	G	0.09	1.44	1.21	1.71	4.19E-05	5.96E-05
rs6880706	5	117677374	intergenic	DTWD2	G	A	0.47	1.22	1.11	1.35	4.31E-05	6.12E-05
rs13404327	2	27372657	intronic	TRIM54	C	A	0.24	1.26	0.71	0.89	4.43E-05	6.28E-05
rs563881	15	52449942	intronic	UNC13C	A	G	0.29	1.25	1.12	1.38	4.52E-05	6.40E-05
rs4665963	2	27382196	intronic	TRIM54	A	G	0.25	1.26	0.71	0.89	4.73E-05	6.69E-05
rs10858918	12	88620476	intergenic	LOC338758	G	A	0.37	1.23	0.73	0.90	4.74E-05	6.71E-05
rs7575245	2	27380825	intronic	TRIM54	G	A	0.24	1.26	0.71	0.89	4.75E-05	6.72E-05
rs10264581	7	27221942	intergenic	HOXA13	A	G	0.04	1.65	0.48	0.77	4.85E-05	6.86E-05
rs4789997	17	74799181	intronic	HRNBP3	A	G	0.34	1.23	0.74	0.90	4.87E-05	6.88E-05
rs9849845	3	109031479	intergenic	BBX	A	C	0.49	1.22	0.75	0.90	4.92E-05	6.94E-05
rs813641	6	161073980	intronic	PLG	A	G	0.15	1.32	1.15	1.51	5.05E-05	7.13E-05
rs1533605	2	85945764	intronic	ST3GAL5	A	G	0.35	1.23	0.73	0.90	5.16E-05	7.28E-05
rs1517331	2	168710837	intronic	STK39	G	A	0.13	1.35	1.17	1.55	5.36E-05	7.55E-05
rs7751252	6	79019345	intergenic	IRAK1BP1	G	A	0.08	1.44	1.21	1.72	5.49E-05	7.73E-05
rs4665965	2	27389884	intronic	MPV17	A	G	0.24	1.26	0.71	0.89	5.68E-05	7.98E-05
rs9888336	12	132073327	5UTR	ZNF26	A	G	0.03	1.82	1.36	2.44	6.03E-05	8.45E-05
rs1993643	10	107247019	intergenic	SORCS3	A	G	0.31	1.24	1.12	1.37	6.14E-05	8.60E-05
rs3769143	2	27304228	intronic	CAD	G	A	0.29	1.24	0.72	0.90	6.20E-05	8.68E-05
rs529565	9	135139321	intronic	ABO	A	G	0.32	1.23	0.73	0.90	6.25E-05	8.75E-05
rs1401283	2	170843292	intronic	MYO3B	G	A	0.38	1.23	0.74	0.90	6.26E-05	8.76E-05
rs1275522	2	27280382	intronic	SLC5A6	A	G	0.29	1.24	0.72	0.90	6.33E-05	8.85E-05
rs11996335	8	77536681	intergenic	ZFHX4	G	A	0.49	1.22	1.10	1.34	6.33E-05	8.86E-05
rs2847579	18	46616662	intergenic	MRO	C	A	0.41	1.22	1.11	1.34	6.34E-05	8.87E-05
rs7221079	17	72856622	intronic	SEPT9	A	G	0.35	1.23	0.73	0.90	6.48E-05	9.06E-05
rs13241373	7	5789747	intergenic	RNF216	A	G	0.10	1.40	1.19	1.66	6.58E-05	9.19E-05
rs608270	12	132143532	intronic	ZNF84	A	G	0.03	1.84	1.36	2.49	6.62E-05	9.25E-05
rs601679	12	132145686	3UTR	ZNF84	A	G	0.03	1.84	1.36	2.49	6.62E-05	9.25E-05

Genome wide association results for hypertension-associated SNPs with pvalue < 1e-04 sorted by hypertension analysis

SNP	CHR	Position (bp)	Location	Nearest gene	Coded Allele	Not coded Allele	MAF	OR	Lower limit, CI 95	Upper limit, CI 95	P	P adjusted for GC
rs11655079	17	72860270	intronic	SEPT9	G	A	0.35	1.23	0.74	0.90	6.65E-05	9.29E-05
rs7794193	7	27261226	intergenic	EVX1	G	A	0.04	1.66	0.47	0.77	6.71E-05	9.37E-05
rs4680062	3	154615406	intergenic	LOC152118	A	G	0.14	1.33	1.16	1.53	6.72E-05	9.38E-05
rs11147244	12	132214574	intergenic	ZNF10	G	A	0.03	1.85	1.37	2.51	6.83E-05	9.53E-05
rs1275501	2	27272786	intergenic	SLC5A6	C	A	0.29	1.24	0.72	0.90	6.92E-05	9.65E-05
rs4722670	7	27196074	intergenic	HOXA11S	A	G	0.08	1.44	0.58	0.83	6.93E-05	9.67E-05
rs11608	2	27288878	5UTR	C2orf28	A	G	0.29	1.24	0.73	0.90	7.01E-05	9.77E-05
rs573740	15	52470723	intronic	UNC13C	A	G	0.30	1.24	1.11	1.37	7.10E-05	9.90E-05
rs623100	12	132144523	coding	ZNF84	A	C	0.03	1.84	1.36	2.48	7.12E-05	9.92E-05
rs6709602	2	27259001	intergenic	SLC5A6	G	A	0.22	1.27	0.70	0.89	7.23E-05	1.01E-04
rs7310221	12	132105704	intergenic	ZNF26	G	A	0.03	1.82	1.36	2.45	7.27E-05	1.01E-04
rs4758930	12	132088232	intronic	ZNF26	A	G	0.03	1.81	1.35	2.42	7.28E-05	1.01E-04
rs783146	6	161058838	intronic	PLG	C	G	0.15	1.31	1.15	1.50	7.32E-05	1.02E-04
rs2675124	2	236295779	intronic	CENTG2	G	A	0.21	1.26	0.71	0.89	7.39E-05	1.03E-04
rs1911588	4	148240594	intergenic	TTC29	G	A	0.35	1.22	1.11	1.35	7.42E-05	1.03E-04
rs1047389	4	11010185	coding	HS3ST1	A	G	0.20	1.27	0.70	0.89	7.81E-05	1.08E-04
rs1474477	14	21856055	intergenic	TRAV41	G	A	0.16	1.29	0.68	0.88	7.81E-05	1.08E-04
rs17819224	17	50923697	intergenic	MMD	A	G	0.36	1.23	0.74	0.90	7.82E-05	1.09E-04
rs2038860	14	56783175	intronic	EXOC5	A	G	0.07	1.46	1.21	1.76	8.32E-05	1.15E-04
rs1652471	6	161174393	intergenic	PLG	G	A	0.46	1.21	0.75	0.91	8.38E-05	1.16E-04
rs11128329	3	73494360	intergenic	PDZRN3	A	G	0.14	1.32	1.15	1.51	8.48E-05	1.17E-04
rs1870461	10	107242452	intergenic	SORCS3	A	G	0.31	1.23	1.11	1.37	8.54E-05	1.18E-04
rs7808467	7	27269575	intergenic	LOC392008	A	G	0.04	1.64	0.47	0.78	8.63E-05	1.19E-04
rs10262140	7	27222989	intergenic	HOXA13	G	A	0.04	1.61	0.49	0.79	8.63E-05	1.19E-04
rs11755444	6	7599162	intergenic	C6orf151	G	A	0.12	1.34	1.16	1.54	8.65E-05	1.20E-04
rs10436976	1	210427031	intergenic	LOC643143	G	A	0.38	1.22	1.10	1.34	8.73E-05	1.21E-04
rs9355841	6	161187325	intergenic	PLG	G	A	0.46	1.21	0.75	0.91	8.73E-05	1.21E-04

Genome wide association results for hypertension-associated SNPs with pvalue < 1e-04 sorted by hypertension analysis												
SNP	**CHR**	**Position (bp)**	**Location**	**Nearest gene**	**Coded Allele**	**Not coded Allele**	**MAF**	**OR**	**Lower limit, CI 95**	**Upper limit, CI 95**	**P**	**P adjusted for GC**
rs1275528	2	27284285	intronic	SLC5A6	G	A	0.29	1.24	0.73	0.90	8.79E-05	1.21E-04
rs10516593	4	114298261	intronic	ANK2	C	A	0.43	1.22	0.74	0.91	8.83E-05	1.22E-04
rs17728780	6	79019012	intergenic	IRAK1BP1	A	G	0.08	1.43	1.20	1.71	8.83E-05	1.22E-04
rs4758928	12	132109189	intergenic	ZNF26	A	G	0.03	1.81	1.35	2.44	8.84E-05	1.22E-04
rs167132	5	57941231	intronic	RAB3C	A	C	0.20	1.27	0.70	0.89	8.97E-05	1.24E-04
rs2042525	1	215805659	intronic	GPATCH2	G	A	0.22	1.25	1.12	1.40	9.02E-05	1.25E-04
rs472174	18	11554905	intergenic	AMAC1L1	G	A	0.17	1.29	0.68	0.88	9.08E-05	1.25E-04
rs4485303	15	91891039	intergenic	LOC390641	G	A	0.08	1.43	0.59	0.84	9.10E-05	1.26E-04
rs1035421	5	167700692	intronic	WWC1	G	A	0.27	1.24	1.11	1.38	9.21E-05	1.27E-04
rs10741150	10	128669948	intronic	DOCK1	G	A	0.38	1.22	0.74	0.91	9.22E-05	1.27E-04
rs722212	4	11004173	intergenic	HS3ST1	G	A	0.16	1.30	0.68	0.88	9.29E-05	1.28E-04
rs1044988	3	197446082	intergenic	OSTalpha	G	A	0.19	1.27	1.13	1.43	9.38E-05	1.29E-04
rs4665958	2	27296700	intronic	CAD	A	G	0.29	1.24	0.73	0.90	9.54E-05	1.32E-04
rs1940604	18	5903606	intergenic	LOC729309	G	A	0.10	1.37	0.62	0.86	9.63E-05	1.33E-04
rs4425356	4	175819559	intronic	GLRA3	G	A	0.35	1.22	1.11	1.35	9.63E-05	1.33E-04
rs10985085	9	122705118	3UTR	TRAF1	A	G	0.06	1.50	1.22	1.84	9.94E-05	1.37E-04

Table S4. List of Best discovery results (p-value adjusted for Genomic Control< 10^{-4}). To retrieve information about SNPs and their genomic context (the nearest gene) we used the hg18 (NCBI 36) assembly.

SNP	Alleles (coded / other)	CHR	GENE	STUDY (SAMPLE)	OR	P VALUE	OR HYPERGENES	P HYPERGENES	OR COMBINED	P COMBINED
rs2681472	A/G	12	ATP2B1	Levy et Al [32] (CHARGE)	1.17	1.65E-08	1.14	4.00E-02	1.16	1.89E-09
rs11105354	G/A	12	ATP2B1	Levy et Al [32] (CHARGE)	0.85	1.80E-08	0.88	3.79E-02	0.86	1.97E-09
rs2681492	G/A	12	ATP2B1	Levy et Al [32] (CHARGE)	0.87	8.40E-08	0.88	4.04E-02	0.87	9.76E-09
rs11105328	G/A	12	WDR51B	Levy et Al [32] (CHARGE)	0.86	7.10E-07	0.90	8.74E-02	0.87	1.56E-07
rs653178	A/G	12	ATXN2	Newton-Cheh et Al[33] (Global Bpgen)	0.93	7.00E-07	0.87	2.81E-03	0.93	1.69E-08
rs17367504	A/G	1	MTHFR	Newton-Cheh et Al[33] (Global Bpgen)	0.89	2.00E-09	0.89	1.39E-01	0.89	7.19E-10
rs1378942	C/A	15	CSK	Newton-Cheh et Al[33] (Global Bpgen)	1.1	2.00E-14	1.03	5.60E-01	1.06	8.80E-14
rs13333226	G/A	16	UROMOD	Padmanabhan et Al[34]	0.85	1.50E-13	0.84	8.72E-03	0.85	4.88E-15

Table S5. meta-analysis of top SNPs previously published in literature as associated with Hypertension in Genome-wide association studies and HYPERGENES results.

rsSNP1-allele	Gene1	rsSNP2-allele	Gene2	Interaction effect size	Interaction p-value (20% FDR)
rs13447427-G	ACTB	rs3918226-T	NOS3	0.47	1.34E-03
rs7503750-G	ACTG1	rs3918226-T	NOS3	-0.42	1.57E-03
rs4922796-G	HSP90AA2	rs3918226-T	NOS3	-0.36	3.47E-03
rs17309979-G	HSP90AA2	rs3918226-T	NOS3	0.35	4.88E-03

Table S6. Interaction analysis of *ACTB* (Beta Actin), *ACTG1* (Gamma 1 Actin) and *HSP90AA2* (heat shock protein 90kDa alpha (cytosolic) with eNOS. Interacting alleles, effects size and P-values are reported. The reported SNPs were selected, controlling the False discovery Rate at 20%.

A.

SBP			
rs3918226	Mean	Std. Dev.	Freq.
GG	125.4	16.2	1523
GT	126.7	16.7	276
TT	131.9	19.2	21
Total	125.7	16.4	1820

B.

DBP			
rs3918226	Mean	Std. Dev.	Freq.
GG	78.7	10.6	1523
GT	79.6	10.7	276
TT	85.3	12.6	21
Total	78.9	10.7	1820

Table S7. BP distribution according to rs3918226 genotype. The table reports the mean values, the standard deviations and the frequencies of SBP (**A**) and DBP (**B**) for each genotype. In the linear regression analysis, accounting for sex, bmi, age and age^2, we obtained a β coefficient of 1.91 (95% CI 0.16-3.66, p-value= 0.032) for SBP (**A**) and of 1.40 (95% CI 0.25-2.55, p-value=0.017) for DBP (**B**), respectively.

Marker Name	Alleles	OR (Discovery)	P (Discovery)	OR (Validation)	P (Validation)	OR (Combined)	CI (Combined)	Inverse variance weighted P (Combined)	Z-score P (Combined)
rs2070744	C/T	1.088	9.40E-02	1.162	7.51e-03	1.04	1.038-1.041	6.88E-04	6.42E-04
rs3918226	T/C	1.425	4.81E-06	1.71	2.55E-09	1.538	1.372-1.726	1.98E-13	2.58E-13
rs1799983	T/G	1.202	4.08E-04	1.068	2.66E-01	1.038	1.034-1.043	2.40E-03	2.63E-03

Table S8. Meta-analysis results for eNOS polymorphisms: rs2070744 (T-786C, 150321012 bp), rs3918226 (T-665C, 150321109 bp) and rs1799983 (G894T, 150327044 bp). The SNPs are ranked by position (BP). rs2070744 has a Minor Allele Frequency (MAF) of 0.47 in discovery and 0.45 in validation, rs3918226 has a MAF of 0.12 both in discovery and in validation and rs1799983 of 0.41 in discovery and 0.40 in validation. The table shows association results (OR and p-values) for Discovery (n=3615), Validation (n=2610) 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.

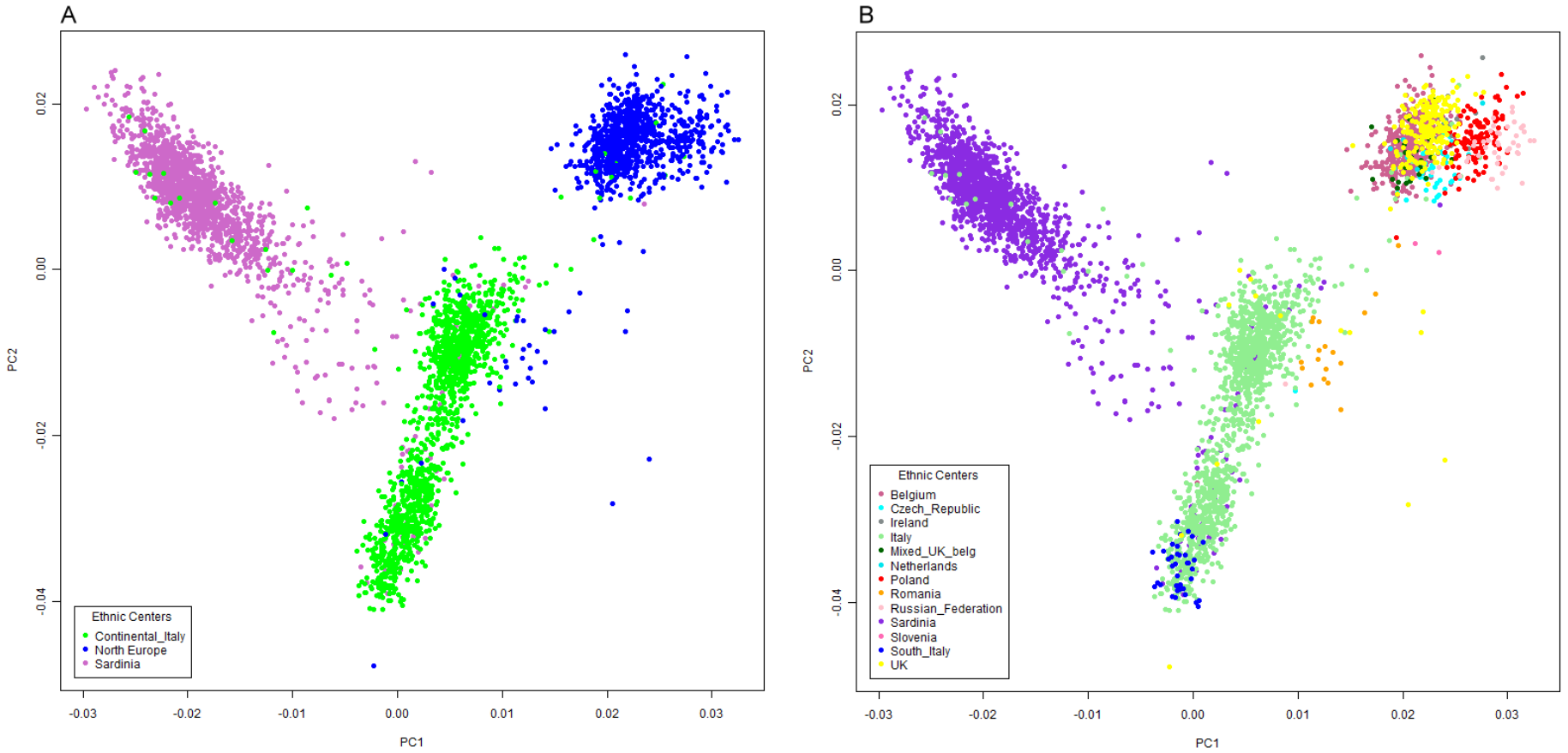

Figure S1. Principal component plot of discovery sample. A. Samples are represented as macro-groups: North Europe in blue, Continental Italy in green and Sardinia in purple. **B:** Samples are marked according to the recruitment centres.

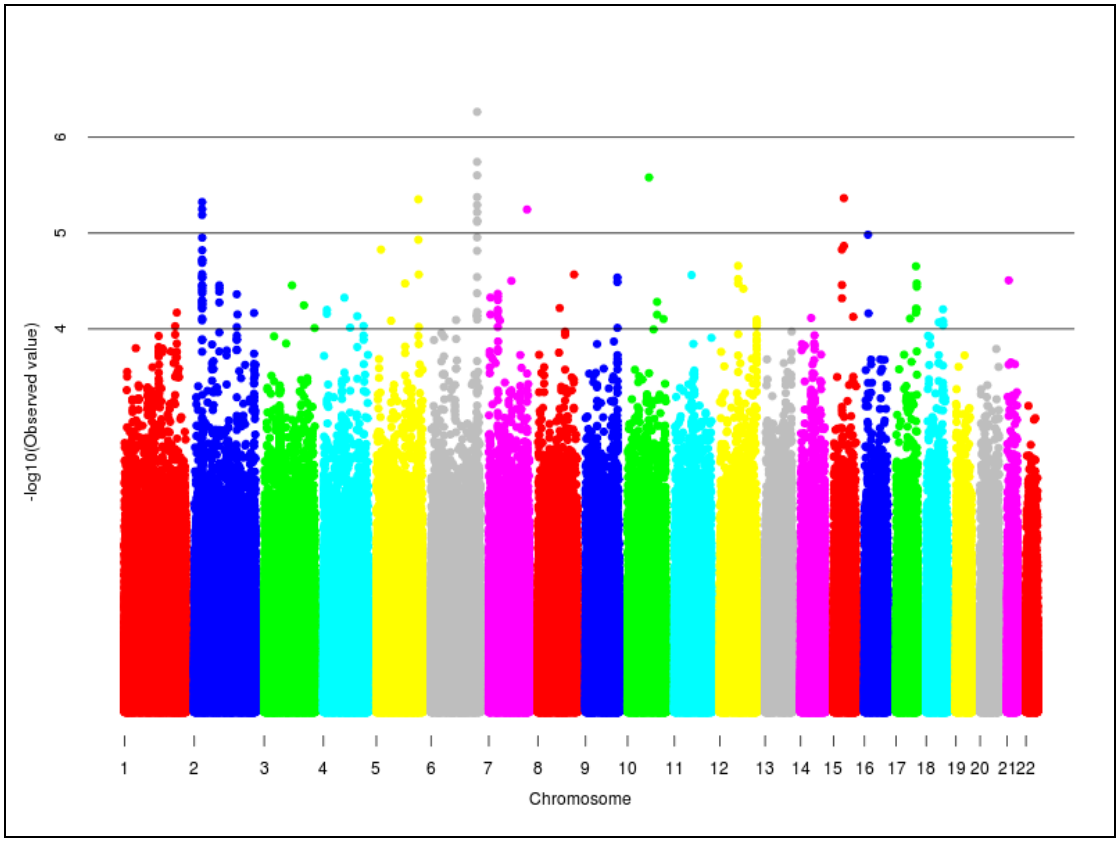

Figure S2. Manhattan plot of single SNP logistic regression test statistics, adjusted for gender and PCs, in discovery GWA analysis. Results are reported as –log10 (P-value) by genomic position.

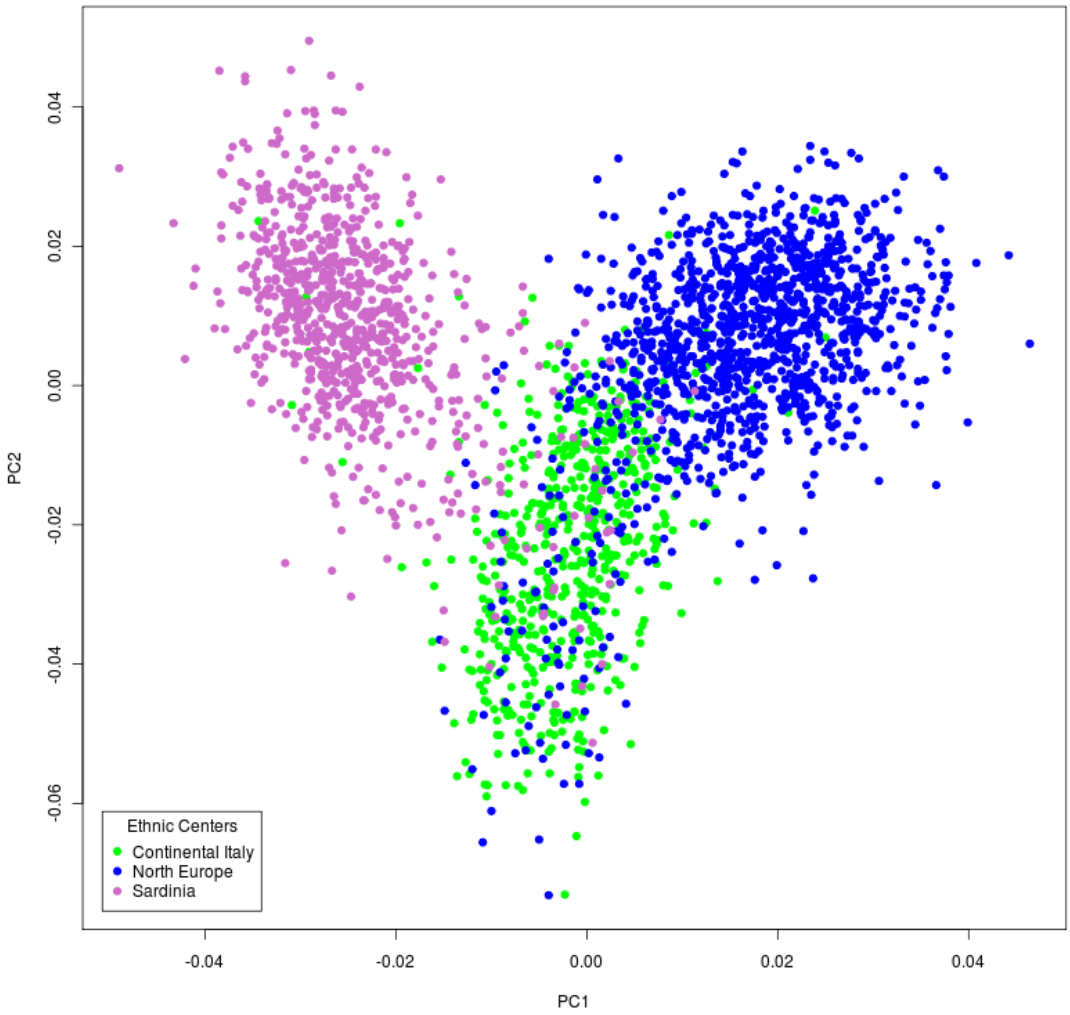

Figure S3. Principal component plot of Validation sample. Samples are represented as macro-groups: North Europe in blue, Continental Italy in green and Sardinia in purple.

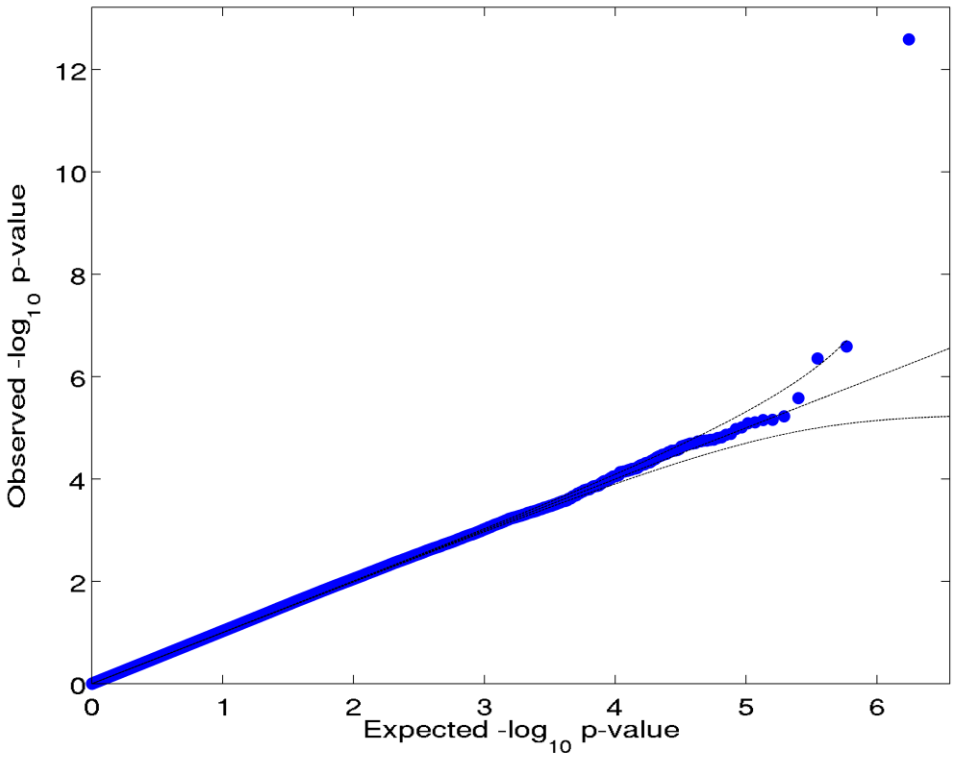

Figure S4: Q-Q plot of combined analysis.

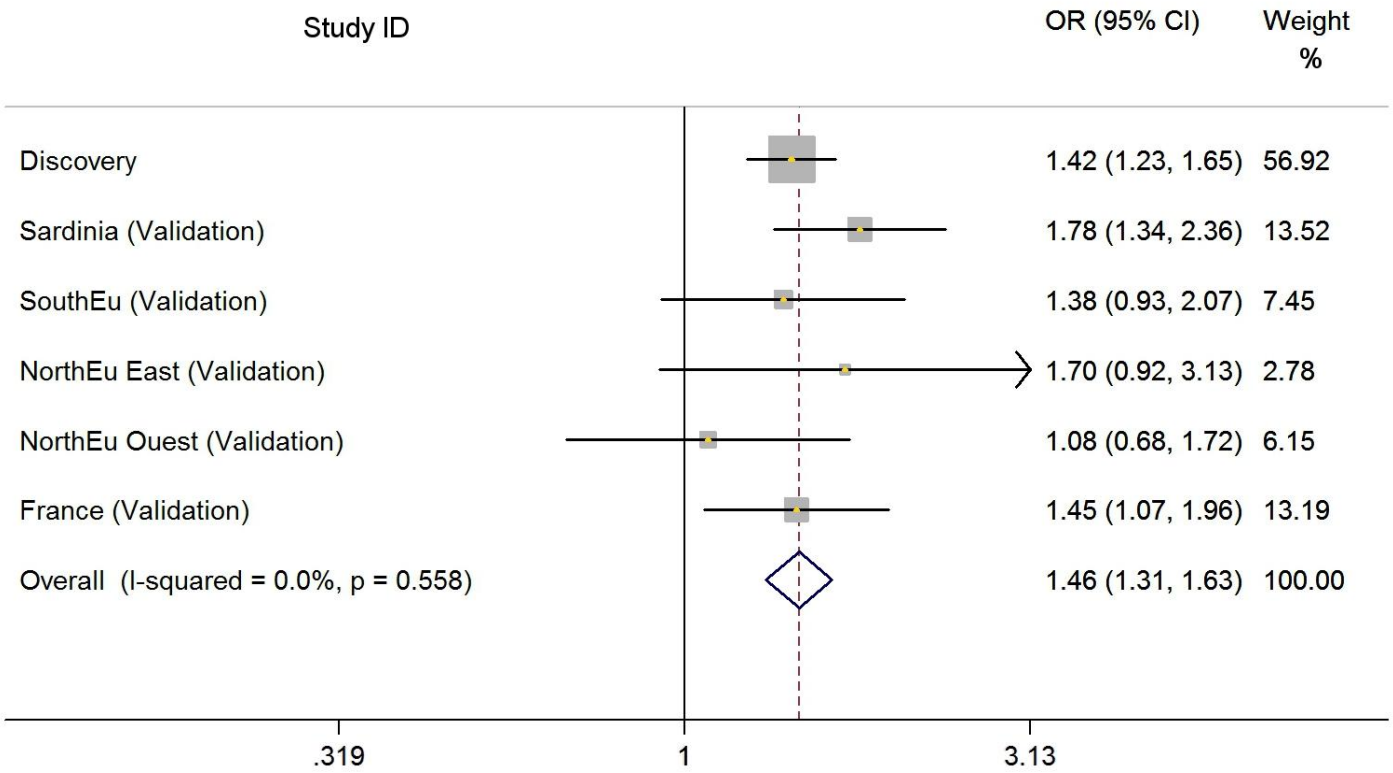

Figure S5: Forest plot of meta-analysis between discovery samples and Validation sub-samples. The Validation sample is divided in Sardinia, Continental Italy, EPOGH, Progress with Victorian Family Heart Study cohort and France subgroups. The squares and the horizontal lines correspond to the OR and 95% CI, and the diamond represents the combined OR and 95% CI. The test of heterogeneity was applied (I-squared and p-value).

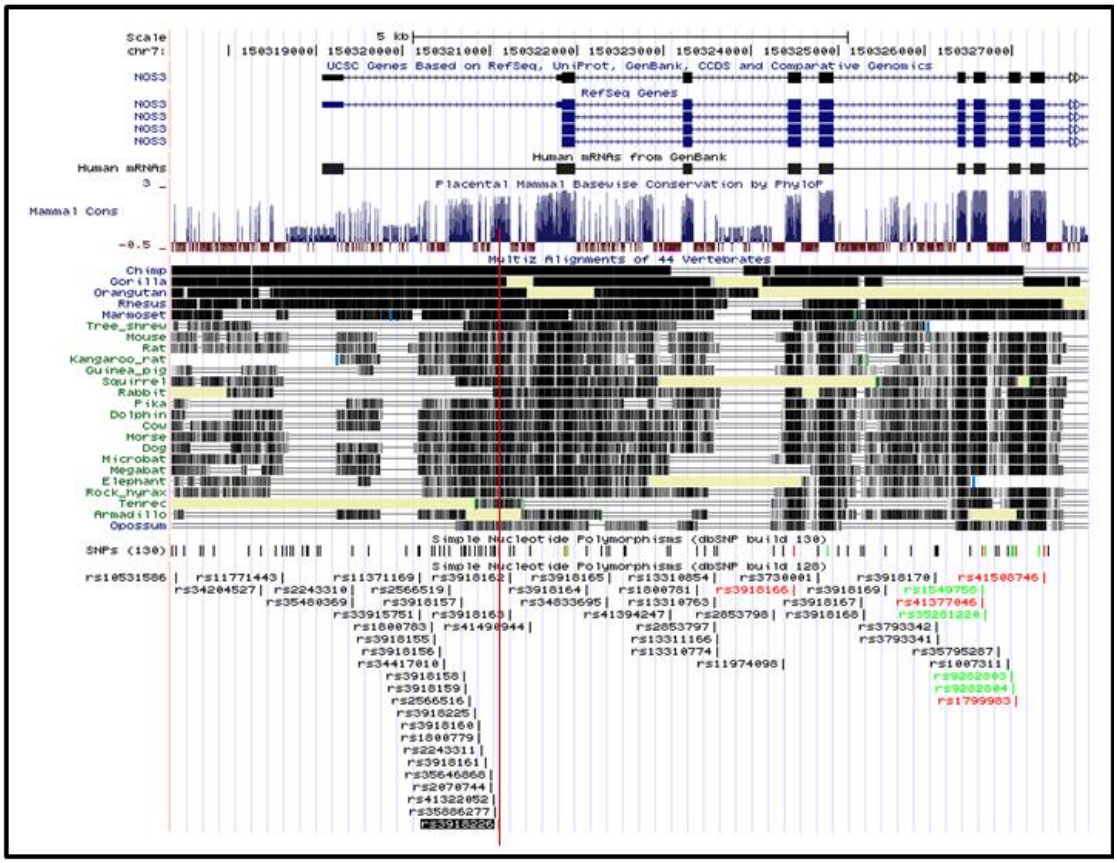

Figure S6. Conservation of eNOS3 region. Measure of evolutionary conservation in Primate and Placental Mammals using the conservation track of UCSC genome browser. The red line represents the position of rs3918226. The black rectangles underneath indicate that the locus is conserved along the different species.