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ΕΡΓΑΣΤΗΡΙΟ ΒΙΟΜΑΘΗΜΑΤΙΚΩΝ
ΤΜΗΜΑ ΙΑΤΡΙΚΗΣ

ΔΙΠΛΩΜΑΤΙΚΗ ΕΡΓΑΣΙΑ:
«ΑΞΙΟΛΟΓΗΣΗ ΤΩΝ ΜΕΛΕΤΩΝ ΓΕΝΕΤΙΚΗΣ ΣΥΣΧΕΤΙΣΗΣ ΤΟΥ ΠΟΛΥΜΟΡΦΙΣΜΟΥ ADRB1 p.Arg389Gly ΜΕ ΤΗΝ ΥΠΕΡΤΑΣΗ ΜΕΣΩ ΤΗΣ ΔΗΛΩΣΗΣ STREGA»

ΕΠΙΒΛΕΠΩΝ ΚΑΘΗΓΗΤΗΣ: Ι. ΣΤΕΦΑΝΙΔΗΣ
ΑΞΙΟΛΟΓΗΤΕΣ: Ι. ΣΤΕΦΑΝΙΔΗΣ-Ε. ΖΙΝΤΖΑΡΑΣ-Γ. ΧΑΤΖΗΓΕΩΡΓΙΟΥ

Ονοματεπώνυμο φοιτητή: ΔΗΜΗΤΡΗΣ ΜΠΑΣΔΕΚΗΣ

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MASTER THESIS:
«ASSESSMENT OF THE GAS FOR THE VARIANT ADRB1 p.Arg389Gly IN HYPERTENSION USING THE STREGA STATEMENT »

SUPERVISOR: I. STEFANIDIS
EVALUATORS: I. STEFANIDIS-E.ZINTZARAS-G. HATZIGEORGIOU

Student's name: DIMITRIS MPASDEKIS

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ABSTRACT

A meta-analysis titled as “b1-adrenoceptor gene Arg389Gly polymorphism and essential hypertension risk in general population: a meta-analysis” was used as basis of our study. The meta-analysis was published in 2013 on Mol Biol Rep and included eight articles, published before June 2012. Also, we searched the PubMed database for other studies published after June 2012, but nothing else returned as result.

Our goal was to assess the studies included in the above meta-analysis by using the STREGA Statement. Nine case-controls or cross-sectional studies were assessed for their conformity to the recommendations of the STREGA Statement.

Finally, a qualitative overall analysis was performed. The results indicated high degree of conformity to the STREGA Statement concerning to Hardy-Weinberg equilibrium, selection criteria of participants, statistical methods and reporting of software used, reporting of descriptive and outcome data, source and method genotyping of DNA and statement for replication. Less encouraging findings concern to population stratification and number of successful genotyping, On the contrary, low conformity was revealed for the genotyping errors, modelling haplotype variation and control for relatedness.

In conclusion, the Genetic Association Studies (GAS) for the variant ADRB1 p.Arg389Gly in hypertension might be improved in reference to the STREGA Statement, for greater transparency and more reliability.

INTRODUCTION

HYPERTENSION AND VARIANT ADRB1 p.Arg389Gly

Hypertension is one of the most common chronic illnesses effecting more than one billion people worldwide and is one of the primary risk factors for coronary artery disease and myocardial infarction, heart failure, stroke and renal failure. By the year 2025, the global prevalence of hypertension is projected to increase to 29.2% in adult population (Kearney et al., 2005). It is well established that reduction in blood pressure is associated with decreased cardiovascular morbidity and mortality (Lewington, Clarke, Qizilbash, Peto, & Collins, 2002). Despite the availability of several antihypertensive drugs which include thiazide diuretics, beta blockers, Angiotensin-Converting Enzyme (ACE) inhibitors, angiotensin receptor blockers (ARB) and calcium channel blockers, global estimates suggest that less than 35% of hypertensives are able to achieve their target systolic and diastolic blood pressure with these drugs (Thoenes et al., 2009).

Essential hypertension (EH) is widely accepted as a multifactorial disorder caused by both genetic and environmental influences. Genetic factors have been reported to take up 20–60 %. Consequently, hypertension susceptibility genes have been being under exploration, to identify the candidate determinants of the risk for hypertension.

The adrenergic receptors (adrenoceptors) family genes have emerged as logical candidate genes for hypertension based on experimental evidence showing involvement of the SNS in hypertension and on positional cloning findings from genome -wide linkage studies. The adrenoceptors belong to the G-protein coupled receptors superfamily, which are integral membrane proteins with seven transmembrane helices, responsible for the signal transduction of a variety of extracellular signals. Neuronally released and circulating catecholamines bind to adrenoceptors to stimulate the intracellular signal transduction cascade and finally exert their biologic effect. The adrenoceptors family is sub-classified into *α*1-, *α*2- or *β*- adrenoceptors, although each of these classes has multiple subtypes so that a total of nine subtypes have been characterized: α1A-, α1B-, α1D-, α2A-, α2B-, α2C-, β1-, β2- and β3-adrenoceptors. Each of these adrenoceptors subtypes is coded by a separate gene (ADRA1A, ADRA1B, ADRA1D, ADRA2A, ADRA2B, ADRA2C, ADRB1, ADRB2 and *ADRB3*, respectively) and has a different tissue distribution and function. Overall, 2250 genetic variants have been annotated to the adrenoceptors family (Kitsios & Zintzaras, 2010).

The genetic candidate, that interests us, is the gene β1-adrenoceptor (ADRB1). The human β1-adrenoceptor (ADRB1) is a key cell surface signaling protein expressed in multiple organs and tissues including heart, kidney, brain and pineal gland, which

mediates the actions of catecholamines like epinephrine and norepinephrine in the sympathetic nervous system (SNS). The β1-adrenergic receptor (ADRB1) belongs to the family of guanine nucleotide binding regulatory protein coupled receptors (GPCRs). ADRB1 is expressed in the heart and mediates the physiological effects of catecholamines. The human β1-adrenergic receptor is encoded by an intronless gene with 45 aminoacids located on chromosome 10q24-262. Of the 73 polymorphisms of ADRB1 gene identified so far, 13 of these result in change of amino acid in the ADRB1 protein.

Two nonsynonymous single nucleotide polymorphisms (SNP) have been identified in ADRB1: Arg389Gly, which causes a substitution of arginine by glycine at amino acid position 389 and Ser49Gly, which replaces serine with glycine at position 49.

The following figure shows the structure of β1-Adrenoceptor single nucleotide polymorphisms.

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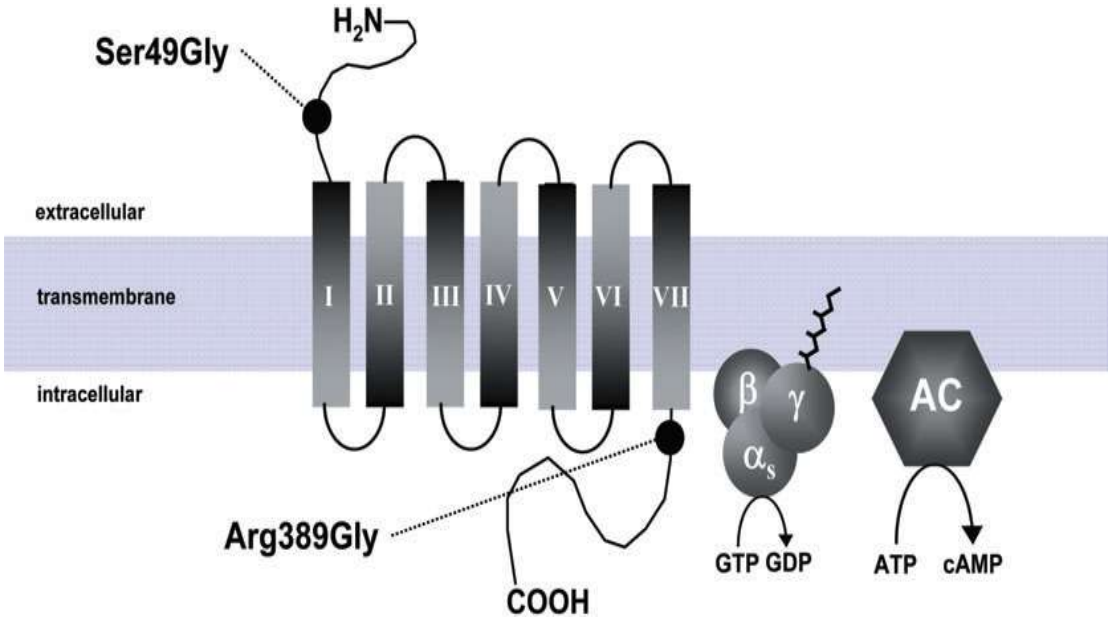

Figure 1: b1-Adrenoceptor single nucleotide polymorphisms.

AC : adenylyl cyclase
ATP : adenosine triphosphate
cAMP : cyclic adenosinemonophosphate
GDP : guanosine diphosphate
GTP : guanosine triphosphate

At the molecular level, the role of ADRB1 in EH has been extensively evaluated, with particular attention on Arg389Gly (rs1801253) single-nucleotide polymorphisms (SNP). However, the results of these genetic association studies(GAS) are controversialand inconclusive, possibly due to methodological limitations, including

inadequate samplesize, patient selection, ethnicity of the populations studied and lack of adjustments forconfounders.

THE STREGA STATEMENT

The rapidly evolving evidence on genetic associations is crucial to integrating human genomics into the practice of medicine and public health. Genetic factors are likely to affect the occurrence of numerous common diseases, and therefore identifying and characterizing the associated risk (or protection) will be important in improving the understanding of etiology and potentially for developing interventions based on genetic information.Assessment of the strengths and weaknesses of this evidence, and hence the ability to synthesize it, has been limited by inadequate reporting of results. The number of publications on the associations between genes and diseases has increased tremendously; with more than 34000 published articles, the annual number has more than doubled between 2001 and 2008. Articles on genetic associations have been published in about 1500 journals and in several languages.

The quality of reporting genetic association studies needs to be improved. Lack of transparency and incomplete reporting have raised concerns in a range of health research fields and poor reporting has been associated with biased estimates of effects in clinical intervention studies. At first, the epidemiology community has developed the Strengthening the Reporting of Observational studies in Epidemiology (STROBE) Statement for cross-sectional, case-control and cohort studies. The recommendations of the STROBE Statement have a strong foundation because they are based on empirical evidence on the reporting of observational studies and they involved extensive consultations in the epidemiologic research community. Given the relevance of general epidemiologic principles for genetic association studiesand the fact that genes may operate in complex pathways with gene-environment and gene-gene interactions STREGA recommendations as an extension of the STROBE Statement were necessary. So, the epidemiology community has developed **The STrengthening the REporting of Genetic Association studies (STREGA),** with main goal to propose and justify a set of guiding principles for reporting results of genetic association studies.

The STrengthening the REporting of Genetic Association studies (STREGA) initiative builds on the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement and provides additions to 12 of the 22 items on the STROBE checklist. Five main areas of special interest that are specific to, or especially relevant in, genetic association studies are identified: genotyping errors, population stratification, modelling haplotype variation, HWE and replication. Each of these areas with the corresponding STREGA recommendations, is followed by an explanation for their necessity. Complementary information on these areas and the

rationale for additional STREGA recommendations relating to selection of participants, choice of genes and variants selected, treatment effects in studying quantitative traits, statistical methods, relatedness, reporting of descriptive and outcome data and issues of data volume, are given.

The additions concern: population stratification, genotyping errors, modelling haplotype variation, Hardy-Weinberg equilibrium, replication, selection of participants, rationale for choice of genes and variants, treatment effects in studying quantitative traits, statistical methods, relatedness, reporting of descriptive and outcome data and the volume of data issues that are important to consider in genetic association studies.
The STREGA recommendations do not prescribe or dictate how a genetic association study should be designed but seek to enhance the transparency of its reporting, regardless of choices made during design, conduct, or analysis.

Below, the five main areas of special interest of STREGA are described:

1. Genotyping Errors

Recommendation for reporting of methods (Table 1, item 8(b)): Describe laboratory methods, including source and storage of DNA, genotyping methods and platforms (including the allele calling algorithm used and its version), error rates and call rates. State the laboratory/center where genotyping was done. Describe comparability of laboratory methods if there is more than one group. Specify whether genotypes were assigned using all of the data from the study simultaneously or in smaller batches.

Recommendation for reporting of results (Table 1, item 13(a)): Report numbers of individuals in whom genotyping was attempted and numbers of individuals in whom genotyping was successful.

Genotyping errors can occur as a result of effects of the DNA sequence flanking the marker of interest, poor quality or quantity of the DNA extracted from biological samples, biochemical artefacts, poor equipment precision or equipment failure, or human error in sample handling, conduct of the array or handling the data obtained from the array. A commentary published in 2005 on the possible causes and consequences of genotyping errors observed that an increasing number of researchers were aware of the problem, but that the effects of such errors had largely been neglected.The magnitude of genotyping errors has been reported to vary between 0.5% and 30%. In high-throughput centers, an error rate of 0.5% per genotype has been observed for blind duplicates that were run on the same gel. This lower error rate reflects an explicit choice of markers for which genotyping rates have been found to be highly repeatable and whose individual polymerase chain

reactions (PCR) have been optimized. Non-differential genotyping errors, that is, those that do not differ systematically according to outcome status, will usually bias associations towards the null, just as for other non-differential errors. The most marked bias occurs when genotyping sensitivity is poor and genotype prevalence is high (>85%) or, as the corollary, when genotyping specificity is poor and genotype prevalence is low (<15%). When measurement of the environmental exposure has substantial error, genotyping errors of the order of 3% can lead to substantial underestimation of the magnitude of an interaction effect. When there are systematic differences in genotyping according to outcome status (differential error), bias in any direction may occur. Unblinded assessment may lead to differential misclassification. For genome-wide association studies of SNPs, differential misclassification between comparison groups (for example, cases and controls) can occur because of differences in DNA storage, collection or processing protocols, even when the genotyping itself meets the highest possible standards. In this situation, using samples blinded to comparison group to determine the parameters for allele calling could still lead to differential misclassification. To minimize such differential misclassification, it would be necessary to calibrate the software separately for each group. The recommendation is to specify whether genotypes were assigned using all of the data from the study simultaneously or in smaller batches.

2. Population Stratification

Recommendation for reporting of methods (Table 1, item 12(h): Describe any methods used to assess or address population stratification.

Population stratification is the presence within a population of subgroups among which allele (or genotype; or haplotype) frequencies and disease risks differ. When the groups compared in the study differ in their proportions of the population subgroups, an association between the genotype and the disease being investigated may reflect the genotype being an indicator identifying a population subgroup rather than a causal variant. In this situation, population subgroup is a confounder because it is associated with both genotype frequency and disease risk. The potential implications of population stratification for the validity of genetic association studies have been debated. Modelling the possible effect is likely to be small in most situations. Meta-analyses of 43 gene-disease associations comprising 697 individual studies showed consistent associations across groups of different ethnic origin and thus provide evidence against a large effect of population stratification, hidden or otherwise. However, as studies of association and interaction typically address moderate or small effects and hence require large sample sizes, a small bias arising from population stratification may be important. Study design (case-family control studies) and statistical methods have been proposed to address population stratification, but so far few studies have used these suggestions. Most of the early

genome-wide association studies used family-based designs or such methods as genomic control and principal components analysis to control for stratification. These approaches are particularly appropriate for addressing bias when the identified genetic effects are very small (odds ratio <1.20), as has been the situation in many recent genome-wide association studies. In view of the debate about the potential implications of population stratification for the validity of genetic association studies, we recommend transparent reporting of the methods used, or stating that none was used to address this potential problem. This reporting will enable empirical evidence to accrue about the effects of population stratification and methods to address it.

3. Modelling Haplotype Variation

Recommendation for reporting of methods (Table 1, item 12(g):Describe any methods used for inferring genotypes or haplotypes.

A haplotype is a combination of specific alleles at neighbouring genes that tends to be inherited together. There has been considerable interest in modelling haplotype variation within candidate genes. Typically, the number of haplotypes observed within a gene is much smaller than the theoretical number of all possible haplotypes. Motivation for utilizing haplotypes comes, in large part, from the fact that multiple SNPs may “tag” an untypical variant more effectively than a single typed variant. The subset of SNPs used in such an approach is called “haplotype tagging” SNPs. Implicitly, an aim ofhaplotype tagging is to reduce the number of SNPs that have to be genotyped, while maintaining statistical power to detect an association with the phenotype. Maps of human genetic variation are becoming more complete, and large scale genotypic analysis is becoming increasingly feasible. In consequence, it is possible that modelling haplotype variation will become more focused on rare causal variants, because these may not be included in the genotyping platforms

4. Hardy-Weinberg Equilibrium

Recommendation for reporting of methods (Table 1, item 12(f)):State whether Hardy-Weinberg equilibrium was considered and, if so, how.

Hardy-Weinberg equilibrium has become widely accepted as an underlying model in population genetics after Hardy and Weinberg proposed the concept that genotype frequencies at a genetic locus are stable within one generation of random mating; the assumption of HWE is equivalent to the independence of two alleles at a locus. Views differ on whether testing for departure from HWE is a useful method to detect errors or peculiarities in the data set, and also the method of testing In particular, it has been suggested that deviation from HWE may be a sign of genotyping errors. Testing for departure from HWE has a role in detecting gross errors of genotyping in

large-scale genotyping projects such as identifying SNPs for which the clustering algorithms used to call genotypes have broken down. However, the statistical power to detect less important errors of genotyping by testing for departure from HWE is low and, in hypothetical data, the presence of HWE was generally not altered by the introduction of genotyping errors. Furthermore, the assumptions underlying HWE, including random mating, lack of selection according to genotype, and absence of mutation or gene flow, are rarely met in human. Moreover, exclusion of HWE-violating studies may result in loss of the statistical significance of some postulated gene-disease associations and that adjustment for the magnitude of deviation from the model may also have the same consequence for some other gene-disease associations. Given the differing views about the value of testing for departure from HWE and about the test methodstransparent reporting of whether such testing was done and, if so, the method used, is important for allowing the empirical evidence to accrue.

5. Replication

Recommendation (Table 1, item 3): State if the study is the first report of a genetic association, a replication effort, or both.

Articles that present and synthesize data from several studies in a single report are becoming more common. In particular, many genome-wide association analyses describe several different study populations, sometimes with different study designs and genotyping platforms, and in various stages of discovery and replication. When data from several studies are presented in a single original report, each of the constituent studies and the composite results should be fully described. For example, a discussion of sample size and the reason for arriving at that size would include clear differentiation between the initial group (those that were typed with the full set of SNPs) and those that were included in the replication phase only (typed with a reduced set of SNPs).Describing the methods and results in sufficient detail would require substantial space in print, but options for publishing additional information on the study online make this possible.

The STREGA Statement has several strengths. First, it is based on existing guidance on reporting observational studies (STROBE). Second, it was developed from discussions of an interdisciplinary group that included epidemiologists, geneticists, statisticians, journal editors, and graduate students, thus reflecting a broad collaborative approach in terminology accessible to scientists from diverse disciplines. Finally, it explicitly describes the rationale for the decisions (Table 2) and has a clear plan for dissemination and evaluation.

Adherence to the recommendations may make some manuscripts longer, and this may be seen as a drawback in an era of limited space in a print journal. However, the

ability to post information on the Web should alleviate this concern. The place in which supplementary information is presented can be decided by authors and editors of the individual journal.

As basis of the aforementioned was used the article with the title: "STrengthening the REporting of Genetic Association Studies (STREGA) – An Extension of the STROBE Statement", that was published in 2009 by a team of significant scientists and researchers from various centers and countries. The workshop team consisted of Julian Little, Julian P.T. Higgins, John P.A. Ioannidis David Moher, France Gagnon, Erik von Elm, Muin J. Khoury, Barbara Cohen, George Davey-Smith, Jeremy Grimshaw, Paul Scheet, Marta Gwinn, Robin E. Williamson, Guang Yong Zou, Kim Hutchings, Candice Y. Johnson, Valerie Tait, Miriam Wiens, Jean Golding, Cornelia van Duijn, John McLaughlin, Andrew Paterson, George Wells, Isabel Fortier, Matthew Freedman, Maja Zecevic, Richard King, Claire Infante-Rivard, Alex Stewart, Nick Birkett.

The head of team was Julian Little, Canada Research Chair in Human Genome Epidemiology, from Department of Epidemiology and Community Medicine University of Ottawa.

In order to encourage dissemination of the STREGA Statement, the article has also been published by the *Annals of Internal Medicine, European Journal of Epidemiology, European Journal of Clinical Investigation, Genetic Epidemiology, Human Genetics, Journal of Clinical Epidemiology as well as PLoS Medicine*. The article has been placed in the public domain and can be freely reproduced, distributed, transmitted, modified, built upon, or otherwise used by anyone for any lawful purpose.

The STREGA recommendations are available at www.strega-statement.org

METHODS

Selection of studies

A meta-analysis titled as **"b1-adrenoceptor gene Arg389Gly polymorphism and essential hypertension risk in general population: a meta-analysis"** was used as basis of our study. It was performed by Hao Wang, Jielin Liu, Kuo Liu, Ya Liu, Zuoguang Wang, Yuqing Lou, QiuliNiu, Wei Gu, Lijuan Wang, Mei Li, Xiaoling Zhu and Shaojun Wen and was published in 2013 on MolBiol Rep. In this meta-analysis, eight (8) articles, published before June 2012, were included.

Also, we searched the PubMed database for other studies published after June 2012, but nothing else returned as result.

Our goal was to assess the genetic association studies (GAS) included in the above meta-analysis by using the STREGA statement.

The articles included in the meta-analysis were:

1. Bengtsson K, Melander O, Orho-Melander M et al (2001) Polymorphismin the beta(1)-adrenergic receptor gene and hypertension. *Circulation 104:187–190*

2. Filigheddu F, Reid JE, Troffa C et al (2004) Genetic polymorphismsof the beta-adrenergic system: association with essentialhypertension and response to beta-blockade. *Pharmacogenomics J 4:154–160*

3. Nieminen T, Lehtimaki T, Laiho J et al (2006) Effects of polymorphisms in β1-adrenoceptor and α-subunit of G protein on heart rate and blood pressure during exercise test. The FinnishCardiovascular Study. *J Appl Physiol 100:507–511*

4. Hu R, Zhao S, Niu G et al (2006) Association between essentialhypertension and polymorphisms of beta 1 adrenergic receptorgene G1165C (Gly389Arg) in Chinese Mongolian population.*Neural Regen Res 1:226–229*

5. Borgel J, Schulz T, Bartels NK et al (2006) Modifying effects ofthe R389G beta1-adrenoceptor polymorphism on resting heartrate and blood pressure in patients with obstructive sleep apnoea. *Clin Sci (Lond) 110:117–123*

6. Gjesing AP, Andersen G, Albrechtsen A. et al (2007) Studies ofassociations between the Arg389Gly polymorphism of the beta1-adrenergic receptor gene (ADRB1) and hypertension and obesityin 7677 Danish white subjects. *Diabet Med 24:392–397*

7. Ramu P, Rajan S, Shewade DG et al (2009) Genetic variants ofbeta(1)-adrenoceptor gene polymorphisms (Ser49Gly andArg389Gly) and essential hypertension in a south Indian Tamilpopulation. *Clin Exp Pharmacol Physiol 36:576–582*

8. Peng Y, Xue H, Luo L et al (2009) Polymorphisms of the beta1-adrenergic receptor gene are associated with essential hypertensionin Chinese. *Clin Chem Lab Med 47:1227–1231*

Studies' abstracts

Initially, we present an abstract for each study. In all studies, hypertension wasdefined as: systolic blood pressure (SBP)>140 mmHg and/or diastolic blood pressure (DBP)>90 mmHg and/ortreatment with anti-hypertensive medicationaccording to World Health Organization (WHO) criteria, except the study by Bengtsson et al., in which the diagnostic standards were 160/90 mmHg for hypertensive patients and150/80 mmHg for normotensive controls, whichwere differentfrom other eligible studiesand likely this fact leads to heterogeneity, as the meta-analysis showed. Also, in one of the studies [6], both criteria for hypertension were used but there was no significant difference observed. Five of the articles were referring to Europeans (Italians, Scandinavians (2), Danish, Germans) and three of them in Asians (Mongolians, Chinese and Indians).

In all studies, the protocols were approved by the local competent Ethical Committees, and all patients gave informed consent before the study initiation, as stipulated in the Declaration of Helsinki.

1. Bengtsson K., Melander O., Orho-Melander M. et al (2001) **Polymorphismin the beta(1)-adrenergic receptor gene and hypertension**. *Circulation 104:187–190*

The present study is the first study that investigated whether the Arg389Gly polymorphism is associated with hypertension. It was conducted in Scandinavians. 292 unrelated, nondiabetic, hypertensive patients and 265 unrelated healthy controlsubjects from Swedish were included in a case-control association study. Allele and genotype frequencies of the Arg389Gly polymorphisms were compared between hypertensive patients and normotensive control subjects.

The Arg389 allele and the Arg389Arg genotype of the b_1-adrenergic receptor gene were more common in patientswith hypertension than in controls.The age-, sex- and body mass index- adjusted odds ratio for hypertension in subjects homozygous for the Arg389 allele was 1.9 (95% confidence interval=1.3 to 2.7; P=0.0005) when compared with carriers of one or two copies of the Gly389 allele. SBP, DBP did not differ between carriers of the different Arg389Gly genotypes within the treated hypertension group or within the control group.

Also, for confirmation of results, a genotype-discordant sibling pair analysis was performed on 102 nondiabetic sibling pairs without antihypertensive medication. This genotype-discordant sibling pair analysis revealed that siblings homozygous for the Arg389 allele had significantly higher diastolic blood pressures DBP (P= 0.003) than siblings carrying one or two copies of the Gly389 allele.
In conclusion, data suggest that individuals homozygous for the Arg389 allele of theb_1-adrenergic receptor gene are at increased risk to develop hypertension. This is the single study, proposing positive association between the Arg389Arg genotype and hypertension. So, in the sensitivity analysis of the aforementioned meta-analysis this is a source of heterogeneity.

2. Filigheddu F, Reid JE, Troffa C et al (2004) **Genetic polymorphisms of the beta-adrenergic system: association with essential hypertension and response to beta-blockade**. *Pharmacogenomics J 4:154–160*

The objectives of the present study were:
(1) to investigate the association of known polymorphisms of b1ARs(as R389G^{b1AR}) with EH in a cohort of genetically homogeneous EH patients and normotensive controls (case–control study)
(2) to analyze the BP fall after 4 and 8 weeks of therapy with b-blockers according to genotype (pharmacogenetics study) in essential hypertensive patients never treated or untreated for at least 6 months.
So, the study consists of two phases:a case-control study and a cohort pharmacogenetics study.
In the case-control study, 526 cases (hypertensive patients) from the Hypertension and Cardiovascular Prevention Center, Sassari, Italy were recruited. Controls were 192 normotensives white, unrelated North Sardinians. Due to laboratory failures, the final numbers of individuals in whom genotyping was successful were: 506 cases and 175 controls. Cases and controls were not matched by age and BMI. So, stratification for gender and including these variables in the regression models were used to address this problem.
In the pharmacogenetics study, 270 patients were treated with atenolol and the BP fall after 4 and 8 weeks of therapy was examined according to genotype.
In conclusion, no association between *ADRB1 Arg389Gly*(R389G^{b1AR})polymorphisms at the b1AR and EH was found, even considering gender specificity. Also no effect of the R389G^{b1AR}to the BP fall after 4 and 8 weeks of therapy with b-blockers was found in essential hypertensive patients never treated or untreated for at least 6 months.

3. Nieminen T, Lehtimaki T, Laiho J et al (2006) **Effects of polymorphisms in β1-adrenoceptor and α-subunit of G proteinon heart rate and blood pressure**

during exercise test. The FinnishCardiovascular Study. *J ApplPhysiol 100:507–511*

In this study, it was tested whether the *Arg389Gly* polymorphism of the β_1-adrenergic receptor gene *ADRB1* modulates blood pressure responses during an exercise stress test. The study is a H-B study and the patients underwent exercise stress tests at the Tampere University Hospital between October 2001 and January 2003. Finally, 890 patients with technically successful exercise tests and acquired genotypic samples were included in the study (563 men and 327 women). The study population was analyzed as a whole and divided based on gender.
In all subjects, and in men and women separately, no statistically significant interaction was found for the *ADRB1 Arg389Gly* polymorphism in relation to SAP and DAP responses over the three study phases (rest, exercise, recovery) (RANOVA, *P*=0.10 for interaction in all analyses where age, BMI, and β-adrenergic antagonism were used as covariates). The *Gly389* homozygotes had higher maximal SAP during exercise than those with at least one *Arg389* allele; consequently, the change of SAP from the resting state to the maximal had the same pattern. Also, this polymorphism did not differentiate the blood pressure response during β-blocker treatment.

4. Hu R, Zhao S, Niu G et al (2006) **Association between essential hypertension and polymorphisms of beta 1 adrenergic receptorgene G1165C (Gly389Arg) in Chinese Mongolian population.** *Neural Regen Res 1:226–229*

The goal was to analyze the association between Arg3896Gly polymorphism and essential hypertension in Mongolian population, where the prevalence of hypertension, cerebrovascular diseases etc. are higher because of the influence of various factors (environmental, genetic, diet etc.).A cross-sectional study, with 239 Mongolian participants, in three groups (122 essential hypertension patients,51 subjects with simple high SBP and 117 subjects with normal BP) was carried out.
The distribution of the genotypes and alleles of the ADRB1 Arg389Gly polymorphism have no obvious differences between normotensives, patients with essential hypertension and subjects with simple high SBP. So, in Mongolians, the ADRB1 Arg389Gly polymorphism may be not a genetic mark of essential hypertension and simple high SBP.

5. Borgel J, Schulz T, Bartels NK et al (2006) **Modifying effects ofthe R389G beta1-adrenoceptor polymorphism on resting heartrate and blood pressure in patients with obstructive sleep apnoea.** *Clin Sci (Lond) 110:117–123*

OSA (obstructive sleep apnoea) stimulates sympathetic nervous activity and elevates resting blood pressure. In the present study in a cohort of untreated OSA patients,

the resting BP during the daytime was correlated with AHI (apnoea/ hypopnea index) and compared with patients with different genotypes of the β_1-adrenoreceptor R389G polymorphism. 309 untreated OSA patients were consecutively enrolled from 1999–2001 in the sleep laboratory of the Marienhospital Herne (Ruhr University Bochum, Germany). Arterial hypertension was diagnosed in 167 patients. So, the study was performed on 167 cases and 142 controls. Data showed that the R389R polymorphism is not associated with higher BP in untreated OSA patients.

Also, the impact of the the β_1-adrenoreceptorR389G polymorphism on the decline of BP in a subgroup of 148 patients during a 6-month follow-up period under CPAP (continuous positive airway pressure) therapy was examined. In this follow-up cohort, the R389G polymorphism of the β1-adreno-ceptor appeared to have an impact on BP response to CPAP therapy. Carriers of the Gly389 (homozygotes and heterozygotes) exhibited a greater and significant decrease in DBP and SBP during CPAP therapy. However, differences in the BP-lowering effects of CPAP therapy between the genotype groups (R389R, R389G and G389G) did not reach statistical significance.

6. Gjesing AP, Andersen G, Albrechtsen A et al (2007) **Studies of associations between the Arg389Gly polymorphism of the beta1-adrenergic receptor gene (ADRB1) and hypertension and obesity in 7677 Danish white subjects.** *Diabet Med 24:392–397*

Case-control study and quantitative trait analyses were carried outin 7677 Danish Caucasians who were genotyped for the Arg389Gly variant.

A case-control study exams the effect of Arg389Gly polymorphism on hypertension in Danish white subjects, in a relatively large-scale population.No association of the hypertension with allele frequencies (P = 0.3) or genotype distribution (P = 0.5) was found; however, in the quantitative trait analyses, individuals carrying the Gly allele had slightly but significantly lower diastolic and systolic blood pressure as well as a lower mean arterial blood pressure.So, the *ADRB1* Arg389Gly variant is most likely not to be a major contributor to the development of hypertension. However, it is possible that the Arg allele may contribute by causing a minor increase in systolic and diastolic blood pressure levels in a middle-aged white population, although the effect is not powerful enough to cause hypertension.

7. Ramu P, Rajan S, Shewade DG et al (2009) **Genetic variants of beta(1)-adrenoceptor gene polymorphisms (Ser49Gly and Arg389Gly) and essential hypertension in a south Indian Tamil population**. *Clin Exp Pharmacol Physiol 36:576–582*

The aim of the present study was to determine the association between β_1-adrenoceptor Arg389Glygene polymorphism and the susceptibility of individuals to

essential hypertension in a south Indian Tamil population. The participants were 438 unrelated essential hypertensive patients(cases) and 444 healthy volunteers (controls).
There were no significant differences in the genotypes and allele frequencies for Arg389Gly between hypertensive patients and controls. The adjusted ORs were 1.1(95% CI: 0.8–1.7, P=0.5) for Arg/Gly and 1.4 (95% CI: 0.8–2.3, P=0.2) for Gly/Gly. The results remained the same after sub-grouping the sample by gender.

8. Peng Y, Xue H, Luo L et al (2009) **Polymorphisms of the beta1-adrenergic receptor gene are associated with essential hypertensionin Chinese**. *Clin Chem Lab Med 47:1227–1231*

The goal of the present study was to investigate whether the functionally important Arg389Gly polymorphism of the *ADRB1* gene was associated with hypertensionin Chinese.The hypothesis was tested in two independent case-control studies, one comprised 481 patients with hypertension and 529 control subjects, and the other study comprised 212 patients and 325 control subjects. The controls were recruited from age- and gender-matched healthy subjects from the same city. This is the first investigation to test the association between Arg389Gly or Ser49Gly *ADRB1* polymorphisms with hypertension in two independent populations. All participants were of the Han ethnic group. The present studies found that the Arg389Gly polymorphism of *ADRB1* is associated with hypertension in Chinese. The first study showed that the Arg389Arg genotype of the *ADRB1* gene was associated with risk of hypertension with odds ratio (OR) 1.77, (95% confidence interval (CI) 1.09–2.98; p=0.008), and the association was replicated in the second independent population (OR 1.65, 95% CI 1.07–2.89, ps0.01). Also, hypertensive patients with Arg389Arg variants had significantly increased DBP, in both studies.

Data analysis

We attempted to assess the studies mentioned on the above articles by using the STREGA statement. Each studywas analyzed according to the recommendations of the STREGA Statement, item by item. This analysis is presented in separate table for each study, presented in the Appendix.Some articles include more than one studies [1, 2, 5,8].Only the case-controls and cross-sectional studies included in the mentioned above meta-analysis, were assessed using the STREGA Statement. An overall assessment of conformity to the recommendations of the STREGA Statement was performed and presented.We attempted to assess studies, in conformity to the five main areas of special interest of STREGA. Particularly, we examined whether the studies satisfied the twelve additional items of STREGA, but also the items of STROBE. Finally, a qualitative analysis was conducted.

RESULTS

Nine studies included in the aforementioned articles were assessed by using the STREGA Statement. We attempted to assess studies, in conformity to the five main areas of special interest of STREGA. Particularly, we examined whether the studies satisfied the twelve additional items of STREGA, but also the items of STROBE, as basis.

In most of the studies,laboratory methods, including source, extraction and storage of DNA, genotyping methods and platforms (including the allele calling algorithm used and its version) were described. On the contrary, no information for storage of DNA was given. Also, error rates and call rates of genotyping are not given except in one study [6]. In the study [2] the genotyping tests were duplicated, for excluding genomic errors and in the study [3] it was reported that negative and positive controls (known genotypes) and random duplicates were used as quality control.

The numbers of individuals in whom genotyping was attempted and numbers of individuals in whom genotyping was successful were reported in studies [2, 3, 6, 7]. Numbers of participants for each genotype category were reported in all studies.

The observed genotype frequencies in all studies, where population'sgenetic profile was documented, obeyed Hardy-Weinberg equilibrium (HWE). Besides, one of the inclusion criteria of the meta-analysis was genotype distribution among control populations must be in Hardy–Weinberg equilibrium.However, the genetic profile of the β_1-ADRB has not yet been documented for any Indian population; so, in the relevant study [7] no statement for HWE existed.

Software version used for the statistical analysis was stated in eight from those nine studies (89%). Only in one [5] of them, it was not stated and so deviation from this requirement was observed in this study. SPSS-in various editions- was used as software for the statistical analysis in six studies (78%), Intercooled Stata 7.0for Windows in one [2] (11%) and also NCSS 6.0.21i n one [1] (11%). The two last programs were used in the oldest studies [1, 2].

In most studies, the statement if the study is the first report of a genetic association, a replication effort or both was given. In cases, where no statement was expressed [5], the kind of the study was inferred.

No information for potential sources of bias and bias resulting from pharmacotherapy and addressing them was given in most studies, except studies

[2,3].

Population stratification was excluded in studies [2, 3, 7, 8a, 8b] by the same ethnic origin and the homogeneity of population, or by using statistical methods. In the rest studies, none was used to address this potential problem.

All studies, simply, reported unrelated participants but no method to address relatedness among subjects was presented.

In three studies [4, 8a, 8b] no information for funding was given. Likely, it is interesting to be mentioned that all these studies refer to Asians (Chinese and Mongolians). So, 33% of the studies were not in conformity with that crucial for transparency requirement

The findings of the assessment by the STREGA Statement are presented in the Table, below.

Table: Findings of the assessment by the STREGA Statement

STREGA recommendations	Number of studies in conformity	% of studies in conformity	Comments
Replication	7	78	[1,3,5,6, 8a, 8b]
Selection criteria	9	100	All
Genotyping			
Source	8	89	All except [6]
Storage	1	11	[2]
Genotyping Method/ platforms	8	89	All except [6]
Error and Call rate	2	22	[2,6]
Potential sources of bias	1	11	[3]
Potential bias resulting from pharmacotherapy	2	22	[2,3]
Effects of treatment	3	33	[2, 3, 5]
Software version used / settings chosen	8 9	89 100	All except [5]
Hardy-Weinberg equilibrium	8	89	All except [7]
Inferring genotypes or haplotypes	1	11	[7]
Population stratification	5	55	[2, 3, 7, 8a, 8b]
Control risk of false positive findings	2	22	[2,6]
Control of Relatedness among subjects	0	0	None
Number of successful genotyping	4	44	[2, 3, 6,7]
Information by genotype	8	89	All except [4]
Outcomes or numbers for each genotype category	9	100	All
Detailed results elsewhere	2	22	[1,2]
Funding	6	67	[1,2,3,5,6,7]

CONCLUSION

The assessment of Genetic Association Studies for the variant ADRB1 p. Arg389Gly in hypertension indicated both positive and negative findings concerning to conformity to the STREGA Statement.The degree of conformity varied from item to item of the STREGA table, still in the same area (i.e.genotyping).

High conformity (>75%) was observed for Hardy-Weinberg equilibrium, selection criteria of participants, statistical methods and reporting of software programs used, reporting of descriptive and outcome data (as outcomes for each genotype category, information by genotype), source and method genotyping of DNA and statement for replication or first effort.
Medium degree of conformity (40-75%) appeared in population stratification and reporting of number of successful genotyping,
On the other hand, low conformity (<40%) was revealed for the error rates and call rates of genotyping, the storage of DNA, the effects in studying quantitative traits, modelling haplotype variation and control for relatedness among subjects.

In conclusion, further improvement of GAS for the variant ADRB1 p. Arg389Gly in hypertension must be done, in reference to the STREGA recommendations. This is more necessary due to controversial results of several studies. This improvement will lead to greater transparency and more reliability.

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ABBREVIATIONS

ADRB1 :β_1-Adrenergic Receptor

AHI : Apnoea/ Hypopnea Index

BMI : Body Mass Indes

DAP : Diastolic Arterial Pressure

DBP : Diastolic Blood Pressure

EH : Essential Hypertension

GAS :Genetic Association Studies

MABP : Mean Arterial Blood Pressure

OSA : Obstructive Sleep Apnoea

SAP : Systolic Arterial Pressure

SBP : Systolic Blood Pressure

SNP : Single Nucleotide Polymorphism

STREGA: STrengthening the REporting of Genetic Association Studies

STROBE : Strengthening the Reporting of Observational Studies in Epidemiology

APPENDIX

TABLE 1: ASSESSMENT OF 1st STUDY

Item	Item number	Description	Conformity with STREGA
Title	1	**Polymorphism in the β_1-Adrenergic Receptor Geneand Hypertension**	
Introduction			
Background rationale	2	The Arg389 variant of theb_1-adrenergic receptor gene mediates a higher isoproterenol-stimulated adenylate activity than the Gly389 variant in vitro.	
Objectives	3	The aim of the present study was to investigate whether the functionally important Arg389Gly polymorphism of the b_1-adrenergic receptor gene was associated with hypertension in a case-control study. For confirmation of results, an additional independent sibling-pair study was performed. First attemption	YES
Methods			
Study design	4	Consists of two studies: <u>1st study: a case-control study.</u> It is the first study which examines the association between Arg389Gly and hypertension. For confirmation of results, an additional independent sibling-pair study was performed. <u>2nd study: Sibling-Pair Study</u> The local ethics committee approved the study, and written informed consent was obtained from all the participants	YES
Setting	5	All study subjects were from southern Sweden and participated in either the Skaraborg hypertension project (n5353) or in a family study (n5204)	No dates determined
Participants	6	<u>1st study: case-control study.</u>	

		Cases: 292 unrelated no diabetic hypertensive patients Selection criteria: (1) age at diagnosis of hypertension ≤60 years (2) presence of chronic antihypertensive treatment (3) absence of diabetes mellitus. *Controls:* 265 unrelated healthy subjects Selection criteria: (1) age at the time of the study ≥40 years (2) SBP≥150 mm Hg and DBP≥80 mm Hg (3) no personal history of elevated blood pressure or diabetes mellitus (4) no antihypertensive medication use (5) no family history of hypertension in first-degree relatives 2nd study: Sibling-Pair Study 491 siblings without antihypertensive medication were ascertained from 118 families comprising 189 sibships from the Botnia study in Finland. Altogether, 455 sibling-pair combinations were identified. (note: one sibling can appear in more than one sibling-pair)	YES
Variables	7	BP as: SBP(systolic), DBP(diastolic) Diagnostic Criteria: 140/90 mmHg As confounders: Age, Sex and BMI	
		Genotype	
Data sources measurement	8	1st study: case-control study. Blood pressure was measured in the supine position with a sphygmomanometer after 5 minutes of rest	
		Total genomic DNA was extracted from whole blood by standard methods. Arg389Gly polymorphism in the b_1-adrenergic receptor gene was genotyped by polymerase chainreaction and restriction fragment length polymorphism methods, as described elsewhere	NO No information for storage of DNA, call rates, error rates.

			References given for more details.
Bias	9	The selection of the control subjects. All patients were on antihypertensive medication.-	
		2nd study: Sibling-Pair Study The genotype-discordant sibling analysis is very powerful because siblings often share lifestyle factors influencing blood pressure and the risk for hypertension, such as diet, exercise habits, and socioeconomic status	YES
Study size	10	1st study: Case-control study *Cases:* 292 *Controls:*265	
Quantitative Variables	11	----------	
Statistical methods	12	Analyses were performed using NCSS 6.0.21 (Statistical Solutions, Ltd). 1st study: Case-control study Continuous variables are presented as means6SD. Differences in proportions were tested by the x^2 test Differences between means were tested by *t* test, ANOVA, or Kruskal-Wallis test, where appropriate. Multiple logistic regression was performed with hypertension as the dependent variable and age, BMI, sex, and codon 389 genotype (Gly389Gly and Arg389Gly versus Arg389Arg) as independent variables. A 2-sided P=0.05 was considered statistically significant.	YES
		No data missing.	
		No sensitivity analysis was conducted.	
		All genotype distributions adhered to the Hardy-Weinberg equilibrium.	YES
		No method to infer genotypes or haplotypes	NO
		No method to address population stratification	NO

		No method to control risk of false positive findings	NO
		No method to address relatedness among subjects	NO
Results			
Participants	13	Case-Control Study: *Cases:* 292 *Controls:* 265	
		No changes in number of participants	
Descriptive data	14	Information by genotype is given	YES
Outcome data	15	Number in each genotype category is reported	YES
Main results	16	1st study: Case-control study The Arg389 allele and the Arg389Arg genotype of the b_1-adrenergic receptor gene were more common in patients with hypertension than in controls . The age-, sex-, and BMI-adjusted odds ratio for treated hypertension in subjects homozygous for the Arg389-allele was 1.9 (95% CI: 1.3 to 2.7; *P*=0.0005) when compared with carriers of 1 or 2 Gly389 alleles. SBP, DBP did not differ between carriers of the different Arg389Gly genotypes within the treated hypertension group or within the control group.	
Other analyses	17	2nd study: Sibling-Pair Study In the 102 sibling-pairs discordant for the Arg389Gly polymorphism, the siblings homozygous for the Arg389 allele had a significantly higher DBP (*P*=0.003) and heart rate (*P*=0.02) than carriers of 1 or 2 Gly389 alleles, but there was no difference in SBP. Age and BMI were similar between siblings	
Discussion			
Key results	18	The Arg389Arg genotype of the b_1-adrenergic receptor gene confers an increased risk of developing hypertension.	
Limitations	19	A weakness of the case-control design is the selection of the control subjects. In addition, all patients were on	

		antihypertensive medication.	
Interpretation	20	In vitro studies have shown that the Arg389 variant of the b_1-adrenergic receptor gene mediates an increased responseto agonist stimulation compared with the Gly389 variant, suggesting that the Arg389Gly polymorphism is of functional importance. The increased activity of the Arg389 variant of the b_1-adrenergic receptor in vivo could be expected to lead to a higher cardiac output and could therefore explain our association between the Arg389 allele and hypertension.	
Generalizability	21	----------	
Other Information			
Funding	22	The research was funded by the Swedish Heart Lung Foundation; the Swedish Medical Research Council; the National Public Health Institute; the Skaraborg Institute; the Skaraborg County Council; the West Region County; the Region Skane; the Faculty of Medicine at Lund University; the PåhlssonFoundation, Malmö University Hospital; the ErnholdLundström Research Foundation; the Crafoord Foundations; and the NEPI Foundation (The Swedish Network of Pharmacoepidemiology).	

TABLE 2: ASSESSMENT OF 2nd STUDY

Item	Item number	Description	Conformity with STREGA
Title	1	**Genetic polymorphisms of the b-adrenergic system: associationwith essential hypertension and response to b-blockade**	
Introduction			
Background rationale	2	Beta adrenergic receptors are involved in several pathophysiological functions such as blood pressure (BP). Genetic polymorphisms of the bARs could lead to functionally different products, higher/lower BP values or greater/smaller BP fall after treatment with b-blockade	
Objectives	3	The objectives of the study were: (1) to investigate the association of polymorphism R389G^{b1AR} with EH in a case–control study and (2) to analyze the BP fall after 4 and 8 weeks of therapy with b-blockers according to genotype (pharmacogenetic study) in essential hypertensive patients never treated or untreated for at least 6 months	NO stated replication
Methods			
Study design	4	The study has two phases: 1st phase: a case-control study. Cases: hypertensives Controls: 2nd phase: Pharmacogenetics Study (a cohort study) Hypertensives were treated with atenolol (pharmacogenetic cohort) and the BP fall after 4 and 8 weeks of therapy was examined according to genotype The study was carried oytwith the approval of the local Ethical Committee and the patients' written informed consent	
Setting	5	At the Hypertension and	

		Cardiovascular Prevention Center, Sassari, Italy	
Participants	6	1st phase: a case-control study. Cases:Hypertensive patients (n = 526) white, unrelated, coming from genetically homogeneous areas of North Sardinia. Selection criteria: 1) either never treated or out of antihypertensive treatment for at least 6 months. 2) their high BP had occurred before the age of 60 years. Controls: (n=192) Normotensives white, unrelated North Sardinians, Selection criteria: 1) with history of normotension and BP p135/85 on the day of the blood sampling. 2) age: over 60 years. 3)not having a personal or familiar history of both hypertension and cardiovascular disease. 2nd phase: Pharmacogenetic Study 270 hypertensives, were treated with atenolol (pharmacogenetic cohort)	YES
Variables	7	1st phase: a case-control studyBP as: SBP(systolic), DBP(diastolic) and MBP (mean blood pressure) Diagnostic Criteria: 140/90 mmHg 2nd phase: Pharmacogenetic Study Fall of BP as: DSBP, DDBP and DMBP	
		Genotype	
Data sources measurement	8	BP was measured in the morning every 2 weeks under patients' usual diet. Three measurements per visit were taken by the same nurse in a quiet room by using automated electronic device. The values of BP were the average of the three measurements	
		Genotyping: Genomic DNA was extracted from leukocytes (Talent extraction kit, Trieste, Italy). The polymorphisms studied were: b1AR: R389G,. The primers for R389G^{b1AR}: were as follows: sense—5′-	NO No informationsfor: Storage of DNA

		CCTCTTCGTCTTCTTCAACT-3′, antisense—5′-CGGCCGGTCTCCGTG-3′ The PCR product was sequenced on ABI 377. Doubtful calls were double-checked and, in case of dubious results, repeated or excluded.	
Bias	9	No matching between cases and controls- Stratification by gender and including confounders as variables in the regression models.	YES
		No investigation of potential bias resulting from pharmacotherapy	NO
Study size	10	1st phase: a case-control study Initially, included in the study: Cases:(Hypertensive patients) n = 526 Controls: (normotensives) n=192 Due to laboratory failures, the final numbers of individuals in whom genotyping was successful were: Cases:(Hypertensive patients) n= 506 Controls: (normotensives) n=175 2nd phase Pharmacogenetic Study) n= 270 were treated with atenolol 50 mg b.i.d. (pharmacogenetic cohort). The number of genotypes for $R389G^{b1AR}$ was255	YES
Quantitative Variables	11	Effect of β-blockade	YES
Statistical methods	12	Intercooled Stata 7.0 for Windows was used. Normality was tested by Shapiro–Wilk and Shapiro–Francia: for non-normally distributed variables mathematical transformation was attempted and parametric/nonparametric tests used accordingly Two-tailed t-test and Wilcoxon's rank-sum test were chosen as appropriate. Genotype frequencies were examined by w^2 analysis; when expected values were below 5, mutant homozygotes and heterozygotes were pooled and	YES

		Yates correction was applied. SBP, DBP and MBP were analyzed also as a quantitative trait in the combined normotensive and hypertensive population introducing age, BMI and gender as confounders (ANCOVA); For the sex-stratified analysis, the same independent variables with the exception of gender were used. For the post hoc analysis, a P-value of 0.017 (ie 0.05/3) was considered as significant. The association between genotype and response to treatment was evaluated by the change in SBP, DBP and MBP vs baseline (DSBP, DDBP and DMBP: normal distributions) at 4 and 8 weeks, adjusted for sex, baseline BPs, heart rate and age (ANCOVA).	
		No data missing.	
		Case-control study: No matching between cases and controls for age, BMI Stratification for gender and including these variables in the regression models were used to address this problem.	
		No sensitivity analysis was conducted.	
		All genotype distributions (for both cases and controls) adher to the Hardy-Weinberg equilibrium.(P=0,45)	YES
		No method to infer genotypes or haplotypes	NO
		Population stratification by selecting cases and controls from the same areas	YES
		Method to control risk of false positive findings (double check)	YES
		No method to address relatedness among subjects	NO
Results			
Participants	13	1) 1st phase: a case-control studyInitially (genotyping attempted) Cases:(Hypertensive patients) n= 526 Controls: (normotensives) n=192	YES

		Genotyped ((genotyping successful) Cases:(Hypertensive patients) n= 506 Controls: (normotensives) n=175 2) 2nd phase: Pharmacogenetic Study(a cohort study)	
		1st phase: a case-control study Due to laboratory failures 2nd phase: Pharmacogenetic Study(a cohort study) Due to: laboratory failures lost to follow-up	
Descriptive data	14	Basic characteristics: given Genotyping characteristics: given (cases: Arg-Arg:285, Arg-Gly:181, Gly-Gly:40, total:506 controls: Arg-Arg:89, Arg-Gly:71, Gly-Gly:15, total:175)	YES
		There are no missing data for participants	
		1st phase: a case-control studyOne-stage study. No long-term follow-up. 2nd phase: Pharmacogenetic Study(a cohort study) 4 and 8 weeks	
Outcome data	15	1st phase: a case-control study Total outcomes: 681 Cases Controls Arg-Arg: 285 (56.3) 89(50,8) Arg-Gly: 181 (35,8) 71(40,6) Gly-Gly: 40 (7,9) 15 (8,6) Total: 506 175	YES
Main results	16	No association between *ADRB1 Arg389Gly*(R389G^{b1AR})polymorphisms at the b1AR and EH was found, even considering gender specificity.	
		Age was used either as a continuous variable or as a categorical with two age groups (<50 yr, and >50 yr)	
		Post hoc analysis	YES
		Reference for detailed results available elsewhere	YES
Discussion			

Key results	18	No association between *ADRB1 Arg389Gly*(R389G^{b1AR})polymorphisms at the b1AR and EH was found, even considering gender specificity. Also no effect of the R389G^{b1AR}to the BP fall after 4 and 8 weeks of therapy with b-blockers was found in essential hypertensive patients never treated or untreated for at least 6 months.	
Limitations	19	1. Baseline characteristics (age, sex, BMI) were different between hypertensives and normotensives: No matching between cases and controls. 2. Haplotype analysis was not performed	
Interpretation	20	----------	
Generalizability	21	----------	
Other Information			
Funding	22	The work was supported in part by a Research Grant of the Italian Society of Hypertension (FF) and by a Grant of the Ministry of University and Research (MIUR: FIRB #RBNE01724C).	

TABLE 3: ASSESSMENT OF 3th STUDY

Item	Item number	Description	Conformity with STREGA
Title	1	**Effects of polymorphisms in β_1-adrenoceptor and α-subunit of G protein on heart rate and blood pressure during exercise test. The Finnish Cardiovascular Study**	
Introduction			
Background rationale	2	The Regulation of Cardiovascular responses, i.e., blood pressure during physical stress is influenced by several environmental and genetic factors.	
Objectives	3	It was tested whether the *Arg389Gly* polymorphism of the β_1-adrenergic receptor gene *ADRB1* modulates blood pressure responses during an exercise stress test. This is the first study to test the association between Arg3896Gly polymorphism and BP during exercise.	YES
Methods			
Study design	4	The study is a H-B study. The study protocol was approved by the Ethical Committee of the Hospital District of Pirkanmaa, Finlandand all patients gave informed consent before the study initiation	
Setting	5	The patients underwent exercise stress tests at the Tampere University Hospital between October 2001 and January 2003.	
Participants	6	This is a Cohort study. The selection criteria were the participants to have technically successful exercise test and acquired genotypic samples.	YES
Variables	7	BP as: SAP and DAP Diagnostic Criteria: 140/90 mmHg	
		Genotype	
Data sources measurement	8	Three values of SAP and DAP were taken for analysis: resting, maximal during the exercise and 4 min after the test (recovery). During the exercise test (using a bicycle	

		ergometer with electrical brakes), SAP and DAP were measured with a brachial cuff every second minute.	
		Genomic DNA was extracted from peripheral blood leukocytes using a commercially available kit and QiagenBioRobot M48 Workstation, according to the manufacturer's instructions (Germany). DNA samples were genotyped by employing the 5 nuclease assay and fluorogenic allele-specific TaqMan MGB probes, using the ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA). The nucleotide sequences of primers and probes used in the PCR were deduced from published sequences deposited in the GenBank and Celera databases and synthesized by Applied Biosystems. PCR reaction containing genomic DNA, 1 Universal PCR Master Mix, 900 nM of each primer, and 200 nM of each probe was performed in 384-well plates by using the standard protocol in a total volume of 5 l. End-point fluorescence was measured and genotype calling was carried out by the allelic Error discrimination analysis module after the PCR resulted in clear identification of *Arg389Gly* polymorphisms of *ADRB1*. Negative and positive controls (known genotypes) and random duplicates were used as quality control.	NO No information for: Storage of DNA Error rates Call rates
Bias	9	No information for potential sources of bias and addressing them	NO
		No investigation of potential bias resulting from pharmacotherapy	NO
Study size	10	The participant pool consisted of the patients undergoing exercise stress tests at the Tampere University Hospital between October 2001 and January 2003. All of the consecutive patients coming to take an exercise stress test and willing to participate	

		in the study were recruited. Finally, the 890 patients with technically successful exercise tests and acquired genotypic samples were included in the study (563 men and 327 women).	
Quantitative Variables	11	Effect of β-blocking agents: The patients takingβ–blockingagents had a lower SAP (95% CI of difference: 11 to 19 mmHg) and tended to have lower DAP (95% CI of difference: 0 to 3 mmHg) at maximal load, than those not taking -blockers	YES
Statistical methods	12	Statistical analyses were performed with the SPSS release 12.0.1 forWindows (SPSS, Chicago, IL). The longitudinal exercise stress test data were analyzed by repeated-measurement analyses of variance (RANOVA) using the genotypes as categorical factors and the HR, SAP, and DAP values measured at different points in time (resting, exercise, and recovery) as dependent repeated variables The SAP and DAP values were compared between the genders with Student's *t*-test for independent samples.	YES
		The study population was analyzed as a whole and divided based on gender. Since BMI is often an explaining factor for different results in men and women, BMI was used as covariate in the analyses. Age, BMI, β-adrenergic antagonism were used as covariates. The interaction between these covariates and the variant was calculated. *A P*= 0.05 was considered statistically significant, and 95% confidence intervals (CI) were calculated	
		No data missing.	
		Cohort Study: no probability of loss to follow-up, because of the study design	

		No sensitivity analysis was conducted.	
		All genotype distributions adhered to the Hardy-Weinberg equilibrium.	YES
		No method to infer genotypes or haplotypes	NO
		Homogeneity of Finnish population	YES (although it was not stated)
		No method to control risk of false positive findings	NO
		No method to address relatedness among subjects	NO
Results			
Participants	13	890 patients: 563 men and 327 women, during the whole study	
		One-stage study.	
		One-stage study. No changes in the participants number, during the study	
Descriptive data	14	Patient characteristics: given Genotyping characteristics: given (Arg-Arg:523, Arg-Gly:307, Gly-Gly:60)	YES
		No missing data for participants	
		One-stage study. No long-term follow-up.	
Outcome data	15	Total outcomes: 890 Arg-Arg:523 Arg-Gly:307 Gly-Gly:60	YES
Main results	16	Among all subjects, *Gly389* homozygotes tended to be more likely to have hypertension (OR- 1.69, 95% CI 1.00 –2.86, *P*= 0.050) compared with *Arg389* carriers. Arg389Gly polymorphism of ADRB1 affected maximal SAP during exercise (P 0.04, ANOVA,) and the change in SAP from rest to maximal (P 0.03, ANOVA,). Age, BMI, β-adrenergic antagonism were considered as confounders and used as covariates. The interaction between these covariates and the variant was calculated. Since BMI is often an explaining factor for different results in men and women, BMI was used as covariate in	

		the analyses.	
		Age was used either as a continuous variable or as a categorical with three age groups (<40 yr, 40 – 60 yr, and >60 yr)	
Other analyses	17	*Effect of gender.* Men had a lower HR than women at rest(CI: 0.3 to 3.2 beats/min) and during maximal exercise (0.7 to 7.8 beats/min); they also had lower SAP at rest (CI: 4 to 9 mmHg) but not at exercise (CI: 0 –7 mmHg). DAP did not differ between the genders. *Effect of age.* The higher the age group, the lower the HR atall three phases. At rest and recovery, the SAP values increased in line with increasing age: the higher the age group, the higher the SAP (*P* 0.001 for both time points, ANOVA). However, during exercise, the middle-aged subjects had the highest SAP (P=0.01, ANOVA). DAP was higher in the middle-aged than the younger and older age groups at rest as well as during exercise (P=0.01, ANOVA), and the two oldest groups had higher DAP than the youngest (40 yr) at recovery	
		No reference for detailed results available elsewhere	
Discussion			
Key results	18	In all subjects, and in men and women separately, no statistically significant interaction was found for the *ADRB1 Arg389Gly* polymorphism in relation to SAP and DAP responses over the three study phases (RANOVA, *P*=0.10 for interaction in all analyses where age, BMI, and β-adrenergic antagonism were used as covariates). The *Gly389* homozygotes had higher maximal SAP during exercise than those with at least one *Arg389* allele; consequently, the change of SAP from the resting state to the maximal had the same pattern.	
Limitations	19	No limitation is referred	

Interpretation	20	----------	
Generalizability	21	The largest available study populations, endorse the negative finding, but they also support the view that the *ADRB1 Arg389Gly* polymorphism may modulate hemodynamics in certain situation..	
Other Information			
Funding	22	Financial support was received from the Medical Research Fund of Tampere University Hospital, the Finnish Foundation for Cardiovascular Research, the Academy of Finland (Grant 104821), and the Emil Aaltonen Foundation, Finland.	

TABLE 4: ASSESSMENT OF 4th STUDY

Item	Item number	Description	Conformity with STREGA
Title	1	**Association between essential hypertension and polymorphisms of beta 1-adrenergic receptor gene G1165C (Gly389Arg) in Chinese Mongolian population**	
Introduction			
Background rationale	2	The prevalence of hypertension, cerebrovascular diseases etc. are higher in Mongolian population because of the influence of various factors including environmental, genetic, diet etc.	
Objectives	3	To analyze the association between Arg3896Gly polymorphism and essential hypertension in Mongolian population	NO NO stated replication
Methods			
Study design	4	A cross-sectional study	
Setting	5	The study was carried out in the Department of Neurology, The First Affiliated Hospital of Inner Mongolia Medical College, from February 2003 to March 2005	
Participants	6	239 Mongolian residents, in three groups 122 :essential hypertension group 51: simple increase of SBP 117 :normal BP group	YES
Variables	7	BP as: SBP Diagnostic Criteria: 140/90 mmHg	
		Genotype	
Data sources measurement	8	Three values of BP were taken continuously with a hemomanometer	
		Peripheral venous blood (5ml) was drawn, anticoagulated with heparin, red blood cells were destroyed with low-permeability method and white blood cellswere separated. DNA was extracted from peripheral white blood cells using a kit.	NO No information for: Storage of DNA Error rates Callrates
Bias	9	No information for potential sources	NO

		of bias and addressing them	
		No investigation of potential bias resulting from pharmacotherapy	NO
Study size	10	239 participants	
Quantitative Variables	11	----------	
Statistical methods	12	Statistical analyses were performed with the SPSS release 11.5 The measurement data were expressed as Mean± SD. The univariate analysis of variance was applied in the sample comparison among groups. Genotypes and allele frequencies were compared with the chi-square test.The odd ratio (OR) and 95% confidence intervals (CI) were calculated	YES
		No data missing.	
		Cross-sectional Study: no probability of loss to follow-up, because of the study design	
		No sensitivity analysis was conducted.	
		All genotype distributions adhered to the Hardy-Weinberg equilibrium.	YES
		No method to infer genotypes or haplotypes	NO
		No method to address population stratification	NO
		No method to control risk of false positive findings	NO
		No method to address relatedness among subjects	NO
Results			
Participants	13	239 Mongolians	
		Cross-sectional Study	
		No one missed	
Descriptive data	14	Demographic characteristics: given Genotyping characteristics:given	YES
		There are no missing data	
		One-stage study. No long-term follow-up.	
Outcome data	15	Number in each genotype category is	YES

		reported	
Main results	16	In Mongolian Population, the distribution of the genotypes and alleles ofthe *ADRB1 Arg389Gly* polymorphismhave no obvious differences between normotensives, patients with essential hypertensionand subjects with simple increase of SBP.	
Other analyses	17	----------	
		No reference for detailed results available elsewhere	
Discussion			
Key results	18	In Mongolian Population, the *ADRB1 Arg389Gly* polymorphism may be not a genetic mark of essential hypertensionand simple high SBP	
Limitations	19	No limitation is referred	
Interpretation	20	----------	
Generalizability	21	----------	
Other Information			
Funding	22	No source of funding for the present study is included in the article.	NO

TABLE 5: ASSESSMENT OF 5th STUDY

Item	Item number	Description	Conformity with STREGA
Title	1	**Modifying effects of the R389G β_1-adrenoceptor polymorphism on restingheart rate and blood pressure in patients with obstructive sleep apnoea (OSA)**	
Introduction			
Background rationale	2	OSA stimulates sympathetic nervous activity and elevates resting blood pressure, likely related with genotypes of the β_1-adrenoreceptorR389G polymorphism	
Objectives	3	The aim was to investigate whether theArg$^{389}\beta_1$-adrenoceptor phenotype modifies blood pressure inpatients with moderate-to-severe OSA. Also, a second goal was to examine whether the effect of CPAP (continuous positive airway pressure) therapy on BP in a subgroup of OSA patients, was modulated by this polymorphism First attemption in this field	YES
Methods			
Study design	4	The study protocol was approved by the Local Ethics Committee at Ruhr University Bochum in Germany. Written consent for participation was available.	
Setting	5	Patients were consecutively enrolled from 1999–2001 in the sleep laboratory of the Marienhospital Herne (Ruhr University Bochum, Bochum, Germany).	
Participants	6	309 untreated OSA patients. Arterial hypertension was diagnosed in 167 patients. So: Cases: 167 Hypertensive Controls : 142 Normotensives	YES
Variables	7	BP as: SBP (systolic) and DBP(diastolic) Diagnostic Criteria: 140/90 mmHg	
		Genotype	

Data sources measurement	8	BP was measured three times a day (08:00, 14:00 and 19:00 hours) for1 min during the hospital visits for polysomnography bypalpitation (1 min) and the Riva Rocci method. Patientswere rested for at least 10 min before measurements wereperformed. by thenurses/technical assistants in the sleep laboratory, whowere introduced to the procedures but were blinded to thepurpose of the study. The means of three measurementswas used for further calculations.	
		Genotyping: Peripheral blood samples from the patients were obtained with their informed consent. Primers were synthesized in order to amplify the 522 bpfragment in which the R389G polymorphism was at position 159: 5⁻-CGCTCTGCTGGCTGCCCTTCTTCC-3⁻ (sense)and 5⁻TGGGCTTCGAGTCCTGCTATC-3⁻ (anti-sense). PCR was carried out in a final volume of 10 μl with 50 ng of DNA, 200 μM dNTP and 1 unit of *Taq* polymerase. PCR cycling started with initial denaturation for 5 min at 94 $^{\circ}$ C. The annealing temperature of the first cycle was 61^{o} C, second cycle was 58 ^{o} C, and the remaining 26 cycles were 55 ^{o} C. The annealing time was 1 min. Extension was performed at 72 ^{o} C, for 1 min (final extension, 5 min). The PCR fragment was treated with the restriction endonuclease Mva1 (EcoR11) which fails to digest the Arg^{389} allele because of the G → C transition. Digested DNA was electrophoresed on 1.5% (w/v) agarose gels. The restriction fragments of the Arg^{389} allele were 311, 84, 58, 45 and 24 bp long. The Gly^{389} allele harbours an additional	

		restriction site so that the 311 bp fragment is digested into 135 and 176 bp fragments	
Bias	9	No information for potential sources of bias and addressing them	NO
		No investigation of potential bias resulting from pharmacotherapy	NO
Study size	10	309 subjects, from which 167 Hypertensive (cases) 142 Normotensives (controls)	
Quantitative Variables	11	The effect of CPAP (continuous positive airway pressure) therapy on BP in a subgroupof OSA patients	
Statistical methods	12	Demographic characteristics of the OSA patients were compared with the different genotypes by using a 2 × 2 contingency table and χ^2test, Mean values of numeric variables were compared for each genotype by ANOVA. The homogeneity of the variances was confirmed by the Levene's test. A multi-variate linear/logistic regression model was established to investigate an independent influence of the severity of OSA (represented by AHI) and the R389G poly-morphism on BP and the prevalence of hypertension. In the follow-up cohort of 148 patients, the number of Gly^{389} homozygotes was small (*n*= 8). Therefore, in an additional model, patients with R389G and G389G genotypes were combined into one group. Differences between the two genotype groups [R389R and (R389G + G389G)] were calculated by Student's *t*-test.	NO **Software used not stated**
		Method to infer genotypes or haplotypes	YES
		No method to address population stratification	NO
		No method to control risk of false	NO

		positive findings	
		No method to address relatedness among subjects	NO
Results			
Participants	13	309 subjects, from which 167 Hypertensive (cases) 142 Normotensives (controls)	
		No changes in the participants number	
Descriptive data	14	Information by genotype is given	
Outcome data	15	Number in each genotype category is reported	YES
Other analyses	17	----------	
		No reference for detailed results available elsewhere	
Main results	16	The *β*1-adrenoceptor R389G polymorphism did not influence the BP in untreated OSA patients. However, it may modify the beneficial effects of CPAP therapy on BP	
		No reference for detailed results available elsewhere	
Discussion			
Key results	18	The R389R polymorphism was not associated with higher BP in untreated OSA patients.	
Limitations	19	1)The study was not designed to dissect thediffering influence of the R389G polymorphism on BP. 2)Comparatively few measurements per patient.	
Interpretation	20	This finding could be interpreted as further evidence that the R389G polymorphism of the β_1-adrenoceptor gene has only minor relevance *in vivo*.	
Generalizability	21	----------	
Other Information			
Funding	22	The study was supported by FoRUM (Forschungs-forderung¨ der Ruhr-Universitat¨ Bochum, Medizinische Fakultat)¨.	

TABLE 6: ASSESSMENT OF 6th STUDY

Item	Item number	Description	Conformity with STREGA
Title	1	**Studies of associations between the Arg389Gly polymorphism of the β1-adrenergic receptor gene (*ADRB1*) and hypertension and obesity in7677 Danish white subjects**	
Introduction			
Background rationale	2	Activation of the β_1-adrenergic receptor (*ADRB1*) enhances cardiac output. Analysis of the association of the functional *ADRB1* Arg389Gly variant with hypertension has given ambiguous results.	
Objectives	3	To clarify the potential impact of the Arg389Gly variant on hypertension in the general population. It is a replication	YES
Methods			
Study design	4	Case-control studies and quantitative trait analyses were carried outin 7677 Danish Caucasians who were genotyped for the Arg389Gly variant	
Setting	5		
Participants	6	The *ADRB1* Arg389Gly (dbSNP rs1801253) variant was genotyped in 7677 Danish whites subjects from three study groups: (i) a population-based cohort (Inter99) of middle-aged Danish white subjects living in the greater Copenhagen area and studied at the Research Centre for Prevention and Health (*n* = 6257) (ii) a group of Type 2 diabetic patients identified through the outpatient clinic at Steno Diabetes Center (*n* = 1088) (iii) a population-based group of middle-aged glucose-tolerant subjects recruited from the Research Centre for Prevention and Health (*n* = 346).	YES
Variables	7	Blood pressure (BP)as:Systolic(SBP)	

		Diastolic(DBP) and mean arterial BP MABP =2 × [diastolic blood pressure + systolic blood pressure] /3 Diagnostic Criteria: 140/90 mmHg	
		Genotype	
Data sources measurement	8	Blood pressure was measured twice in the morning after 10 min of rest before blood drawing, with the participant in a supine position with slightly elevated head using a Hawksley random zero mercury sphygmomanometer with an appropriate cuff size. Systolic blood pressure was taken at the return of arterial sounds (Korotkoff phase I) and diastolic blood pressure at the disappearance of sounds (Korotkoff phase V).MABP was calculated	
		Genotyping of the *ADRB1* Arg389Gly variant (dbSNP rs1801253) was performed using a chip-based matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (DNA MassARRAY; Sequenom, San Diego, CA, USA) of PCR-generated primer extension products. The overall success rate for the genotyping was> 98%. Of 182 samples genotyped in duplicates, no genotype discrepancies were observed.	NO No information for: Extraction of DNA Storage of DNA Genotyping Platform YES for errors
Bias	9	No information for potential sources of bias and addressing them	NO
		No investigation of potential bias resulting from pharmacotherapy	NO
Study Size	10	Final number: 6499 (cases *n* = 2518; control *n* = 3981)	
Quantitative Variables	11	----------	
Statistical methods	12	All analyses were performed using Statistical Package for Social Science (SPSS; Chicago, IL, USA) version 13.0and RGui version 2.10. Fisher's exact test was applied to examine differences in minor allele frequencies (MAF) and genotype distributions between affected and	YES

		unaffected subjects. Logistic regression with adjustment for sex and age was also used to test for differences in genotype distribution. A general linear model was used to test quantitative variables (or transformed variables) for differences between genotype groups. Genotype and sex were considered as fixed factors and age as covariates. A *P*-value <0.05 was considered to be significant.	YES
		No data missing.	
		Case-control Study	
		No sensitivity analysis was conducted.	
		All genotype groups obeyed Hardy–Weinberg equilibrium.	Yes
		No method to infer genotypes or haplotypes	NO
		No method to address population stratification	NO
		Method to control risk of false positive findings (Of 182 samples genotyped in duplicates, no genotype discrepancies were observed)	YES
		No method to control relatedness	NO
Results			
Participants	13	Total number: 6499 (cases *n* = 2518; control *n* = 3981)	
		Number of missing participants is given	
Descriptive data	14	Patient characteristics: given	
		Genotyping characteristics: given	YES
Outcome data	15	Number in each genotype category is reported	YES
Main results	16	No association of the hypertension with allele frequencies (*P* = 0.3) or genotype distribution (*P* = 0.5) was found.	
Other analyses	17	In the quantitative trait analyses, individuals carrying the Gly allele had slightly but significantly lower diastolic (Arg/Arg = 81.9 mmHg vs. Gly-allele carriers = 81.5 mmHg) and systolic (Arg/Arg = 129.4 mmHg vs. Gly-allele	

		carriers = 128.8 mmHg) blood pressure as well as a lower mean arterial blood pressure.	
Discussion			
Key results	18	The *ADRB1* Arg389Gly variant is most likely not to be a major contributor to the development of hypertension. However, it is possible that the Arg allele may contribute by causing a minor increase in systolic and diastolic blood pressure levels in a middle-aged white population, although the effect is not powerful enough to cause hypertension.	
Limitations	19	----------	
Interpretation	20	----------	
Generalizability	21	----------	
Other Information			
Funding	22	The study was supported by the Danish Medical Research Council, the Danish Diabetes Association, the Danish Heart Foundation, the Velux Foundation, and the European Economic Community (EUGENE2 LSHM-CT-2004–512013).	

TABLE 7: ASSESSMENT OF 7th STUDY

Item	Item number	Description	Conformity with STREGA
Title	1	**Genetic variants of β_1-adrenoceptor gene polymorphisms (Ser49Gly and Arg389Gly) and essential hypertension in a South Indian Tamil population**	
Introduction			
Background rationale	2	Very few studies have described the role of β_1-adrenoceptor gene polymorphisms in hypertension worldwide and so far there are few data relevant to the Indian population. The genetic profile of the β_1-ADRB has not yet been documented for any Indian population.	
Objectives	3	The aim was to determine the association between β_1-adrenoceptor Arg389Glygene polymorphism and the susceptibility of individuals to essential hypertension in a south Indian Tamil population. Replication	YES
Methods			
Study design	4	The present case-control study included patients with essential hypertension (cases) and healthy volunteers (controls) from the Tamil population.	
Setting	5	The present study was performed from January 2005 to February 2008 from outpatient clinics of hypertension and internal medicine (Jawaharlal Institute of Post graduate Medical Education and Research (JIPMER) Hospital, Pondicherry, India). The control group consisted of 444 healthy volunteers (201 men and 243 women) aged 30–60 years. These subjects had no personal or family history of hypertension in first-degree relatives. Patient characteristics, such as bodyweight, height and drug history, were recorded. All participants were interviewed using a standardized questionnaire with regard to their lifestyle, smoking, alcohol consumption and drug intake.	
Participants	6	*Cases:*438 unrelated essential hypertensive patients (213 men /225 women) aged 30–60 ys, being resident in Tamilnadu &Pondicherry going back for at least three generations. Selection criteria: Patients receiving	YES

		antihypertensive medications for more than 3 months or newly diagnosed hypertensive patients with systolic blood pressure (SBP) > 140 mmHg and/or diastolic blood pressure (DBP) > 90 mmHg on two or more visits <u>*Controls:*</u> 444 healthy volunteers (201 men/243 women) aged 30–60 yrs, with no personal or family history of hypertension in first-degree relatives and had SBP < 130 mmHg and DBP <85 mmHg	
Variables	7	BP as: SBP and DBP Diagnostic Criteria: 140/90 mmHg (hypertensive) and 130/85 mmHg (non-hypertensive)	
		Genotype	
Data sources measurement	8	Blood pressure was measured in the right arm after subjects had rested for 10 min using a standard sphygmomanometer; the average of three readings, taken 2 min, apart was recorded.	
		Genotyping: A 5 mL venous blood sample was collected using EDTA as an anticoagulant. Genomic DNA was extracted from peripheral leucocytes using the standard phenol: chloroform method. Genotyping for Arg389Gly was performed using a Real Time Thermocycler (ABI Prism 7700; ABI, Foster City, CA, USA) with a Taqman SNP genotyping assay method. This technique uses fluorogenic 5' nuclease chemistry (also known as Taqman probe-based chemistry) to enable detection of specific polymerase chain reaction (PCR) products. The SNP genotyping assay identification details used (Applied Biosystems) were and C__8898494-10 for Arg389Gly.The PCR reaction was performed in duplicate in 20L final volume, containing 10L Taqman Universal PCR master mix (2×), 0.75 L of 20× working stock of SNP genotyping assay and 4.5 L genomic DNA (diluted in DNase-free water and 4.75 L deionized water). The thermocycler conditions included one cycle at 50°C for 2 min and one cycle at 95°C for 10 min to activate the AmpliTaq Gold polymerase, followed by 40 cycles of denaturation at 92°C for 15 s and annealing/extension at 60°C for 1 min. The allelic discrimination analysis was performed using 7700 SDS software (Applied Biosystems, Foster City, CA, USA).	
Bias	9	No information for potential sources of bias and	NO

		addressing them	
		No investigation of potential bias resulting from pharmacotherapy	NO
Study size	10	The study size remained the same during the whole time.	
Quantitative Variables	11	----------	
Statistical methods	12	Statistical analysis was performed using the SPSS software (Windows version release 13; SPSS, Chicago, IL, USA) and GraphPadInstat software (GraphPad Software, San Diego, CA, USA). Demographic details of hypertensive patients and controls that were continuous variables were compared using Student's unpaired *t*-test, whereas dichotomous variables were analyzed using χ^2 and/or Fisher's exact test. Differences in allele frequencies and genotype distributions between hypertensive patients and normotensives controls were compared by χ^2 and/or Fisher's exact test. The association between genotypes and the risk of hypertension was analyzed by calculating the crude odds ratio (OR) and 95% confidence interval (95% CI) using χ^2 and/ or Fisher's exact test. The adjusted OR was calculated using unconditional logistic regression and the low-risk genotype was designated as the reference category. For analyzing gene–gene interactions, stratified variables were generated and included the logistic model, simultaneously with appropriate indicator variables. Linkage disequilibrium values for the pair of dimorphisms were measured using Helix Tree software (Golden Helix, Bozeman, MT, USA). The haplotype of Arg389Gly was constructed using Helix tree software using the EM algorithm. The genotype frequencies observed were compared with the expected frequencies to check for Hardy– Weinberg equilibrium $P < 0.05$ was set as the level of significance.	YES
		<u>Matching of cases and controls:</u> No significant differences were observed in sex distribution, BMI, alcohol consumption, triglycerides and HDL- cholesterol levels between patients and controls.	

		Patients and controls were not age matched.	
		No sensitivity analysis was conducted.	
		No genetic information for Indian , yet	NO
		No method to infer genotypes or haplotypes	NO
		The subjects were all of the same ethnic group to control for population stratification.	YES
		No method to control risk of false positive findings	NO
		No method to address relatedness among subjects	NO
Results			
Participants	13	The study size remained the same during the whole time the study was conducted.	
Descriptive data	14	Patient characteristics: given	
		Genotyping characteristics: given	
Outcome data	15	Number in each genotype category is reported	YES
Main results	16	There were no significant differences in the genotypes and allele frequencies for Arg389Gly between hypertensive patients and controls. The unadjusted ORs were 1.1(95% CI: 0.8–1.4, P=0.7) for Arg/Gly and 1.3(95% CI: 0.8–2.2, P=0.3) for Gly/Gly	
Other analyses	17	Gender-specific analysis was performed by comparing the hypertensive men / women with their respective control	
		Potential confounders: sex, age, BMI	
		No reference for detailed results available	
Discussion			
Key results	18	There were no significant differences in the genotypes and allele frequencies for Arg389Gly between hypertensive patients and controls.	
Limitations	19	A limitation of the present study is that hypertensive patients and controls were not age matched. This does not appear to be a major drawback because the age of the control group was higher(47.4±0.4 years) than that of the hypertensive patient group (44.9±0.4 years)	
Interpretation	20	The results of the present study deviate from those reported in previous case-control study. This could be attributed to variations in environmental factors, in addition to differences in the selection of hypertensive patients and controls, sample size, age, BMI and other environmental factors.	

Generalizability	21	The strength of the present study lies in its judicious selection of unrelated hypertensive patients and controls from the homogeneous population.	
Other Information			
Funding	22	This study was funded by the Department of Biotechnology–NewDelhi (D.O.No.BT/PR4076/Medical/12/163/2003 dated 1/12/2004).	

TABLE 8: ASSESSMENT OF 8th STUDY

Item	Item number	Description	Conformity with STREGA
Title and Abstract	1	**Polymorphisms of the b1-adrenergic receptor gene are associated with essential hypertension in Chinese.**	
Introduction			
Background rationale	2	Blood pressure is determined by two factors: cardiac output and peripheral resistance, which are regulated by the sympathetic nervous system. The b1-adrenergic receptor (ADRB1) plays a pivotal role in mediating signal transduction of the sympathetic-adrenal system, which is involved in the regulation of cardiac output and peripheral resistance.	
Objectives	3	The goal of the present study was to investigate whether the functionally important Arg389Gly polymorphism of the *ADRB1* gene was associated with hypertension in Chinese. First, with two studies in two independent populations	YES
Methods			
Study design	4	The hypothesis was tested in two independent case-control studies, one comprised 481 patients with hypertension and 529 control subjects, and the other study comprised 212 patients and 325 control subjects. Informed consent was obtained from all individuals	
Setting	5	The subjects of the first study were comprised of outpatients from a hospital in Shijiazhuang city, Hebei province, People's Republic of China. In the second study, the population was from a community center in Beijing Fengtai District.	
Participants	6	**1st Case-control study :**The subjects consisted of 481 patients with mild and moderate EH and 529 age- and gender-matched controls from the	YES

		same area. **2nd Case-control study :**The population was from a community center in Beijing Fengtai District. The study population was comprised of 212 cases with hypertension and 325 control subjects and was recruited using the same criteria as the first study.	
Variables	7	BP (blood pressure),	
		Genotype	
Data sources measurement	8	Blood pressure was measured by the same investigator using the right arm with a mercury sphygmomanometer and standard techniques after at least 5 min of rest in a sitting position.	
		Genotyping Peripheral blood (10 mL) was collected into tubes containing trisodium citrate (final concentration in blood, 0.026 mol/L), and centrifuged at 3000 *g* for 10 min at room temperature. The plasma and ‘‘buffy-coat’’ were separated and stored in a 1.5-mL EP tube at –708C. All assays were performed in duplicate. DNA was extracted from the ‘‘buffy-coat’’ as described previously (13), and stored at –708C before use. Single nucleotide polymorphism Arg389Gly was analyzed by amplification of a 530-base pair (bp) sequence with primers: 59-CGC TCT GCT GGC TGC CCT TCT TCC -39 and 59-TGG GCT TCG AGT TCA CCT GCT ATC-39 (6). The polymerase chain reaction (PCR) products were digested with BcgI (New England Biolabs, Beverly, MA, USA); only one band for the Gly389Gly homozygote, two DNA fragments of 376 bp and 154 bp were obtained for the Arg389Arg homozygote on 3% agarose gel and three bands for the Arg389Gly heterozygote. The sequence was confirmed by	

		bidirectional sequencing of 200 samples with ABI Prism 3730 Genetic Analyzer (Applied Biosystems Inc.) (14), and the reproducibility was 100%.	
Bias	9	No information for potential sources of bias and addressing them	
		No investigation of potential bias resulting from pharmacotherapy	
Study Size	10	**1st Case-control study** 481 cases 529 controls **2nd Case-control study** 212 cases 325 control	
Quantitative Variables	11	----------	
Statistical methods	12	Statistical analysis was performed with the SPSS 13.0 package. Data are expressed as means±SD. The x^2-test was used for testing categorical variables, the Hardy-Weinberg equilibrium of the polymorphisms, and genotype/allele frequencies. Quantitative variables between groups were tested with Student's test. The association of SNPs with hypertension was analyzed using multivariate logistic regression. The analysis was adjusted for age, gender, BMI, smoking, glucose, HDL-C, LDL-C, TC, TG alcohol consumption, and family history of hypertension. A two-tailed p=0.05 was considered significant.	YES
		No data missing.	
		No sensitivity analysis was conducted.	
		All genotype distributions adhered to the Hardy-Weinberg equilibrium.	YES
		No method to infer genotypes or haplotypes	NO

		The subjects were all of Han ethnic group to control for population stratification.	YES
		No method to control risk of false positive findings	NO
		No method to address relatedness among subjects	NO
Results			
Participants	13	**1st Case-control study** 481 cases 529 controls **2nd Case-control study** 212 cases 325 control	
		No changes in the participants number, during the study	
Descriptive data	14	Information by genotype is given	YES
		No missing data for participants	
Outcome data	15	Number in each genotype category is reported	YES
Main results	16	**1st Case-control study** The Arg389Arg genotype of the *ADRB1* gene was associated with risk of hypertension (odds ratio (OR)= 1.77, 95% confidence interval (CI) 1.09–2.98) **2nd Case-control study** The association was replicated in the second independent population (OR 1.65, 95% CI 1.07–2.89)	
		The patients with the Arg389Arg genotype had significantly higher diastolic blood pressure (DBP) than did those with Arg389Gly genotype as well as those with Gly389Gly genotype, in both studies No association was seen between systolic blood pressure (SBP) and any of the three genotypes at amino acid position 389 in hypertensive patients	
Other analyses	17		
Discussion			
Key results	18	The Arg389Gly polymorphism of the	

		ADRB1 gene confers higher risk for hypertension. Hypertensive patients with Arg389Arg variants had significantly increased DBP.	
Limitations	19	Results need to be confirmed in a larger sample of individuals.	
Interpretation	20	The maximal increase in cyclic adenosine monophosphate caused by isoprenaline was significantly larger in Arg389 than in Gly389 *ADRB1* cells. The increased in vivo activity of the Arg389 variant of *ADRB1* could be expected to lead to higher cardiac output, and could therefore explain the association between the Arg389 allele and hypertension	
Other Information			
Funding	22	No source of funding for the present study is included in the article.	NO

ABBREVIATIONS

ADRB1 :β_1-Adrenergic Receptor

AHI : Apnoea/ Hypopnea Index

BMI : Body Mass Indes

DAP: Diastolic Arterial Pressure

DBP: Diastolic Blood Pressure

EH : Essential Hypertension

GAS :Genetic Association Studies

MABP : Mean Arterial Blood Pressure

OSA: Obstructive Sleep Apnoea

SAP: Systolic Arterial Pressure

SBP: Systolic Blood Pressure

SNP : Single Nucleotide Polymorphism

STREGA: STrengthening the REporting of Genetic Association Studies