UCSD twins	N	Male	N	Female	p-value
Age (years)	80	40.7±1.2	282	40.7±0.2	0.244
BP status, NT/HTN (%)	80	69(86.3)/11(13.8)	282	256(90.8)/26(9.2)	0.238 (Chi-sq)
Physical					
Body mass index, kg/m^2	80	25.9±0.6	282	24.7±0.4	0.098
Physiological					
SBP, mmHg	77	135±1.7	277	129±1.1	0.005*
DBP, mmHg	77	72.8±1.4	277	70.7±0.6	0.160
Metabolic					
Plasma glucose, mg/dl	80	83.5±1.8	282	81.5±1.3	0.368
Plasma insulin, µUnit/ml	80	13.3±1.5	280	13.6±0.8	0.852
QUICKI (insulin sensitivity)	80	0.34±0.004	280	0.35±0.004	0.183
HOMA (insulin resistance)	80	2.89±0.4	280	2.90±0.2	0.991
Plasma leptin, ng/ml	80	5.96±0.78	282	13.5±0.7	7.39E-12*
Biochemical					
Epinephrine in urine, ng/gm	74	13314±795	261	13342±461	0.973
Norepinephrine in urine, ng/gm	74	26346±1067	261	30258±956	3.10E-03*
$CHGA_{116-439}$, nmol/L	75	3.98±0.27	278	3.99±0.20	0.988
$CHGA_{361-372}$, nmol/L	75	1.18±0.05	275	1.33±0.05	3.00E-02*
C-reactive protein, ng/ml	77	1468±234	278	2702±271	5.55E-04*
Australian (QIMR) twins					
Age (years)	630	43.7±0.5	1295	46.0±0.4	1.76E-04*
BP status, NT/HTN (%)	497	451(90.7)/46(9.3)	968	859(88.7)/109(11.3)	0.245 (Chi-sq)
Physical					
Body mass index, kg/m^2	630	25.7±0.16	1295	25.0±0.15	1.00E-03*

On-line Table 1. Descriptive statistics for twin study population samples: UCSD and Australian/QIMR twins, stratified by sex. Values are mean ± standard error of the mean (or n and %) derived from GEE (age and sex adjusted). *: P<0.05.

SUPPLEMENTARY METHODS.

Molecular methods: Function of *CHGA* promoter variant G-462A.

Genomics. Genomic DNA was prepared from leukocytes in EDTA-anticoagulated blood, using PureGene extraction kits (Gentra Biosystems, Minnesota). *CHGA* promoter genotypes in twin and population samples were scored on amplified DNA by extension-based methods: mass spectrometry (Sequenom; La Jolla, CA), or Pyrosequencing (Biotage; Uppsala, Sweden), as previously described [1].

Bioinformatics. Promoter motif matches used the TRANSFAC®-7.0-Public-2005 [2] position weight matrix database <http://www.gene-regulation.com>, accessed by the graphical user interfaces at Chip Mapper [3] <http://mapper.chip.org/mapper> or JASPAR [4] at <http://jaspar.genereg.net/>. Inter-species multiple sequence alignments were done at Clustal-W version-2.0.10 at <http://www.ebi.ac.uk/Tools/clustalw2/index.html>.

Cell culture. The rat adrenal medullary chromaffin cell line PC12 was grown on 24-well polystyrene plates pre-coated with poly-L-lysine, in high-glucose Dulbecco's modification of Eagle's medium, with penicillin G (100 U/ml) and streptomycin sulfate (100 mg/ml). The medium for PC12 cells was supplemented with 10% horse serum and 5% fetal bovine serum.

***CHGA* promoter/luciferase reporter plasmids.** Human *CHGA* promoter/luciferase reporter plasmids were constructed essentially as previously described [5]. Haplotype-specific promoter fragments corresponding to CHGA -1142/+54 bp (-/+ with respect to the cap site) were amplified from genomic DNA of known homozygotes (or heterozygotes for the two least common haplotypes), and subcloned into the upstream promoter polylinker site of pGL3-Basic (Promega Inc., Madison, WI). Synthetic replacements were made by site-directed mutagenesis (Quik-Change; Stratagene, La Jolla, CA). Promoter fragments were sequence-verified before use. Promoter positions (e.g., G-462A) are numbered upstream (-) or downstream (+) of the cap site. Plasmids were purified on columns (Qiagen, Valencia, CA) before transfection. To probe the specific role of G-462A, here we focused on haplotype-A (T-1014→T-988→**G**-462→T-415→C-89), or its G-462A point mutant (T-1014→T-988→**A**-462→T-415→C-89) as previously described [1].

Transfection, transcriptional stimulation, and reporter assay. Cell lines were transfected (at 50–60% confluence, 1 day after 1:4 splitting), by the cationic liposome method (TransFectin; BioRad). Human *CHGA* promoter haplotype/reporter plasmids (500 ng) were transfected into PC12 cells, along with 50 ng of a PPARγ expression plasmid (in the

cytomegalovirus promoter vector pcDNA3.1; Invitrogen, Carlsbad, CA), or the insert-less control vector (pcDNA3.1). After 5 hours, the PPARγ/RXRa ligands rosiglitazone (10 μM) alone, or rosiglitazone plus retinoic acid (1 μM), versus mock (DMSO vehicle), were added to the medium. Firefly luciferase activity in cell lysates was measured 24 h after transfection, and the results were expressed as firefly luciferase activity/cell protein. Each experiment was repeated a minimum of three times.

Chromatin ImmunoPrecipitation (ChIP). ChIP was accomplished by modification of procedures previously described by us[54]. PC12 chromaffin cells were transfected with particular CHGA promoter haplotype/reporters to obtain G versus A alleles for the G-462A variant. ChIP assays were carried out using the Imprint® ChIP kit (CHP1; Sigma, St. Louis, MO). Cells (~3×10^6 in transfected 10-cm plates) were cross-linked in 1% formaldehyde for 10 min at room temp and washed x3 with ice-cold PBS, then resuspended in nuclear preparation buffer. Chromatin was sonicated to achieve inter-nucleosomal cleavage (Branson Sonifier) until DNA was fragmented to ~500-1000 bp size. After 10-min centrifugation, samples were incubated with specific or control antibodies pre-adsorbed to polystyrene wells at room temp for 1.5 hours with rotation. The antibody against PPARg was from Santa Cruz Biotechnology. Control antibodies were from the Sigma ChIP kit: pre-immune normal mouse IgG (as a negative control), and anti-RNA polymerase II (as a positive control). The adsorbed immune complex washed 6-7 times and eluted by “DNA release buffer” including proteinase K digestion at 65°C for 15 min, then cross-links were reversed with “reversing solution” with heating in at 65°C for 1.5 hours. The DNA was subsequently extracted and purified with GenElute Binding Column G (Sigma). Immunoprecipitated nucleosomal DNA samples were analyzed by PCR using primers forming a 152-bp amplicon that bracketed the G-462A (sense: 5’-AGAGAGAAGCCTCACTCAGACAG-3’, antisense: 5’-CACCCCGTGCTATTTTTCCTA-3’) site in the human *CHGA* promoter. Extracted DNA from the chromatin fractions before antibody adsorption/elution was used as a positive control ("input DNA"). To ensure that the PCR amplification was in the linear range, reactions with different amounts of input DNA samples were carried out for various (typically 15-30) cycle numbers; a linear range of amplification typically occurred at ~25 cycles. After amplification, PCR products were separated on 1.5% agarose TBE gels. In this reporter system, the transfected plasmid is incorporated into the chromatin fraction of the cell[54].

REFERENCES.

1. Chen Y, Rao F, Rodriguez-Flores JL, Mahapatra NR, O'Connor DT. Common genetic variants in the chromogranin a promoter alter autonomic activity and blood pressure. Kidney International 2008;74:115–125
2. Knuppel R, Dietze P, Lehnberg W, Frech K, Wingender E. Transfac retrieval program: A network model database of eukaryotic transcription regulating sequences and proteins. J Comput Biol. 1994;1:191-198
3. Marinescu VD, Kohane IS, Riva A. Mapper: A search engine for the computational identification of putative transcription factor binding sites in multiple genomes. BMC Bioinformatics. 2005;6:79
4. Wasserman WW, Sandelin A. Applied bioinformatics for the identification of regulatory elements. Nat Rev Genet. 2004;5:276-287
5. Wen G, Mahata SK, Cadman P, Mahata M, Ghosh S, Mahapatra NR, Rao F, Stridsberg M, Smith DW, Mahboubi P, Schork NJ, O'Connor DT, Hamilton BA. Both rare and common polymorphisms contribute functional variation at chga, a regulator of catecholamine physiology. Am J Hum Genet. 2004;74:197-207