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Naturally Occurring Human Genetic Variation in the 3′-Untranslated Region of the Secretory Protein Chromogranin A Is Associated With Autonomic Blood Pressure Regulation and Hypertension in a Sex-Dependent Fashion

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Objectives We aimed to determine whether the common variation at the chromogranin A (*CHGA*) locus increases susceptibility to hypertension.

Background *CHGA* regulates catecholamine storage and release. Previously we systematically identified genetic variants across *CHGA*.

Methods We carried out dense genotyping across the *CHGA* locus in >1,000 individuals with the most extreme blood pressures (BPs) in the population, as well as twin pairs with autonomic phenotypes. We also characterized the function of a trait-associated 3′-untranslated region (3′-UTR) variant with transfected *CHGA* 3′-UTR/luciferase reporter plasmids.

Results *CHGA* was overexpressed in patients with hypertension, especially hypertensive men, and *CHGA* predicted catecholamines. In individuals with extreme BPs, *CHGA* genetic variants predicted BP, especially in men, with a peak association occurring in the 3′-UTR at C+87T, accounting for up to ~12/~9 mm Hg. The C+87T genotype predicted *CHGA* secretion in vivo, with the +87T allele (associated with lower BP) also diminishing plasma *CHGA* by ~10%. The C+87T 3′-UTR variant also predicted the BP response to environmental (cold) stress; the same allele (+87T) that diminished basal BP in the population also decreased the systolic BP response to stress by ~12 mm Hg, and the response was smaller in women (by ~6 mm Hg). In a chromaffin cell-transfected *CHGA* 3′-UTR/luciferase reporter plasmid, the +87T allele associated with lower BP also decreased reporter expression by ~30%. In cultured chromaffin cells, reducing endogenous *CHGA* expression by small interfering ribonucleic acid caused approximately two-thirds depletion of catecholamine storage vesicles.

Conclusions Common variant C+87T in the *CHGA* 3′-UTR is a functional polymorphism causally associated with hypertension especially in men of the population, and we propose steps ("intermediate phenotypes") whereby in a sex-dependent fashion this genetic variant influences the ultimate disease trait. These observations suggest new molecular strategies to probe the pathophysiology, risk, and rational treatment of hypertension. (J Am Coll Cardiol 2008;52:1468–81) © 2008 by the American College of Cardiology Foundation

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Chromogranin A (*CHGA*), a 48-kDa acidic polypeptide (1,2), is the major protein costored and coreleased with catecholamines from secretory vesicles in adrenal medulla and postganglionic sympathetic axons (3). Catecholamine storage vesicles (or chromaffin granules) of the adrenal medulla contain remarkably high concentrations of *CHGA*, catecholamines, adenosine triphosphate, and Ca^{2+}, and *CHGA* seems to bind and store both catecholamines and Ca^{2+} (4). *CHGA* also binds to the vesicle membrane, where it may influence the release of calcium from secretory granules to the cytosolic exocytotic machinery through the inositol 1,4,5-trisphosphate receptor/Ca^{2+} channel (5). *CHGA* is required for formation of catecholamine secretory vesicles in chromaffin cells, and its expression may be sufficient to induce a regulated secretory system in nonsecretory cells (6). *CHGA* is also a pro-hormone that gives rise to biologically active peptides such as the dysglycemic peptide pancreastatin (7,8), the antimicrobial peptide chromacin (9), the vasodilator vasostatin (10), and catestatin, which acts to inhibit catecholamine release (11,12).

Essential hypertension is a complex trait (13), with contributions from multiple factors: cardiovascular, neuronal, renal, and adrenal. Over the past ~20 years, phenotypic links between *CHGA* and essential (idiopathic, genetic) human (14–17) and rodent (18) hypertension have been repeatedly observed. Plasma *CHGA* concentration correlates with catecholamine release rates (19), and increases in blood pressure (BP) caused by the action of catecholamines are likely to be coupled to the formation of dense-core secretory granules, whose biogenesis is regulated in vivo by *CHGA* (20). Recently, we systematically identified common genetic variation in human *CHGA* by resequencing the gene in several human populations (21); here we explored whether common genetic variation at the *CHGA* locus is associated with BP, beginning with a large population-based sample of extreme BPs, in which we found that a 3′-untranslated region (3′-UTR) polymorphism (C+87T) is substantially associated with both diastolic blood pressure (DBP) and systolic blood pressure (SBP). We then established its influence on an earlier pathogenic phenotype (environmental stress-evoked change in BP), and finally documented its effect on gene expression in a transfected reporter system.

Abbreviations and Acronyms

BP = blood pressure
CHGA = chromogranin A
DBP = diastolic blood pressure
LD = linkage disequilibrium
PCR = polymerase chain reaction
SBP = systolic blood pressure
siRNA = small interfering ribonucleic acid
SNP = single nucleotide polymorphism
SNPEM = single nucleotide polymorphism expectation maximation
3′-UTR = 3′-untranslated region

Methods

Subjects and Clinical Characterization

Hypertension. DIAGNOSIS OF HYPERTENSION. Since hypertension is part of a larger syndrome, all individuals of diverse ancestries were phenotyped for not only BP, but also associated traits, both metabolic and renal (Online Tables 1 and 2).

PHENOTYPE (*CHGA*, CATECHOLAMINE) AND BP STUDY. In the first (purely phenotypic) study, we measured the plasma concentrations of *CHGA*, norepinephrine, lipids, and creatinine (see the following text) in 724 individuals with normal renal function (serum creatinine ≤1.5 mg/dl), stratified by BP status: normal BP (documented at <135/<85 mm Hg, on no medications), versus a diagnosis of essential hypertension (documented at DBP ≥90 mm Hg). Of those with hypertension, 75% were treated with antihypertensive medications. BPs were determined in triplicate (and then averaged) in seated subjects with an oscillometric device (DynaPulse, PulseMetric, Vista, California), validated, and calibrated as described previously (22). During the same visit, the same subjects also underwent prolonged (5-min, ~400 beats), noninvasive monitoring of BP with a radial artery applanation tonometer (Colin Pilot, Colin Instruments, San Antonio, Texas); such prolonged radial arterial readings correlated with both SBP (Spearman rho = 0.57; $p < 0.001$) and DBP (Spearman rho = 0.53; $p < 0.001$) obtained by the DynaPulse brachial cuff.

CHGA GENOTYPE AND BP STUDY. In the second (genotype/phenotype) study, a population cohort with extreme BPs consisted of 470 male and 558 female white (European ancestry, by self-identification) subjects. These participants were selected based on DBP in the upper or lower most extreme (fifth) percentiles of DBP distribution in 25,599 men and 27,479 women in a primary care practice at Kaiser-Permanente of Southern California medical group (23,24). Subjects were ascertained on the DBP trait, because twin and family studies have provided evidence that DBP is substantially heritable (25), and SBP correlates highly with DBP. BP was measured in seated individuals with an aneroid sphygmomanometer in a single health appraisal clinic site by trained, long-term personnel, and BP measurement was repeated if elevated on initial reading. The health appraisal visit included measurement of vital signs, extended questionnaire, and clinical laboratory evaluation, including hemogram, chemistry panel, glucose, and lipids. Individuals in the upper DBP percentiles were age-matched to subjects in the lower extreme percentiles. We ascertained 189 men (age 58.5 ± 10.4 [SD] years) with DBP ≥96 mm Hg and 281 men (age 57.7 ± 15.8 years) with DBP ≤61 mm Hg. Among the women, 175 were ascertained with high DBP (≥92 mm Hg; age 61.4 ± 11.2 years), along with 383 age-matched women with low DBP (DBP ≤59 mm Hg; age 53.9 ± 14.0 years). BP was treated by antihypertensive medications in 48% of subjects with hypertension. Thus, the DBP group separation for men was >35 mm Hg, while that for women was >33 mm Hg. Power calculations were performed using the on-line genetic power calculator for quantitative trait loci (26) according to the method described by Schork et al. (27). The power of an association