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Human Heart Rate

Heritability of Resting and Stress Values in Twin Pairs, and Influence of Genetic Variation in the Adrenergic Pathway at a Microribonucleic Acid MicroRNA Motif in the 3′-UTR of Cytochrome b561

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Objectives The goal of this study was to understand the role of genetic variation in the catecholamine biosynthetic pathway for control of human heart rate (HR).

Background Human HR is an integrated cardiovascular trait predictive of morbidity and survival. Because the autonomic pathway exerts rapid control over the heart, we probed the role of heredity in the control of HR, focusing on a component of the autonomic sympathetic pathway already predictive of outflow responses: cytochrome b561 (*CYB561*), the electron shuttle in catecholamine vesicle membranes for transmitter biosynthesis.

Methods We studied hereditary control of HR with the twin pair design, at rest and during environmental (cold) stress. Single nucleotide polymorphism disruption of a microribonucleic acid (microRNA) recognition motif in the human *CYB561* 3′-UTR was identified computationally, and its differential effect on gene expression was demonstrated in a transfected luciferase reporter/3′-UTR variant. We exposed stem cell–derived human embryoid bodies to the microRNA mimic or antagomir oligonucleotides, and we observed the effects on contraction rate in proto-hearts.

Results Substantial heritability (h^2) was demonstrated by using twin pair variance components for both basal/resting HR (h^2 50.9 ± 6.4% of trait variation, $p = 2.47 \times 10^{-10}$) and stress-augmented HR (h^2 55.1 ± 5.9%, $p = 8.79 \times 10^{-13}$), and the 2 HR traits shared genetic determination (genetic covariance ρG 0.747 ± 0.058, $p = 2.85 \times 10^{-9}$). *CYB561* displayed 1 common genetic variant in the transcript region: A+1485G (rs3087776), in the 3′-UTR, 1485 bp downstream of the termination codon, in a conserved region, with the A-allele ancestral in primates. In a twin/sibling sample (n = 576), A+1485G influenced HR, both at rest ($p = 0.010$) and after environmental stress ($p = 0.002$), with the minor (A) allele displaying a recessive effect with lower HR. The effect of A+1485G on HR was extended by meta-analysis into 2 additional population samples (total n = 2,579), and the influence remained directionally consistent and significant ($p = 0.007$). A+1485G disrupted a microRNA (human microribonucleic acid-1294 [hsa-miR-1294]) recognition motif in the 3′-UTR, as demonstrated by a transfected luciferase reporter/human 3′-UTR variant system in 2 different neuronal/neuroendocrine cell types. The microRNA effect was further documented by cotransfection of an hsa-miR-1294 mimic, yielding an exaggerated decline in expression of the A-allele (better match) reporter ($p = 4.3 \times 10^{-5}$). Similar findings of differential 3′-UTR allelic susceptibility to hsa-miR-1294 were noted during expression of the full-length human *CYB561* messenger ribonucleic acid with its cognate 3′-UTR. Finally, exposure of stem cell–derived human embryoid bodies to hsa-miR-1294 mimic or antagomir oligonucleotides yielded directionally opposite effects on contraction rate in proto-hearts.

Conclusions HR is a substantially heritable trait, with genetic influence by variation in the adrenergic pathway, here shown for messenger ribonucleic acid translational control at the *CYB561* step of transmitter formation. The results have implications for potentially modifiable autonomic pathways that influence this risk trait in the population.
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Human heart rate (HR) is an integrated cardiovascular trait that has assumed epidemiological importance because it may be predictive of premature mortality (both cardiovascular and noncardiovascular) and, consequently, lifespan (1,2). The HR trait is complex, reflecting not only cardiovascular but also metabolic adjustments (2). Rapid control of HR is achieved by the autonomic nervous system, in both its sympathetic (catecholaminergic, stimulatory) (3) and parasympathetic (vagal, inhibitory) branches. The role of heredity in control of HR has not been exhaustively examined, but genetic variation in the sympathetic pathway, including "tagging" (intronic) variation at the cytochrome b561 (*CYB561*) locus, reportedly influences cardiovascular responses to sympathetic activation (4). Indeed, naturally-occurring genetic variation at every other point in the adrenergic pathway (*GCH1* [5], *TH* [6], *DBH* [7], *PNMT* [8], and *ADRB1* [9]) has been associated with altered HR control, yet the interaction of *CYB561* and HR is still unexplored.

CYB561 is an electron transfer protein unique to catecholamine and neuropeptide secretory vesicles of the adrenal medulla, pituitary gland, and other neuroendocrine tissues (10,11). The 30-kDa protein may comprise as much as ~15% of the hormone storage vesicle membrane protein (12), and its role is to supply reducing equivalents to 2 monooxygenases, dopamine beta-hydroxylase (DBH) in chromaffin granules, and peptidylglycine alpha-amidating monooxygenase in neurosecretory vesicles (13). The cytochrome fulfills this role by catalyzing the transfer of electrons from a cytoplasmic donor, ascorbate, across a phospholipid bilayer to the luminal acceptor, semidehydroascorbate, in the interior of the vesicles. The continuously regenerated ascorbate within these vesicles is the immediate donor for the monooxygenases within the neuroendocrine secretory vesicles. Thus, cytochrome b561 is a transmembrane electron channel.

Because the sympathetic system exerts substantial control over HR, we first used the twin pair approach to determine whether HR responses were heritable; twin studies have successfully revealed hereditary contributions to cardiovascular stress traits, including HR (14). We then probed the role of genetic variation at the *CYB561* locus in such responses. We next used a transfected reporter system to examine the role of common variation in the 3′-UTR of the gene, which disrupted a microribonucleic acid (microRNA) recognition motif, a potential control point for messenger ribonucleic acid (mRNA) translation. Finally, we explored whether microRNA influenced the contraction rate in cultured human embryoid bodies (EBs).

MicroRNAs are emerging as a widespread endogenous mechanism for control of gene expression at the post-translational level (15), wherein the ~22-nucleotide mature/processed microRNAs bind to particular motifs in the 3′-UTRs of target mRNAs, inhibiting translation by catalyzing transcript scission/degradation or by steric hindrance. Indeed, the human genome harbors more than 1,000 microRNA-encoding loci (http://www.microrna.org).

Methods

Subjects and characterization. Subjects were volunteers drawn largely from southern California, and each subject provided written informed consent; the protocol was approved by the institutional review board of the University of California at San Diego. Recruitment procedures, definitions, and confirmation of subject diagnoses are according to previous reports from our group.

Initial: twins and siblings. From 235 nuclear families, 576 individuals from twin and sibling pairs were recruited to conduct the following study. Zygosity was confirmed by extensive microsatellite and single nucleotide polymorphism (SNP) genotyping, as described previously (16). Twins ranged in age from 15 to 84 years. Individuals of white (European-American, 87%) or Hispanic (Mexican-American, 13%) biogeographic ancestry/ethnicity (according to self-identification) were included.

Physiological phenotyping in vivo (twins and siblings): environmental (cold) stress. To probe the functional significance of common variation at *CYB561*, we examined the potential influence of 1 common *CYB561* polymorphism on HR before and during environmental (cold) stress testing (17) on 576 twin (monozygotic or dizygotic) and sibling individuals. Resting HR was recorded continuously for ~5 min before cold stress. During the stressor, the subject immersed the nondominant hand into ice (0°C) water for 1 min, with averaged measurements of HR stable over at least 3 beats before and then again toward the end of the 60-s procedure.

Extension: primary care population samples. From a pool of >53,000 subjects in a primary care (Kaiser Permanente) database in southern California, we ascertained 2 samples (Kaiser-1 and Kaiser-2) of European-ancestry individuals of both sexes (18). Evaluation included physical examination (with vital signs), blood chemistry screening, hemogram, and medical history questionnaire. HR was measured by manual palpation of a radial artery for 30 s (then multiplying by 2 to obtain beats/min) in seated, resting subjects just before measurement of blood pressure.

Genotyping *CYB561* variants. BIOINFORMATICS OF POLYMORPHISM. The extent of polymorphism at the *CYB561* locus was visualized at the National Center for

Abbreviations and Acronyms

A+1485G = single nucleotide polymorphism (rs3087776) in the human *CYB561* 3′-UTR

CYB561 = cytochrome b561 (electron shuttle for dopamine beta-hydroxylase)

DBH = dopamine beta-hydroxylase

DNA = deoxyribonucleic acid

EB = embryoid body

ESC = embryonic stem cells

hsa-miR-1294 = human micro-ribonucleic acid-1294

h^2 = heritability

HR = heart rate

MAF = minor allele frequency

microRNA = microribonucleic acid

mRNA = messenger ribonucleic acid

PCR = polymerase chain reaction

SNP = single nucleotide polymorphism

3′-UTR = 3′ (downstream of the open reading frame) untranslated region (of the human *CYB561* messenger ribonucleic acid)