A genetic polymorphism of the $\alpha_2$-adrenorenergic receptor increases autonomic responses to stress

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Finley, J. Clayton, Jr., Michael O’Leary, Derin Wester, Steven MacKenzie, Neil Shepard, Stephen Farrow, and Warren Lockette. A genetic polymorphism of the $\alpha_2$-adrenorenergic receptor increases autonomic responses to stress. J Appl Physiol 96: 2231–2239, 2004. We hypothesized that individual differences in autonomic responses to psychological, physiological, or environmental stresses are inherited, and exaggerated autonomic responsiveness may represent an intermediate phenotype that can contribute to the development of essential hypertension in humans over time. $\alpha_2$-Adrenergic receptors ($\alpha_2$-ARs), encoded by a gene on chromosome 10, are found in the central nervous system and also mediate release of norepinephrine from the presynaptic nerve terminals of the peripheral sympathetic nervous system and the exocytosis of epinephrine from the adrenal medulla. We postulated that, because this receptor mediates central and peripheral autonomic responsiveness to stress, genetic mutations in the gene encoding this receptor may explain contrasting activity of the autonomic nervous system among individuals. The restriction enzyme Dra I identifies a polymorphic site in the 3'-transcribed, but not translated, portion of the gene encoding the chromosome 10 $\alpha_2$-AR. Southern blotting of genomic DNA with a cDNA probe after restriction enzyme digestion results in fragments that are either 6.7 kb or 6.3 kb in size. Transfection studies of these two genotypes resulted in contrasting expression of a reporter gene, and it is suggested from these findings that this is a functional polymorphism. In a study of 194 healthy subjects, we measured autonomic responses to provocative motion, a fall in blood pressure induced by decreasing venous return and cardiac output, or exercise. Specifically, we measured reactions to 1) Coriolis stress, a strong stimulus that induces motion sickness in man; 2) heart rate responses to the fall in blood pressure induced by the application of graded lower body negative pressure; and 3) exercise-induced sweat secretion. In all of these paradigms of stress, subjective and objective evidence of increased autonomic responsiveness was found in those individuals harboring the 6.3-kb allele. Specifically, volunteers with the 6.3-kb allele had greater signs and symptoms of motion sickness mediated by the autonomic nervous system after off-axis rotation at increasing velocity (number of head movements a subject could complete during rotation before emesis ± SE: 295 ± 18 vs. 365 ± 11; P = 0.001). They also had greater increases in heart rate in responses to the lower body negative pressure-induced fall in blood pressure (increase in heart rate ± SE: 3.0 ± 0.4 vs. 1.8 ± 0.3; P = 0.012), and the 6.3-kb group had higher sweat sodium concentrations during exercise (mean sweat sodium concentration in meq/l over 30 min of exercise ± SE: 43.2 ± 7.1 vs. 27.6 ± 3.4; P < 0.05). This single-nucleotide polymorphism may contribute to contrasting individual differences in autonomic responsiveness among healthy individuals.

HYPERTENSION RARELY RESULTS from the Mendelian inheritance of a single abnormal gene in humans. High blood pressure is usually caused by a number of independently inherited traits, each encoded by an individual gene, which, when combined contribute to pathological elevations in vascular resistance. Also, the environment can affect the expression of these inherited traits. Because of the difficulty in sorting out the role of genes and the environment in the development of human hypertension, investigators often study “intermediate phenotypes.” These separate traits result from the biochemical and physiological expression of mutations in genes whose products alone do not raise blood pressure, but when coupled with other environmental or epistatic forces, over time, increase blood pressure. Once these intermediate phenotypes are identified, the degree to which these traits are inherited, and their genetic bases, can be determined. Humans carry their genetic makeup throughout life, yet essential hypertension frequently does not present until advancing age. The identification and study of these intermediate phenotypes is important because their presence may help identify those individuals at risk for the development of hypertension. It is possible that intermediate phenotype traits may be identified as being abnormal before a frank elevation in blood pressure in the individual is noted.

Augmented autonomic responses to a number of physiological and psychological stresses have been reported not only in laboratory animals and patients with hypertension but also in healthy individuals who have a family history and, hence, a genetic predisposition to high blood pressure. These paradigms of environmental stress include such techniques as measuring the heart rate and vascular resistance responses to mental arithmetic, pain, or exposure to cold (6, 17, 24, 33). We postulated that individual differences in autonomic responses to psychological, physiological, or environmental stresses are inherited, and exaggerated autonomic responsiveness may represent an intermediate phenotype that can contribute to the development of essential hypertension in humans over time.

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α₂-Adrenergic receptors (AR), encoded by a gene on chromosome 10 (C10), are found primarily in the brain stem, and they decrease the release of norepinephrine from peripheral presynaptic nerve terminals of the autonomic nervous system and the exocytosis of epinephrine from the adrenal medulla in the peripheral nervous system (21).

There is substantial evidence that these α₂-ARs in the central nervous system affect the level of tonic sympathetic drive to the heart and blood vessels. An α₂-AR agonist, clonidine, has been shown to cause hypotension and bradycardia when administered directly into the cerebral ventricles, and conversely, intracisternal administration of an α₂-AR antagonist yohimbine increases blood pressure, heart rate, and sympathetic activity in conscious rabbits. (3, 10). We postulated that, because this receptor helps mediate central autonomic responsiveness, genetic mutations in the gene encoding this receptor may explain contrasting activity of the autonomic nervous system in humans in response to stress. We tested our hypothesis that, by regulating the expression of central α₂-ARs and hence the release of norepinephrine from sympathetic nerve terminals, genetic polymorphisms of the α₂-AR could account for the increased autonomic reactivity and response to stress that may predispose some healthy individuals to hypertension.

The restriction enzyme DraI identifies a restriction fragment length polymorphism in the C10 α₂-AR gene. Genetic polymorphisms are mutations in a gene that occur relatively frequently within a population; i.e., the mutation is generally found in >1% of the population. Southern blotting of genomic DNA with a cDNA probe after restriction enzyme digestion results in fragments of 6.3 or 6.7 kb in size. We previously reported that homozygosity for the 6.3-kb allele, which is more prevalent in blacks, was associated with hypertension in blacks (19). However, that finding was based on a population-based study, and it was unknown as to whether this mutation was functional and whether expression of this mutation was causally related to the elevation in blood pressure of some (14, 19). To first determine whether this mutation results in real differences in gene expression, we sequenced this region of the C10 α₂-AR gene, and we sought to determine whether this mutation changed α₂-AR gene transcription.

We also postulated that autonomic responsiveness to physiological stressors should be uniformly altered in healthy individuals harboring a particular genotype. To study the effect of contrasting genotypes on autonomic responsiveness to environmental stress, we selected a novel paradigm, Coriolis stress, to activate many arms of the sympathetic nervous system. Coriolis stress results when an individual undergoes off-axis rotation at increasing velocity; in essence, it provokes an extreme form of motion sickness. All of the signs and symptoms of Coriolis-induced motion sickness are mediated by the efferent limbs of the autonomic nervous system (16, 25).

We proposed that the C10 α₂-AR genotype would also correlate with autonomic responsiveness to other physiological or environmental stimuli besides Coriolis stress. A fall in blood pressure activates cardiovascular baroreceptors, and the physiological response to this type of stress is also mediated by central and peripheral α₂-ARs of the autonomic nervous system (2, 12, 30). We used the technique of graded lower body negative pressure (LBNP) to safely induce a fall in blood pressure, and we measured heart rate responses to LBNP-induced decreases in venous return and blood pressure in individuals with contrasting genotype. We determined whether an individual’s complement of C10 α₂-AR also correlated with his or her heart rate responses to the fall in blood pressure induced by the application of graded LBNP.

Whereas activation of effenter sympathetic adrenergic neurons is reflected by increases in heart rate and vascular resistance, the α₂-ARs also mediate stimulation of central sympathetic, cholinergic efferent neurons that innervate the sweat glands (13, 22). We reasoned that functional differences in α₂-AR gene expression should also result in contrasting exercise-induced sweat secretion as well. We measured sympathetic cholinergic activation by comparing the concentration of sweat sodium during exercise and the sweat sodium concentration that resulted from direct peripheral muscarinic-receptor activation with the iontophoretic application of pilocarpine.

We present evidence that a functional genetic polymorphism of C10 α₂-AR correlated with increased autonomic responsiveness to undervest motion, LBNP-induced decrements in blood pressure, and augmented exercise-induced sweat sodium concentrations. These findings support the possibility that some healthy individuals may be genetically predisposed to exaggerated autonomic responsiveness to a variety of environmental stressors as a result of their α₂-AR gene complement.

METHODS

We recruited healthy men and women, and many first-degree relatives of these volunteers, for study from either the campus of Wayne State University (Detroit, MI), the University of Michigan (Ann Arbor, MI), or the Naval Medical Center (San Diego, CA). These protocols were approved by our institutional committees for the protection of human subjects in research, and all volunteers gave informed consent. All subjects were healthy, and they were taking no prescription or over-the-counter medications. None of the subjects studied in this work had any history of hypertension, and all had normal blood pressures. A small sample of blood was collected, and genomic DNA was isolated from lymphocytes using a standard isolation technique. Initially, subjects were genotyped after restriction digestion of their genomic DNA with DraI followed by Southern blotting with a C10 α₂-AR probe. Subsequently, we sequenced the portion of this gene responsible for this polymorphism, and subjects were then identified through automated DNA sequencing or after restriction digestion of cDNA generated from primers specifically designed to encompass this polymorphic portion of the C10 α₂-AR gene. Subjects were classified as either 6.7/6.7 homozygotes, 6.7/6.3 heterozygotes, or 6.3/6.3 homozygotes.

DNA sequencing and chloramphenicol acetyltransferase transfection assay. Many polymorphisms that have been identified with various diseases in population-based association studies subsequently prove to be silent mutations; i.e., they do not encode structural or functional differences in the protein translated. It can be surmised that those polymorphisms are simply markers that are in linkage disequilibrium with the truly causative mutation. Alternatively, correlations between genotype and trait may result from nonrandom mating within the population being investigated. Consequently, a trait may be associated with a particular polymorphism simply because the trait and the gene both independently occur more frequently in a particular population. A genetic polymorphism that results in functional differences in its protein product is more likely to result in alterations in physiological activity. To determine whether the known DraI polymorphism was functional, we first had to identify its location within the C10 α₂-AR gene, and we had to determine whether this mutation had functional consequences. We sequenced the polymorphic portion of this single exon gene, and we identified the mutation in the 3’-transcribed, but not translated, region of the gene. The C10 α₂-AR
3′ untranslated region (UTR) was sequenced from a 5.5-kb BamHI genomic fragment that contains ~2.4 kb of DNA downstream from the stop codon. The site of the polyadenylation was characterized using the 3′-rapid amplification of cDNA ends procedure on total RNA from HT29 cultured cells. The Dral site appeared to identify a potential rapid degradation signal for mRNA. Accordingly, we performed transfection studies with genomic DNA isolated from individuals who were homozygous for either the 6.3- or 6.7-kb allele. Chloramphenicol acetyltransferase (CAT) constructs containing a 1,115-bp fragment of the 3′ UTR sequence from individuals homozygous for each genotype were amplified with the polymerase chain reaction (upstream primer, 5′-GTA GAC TCA CGC TGA CTG CAG; downstream primer, 5′-GAA ACT GTA CAG TTT GGC AGG C) and subsequently ligated into pCR II vectors. Each pCR II-α2-AR UTR construct was cut with BamHI and NotI. The fragments were then ligated between the BamHIII/NotI cut sites within the bovine growth hormone pA 3′-UTR of expression vector pcDNAIII/CAT. The resultant vector was termed pcDNA/CAT/α2-AR UTR.

Next, Chinese hamster ovary cells (120,000 cells/well, 6 wells/plate) were transfected with 1 μg of pcDNA III/CAT/α2-AR UTR plasmid and 5 μl of lipofectin in 1 ml of Opti-MEM solution for 24 h. After completion of the transfection, the media was changed to 5% FBS/F-12 media, and the cells were incubated for 48 h. After incubation, the cells were washed with PBS, incubated, for 5 min in cold 1× TNE, and lysed in 100 μl of 0.25 M Tris·HCl, pH 7.8. To assay for CAT activity, 5 μl of a 1:10 dilution of the Tris·HCl extract, 6 μl of [14C]chloramphenicol (57 mCi/mmol, 25 μCi/ml), and 5 μl of N-butyryl-CoA (5 mg/ml) were added to 109 μl of 0.25 Tris·HCl, pH 7.8. This solution was incubated for 1.5 h at 37°C. After the incubation was complete, N-butyryl chloramphenicol was extracted with mixed xylene, and the amount of N-butyryl chloramphenicol production was determined by measuring the beta emissions of the xylene phase. The emissions from the control constructs were adjusted to account for differences in the quantity of gene insert per microgram of plasmid. Plasmids used in each replicate were separately isolated and quantified.

Coriolis stress susceptibility testing. To determine whether increased susceptibility to motion sickness was associated with a particular α2-AR phenotype, 115 unrelated college-age subjects were recruited from the Wayne State University and the University of Michigan, and we measured their susceptibility to Coriolis stress. We recognized that contrasting, individual susceptibility for motion sickness may depend on the different types of movement experienced (e.g., rectilinear acceleration vs. off-axis rotation vs. optokinetic rotation vs. up/down movement, etc.); accordingly, we restricted our study to one specific intermediate phenotype: Coriolis stress. We counted the number of off-axis head movements students could make while being rotated around their vertical axis at increasing velocity (16, 25). The development of signs and symptoms of motion sickness, mediated by the autonomic nervous system, were noted until the subjects reached the “end-point malaise” or maximum chair speed (23 rpm) as described. Subjects were queried about symptomatic responses mediated by the autonomic nervous system they experienced after each set of head movements, and they received one to three points for each symptom developed: lightheadedness, drowsiness, sweating, subjective warmth, increase in salivation, epigastric awareness, or distress, or nausea. The test was terminated when the subject observed eight points or when the chair speed reached 23 rpm. Coriolis stress susceptibility indexes (CSSI) were determined by utilizing a nonlinear transformation that weighted higher numbers of head movements so as to mathematically account for the increasing difficulty of performing this maneuver at higher angular velocities induced by faster chair speeds. The subject’s final score consisted of the number of head movements performed up to a maximum of 480; this was equivalent to a CSSI of 46.9. An increasing CSSI is correlated with greater resistance to the autonomic signs and symptoms that are characteristic of the development of motion sickness, and those individuals who reach the maximum score have relatively little activation of their effector autonomic nervous system.

Heart rate sensitivity. We used the technique of graded LBNP to induce a specific cardiovascular stress (12). With this approach, supine, euvelomeric subjects were encased to the waist in an airtight metal chamber, and a vacuum source was applied at increasing negative pressures relative to the atmosphere. During the application of LBNP, blood pools in the lower extremities and venous return to the heart are decreased; end-diastolic volume, stroke volume, and blood pressure fall; and, subsequently, there is a reflex increase in heart rate and vascular tone that is mediated by the autonomic nervous system. During this time, blood pressures were taken and heart rates and stroke volumes were determined by using cardiac impedance plethysmography (CardioDynamics, San Diego, CA). Forearm blood flow was measured with a mercury-in-Silastic strain gauge (Hokanson Instruments, Seattle, WA). We studied 44 subjects from the campus of the University of Michigan and 72 subjects from the Naval Medical Center. After a rest period, −10, −20, −30, −40, −50, and −60-mmHg vacuum pressure was applied incrementally every 10 min until the subjects reached either −50 mmHg in the University of Michigan study or −60 mmHg in the Naval Medical Center study, or the volunteer reported symptoms of impending syncope, or their diastolic blood pressure fell below 50 mmHg.

Exercised induced sweat excretion. Sweating is under the control of sympathetic, cholinergic fibers that descend from higher brain stem centers. We measured sympathetic-mediated sweat secretion during exercise. Because of difficulty we had in accurately collecting sweat during vigorous exercise, we measured the sodium concentration of sweat during exercise. An acute bout of exercise increases sweat sodium concentration (32). Also, to determine whether phenotypic differences in sweat gland responsiveness to direct cholinergic activation existed in our subjects, we also measured sweat sodium concentration after the iontophoretic application of pilocarpine to the volar aspect of the forearm. Pilocarpine (0.4%) was applied ionto- phonically to cleansed skin at 2–4 mA for 5 min. Sweat was subsequently collected from a 1-cm² area of the forearm with Mac- roduct Sweat Collection devices (Wescor, Logan, UT). To contrast the effect of genotype on sympathetic-dependent sweat sodium, we measured sweat output in a cohort of these subjects that exercised on bicycle ergometers at 85% of their predicted maximum heart rate for 30 min while sweat was similarly collected.

RESULTS

DNA sequencing and transfection studies. Many polymorphisms that have been associated with various diseases subsequently prove to be silent mutations; i.e., they do not encode structural or functional differences in the protein translated. It can be surmised that those polymorphisms are simply markers that are in linkage disequilibrium with the truly causative mutation, which may be found in a nearby gene, or they may be chance associations. We sequenced the polymorphic portion of this single-exon gene, and we identified the mutation in the human 3′-transcribed, but UTR, region of the gene before the polyadenylation signal; the mutation interrupted a mRNA rapid-degradation signal (Fig. 1). We found that the UTR region of the 6.3-kb allele reduced reporter gene expression, and it is likely that this mutation affects transcript levels of the C10 α2-AR. Specifically, we found that insertion of a portion of the 6.3-kb allele 3′ UTR into the 3′ UTR of a CAT reporter gene resulted in a diminished expression of CAT relative to the same construct containing the 6.7-kb allele (Fig. 2).

Coriolis gene distribution, polymorphism, and association within family studies. We found a bimodal distribution of Coriolis stress susceptibility scores in our population of 115
subjects from the University of Michigan; this distribution was suggestive of a major gene effect (Fig. 3). When these subjects were categorized by phenotype, we found that homozygotes for the 6.7-kb allele had markedly decreased susceptibility to Coriolis stress compared with 6.7-/6.3-kb heterozygotes (Fig. 4). From the prevalence rate of the 6.3-kb allele in the general population (20%), we would expect to find at least four homozygotes for the 6.3-kb allele according to Hardy-Weinberg equilibrium. However, in this cohort of subjects, we found no 6.3/6.3 homozygotes. This finding most likely represents the fact that individuals with hypertension were excluded from this study, and we have previously reported that homozygosity for the 6.3-kb allele predisposed some individuals to hypertension (19).

To guard against population bias, we next recruited an additional 79 people, with each study subject having at least one sibling or parent, who underwent a modified Coriolis stress susceptibility testing protocol at the Naval Medical Center. Under this modified protocol, subjects were similarly rotated at increasing velocity while they performed off-axis head movements. However, we increased the rotational speed of the chair.

Fig. 1. Sequence of the 3’-transcribed, but not translated (UTR), region of the chromosome 10 C10\textsuperscript{-AR} gene. Entire nucleotide sequence for the C10\textsuperscript{-AR} 3’ UTR. Numbers correspond to base position downstream of the start codon. The DraI polymorphic site, polyA signal and PCR primers used for transfection studies are indicated by shading or underline.

Fig. 2. The 6.7- and 6.3-kb alleles have functional differences. This presence of a DraI site indicates an AT-rich degradation site for mRNA. Varying levels of chloramphenicol acetyltransferase (CAT) expression from CAT constructs containing the α\textsubscript{2}-AR 3’ UTR that possess, or lack, the DraI polymorphism. The construct contained 1,115 bp of the C10\textsuperscript{-AR} 3’ UTR, 110 bp bovine growth hormone 3’ UTR, and 39 bp of polylinker DNA. Transfection with a CAT construct containing the 3’ UTR of the 6.7-kb allele resulted in decreased expression of CAT compared with cells transfected with a CAT construct containing the 6.7-kb allele. Values represent percent expression ± SE relative to constructs without the α\textsubscript{2}-AR UTR insert.

Fig. 3. Distribution of Coriolis stress susceptibility index (CSSI) scores in 115 healthy Michigan college-age students. A small, but significant, percentage of subjects did not reach “end-point malaise,” and they were able to continue off-axis head movements. However, we increased the rotational speed of the chair.
such that the velocity increased by 3 rpm, rather than 2 rpm, each time a subject completed eight sets of head movements. Because siblings, as well as parents and siblings, share genetic material, a correlation of traits among siblings can be used to study the degree to which certain physical characteristics or traits are passed from a parent to a child.

Those individuals who had high CSSI scores were more likely to have siblings who shared their relative immunity to motion sickness. When we examined volunteers within families where at least two members differed in genotype (11/31) but were expected to share an environment, we found significant decreases in Coriolis stress susceptibility in subjects who did, and did not, have a 6.3-kb allele (values expressed as head movements completed before malaise ± SE): 219 ± 11 vs. 287 ± 17, \( P = 0.003 \) (Fig. 5).

**LBNP gene distribution, polymorphism, and association within family studies.** We found that, in both cohorts of subjects from either Michigan or California, subjects who were heterozygous for the 6.7-/6.3-kb genotype had a greater increase in heart rate in response to the fall in blood pressure in response to maximum LBNP compared with individuals homozygous for the 6.7-/6.7-kb allele (Fig. 6). Similarly, we found greater decrements in forearm blood flow in response to LBNP in subjects heterozygous at the C10 \( \alpha_2 \)-AR allele compared with 6.7-/6.7-kb homozygotes (Fig. 7). Those individuals who are 6.7-kb homozygotes have less exuberant sympathetic responses, as measured by a change in heart rate and vascular reactivity, during the fall in blood pressure that was induced with the application of graded LBNP. Interestingly, those individuals who developed pronounced symptoms of the hypotension induced by LBNP were more likely to note their visceral responses to untoward motion. Those subjects who were most aware of impending syncope were also more likely to report autonomic symptoms to Coriolis stress (Fig. 8).

If the C10 \( \alpha_2 \)-AR indeed mediates both susceptibility to Coriolis stress, heart rate, and vascular contractile responses to orthostatic tolerance, then we should be able to demonstrate an association between Coriolis stress susceptibility and orthostatic tolerance. Indeed, we found a negative correlation between Coriolis stress susceptibility scores and baroreceptor sensitivity. Our retrospective analysis resulted in a relatively weak, but significant, correlation (data pooled from Michigan and San Diego, \( n = 98 \), \( r = -0.21 \), \( P = 0.01 \)) among all subjects.

**Exercise-induced sweating.** Despite comparable ergonomic efforts on stationary bicycles, in a room of constant temperature, we found that individuals carrying at least one allele of
the 6.3-kb C10 α2-AR allele had greater exercise-induced, sympathetic cholinergic sweat sodium concentration than individuals homozygous for the 6.7-kb allele (Fig. 9). These contrasting differences in exercise-induced sweat concentration were not due to intrinsic capacity to secrete sweat under maximum stimulation because genotype had no effect on maximal sweat excretion induced by pilocarpine.

**DISCUSSION**

It is suggested from a significant body of evidence that heightened activity of the sympathetic nervous system contributes to many of the phenotypic characteristics, i.e., measurable traits of human, essential hypertension. Blood pressure and heart rate responses to psychological stress, such as that induced by mental arithmetic or physiological stress resulting from exposure to cold or pain, are increased in individuals predisposed to the development of hypertension and in patients with preexisting “borderline” hypertension (6, 17, 24, 33). A logical extension to these observations is that noxious environmental influences, over a long period of time, lead to chronic, pathological elevations in blood pressure. Similarly, it has been demonstrated that baroreceptor sensitivity is abnormal among individuals predisposed to the development of high blood pressure (1, 28). To maintain adequate tissue perfusion, cardiovascular baroreceptors mediate the heart rate response to variations in blood pressure. Environmental challenge and baroreceptor activation induce changes in heart rate and peripheral blood flow mediated by the autonomic nervous system.

There is substantial evidence that α2-AR mechanisms in the central nervous system affect the level of sympathetic drive to the heart and blood vessels. The α2-AR agonist clonidine has been shown to cause hypotension and bradycardia when administered into the cerebral ventricles, and conversely, intracisternal administration of the α2-AR antagonist yohimbine increases blood pressure, heart rate, and sympathetic activity in conscious rabbits (3, 10). Clearly, central α2-ARs in some way mediate peripheral sympathetic nerve activity. For these reasons, we believed it likely that functional genetic polymorphisms of the central α2-ARs would manifest themselves when the sympathetic nervous system was activated.

There are three different genes on three different chromosomes, each encoding an α2-AR subtype; these receptors have contrasting ligand-binding characteristics and contrasting tissue specificity and expression (29). We first identified a genetic polymorphism of the α2-AR encoded on C10 that was associated with hypertension in blacks (19). In a population-based study of healthy normotensive subjects (14), our laboratory subsequently showed that this mutation was also associated with increased α2-AR-mediated platelet aggregation and decreased α2-AR-mediated natriuresis after expansion of the central blood volume. These two phenotypes are common in essential hypertension, and it was suggested that the tendency toward thrombotic stroke and salt sensitivity were due to the genetic polymorphism of the α2-AR rather than through the elevation of blood pressure per se.

The major limitation from our earlier work was the population-based design of these studies. Such an approach precludes any comment about cause and effect. However, in this report, we demonstrate that this mutation in the C10 α2-AR gene is functional, i.e., it does not encode a silent mutation. Our data with transiently transfected cells show that the 6.3-kb allele appeared to diminish the expression of the α2-AR. It is difficult to ascertain the level of α2-AR gene expression in the human central or peripheral nervous system in humans. In any event, chronic impairment of central α2-AR expression could be expected to result in an increased level of central catecholamines and raise central sympathetic outflow and enhance autonomic responsiveness.

In humans and rodents, the blood pressure response to the intravenous administration of an α2-AR agonist is biphasic. During the initial phase, mean arterial pressure rises transiently as postsynaptic vascular α2-ARs are activated. After a brief, initial hypertensive response, mean blood pressure drops below baseline levels because α2-ARs in the brain stem attenuate
sympathetic outflow and accentuate parasympathetic outflow. To summarize, the activation of brain stem α2-ARs primarily inhibits sympathetic outflow and enhances parasympathetic activity. Accordingly, it is likely that loss of brain stem α2-ARs or a diminished expression of these receptors could be expected to result in increased peripheral sympathetic nerve activity. Indeed, targeted disruption of the C10 α2-AR gene in mice (this homolog is the α2-AR 2A subtype), our gene of interest, resulted in a diminished expression of this receptor subtype in mouse brain stem slices (21). Furthermore, in animals with targeted disruption of the 2A receptor, the hypertensive response mediated by inhibition of the peripheral sympathetic nervous system was completely ablated after administration of an α2-AR agonist. Other studies (23) have also reported that disruption of the mouse equivalent of the human C10 α2-AR gene results in a reduction in the central inhibitory input on sympathetic outflow in mice.

In agreement with animal studies using genetic knockouts, we found that healthy, nonhypertensive individuals carrying the 6.3-kb allele for the C10 α2-AR gene had evidence of increased autonomic outflow (18, 21, 23). Other laboratories (9, 15) have reported abnormalities in α2-AR function in established, human hypertension. Goldstein et al. (15) characterized blood pressure responses to α2-AR inhibition by yohimbine, a pharmacological antagonist of the α2-AR that raises central catecholamines in humans. They reported that blood pressure, cardiac output, and plasma norepinephrine increments were exaggerated in subjects with hypertension and that there was no overlap whatsoever between the pressure responses in the normotensive and hypertensive subjects. Our work shows that these signs of exuberant autonomic responsiveness are likely a function of C10 α2-AR genotype, and this genetic complement may result in intermediate phenotypes associated with many of the characteristics responsible for the morbidity associated with hypertension.

There is evidence, previously unappreciated, that the autonomic responses to untoward motion sickness are, in part, genetically encoded. For example, the squirrel monkey is often used to study motion sickness, and marked strain differences in the susceptibility to motion sickness in this nonhuman primate have always been known. These New World monkeys are clearly inbred strains; they demonstrate other phenotypes with characteristics of polymorphic genetic markers segregating as Mendelian traits in closed populations. These strains display divergent phenotypes that have resulted from rapid gene fixation associated with small populations (7, 8). This contrasting sensitivity to motion in these inbred strains, among similar environmental influences, suggests that a strong genetic component to the propensity for motion sickness may exist in primates.

In an effort to understand the autonomic response to provocative motion, targeted disruption of the POU homeodomain transcription factor Brn-3.1 gene was undertaken to prevent the terminal differentiation of progenitor vestibular hair cells in mice that regulate responses to body position. Mice with disruption of this gene fail to respond with a fall in temperature, as is the case with normal control mice when exposed to off-axis rotation, and temperature regulation is mediated by α2-AR in mice (18). These data from studies of mouse knockouts give further support to our contention that the autonomic responsiveness to vestibular stimulation is mediated by the α2-AR encoded by C10 in humans.

A finding of ethnic differences in motion sickness susceptibility was the first suggestion of a heritable component of this trait (31). Similar to the report of others (16, 25), we found a bimodal distribution of CSSI in our population of volunteers; 15% of the subjects we studied were completely immune to this untoward motion. A plausible explanation for such a distribution of scores would be the influence of a genetic component, in addition to environmental influences, on the development of motion sickness. We also found a strong correlation of Coriolis susceptibility and genotype within family members. Similarly, we found a very tight correlation of heart rate response to LBNP and genotype among family members (not shown). Moreover, these within-family member correlations are highly suggestive of the heritable component of autonomic responsiveness.

In the past, other investigators have noted that challenges such as exposure to cold (cold pressor test) or mental arithmetic both result in exaggerated blood pressure response in individuals predisposed to develop hypertension (6, 17, 24, 33). We postulate that Coriolis stress susceptibility and heart rate response to LBNP represent two intermediate phenotypes that share a common genetic etiology. It can be suggested from our data that healthy individuals who harbor at least one copy of the 6.3-kb allele have a diminished ability to upregulate their α2-AR complement, and the ensuing increase in central catecholamines results in autonomic hyperresponsiveness that is manifested by more pronounced autonomic signs of motion sickness, excessive heart rate responses to LBNP-induced reductions in blood pressure, and enhanced exercise-induced sweat secretion.

It is interesting that Coriolis stress susceptibility scores correlated not only with objective, measurable cardiovascular responses to baroreceptor activation but with subjective responses to hypotension as well. Indeed, in our cohort of subjects in San Diego, we found that those individuals who reported presyncopal symptoms (sweating, light-headedness, tunnel vision, etc.) or had a dramatic decline in blood pressure during LBNP had significantly lower CSSI scores than those individuals who were able to tolerate the full application of LBNP. The number of head movements completed during testing, and CSSI scores, were significantly lower in those subjects who complained of symptoms of near syncope or met the predetermined level for fall in blood pressure that required termination of the LBNP (9.5 ± 1.7 vs. 14.0 ± 2.1; P = 0.028). Neurogenic syncope, a variant of the Bezold-Jarisch reflex, occurs in some individuals who have a reduction in their central blood volume. With a reduction in cardiac filling and blood pressure, those individuals with excessive catecholamine output have increased cardiac contractility and activation of cardiac stretch receptors that result in a central withdrawal of sympathetic tone and bradycardia. It appears that yohimbine-sensitive, brain stem α2-ARs are responsible for this compensatory phase of acute central hypovolemia. It is suggested that genotypic differences in the C10 α2-AR are associated with contrasting susceptibility to the Bezold-Jarisch reflex (11).

Our findings of contrasting sensitivity to LBNP-induced changes in heart rate among different α2-AR genotypes are consistent with our laboratory’s previously published findings.
that possession of the 6.3-kb allele also helps identify those individuals who have a decreased natriuretic response to thermal-neutral water immersion (14). When subjects are immersed to the neck in warm water, there is an expansion of the central blood volume, and cardiovascular baroreceptors are fully loaded. This response is just the opposite of that induced by unloading the baroreceptors with LBNP. This inhibition of cardiovascular baroreceptor activity results in a diminution of renal sympathetic nerve activity and salt-excretion increases (26, 27). Healthy individuals harboring the 6.3-kb allele had renal autonomic responses that were not inhibited by water immersion, and their sodium excretion was much lower than the 6.7/6.7 homozygotes. All of these intermediate phenotypes have been demonstrated to be abnormal in some patients with essential hypertension. Interestingly, our laboratory (20) also showed previously that patients with hypertension were exquisitely more sensitive to untoward motion. However, those investigations were performed before we began genotyping individuals for their C10 α2-AR alleles. In retrospect, those hypertensive individuals we studied were more likely to harbor the 6.3-kb allele. We cannot discount that the association between C10 α2-AR genotype and autonomic responsiveness could be due to another mutation in this gene or mutations in other genes in linkage disequilibrium with the C10 α2-AR. One example of such a linked gene is the β1-AR that is also encoded on C10. We found that the minor allele of an anonymous BglI polymorphism of this gene was also associated with hypertension in our population-based study (unpublished observation). Using discordant sibling pair analysis, other studies (4, 5) recently demonstrated an association with the β1-AR gene and essential hypertension.

In the last set of experiments, we demonstrated that sweat sodium concentrations during exercise differed as a function of the subject’s genotype. Increasing exertion is associated with increasing sweat sodium concentration during acute bouts of exercise (32). Although we cannot state with certainty, it is likely that the greater sweat sodium concentrations obtained from individuals harboring the 6.3-kb allele during exercise reflect greater activation of sympathetic cholinergic nerve fibers that innervate the sweat gland. These genotype-dependent differences in exercise-induced increases in sweat sodium concentration are not likely due to functional differences in sweat glands among individuals with contrasting genotypes, because stimulation of the sweat glands with pilocarpine brought about comparable sweat sodium concentrations.

In summary, we have identified a genetic polymorphism of the C10 α2-AR gene that places carriers at risk for exaggerated autonomic responsiveness to provocative stimuli: untoward motion, lowering of blood pressure, and exercise. These findings suggest the possibility of a pharmacogenomic approach to therapy, where genotypes such as the C10 α2-AR or one of these intermediate phenotypes that place individuals at risk for hypertension can be identified and more rationale treatment strategies planned.

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