

## $\beta$ -2 adrenergic receptor gene variations and blood pressure under stress in normal twins

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### Abstract

We tested the hypothesis that blood pressure (BP) responses to physical and mental stress are associated with polymorphisms in the  $\beta$ -2 adrenergic receptor (AR) gene. We studied normotensive, young, monozygotic (MZ) and dizygotic (DZ) twins. The subjects underwent automated BP measurements at the brachial and digital arteries and were subjected to mental arithmetic and cold pressor stress. We used allele-specific PCR to genotype four single nucleotide polymorphisms in the  $\beta$ -2 AR gene. The most functionally relevant polymorphism in the  $\beta$ -2 AR gene, Arg16/Gly, was associated with systolic and diastolic BP under resting conditions, during mental arithmetic, and during the cold pressor test, as well as with the increase in diastolic BP during both forms of stress. These findings support a role for the  $\beta$ -2 AR gene in BP regulation. They also indicate that the  $\beta$ -2 AR gene influences the level of not only resting but also stress-related BP.

**Descriptors:**  $\beta$ -2 adrenergic receptor, Molecular genetics, Twins, Blood pressure regulation, Stress, Reactivity

Weitz was the first to recognize the heritable nature of blood pressure (Weitz, 1923) and was also the first to study hypertension in twins (Weitz, 1925). Genetic variability of blood pressure is invariably found in twin studies (Ward, 1990). Differences in the heritability estimates may be attributed to population differences, differences in protocols, numbers of subjects, and study design. In addition to the basal level of blood pressure, blood pressure responses to exogenous physical stress are also influenced by genetic variability (Boomsma, Snieder, de Geus, & van Doornen, 1999; Ditto, 1993; Rose, 1992). The heritability estimates of these responses are variable as well, perhaps because the populations differ or the tests employed are insufficiently standardized. Because stress and autonomic hyperactivity have been associated with the development of hypertension later in life (Julius & Johnson, 1985; Manuck, 1994), and because both the blood pressure level and the stress-related reactivity of blood pressure seem to be important (Lambrechtsen, Rasmussen, Hansen, & Jacobsen, 1999; Light, Sherwood, & Turner, 1992), we studied basal blood pressure and heart rate under resting conditions as well as under mental and physical stress in 166 pairs of monozygotic (MZ) and dizygotic (DZ) twins. We employed both a mental arithmetic task and the cold pressor test, because these tests are comparable between studies (Sherwood & Turner, 1992).

The  $\beta$ -2 adrenergic receptor ( $\beta$ -2 AR) has been implicated in the pathogenesis of hypertension, both on the basis of studies suggesting altered  $\beta$ -2 mediated vasodilatation (Skrabal, Kotanko, & Luft, 1989) and on the basis of molecular genetic association studies (Svetkey et al., 1996; Svetkey, Chen, McKeown, Preis, Wilson 1997). Recently, Kotanko et al. (1997) found an association between the Arg16/Gly polymorphism in the  $\beta$ -2 AR gene and hypertension in an African-Caribbean population. They showed that the Gly16 allele was more common in hypertensive subjects than in normotensive African-Caribbean controls. Because the Gly16 allele indicates an increased propensity for downregulation of the receptor (Turki, Pak, Green, Martin, & Liggett, 1995), the authors raised the possibility that an impaired vasodilatation in peripheral arteries in response to  $\beta$ -2 AR agonists may play a role in the hypertension of individuals carrying the Gly16 allele. We subsequently examined the first-born normotensive adult children of couples documented to be normotensive or hypertensive in the Bergen Blood Pressure Study (Timmermann et al., 1998). Offspring of two hypertensive parents had higher blood pressures. Furthermore, in contradistinction to the findings of Kotanko et al., a preponderance of the Arg16 allele, compared to offspring of two normotensive parents, was observed. To further examine the genetic variability at the  $\beta$ -2 AR locus and its relevance for the cardiovascular system in northern Europeans, we performed an association study in normotensive twin subjects. We employed allele-specific PCR, which allowed us to examine other polymorphisms in the  $\beta$ -2 AR gene (Busjahn et al., 2000). This study relied on mercury manometer readings, which can be criticized in terms

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of accuracy. Indeed, we were only able to find associations for systolic, but not for diastolic blood pressures in that study.

In the present study, we measured blood pressure with automated devices relying on different physical principles. We incorporated physical and mental stress tests. Our rationale was that systolic and diastolic blood pressure, blood pressure in response to stressors, and the increase in blood pressure in response to stressors might very well involve different genes. We were able to confirm a role for the  $\beta$ -2 AR gene in blood pressure regulation.

## Methods

We recruited 166 pairs of twins (MZ 100 and DZ 66) by advertisement to participate in studies involving blood pressure regulation and cardiovascular phenotypes (Busjahn, Faulhaber, Viken, Rose, & Luft, 1996; Busjahn et al., 1997). The subjects were all German Caucasians, recruited from various parts of Germany. The protocol was approved by Humboldt University's committee on the protection of human subjects and written informed consent was obtained from all participants. Blood was obtained for the determination of zygosity and other molecular genetic studies from all the twins and the parents of the DZ twins. Each participant underwent a medical history and physical examination. Subjects taking antihypertensive medication or being affected by any other chronic medical illness were excluded.

Blood pressure and heart rate were measured in the nondominant arm by an automated oscillometric method (Dinamap; Tampa, FL) every minute as well as continuously at a finger of the dominant arm by the Finapres (Ohmeda, Louisville, CO) blood pressure monitor. The latter device relies on the Penaz principle, in which the blood pressure is determined in the middle finger. Although the absolute values of this method may be influenced by various factors, intra-individual comparisons within one session are highly reliable (Bos, Imholz, van Goudoever, Weseling, van Montfrans, 1992). All blood pressure level data were based on the averaged Dinamap measurements, and blood pressure reactivity was calculated based on continuous Finapres blood pressure values. This approach allowed us to observe blood pressure differences on a beat-to-beat basis with the Finapres values. We could then check the absolute accuracy of these measurements by means of the Dinamap values. Furthermore, the Dinamap measurements provided highly accurate inter-individual differences, whereas the Finapres values provided unbiased intra-individual differences. The dependent variables that were studied were blood pressure level at rest, during mental arithmetic and cold pressor, and blood pressure increases during mental arithmetic and cold pressor.

The three-minute mental arithmetic task consisted of multiplications. Tasks were presented on a computer screen; the level of tasks was adapted to reaction times, to reduce any possible differences related to education levels as well as to ensure a similar demand level independent of abilities. For exogenous physical stress we relied on the cold pressor test in our studies. This test exhibits considerable standardization and reliability (Mathias & Bannister, 1993) and does not require the subject's cooperation as does the handgrip test. The sympathetic activation of the cold pressor test in terms of increases in plasma norepinephrine values is established (LeBlanc, Cote, & Jobin, 1979). The cold pressor test has been used in earlier twin studies (McIlhany, Shaffer, & Hines, 1975). For the cold pressor test, the subjects rested semi-recumbent in a dental chair. The studies were conducted in a quiet, sound-proof room at 20 °C. The subjects immersed the left hand into cold (<4 °C) water for 2 min after a 5-min rest period. The

mental arithmetic task is primarily a  $\beta$ -adrenergic task, whereas the cold pressor test is a  $\alpha$ -adrenergic task. Thus they are not directly comparable as they invoke different physiological pathways. For the resting blood pressure, we chose to use the averaged blood pressure values obtained continuously for 2 min, 3 min after the cold pressor test to avoid any possible pretest anxiety. These values were lower than pretest values (data not shown), suggesting that indeed the posttest values were obtained with less anxiety. Ditto (1993) also relied on the posttest values in his twin study. Our techniques for zygosity testing and genotyping of microsatellites have been previously described (Becker et al., 1997). We genotyped the functionally relevant polymorphisms of the  $\beta$ -2 AR by means of allele-specific PCR as described elsewhere (Busjahn et al., 2000).

Statistical analysis was conducted using the SPSS program. The  $p$  values were adjusted for multiple testing by Bonferroni's method. Blood pressure values were adjusted for sex and age by multiple linear regression. Heritability was estimated based on variance/covariance matrices by structural equation modeling (Neale & Cardon, 1992) using the MX program (Neale, 1997). Association analysis was based on quantitative measures. As association analysis based on ANOVA or  $t$  test might be prone to false positive results due to population stratification, an analysis based on sib-pair data was carried out (Fulker, Cherny, Sham, & Hewitt, 1999; Neale, 1997; Neale et al. 1999). To test for association, structural equation modeling was used to obtain maximum likelihood estimates for the allelic effect, based on within-family and between-family differences. Significance was tested by computing nested models with and without allelic effects and comparing the log-likelihood between models.  $P$  values were obtained on the basis of the  $\chi^2$  distribution. To test for stratification effects, both estimates of allelic effect were constrained to be equal. In the absence of stratification, both estimates were then set to zero. In the presence of stratification effects, setting only the within-family estimate to zero provides an unbiased test for true allelic association.

## Results

Demographic data, blood pressure values, and heritability estimates of blood pressure in 200 MZ and 132 DZ twins are given in Table 1. There were no significant differences between MZ and DZ twins for any of the variables examined. Height, weight, and body mass index (BMI) were strongly influenced by genetic variance. Furthermore, resting systolic and diastolic blood pressure, heart rate, blood pressure under stress, heart rate and blood pressure increase under stress were all heritable, albeit to variable degrees with the exception of systolic blood pressure increase with mental arithmetic.

We next performed allele-specific PCR for four polymorphisms responsible for amino acid exchanges. The gene frequencies in our subjects are given in Table 2. Table 3 shows the results for the association analysis for the Arg16/Gly polymorphism. We elected to show this polymorphism because it gave the strongest associations. The other three polymorphisms were in linkage disequilibrium with Arg16/Gly to variable degrees. Arg16/Gly showed strong association to systolic and diastolic blood pressure levels, as well as with diastolic blood pressure increases to both stressors. Gln27/Glu was only related to diastolic blood pressure level and to diastolic blood pressure increases during cold pressor and during mental arithmetic. Thr164/Ile and the promotor polymorphism -47C/T were not associated with any measures (data not shown).

**Table 1.** Demographic Data (Mean  $\pm$  SD) and Heritability Estimates

Phenotype	MZ twins	DZ twins	$a^2$ ( $r_{MZ}/r_{DZ}$ )
N	200	132	
Age, y	29 $\pm$ 12	31 $\pm$ 12	
Sex M/F	52/148	85/47	
Height, cm	169 $\pm$ 8	170 $\pm$ 8	0.96 (0.96/0.45)
Weight, kg	65 $\pm$ 11	67 $\pm$ 12	0.88 (0.88/0.42)
BMI, kg/m <sup>2</sup>	22.4 $\pm$ 3.5	22.8 $\pm$ 3.4	0.68 (0.83/0.49)
Systolic BP rest, mmHg	114.5 $\pm$ 17.3	112.1 $\pm$ 13.7	0.24 (0.55/0.43)
Diastolic BP rest, mmHg	63.1 $\pm$ 11.1	61.8 $\pm$ 8.5	0.40 (0.65/0.45)
Heart rate rest, bpm	71.9 $\pm$ 11.0	71.7 $\pm$ 9.5	0.54 (0.53/0.35)
Systolic BP mental arithmetic, mmHg	134.8 $\pm$ 20.1	132.9 $\pm$ 19.5	0.34 (0.59/0.42)
Diastolic BP mental arithmetic, mmHg	75.4 $\pm$ 11.9	74.5 $\pm$ 9.6	0.48 (0.65/0.41)
Heart rate mental arithmetic, bpm	88.8 $\pm$ 16.6	88.8 $\pm$ 15.4	0.42 (0.52/0.31)
Systolic BP cold pressor, mmHg	128.9 $\pm$ 19.2	123.6 $\pm$ 16.9	0.30 (0.55/0.40)
Diastolic BP cold pressor, mmHg	74.7 $\pm$ 11.8	70.9 $\pm$ 10.2	0.59 (0.59/0.31)
Heart rate cold pressor, bpm	77.1 $\pm$ 11.9	76.8 $\pm$ 10.5	0.40 (0.60/0.40)
Systolic BP increase mental arithmetic, mmHg	15.2 $\pm$ 16.1	13.7 $\pm$ 14.6	0.00 (0.17/0.23)
Diastolic BP increase mental arithmetic, mmHg	12.3 $\pm$ 7.4	12.6 $\pm$ 7.7	0.12 (0.25/0.19)
Heart rate increase mental arithmetic, bpm	17.0 $\pm$ 11.2	17.2 $\pm$ 10.1	0.52 (0.52/0.26)
Systolic BP increase cold pressor, mmHg	11.3 $\pm$ 11.9	8.8 $\pm$ 9.6	0.08 (0.29/0.25)
Diastolic BP increase cold pressor, mmHg	11.7 $\pm$ 7.2	9.4 $\pm$ 6.1	0.30 (0.45/0.30)
Heart rate increase cold pressor, bpm	5.2 $\pm$ 5.2	5.1 $\pm$ 4.1	0.40 (0.46/0.26)

**Discussion**

The findings of the present study confirm and extend our earlier report in which we showed linkage of the  $\beta$ -2 AR gene locus to systolic blood pressure in these same twin subjects and associations between the various polymorphisms and systolic blood pressure (Busjahn et al., 2000). In that study, we relied on blood pressure measurements obtained with a mercury manometer. The values tested here were obtained by the Dinamap oscillometric method and with the Finapres device. These more sensitive determinations extend the significance of the observations to diastolic blood pressure, which is more accurately recorded with the automated methods applied in this study. Furthermore, we were now able to extend the observations to blood pressure responses under mental and physical stress conditions.

In an earlier twin study (Busjahn et al., 1996), we had observed that resting levels and stress-related blood pressure increases might be regulated by entirely different genes. Furthermore, the same may very well be true of systolic and diastolic blood pressure. Thus, the finding of consistent Arg16/Gly polymorphism associ-

ations in the  $\beta$ -2 AR gene under both resting and stress conditions was somewhat surprising. The associations were present for both systolic and diastolic blood pressure irrespective of whether the stress was mental or physical in nature. Also unexpected was the finding that the increase in diastolic, but not systolic, blood pressure in response to both stress forms also was associated with this same polymorphism. Systolic blood pressure may be more a function of aortic elasticity and pulse wave velocity, whereas diastolic blood pressure may be more related to peripheral vascular resistance (Safar, London, Asmar, & Frohlich, 1998). Such influences may explain why only diastolic blood pressure increases were associated with the Arg/Gly polymorphism.

We examined four biallelic polymorphisms in the  $\beta$ -2 AR gene, which result in amino acid substitutions (Liggett, 1995). A fifth, Val34/Met, was not encountered in our subjects. We reported only the results for the Arg16/Gly polymorphism because it gave the most consistent results. The polymorphisms are in linkage disequilibrium. The strongest linkage was found between polymorphisms in close spatial relationship to one another, namely -47C/T, Arg16/Gly, and Gln27/Glu. Thr164/Ile was independent of -47C/T, Gln27/Glu, and Arg16/Gly. We believe that our association approach strongly supports an important role for the  $\beta$ -2 AR gene in blood pressure regulation. We base this statement on the association between the genotypes and blood pressure levels at rest and during stress. However, the nature of that role is not entirely clear. Furthermore, only the diastolic blood pressure increases in response to the stress stimuli were associated with the genotypes, which makes any role of  $\beta$ -2 AR in the degree of increase less clear.

In our study, the Gly allele was consistently associated with the lower blood pressure values, irrespective of the conditions. In the report by Kotanko et al. (1997), the Arg allele was associated with the lower blood pressures. Kotanko et al. (1997) interpreted their results by suggesting that because the Gly allele has been shown to feature a receptor that downregulates more effectively when exposed to agonist (Turki et al., 1995), the Arg allele would be more likely to permit vasodilatation in response to  $\beta$ -2 AR stimulation.

**Table 2.** Position, Consequence, and Genotype Frequencies for Polymorphisms

Position (nucleotide base)	Polymorphism and frequencies (allele 1 /allele 2)	Amino acid change/position
-47	C $\rightarrow$ T (38%/62%)	(regulatory region)
+46	A $\rightarrow$ G (47%/53%)	Arg $\rightarrow$ Gly/16
+79	C $\rightarrow$ G (61%/49%)	Gln $\rightarrow$ Glu/27
+491	G $\rightarrow$ A (99%/1%)	Thr $\rightarrow$ Ile/164

**Table 3.** Blood Pressure (BP) and Heart Rate Association Analyses for the Arg16/Gly Polymorphisms in the  $\beta$ -2 AR Gene

Phenotype	Arg/Arg	Arg/Gly	Gly/Gly	<i>p</i>
<i>n</i> (subjects)	86	120	101	
Systolic BP rest, mmHg	116.9 ± 15.5	112.6 ± 16.7	110.6 ± 14.3	<.05
Diastolic BP rest, mmHg	64.2 ± 9.6	62.0 ± 10.3	60.9 ± 9.3	<.05
Heart Rate rest, bpm	72.1 ± 12.2	73.1 ± 9.0	70.9 ± 11.1	n.s.
Systolic BP mental arithmetic, mmHg	137.9 ± 18.7	133.3 ± 20.9	131.6 ± 19.1	<.05
Diastolic BP mental arithmetic, mmHg	76.2 ± 11.	74.8 ± 10.7	74.2 ± 10	<.05
Heart rate mental arithmetic, bpm	88.2 ± 14.8	91.5 ± 15.5	86.7 ± 16.0	n.s.
Systolic BP cold pressor, mmHg	130.1 ± 20.2	126.0 ± 17.9	125.3 ± 17.6	<.05
Diastolic BP cold pressor, mmHg	76.8 ± 13.2	72.6 ± 10.4	71.4 ± 10.3	<.05
Heart rate cold pressor, bpm	77.0 ± 12.6	78.1 ± 9.9	76.0 ± 11.9	n.s.
Systolic BP increase mental arithmetic, mmHg	15.7 ± 17.9	14.2 ± 15.2	14.5 ± 14.2	n.s.
Diastolic BP increase mental arithmetic, mmHg	10.0 ± 10.1	8.0 ± 8.6	7.5 ± 7.5	<.05
Heart Rate increase mental arithmetic, bpm	16.1 ± 10.2	18.5 ± 11.6	15.8 ± 9.3	n.s.
Systolic BP increase cold pressor, mmHg	11.7 ± 13.7	10.7 ± 9.5	10.8 ± 9.4	n.s.
Diastolic BP increase cold pressor, mmHg	8.4 ± 7.6	7.0 ± 5.0	7.0 ± 6.0	<.05
Heart rate increase cold pressor, bpm	4.8 ± 4.5	5.0 ± 5.5	5.1 ± 4.6	n.s.

In support of their interpretation, Kirby, Woodworth, Woodworth, and Johnson (1991) have demonstrated the importance of  $\beta$ -2 AR-mediated vasodilatation in spontaneously hypertensive rats. Gratzke et al. (1999) reported similar findings in normal human subjects, namely, persons with the Arg allele showed more dilatation in response to isoproterenol than persons harboring the Gly allele. We found the opposite in the present and in our earlier studies (Timmermann et al., 1998; Busjahn et al., 2000). We also have a rationale to explain our results. A more downregulated  $\beta$ -2 AR would be consistent with the pharmacological effects engendered by beta blockade. The value of beta blockers in the treatment of hypertension is not disputed. Further support is perhaps related to the fact that the  $\beta$ -2 AR genotypes seemed to influence the blood pressure levels during and after stress. The degree of increase with stress was weak and only apparent for diastolic pressure. Interestingly, beta blockers have little effect on blood pressure responses to various acute stressors, although they obviously affect blood pressure levels (Andren & Hansson, 1981). Finally, Kotanko et al. (1997) studied hypertensive persons of African origin, whereas we investigated normotensive Caucasians. Ethnic differences could perhaps explain the discrepancies. Such differences have been described for beta-receptor activity/sensitivity (Girdler, Hinderliter, & Light, 1993; Lang et al., 1995).

Brain and peripheral adrenergic and noradrenergic receptors undoubtedly participated in the blood pressure responses to mental and physical stress in our subjects. Klimek et al. (1999) studied brain noradrenergic receptors in normal subjects and in patients with major mental illnesses. They found differences in

$\beta$ -1 AR and  $\beta$ -2 AR densities in these subjects, illustrating how subtle changes in noradrenergic function in the central nervous system may influence mental well-being. Furthermore, Tangri, Gupta, Vrat, and Husain (1993) have demonstrated that neurotransmitters administered directly into the lateral cerebral ventricle of humans influence central  $\alpha$  and  $\beta$  adrenergic receptors, thereby influencing blood pressure and temperature regulation. Our subjects were all normal and thus likely represent the normal spectrum of receptor variation. We have not yet tested the hypothesis that genetic variations in adrenergic receptors might influence mood or behavior patterns; however, that hypothesis lends itself to testing with our current techniques. Adrenergic neurotransmission is strongly influenced by presynaptic adrenergic receptors, including the  $\beta$ -2 AR (Westfall, 1977). Genetic modifications in the  $\beta$ -2 AR would be expected to alter those responses.

We have no immediate explanation for why the associations did not robustly extend to blood pressure increases under physical and mental stress. Certainly, our study has limitations. Although our stressors were relatively standard, perhaps they were not sufficiently profound to produce a reliable phenotype. Furthermore, the stressors were necessarily short term. We believe that our measurement techniques, albeit noninvasive, were reliable. We relied only on a single gene to seek associations. Clearly, a dozen or more genes are involved in the efferent arm of the autonomic systems we addressed. To study this issue and to illuminate these responses further, a genetic analysis of other receptors, neurotransmitters, and metabolizing enzymes will be necessary.

## REFERENCES

- Andren, L., & Hansson, L. (1981). Circulatory effects of stress in essential hypertension. *Acta Med Scand Suppl.*, 646, 69–72.
- Becker, A., Busjahn, A., Faulhaber, H.-D., Bähring, S., Schuster, H., & Luft, F. C. (1997). Automated twin zygosity determination with microsatellites. *Journal of Reproductive Medicine*, 42, 260–266.
- Boomsma, D. I., Snieder, H., de Geus, E. J. C., & van Doornen L. J. P. (1999). Heritability of blood pressure increases during mental stress. *Twin Research*, 1, 15–24.
- Bos, W. J. W., Imholz, B. P. M., van Goudoever, J., Weseling, K. H., & van Montfrans G. A. (1992). The reliability of noninvasive continuous finger blood pressure measurement in patients with both hypertension and vascular disease. *American Journal of Hypertension*, 5, 529–535.
- Busjahn, A., Faulhaber, H.-D., Viken, R. J., Rose, R. J., & Luft, F. C. (1996). Genetic influences on blood pressure with the cold pressor test: A twin study. *Journal of Hypertension*, 14, 1195–1199.
- Busjahn, A., Knoblauch, J., Knoblauch, M., Bohlender, J., Menz, M.,

- Faulhaber, H.-D., Becker, A., Schuster, H., & Luft, F. C. (1997). Angiotensin converting enzyme and angiotensinogen gene polymorphisms, plasma levels, and left ventricular size: A twin study. *Hypertension*, *29*, 165–170.
- Busjahn, A., Li, G.-H., Faulhaber, H.-D., Rosenthal, M., Becker, A., Jeschke, E., Schuster, H., Timmermann, B., Hoehe, M. R., & Luft, F. C. (2000).  $\beta$ -2 adrenergic receptor gene variations, blood pressure, and heart size in normal twins. *Hypertension*, *35*, 555–560.
- Ditto, B. (1993). Familial influences on heart rate, blood pressure, and self-reported anxiety responses to stress: Results from 100 twin pairs. *Psychophysiology*, *30*, 635–645.
- Fulker, D. W., Cherny, S. S., Sham, P. C., & Hewitt, J. K. (1999). Combined linkage and association sib-pair analysis for quantitative traits. *American Journal of Human Genetics*, *64*, 259–267.
- Girdler, S. S., Hinderliter, A. L., & Light, K. C. (1993). Peripheral adrenergic receptor contributions to cardiovascular reactivity: Influence of race and gender. *Journal of Psychosomatic Research*, *37*, 177–193.
- Gratze, G., Fortin, J., Labugger, R., Binder, A., Kotanko, P., Timmermann, B., Luft, F. C., Hoehe, M., & Skrabal, F. (1999).  $\beta$ -2 adrenergic receptor variants affect agonist-induced vasodilatation in normotensive caucasians. *Hypertension*, *33*, 1425–1430.
- Julius, S., & Johnson, E. H. (1985). Stress, autonomic hyperactivity and essential hypertension: An enigma. *Journal of Hypertension*, *3*(suppl. 4), S11–S17.
- Kirby, R. F., Woodworth, C. H., Woodworth, G. G., & Johnson, A. K. (1991). Beta-2 adrenoceptor mediated vasodilatation: Role in cardiovascular responses to acute stressors in spontaneously hypertensive rats. *Clinical and Experimental Hypertension A*, *13*, 1059–1068.
- Kotanko, P., Binder, A., Tasker, J., DeFreitas, P., Kamdar, S., Clark, A. J. L., Skrabal, F., & Caulfield, M. (1997). Essential hypertension in African Caribbeans associates with a variant of the  $\beta$ -2 adrenoceptor. *Hypertension*, *30*, 773–776.
- Klimek, V., Rajkowska, G., Luker, S. N., Dilley, G., Meltzer, H. Y., Overholser, J. C., Stockmeier, C. A., & Ordway, G. A. (1999). Brain noradrenergic receptors in major depression and schizophrenia. *Neuropsychopharmacology*, *21*, 69–81.
- Lambrechtsen, J., Rasmussen, F., Hansen, H. S., & Jacobsen, I. A. (1999). Tracking and factors predicting rising in “tracking quartile” in blood pressure from childhood to adulthood: Odense schoolchild study. *Journal of Human Hypertension*, *13*, 385–391.
- Lang, C. C., Stein, C. M., Brown, R. M., Deegan, R., Nelson, R., He, H. B., Wood, M., & Wood, A. J. (1995). Attenuation of isoproterenol-mediated vasodilation in blacks. *New England Journal of Medicine*, *333*, 155–160.
- LeBlanc, T., Cote, T., & Jobin, M. (1979). Plasma catecholamines and cardiovascular responses to cold and mental activity. *Journal of Applied Physiology*, *47*, 1207–1211.
- Liggett, S. (1995). Functional properties of human  $\beta$ 2-adrenergic receptor polymorphisms. *News in Physiological Sciences*, *10*, 265–273.
- Light, K. C., Sherwood, A., & Turner, J. R. (1992). High cardiovascular reactivity to stress. In J.R. Turner, A. Sherwood, & K.C. Light (Eds.), *Individual differences in cardiovascular response to stress* (pp. 281–293). Plenum Press: New York.
- Manuck, St. B. (1994). Cardiovascular reactivity in cardiovascular disease: Once more onto the breach. *International Journal of Behavioral Medicine*, *1*, 4–31.
- Mathias, C. J., & Bannister, R. (1993). Investigation of autonomic disorders. In R. Bannister & C.J. Mathias (Eds.), *Autonomic failure* (3rd ed., p. 266). Oxford: Oxford Medical Publishers.
- McIlhany, M. L., Shaffer, J. W., & Hines, E. A., Jr. (1975). The heritability of blood pressure: An investigation of 200 pairs of twins using the cold pressor test. *Johns Hopkins Medical Journal*, *132*, 57–64.
- Neale, M. C. (1997). *Mx: Statistical modeling*. 4th edition. Richmond, VA: Department of Psychiatry, Medical College of Virginia.
- Neale, M. C., & Cardon, L. R. (1992). *Methodology for genetic studies of twins and families*. Dordrecht, Netherlands: Kluwer Academic Publishers.
- Neale, M. C., Cherny, S. S., Sham, P. C., Whitfield, J. B., Heath, A. C., Birley, A. J., & Martin, N. G. (1999). Distinguishing population stratification from genuine allelic effects with Mx: Association of ADH2 with alcohol consumption. *Behavior Genetics*, *29*, 233–243.
- Rose, R. J. (1992). Genes, stress, and cardiovascular reactivity. In J.R. Turner, A. Sherwood, & K.C. Light (Eds.), *Individual differences in cardiovascular response to stress* (pp. 87–102). New York and London: Plenum Press.
- Safar, M. E., London, G. M., Asmar, R., & Frohlich, E. D. (1998). Recent advances on large arteries in hypertension. *Hypertension*, *32*, 156–161.
- Sherwood, A., & Turner, J. R. (1992). A conceptual and methodological overview of cardiovascular reactivity research. In J.R. Turner, A. Sherwood, & K.C. Light (Eds.), *Individual differences in cardiovascular response to stress* (pp. 3–32). New York and London: Plenum Press.
- Skrabal, F., Kotanko, P., & Luft, F. C. (1989). Minireview: Inverse regulation of  $\alpha$ -2 and  $\beta$ -2 adrenoceptors in salt-sensitive hypertension: An hypothesis. *Life Sciences*, *45*, 2061–2076.
- Svetkey, L. P., Chen, Y. T., McKeown, S. P., Preis, L., & Wilson, A. F. (1997). Preliminary evidence of linkage of salt sensitivity in black Americans at the beta 2-adrenergic receptor locus. *Hypertension*, *29*, 918–922.
- Svetkey, L. P., Timmons, P.Z., Emovon, O., Anderson, N. B., Preis, L., & Chen, Y. T. (1996). Association of hypertension with the  $\beta$ -2 and  $\alpha$  2c10 adrenergic receptor genotype. *Hypertension*, *27*, 1210–1215.
- Tangri, K. K., Gupta, S. K. Vrat, S., & Husain, M. (1993). A study of effects of putative neurotransmitters injected into the lateral cerebral ventricle of man. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *17*, 731–745.
- Timmermann, B., Rune, M., Luft, F. C., Gerds, E., Busjahn, A., Omvik, P., Guo-Hua, L., Schuster, H., Wienker, T. F., Hoehe, M., & Lund-Johansen, P. (1998).  $\beta$ -2 adrenoceptor genetic variation is associated with genetic predisposition to essential hypertension: The Bergen Blood Pressure Study. *Kidney International*, *53*, 1455–1460.
- Turki, J., Pak, J., Green, S. A., Martin, R. J., & Liggett, S. B. (1995). Genetic polymorphisms of the  $\beta$ 2-adrenergic receptor in nocturnal and nonnocturnal asthma: Evidence that Gly16 correlates with the nocturnal phenotype. *Journal of Clinical Investigation*, *95*, 1635–1641.
- Ward, R. (1990). Familial aggregation and genetic epidemiology of blood pressure. In J.H. Laragh & B.M. Brenner (Eds.), *Hypertension: Pathophysiology, diagnosis, and management* (pp. 81–99). New York: Raven Press, Ltd.
- Weitz, W. (1923). Zur Ätiologie der genuinen oder vasculären Hypertension. *Zeitschrift für Klinische Medizin*, *96*, 150–181.
- Weitz, W. (1925). Studien an eineigen Zwillingen. *Zeitschrift für Klinische Medizin*, *101*, 115–135.
- Westfall, T. C. (1977). Local regulation of adrenergic neurotransmission. *Physiological Reviews*, *57*, 659–728.

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