Blood Pressure in Blacks
Twin Studies in Barbados
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We have recently reported that there are significant genetic influences on the population variation in blood pressure in black twins in Los Angeles. The present cross-sectional study was undertaken to replicate these findings in a black twin population that lives in a different biosocial environment. We chose the Caribbean island nation of Barbados, where 96% of the population is black, the literacy rate is 99%, and the access to health care is guaranteed. The goals were 1) to test the feasibility of twin studies in blood pressure research in a developing country and 2) to estimate the relative contribution of genes and environment to blood pressure variability in blacks in the Caribbean. The names of 200 twin sets were obtained with the assistance of community resources including a twin club, by media advertisement, and by asking people at public blood pressure screenings if they knew any twins. By using these methods, we identified 200 sets of twins. Of these, 37.5% (75/200) met our criteria for study. Although 97% of the sets of twins (73/75) said they were willing to participate, only 69% (52/75) were able to be scheduled during the 1 week of the study when the full team of investigators was in Barbados. Of those scheduled, 83% (43/52) were examined. Examination included medical history, physical examination, recumbent blood pressure measurements by two observers, anthropometric measurements, 24-hour urine collections for sodium and potassium tests, and blood tests for zygosity. Zygosity was determined by history, multiple genotyping, or both, which classified 21 pairs as monozygotic (13 male and eight female) and 22 pairs as dizygotic (11 female and 11 male). The average age was 29±9.5 (±SD) years, average height was 169±9.2 cm, average weight was 69±3.8 kg, average triceps skin-fold thickness was 11.3±6.5 mm, average arm circumference was 29±3.9 mm, average systolic blood pressure was 118±103 mm Hg, average sodium excretion was 131±653 mM/24 hours, and average potassium excretion was 46±17 mM/24 hours. In male twins, the quantifiable traits such as height, weight, triceps skin-fold thickness, arm circumference, and systolic blood pressure were all under significant genetic influence. The intraclass correlation for systolic blood pressure in all twin pairs was 0.67 (p<0.001), strong evidence for family aggregation of blood pressure. Twin analysis suggested that the major source of variance for systolic blood pressure between individuals was genetic, and heritability in males was estimated to be 70%. The heritability of blood pressure was not explained by any of the other measured variables. Within-twin-pair comparisons did not suggest that the twin with the highest blood pressure was taller, heavier, or consumed more sodium or less potassium. The twin with the thickest triceps skin folds, however, had a higher blood pressure. These results suggest that in blacks in the Caribbean, as in the United States, the major source of variation in blood pressure in the population is inherited factors and that twin studies are feasible in developing countries. (Hypertension 1990;15:803–809)

Hypertension in blacks has been a topic among those studying hypertension for many years, primarily because of the much higher incidence of high blood pressure and its complications among black Americans when compared with Italy, August 31, 1989.

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Address for correspondence: Clarence E. Grim, MD, Drew/UCLA Hypertension Research Center, Charles R. Drew University of Medicine and Science, 1621 E. 120th St., Mail Point 11, Los Angeles, CA 90059.
white Americans. This observation, first made in 1932 by Adams, has been replicated in numerous studies in the United States. A decade after the Adams study, observers also noted that this higher blood pressure in blacks was not limited to blacks in the United States. In 1942 Saunders and Bancroft reported that high blood pressure was a serious problem in the Virgin Islands, and a recent review by Grell has noted that all West Indian studies have confirmed Saunders and Bancroft's observation. In distinct contrast, however, sub-Saharan African populations exhibit a much lower prevalence of hypertension, a fact known since 1929. The leading explanations for these geographical variations in blood pressure have implicated environmental factors such as psychosocial stress, diet, or both. We have recently suggested, however, that, based on the biohistory of Western Hemisphere blacks, genetic factors might also have a role in blood pressure differences between African and Western Hemisphere blacks. The hypothesis suggests that selective survival based on the ability to conserve sodium during the sodium-depleting conditions of the slavery period of black history in the Western Hemisphere has resulted in the emergence of a population with a better ability to conserve sodium. With today's high sodium intake, these "sodium conservers" are more likely to become hypertensive. Driven by this hypothesis, we are pursuing epidemiological and physiological studies in black populations in and out of the United States to test the relative influence of both environmental and genetic factors on blood pressure and blood pressure-control systems. Our past success with the twin model in US whites and blacks suggested that this would be an efficient design to study questions about genes and environments in black populations worldwide. Because of the limited resources of these countries, the efficiency of twin studies should have added value in understanding the contribution of inherited and environmental factors in health and disease.

Our first effort has been in Barbados, an island country in the Caribbean where the biosocial environment is distinctly different than the United States. These major differences in Barbados include a population that is 96% black, a literacy rate of 99%, and access to health care that is guaranteed. Most blacks currently residing in Barbados are descendants from a population of blacks living in West Africa over 400 years ago, the same parent population as US blacks.

The present study had the following two goals: 1) to test the feasibility of the twin research model in black populations in the developing world and 2) to understand the relative influence of genetic and environmental factors on blood pressure variability in Barbados.

Methods

Study Design

This was a cross-sectional twin study of a volunteer population using a two-by-two factorial design for the analysis of quantitative traits. The factors were twin type (monozygotic [MZ] and dizygotic [DZ]) and sex (male and female).

The protocol was approved by the Chief Medical Officer of the Barbados Ministry of Health and the Human Subjects Committee of the Charles R. Drew University of Medicine and Science.

Recruitment of Twins

Active recruitment of twin sets began on January 11, 1989, for the proposed study in early April 1989. We (T.W.W. and D.M.W.) worked closely with Mr. Piercy Ward, President of the Barbados Twins Association (BTA) and the Heart Foundation of Barbados.

In the fall of 1988, one of us (T.W.W.) met with the BTA and discussed the Barbados Twins for Life research project and received their approval and support. Advertisements were placed in the two national newspapers, and a 20-minute segment was televised on the only national station. At the next meeting of the BTA, many twins volunteered to find more twins for the study by telephoning other known twin sets and friends who would, in turn, do the same. Six sets of identical twins agreed to advertise the project on the busiest shopping street in the capital city, Bridgetown, by handing out small flyers to all interested passersby. Additionally, blood pressure screenings were set up throughout the island, and all screened individuals were asked "Do you know the names of any twins?" These events received considerable media coverage. All these efforts resulted in the identification of 200 sets of twins.

Examination Site

The Chief Medical Officer of the Queen Elizabeth Hospital in Bridgetown agreed to donate examination facilities in a new unopened area of the hospital for the week of the study. This area was air conditioned and there were separate examination rooms for each twin.

Examination

The examination team had been trained in Los Angeles during the previous 6 months. All subjects were examined between April 1, 1989, and April 5, 1989, and followed a protocol that included a questionnaire, blood pressure measurement, height and weight measurement, electrocardiogram, blood and urine analysis, and 24-hour blood pressure monitoring. The latter is not reported here. Both members of the twin sets were seen at the same time. The time allowed for each portion of the examination is shown in parentheses. Twin sets were scheduled every half hour from 8 AM to 4:30 PM. On arrival, they were given a detailed description of the research, and a written informed consent was signed (15 minutes). A standardized questionnaire was used for assessing zygosity, socioeconomic status, lifestyle, alcohol intake, personal medical history, family medical history, and racial awareness (15 minutes). A trained observer measured height, weight, triceps skin-fold
measurements, and upper-arm circumference of both arms (10 minutes). The subjects were then placed in the recumbent position for at least 5 minutes, and time of day, ambient temperature, and three separate blood pressure readings were recorded by observer 1 with a mercury manometer and a blood pressure cuff appropriate for the arm size.15 Korotkoff sound 5 was used as the diastolic pressure. A second observer then recorded three additional blood pressure measurements (10 minutes). The observers (C.E.G. and C.M.G.) are both white and C.E.G. is male. White coats were not worn by any member of the team. Two days before the study, both blood pressure observers took a standardized video test16; both scored at least 95% correct (±2 mm Hg), neither had directional or terminal digit bias, and both had adequate within-observer variation scores. The medical history previously obtained from the questionnaire was reviewed by the examining physician (C.E.G. or G.D.N.), and a standardized examination was performed (5 minutes). While subjects were still in the recumbent position, 25 ml venous blood was obtained (10 minutes). The buffy coat was frozen for future genetic studies. A 24-hour blood pressure device was placed on the nondominant arm, and the subject was instructed in its use and how to keep an activity and food diary (20 minutes). The subject was then instructed (10 minutes) in 24-hour urine collection and had to void before leaving. This time was recorded. Women were given a special device to make voiding into the container easier. The subject returned to the study site approximately 24 hours later, at which time the last urine collection was completed, the 24-hour blood pressure device disconnected, and an exit interview completed (15 minutes).

Twin Data Analysis

Zygosity was based first on a self-reported answer to a question relying on the concept that, as children, identical twins are frequently confused by their family and friends. We used the question, “Did your parents and friends often confuse you two when you were children?” The second question we used was “Do you think you and your twin were born identical twins?” If the twin and cotwin answered “yes” to both questions, they were classified as identical or MZ; if they answered “no,” they were classified as nonidentical or DZ. If blood was drawn, we relied on genotyping. Zygosity determination was first obtained by ABO and Rh blood grouping and hemoglobin electrophoresis at the Queen Elizabeth Hospital. In those pairs without a difference, additional genotyping was performed by the Laboratory of the Department of Human Genetics at the University of California at Los Angeles School of Medicine. The markers used for this report were haptoglobin, transferrin (c1V), complement-3, amylose-2, properdin Factor B, and α1-antitrypsin protease inhibitor. When any marker indicated a difference between the pairs, they were labeled as DZ, and no further genotyping was performed. In the four sets of twins in whom we were not able to get blood samples, we relied on the self-report statements. This self-report method has been shown to be 90% correct in white twins.17

The statistical methods involved in the analysis of MZ and DZ twin data have been reviewed by Christian et al.18 To estimate the variance in the population that might be attributed to genetic sources, intraclass correlations (r) for MZ sets and DZ sets were calculated for each trait, and heritability estimates (h^2) were then calculated by the following formula: h^2=2(r_MZ−r_DZ).

Results

Recruitment

From the original list of 200 twin sets, 75 sets met the following study criteria: 16 years old or older, same-sex twin pairs, and both twins on the island during the 5-day study period. Of the 75 twin sets that met our criteria, 97% (73/75) agreed to take part in the study and 3% (2/75) refused. Of those who agreed to take part, we scheduled 71% (52/73) during the study week. Of the 52 twin sets scheduled, 83% of the twin sets (43/52) were examined, and 17% (9/52) did not appear (seven sent regrets and cancelled). After the study, we asked the subjects why they participated; 46% of the subjects said they participated primarily to “help mankind,” 34% participated to “learn facts about twins,” 12% participated because of “curiosity,” 3% participated for “free medical checkup,” and 4% participated for “other” reasons. All subjects said they would like to participate in any future Twins for Life projects.

Zygosity

The question about being confused as children identified 20 sets as MZ, and genotyping was compatible with this in 85% of the sets (17/20). This question also identified 18 sets as DZ twins, and this was confirmed by genotyping in 68% of the sets (13/18). Twenty-two sets answered “yes” to the question regarding the twins’ MZ status. Genotyping was available from 18 of these sets and was compatible with MZ status in 88% of the sets (16/18). In the 19 sets who thought they were DZ twins, genotyping revealed only 63% of them (13/19) to be correct. Overall, we classified 21 as MZ pairs (eight female and 13 male) and as DZ 22 pairs (11 female and 11 male). Six sets, however, were excluded from further analysis. One MZ female set (age, 25 years) was classified as white, and another set was classified as Asian-Indian. Medical exclusions included an MZ male set (age, 51 years) in which both members had hypertension and were on antihypertensive therapy, a DZ male set (age, 52 years) in which one member had adult onset-insulin-dependent diabetes and hypertension, one female MZ set (age, 31 years) in which one member had had cardiac surgery for rheumatic heart disease, and one female DZ set (age, 22 years) in which one member was taking oral
contraceptives. Thus, the final sex-zygosity breakdown for our analysis was 12 MZ male, 10 DZ male, 5 MZ female, and 10 DZ female sets.

**Anthropometric Variables**

As shown in Table 1, men were older, taller, heavier, and had larger arm circumferences but smaller triceps skin-fold thickness than women. None of these variables differed significantly by twin type (Table 2).

**Blood Pressure**

The average of the three supine systolic pressures taken by C.E.G. was 118.1 ±12 mm Hg, which was not significantly different from those taken by C.M.G. (117.0±12 mm Hg). Therefore, all six supine systolic blood pressure measurements were pooled and used for further analysis. As shown in Table 1, the female twins had lower systolic blood pressure than the male twins (*p*<0.05). In simple and stepwise regression analysis of systolic blood pressure, only sex emerged as a significant correlate. Thus, the older age of the male twins did not explain the higher blood pressure in males. As shown in Table 2, systolic blood pressure was not different by twin type. Figure 1 shows the plotting of the intraclass correlation coefficients for systolic blood pressure for all twin pairs. It is clear that within all twin pairs, the systolic blood pressure was highly correlated

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**TABLE 1. Body Size Variables, Systolic Blood Pressure, and Urine Values in 37 Pairs of Twins**

<table>
<thead>
<tr>
<th>Variable</th>
<th>All subjects (n=74)</th>
<th>Males (n=44)</th>
<th>Females (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>28.8±9.5</td>
<td>32.1±10.0</td>
<td>23.9±6.0</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>169.3±9.2</td>
<td>175.8±4.8</td>
<td>162.6±10.1</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>69.0±3.8</td>
<td>72.6±11.9</td>
<td>63.7±14.9</td>
</tr>
<tr>
<td>Triceps (mm)</td>
<td>11.3±6.5</td>
<td>8.2±4.3</td>
<td>15.8±6.5</td>
</tr>
<tr>
<td>Arm circumference (mm)</td>
<td>29.0±3.9</td>
<td>30.2±3.6</td>
<td>27.4±3.8</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>117.5±10.3</td>
<td>121.8±7.7</td>
<td>111.2±10.4</td>
</tr>
<tr>
<td>Urine volume (ml)*</td>
<td>700.0±460.0</td>
<td>740.0±450.0</td>
<td>610.0±490.0</td>
</tr>
<tr>
<td>ENa+ (mM/24 hr)*</td>
<td>131.2±65.3</td>
<td>146.0±68.1</td>
<td>87.9±27.4</td>
</tr>
<tr>
<td>EK+ (mM/24 hr)*</td>
<td>45.6±17.2</td>
<td>47.5±16.2</td>
<td>40.1±19.8</td>
</tr>
</tbody>
</table>

Values are mean±SEM with the range in parentheses. BP, blood pressure; ENa+, Na+ excretion; EK+, K+ excretion.

*Male, n=36 for volume; female, n=14 for volume.
†Male, n=35; female, n=12.

contraceptives. Thus, the final sex-zygosity breakdown for our analysis was 12 MZ male, 10 DZ male, 5 MZ female, and 10 DZ female sets.

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**TABLE 2. Mean Values and Heritability Analysis of Quantifiable Traits in 37 Sets of Barbados Twins**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Uniform (cm)</th>
<th>Dizygotic (cm)</th>
<th>F*</th>
<th>rMZ</th>
<th>rDZ</th>
<th>h^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height (cm)</td>
<td></td>
<td></td>
<td>0.378</td>
<td>0.97</td>
<td>0.31</td>
<td>≥1†</td>
</tr>
<tr>
<td>Males</td>
<td>168.4</td>
<td>169.9</td>
<td>0.809</td>
<td>0.86</td>
<td>0.20</td>
<td>≥1</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td></td>
<td></td>
<td>0.105</td>
<td>0.92</td>
<td>0.64</td>
<td>0.56</td>
</tr>
<tr>
<td>Males</td>
<td>66.8</td>
<td>71.0</td>
<td>0.040</td>
<td>0.89</td>
<td>0.28</td>
<td>0.34</td>
</tr>
<tr>
<td>Triceps (mm)</td>
<td></td>
<td></td>
<td>0.869</td>
<td>0.91</td>
<td>0.69</td>
<td>0.44</td>
</tr>
<tr>
<td>Males</td>
<td>10.2</td>
<td>12.2</td>
<td>0.142</td>
<td>0.77</td>
<td>0.39</td>
<td>0.76</td>
</tr>
<tr>
<td>Arm circumference (cm)</td>
<td></td>
<td></td>
<td>0.221</td>
<td>0.86</td>
<td>0.68</td>
<td>0.36</td>
</tr>
<tr>
<td>Males</td>
<td>28.7</td>
<td>29.4</td>
<td>0.046</td>
<td>0.84</td>
<td>0.25</td>
<td>≥1.0</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td></td>
<td></td>
<td>0.290</td>
<td>0.68</td>
<td>0.66</td>
<td>0.04</td>
</tr>
<tr>
<td>Males</td>
<td>117.2</td>
<td>117.8</td>
<td>0.747</td>
<td>0.53</td>
<td>0.18</td>
<td>0.70</td>
</tr>
<tr>
<td>Sodium excretion (mM/24 hr)</td>
<td></td>
<td></td>
<td>0.9252</td>
<td>0.2</td>
<td>0.07</td>
<td>...</td>
</tr>
<tr>
<td>Males</td>
<td>133.3</td>
<td>131.6</td>
<td>0.8413</td>
<td>0.19</td>
<td>0.32</td>
<td>...</td>
</tr>
</tbody>
</table>

rMZ, intraclass correlation coefficient for monozygotic twins; rDZ, intraclass correlation coefficient for dizygotic twins; BP, blood pressure.

*Refers to test of equality of total variance for the two types of twins.
†If the calculated heritability exceeds 1, the value of ≥1 is used.
Within-Twin-Pair Analysis of Factors Possibly Related to Blood Pressure

Because same-sex twins are perfectly matched for sex and age, the study of within-twin-pair differences is a powerful way to test for that influence of a variable on blood pressure differences within twin pairs. MZ twin pairs are also perfectly matched for genes. Differences between them, therefore, can only be caused by environmental factors. If a variable is strongly predictive of blood pressure, we would expect that, on the average within each twin pair, the twin with the highest value for that variable should also have the highest blood pressure. Therefore, by correlating the within-twin-pair difference in blood pressure with the within-twin difference in the variable of interest, a significant positive correlation would be strong evidence that this variable was in fact contributing to the difference in blood pressure within twin pairs. Such a plot for weight is shown in Figure 2. As shown, there was no evidence that the heaviest twin had the highest blood pressure. The same was true for height and sodium and potassium excretion. As shown in Figure 3, however, the difference in triceps skin-fold thickness was positively correlated with blood pressure ($r=0.43, p<0.008$); that is, the twin with the thickest skin fold had the highest blood pressure.

**Discussion**

This is the first time that the twin design has been used to investigate the relative contribution of genes and environment to anthropometric and blood pressure measurements in a developing country of African heritage. The success we obtained in recruiting twins suggests that this design is feasible in the third world, and because of the power and economy of twin studies in epidemiological research, they should be considered in other countries with limited resources.

The zyosity testing results, however, suggest that in future twin studies in the Caribbean, the questionnaire method of determining zyosity might be rea-
sonably satisfactory for those subjects who identify themselves as MZ twins because, on genotyping, 89% probably are MZ. In those who believe themselves to be DZ twins, on genotyping, only about 66% will be found to be DZ. In a trait influenced by genetic factors, the effects of classifying MZ twins as DZ based on the history would be to increase the apparent rDZ and decrease h². The effect of misclassifying DZ twins as MZ based on genotyping would be to decrease rMZ and decrease h². In the future, DNA “fingerprinting” will increase the reliability of twin classification and minimize these problems.

This study demonstrated that, in blacks in Barbados, there is strong family aggregation for height, weight, and systolic blood pressure. These findings are similar to what we have found in black twins in Los Angeles. The correlation of triceps skin-fold thickness with blood pressure is interesting and might be related to the observations reported elsewhere in this issue by Harshfield et al.40 that, among black adolescents, the most physically fit had a tendency to have the lowest blood pressure.

Family aggregation of blood pressure in the Caribbean was first reported by Miall et al.21 in 1962, in the study of families in Jamaica, and in the United States by Zinner et al.42 The study of twins in the United States and in Barbados supports the major cause of this familial resemblance of blood pressure is genetic factors. This is similar to what has been reported by the Indiana group using white adult twins23 and adult twins and their families.24 Schicken et al.42 have recently reported similar results in white twin children (age, 11 years) living in Virginia. The most powerful test of the nature-nurture influence on blood pressure can be obtained by comparing twins raised separately.28 This has never been reported for black twins.

The urinary sodium excretion data that we reported is similar to that reported by the Intersalt Cooperative Research Group26 in the neighboring twin-island republic, Trinidad and Tobago, where the 24-hour excretion rates were 117±56 mM (sodium) and 41±17 mM (potassium). In that study, which had a larger sample and included equal numbers of subjects throughout the age span of 20–59 years, a statistically significant regression coefficient of blood pressure (diastolic) and sodium adjusted for age, sex, body mass index, alcohol, and potassium was reported. Our sample size and young age range might have minimized the likelihood we would find a correlation between salt intake and blood pressure in Barbados. Nevertheless, our study does not suggest that, in this younger age group, differences in height, weight, or electrolyte intake contribute to within–twin-pair differences in blood pressure.

Conclusion

In Barbados, as in Los Angeles, twins are cooperative and reliable research subjects for the epidemiological study of blood pressure. In Barbados, which has a biosocial milieu distinctly different from Los Angeles, blood pressure in blacks is strongly influenced by genetic factors, as it appears to be influenced in blacks in Los Angeles. These genetic factors do not appear to operate through genetic effects on height, body weight, arm circumference, and dietary sodium or potassium intake. A much larger sample selected randomly from the Barbados twin population, however, will be necessary to further the conclusiveness of the tests conducted here. When taken together, these results suggest that blacks in the Western Hemisphere have a similar genetic determination of blood pressure variation. Further studies in Africa are necessary to examine the reasons for the lower blood pressure seen in blacks in Africa as compared with the blood pressure seen in blacks in the Western Hemisphere.

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References


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