Y-Chromosome Transfer Induces Changes in Blood Pressure and Blood Lipids in SHR

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Abstract—Previous studies with chromosome-Y consomic strains of spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats suggest that a quantitative trait locus for blood pressure regulation exists on chromosome Y. To test this hypothesis in the SHR–Brown Norway (BN) model and to study the effects of chromosome Y on lipid and carbohydrate metabolism, we produced a new consomic strain of SHR carrying the Y chromosome transferred from the BN rat. We found that replacing the SHR Y chromosome with the BN Y chromosome resulted in significant decreases in systolic and diastolic blood pressures in the SHR.BN-Y consomic strain ($P<0.05$). To elicit possible dietary-induced variation in lipid and glucose metabolism between the SHR progenitor and chromosome-Y consomic strains, we fed rats a high-fructose diet for 15 days in addition to the normal diet. On the high-fructose diet, the SHR.BN-Y consomic rats exhibited significantly increased levels of serum triglycerides and decreased levels of serum HDL cholesterol versus the SHR progenitor rats. Glucose tolerance and insulin/glucose ratios, however, were similar in both strains on both normal and high-fructose diets. These findings provide direct evidence that a gene or genes on chromosome Y contribute to the pathogenesis of spontaneous hypertension in the SHR-BN model. These results also indicate that transfer of the Y chromosome from the BN rat onto the SHR background exacerbates dietary-induced dyslipidemia in SHR. Thus, genetic variation in genes on the Y chromosome may contribute to variation in blood pressure and lipid levels and may influence the risk for cardiovascular disease. **(*Hypertension*. 2001;37:1147-1152.)**

Key Words: hypertension, genetic ■ rats, spontaneously hypertensive ■ cholesterol ■ genes ■ lipids ■ cardiovascular disease

In patients with essential hypertension, the clustering of metabolic cardiovascular risk factors with increased blood pressure (BP) is a well-recognized phenomenon that has major implications for the pathogenesis of atherosclerosis and cardiovascular disease.[1,2] The frequent association of insulin resistance, dyslipidemia, and increased BP may promote susceptibility to target organ damage and partly explain why conventional antihypertensive agents have failed to reduce the risk for coronary heart disease to the extent predicted from epidemiological studies.[2] The basis for this risk factor clustering in essential hypertension is poorly understood and is likely to involve a complex assortment of genetic and nongenetic mechanisms.[3–6] The existence of individual genes or linked genes with multiple effects on BP, lipid metabolism, and carbohydrate metabolism represents one mechanism that might contribute to risk factor clustering in essential hypertension. Indeed, genetic studies in animals have suggested that in certain chromosome regions, genes regulating BP may be linked to genes influencing metabolic risk factors for cardiovascular disease.[7,8] For example, in the spontaneously hypertensive rat (SHR), the most widely studied model of essential hypertension, it has recently been found that a segment of chromosome 4 corresponding to a region of chromosome 7q in humans may be involved in the inherited control of BP, glucose tolerance, and circulating levels of triglycerides and fatty acids.[7,9–11]

Although major efforts are underway to search for autosomal and X-linked genes involved in the regulation of BP and other risk factors for cardiovascular disease, relatively little effort has been made to investigate the role of the Y chromosome in the pathogenesis of hypertension or atherosclerosis. However, studies by Ely and Turner[12] have clearly indicated that in the SHR, the Y chromosome may be an important determinant of BP. These investigators directly

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investigated the role of the Y chromosome in the pathogenesis of hypertension by measuring BP in Y-chromosome consomic strains derived from SHR and normotensive Wistar Kyoto (WKY) rats.[13] Replacing the Y chromosome of the SHR with the Y chromosome of the WKY rat attenuated hypertension. Conversely, replacing the Y chromosome of the WKY rat with the Y chromosome of the SHR induced an increase in BP.[13] These observations provide strong support for the hypothesis that sequence variation on the Y chromosome can influence BP and the pathogenesis of spontaneous hypertension. However, given the known heterogeneity among SHR and WKY from different sources,[14] it is uncertain whether the pioneering findings of Turner and colleagues are unique to their particular colonies of SHR and WKY or whether they extend to hypertensive and normotensive strains of rats from other sources.[15–18] Moreover, in the SHR, the possibility that the Y chromosome may contribute to the regulation of carbohydrate and lipid metabolism as well as BP has not been investigated.

In this study, we exploited the power of consomic strain technology to test the hypothesis that the Y chromosome is involved in the inherited control of lipid and/or carbohydrate metabolism as well as BP. We found that transfer of the Y chromosome of the Brown Norway (BN) rat onto the genetic background of the SHR induced significant effects on both BP and circulating levels of triglycerides and HDL cholesterol but did not affect glucose tolerance in the SHR. These findings confirm the seminal observations of Turner and Ely on the role of the Y chromosome in the pathogenesis of spontaneous hypertension and suggest that sequence variation in the Y chromosome may also be involved in the genetic control of lipid metabolism.

Methods

Animals

The SHR/Ola inbred strain was originally obtained from the National Institutes of Health and has been maintained by strict brother×sister mating at the Czech Academy of Sciences in Prague for more than 17 years.[19] As a donor of the Y chromosome, we used the normotensive BN-Lx/Cub strain[20] (hereafter referred to as BN).

A consomic strain (or chromosome substitution strain) is an inbred strain derived from a progenitor strain that is genetically identical to the progenitor except for the transfer of an entire chromosome from a different strain.[21] In the current study, a selective breeding protocol was used to transfer the Y chromosome from the BN strain onto the genetic background of the SHR to generate the SHR.BN-Y consomic strain. BN males were first crossed with SHR females; then, in each of 8 successive back-cross generations, hybrid male offspring were mated with SHR females. Rats of the N8F5 generation or higher were used in the current studies. Seventy-two dispersed microsatellite markers polymorphic between the SHR and BN progenitor strains and covering all autosomes and the X chromosome were used to confirm the consomic status of the SHR.BN-Y strain.[22,23]

Cytogenetic Analysis of Y Chromosome

To confirm successful transfer of the Y chromosome of the BN rat onto the SHR background, we performed cytogenetic analyses on a subset of the animals used for BP phenotyping. Bone marrow samples for cytogenetic analysis were extracted from the femur. Chromosomes were processed by conventional Wright's staining technique and by a modified Seabright's G-banding technique.[24] G-banded metaphases were observed at ×1500 magnification and evaluated according to the established nomenclature for G-banding rat chromosomes.[25] At least 10 metaphases were evaluated in each animal.

Cardiovascular Phenotyping

BPs and heart rates were measured continuously by radiotelemetry in 13 SHR and 8 consomic SHR.BN-Y males for 8 weeks, beginning at 11 weeks of age as described previously.[26,27] The daytime (6 AM to 6 PM) and nighttime (6 PM to 6 AM) systolic and diastolic BPs and heart rates of each rat were averaged for each day from 11 to 18 weeks of age. Cardiac mass was determined as the ratio of heart weight to body weight.

All rats were maintained on a standard laboratory diet containing 0.58% NaCl and 1.1% KCl, with tap water ad libitum from weaning (4 weeks) through 13 weeks of age. To test for effects of the Y chromosome on salt-induced increases in BP, the rats were given 1% NaCl water to drink for 1 week beginning at 14 weeks of age. After 1 week of the high-salt challenge, the rats were switched back to tap water.

Phenotyping of Metabolic Risk Factors

To test the hypothesis that genes affecting glucose tolerance and lipid phenotypes might be located on chromosome Y, we tested for the presence of hyperglycemia, hyperinsulinemia, and dyslipidemia in additional groups of 8- to 10-week-old male SHR progenitor and Y-chromosome consomic rats under different dietary conditions. After obtaining baseline fasting blood samples, we fed SHR (n=9) and Y-consomic rats (n=6) a high-fructose diet (60% fructose) for 15 days to provoke insulin resistance.[28] A standard intraperitoneal glucose tolerance test was performed on day 13 of fructose feeding as described previously.[28] In addition, to test for the effect of high-fat intake on serum lipid profiles, we fed 8-week-old SHR progenitor (n=6) and Y-consomic (n=6) rats a 2% added cholesterol, 5% added olive oil, high-fat diet for 4 weeks and then collected fasting blood samples for analysis of serum cholesterol, triglycerides, and lipoprotein fractions.

Glucose was measured by the glucose oxidase technique; rat insulin was measured by radioimmunoassay (Amersham); and total cholesterol, triglycerides (without glycerol blanking), and total HDL cholesterol were measured by standard enzymatic techniques.[8,29] Lipoprotein fractions including VLDL, IDL, LDL, and HDL_2 were isolated by density gradient ultracentrifugation as previously described.[8]

Statistical Analysis

All data are expressed as mean±SEM. Daytime and nighttime BPs, heart rates, and body weights over the course of the study were separately analyzed by repeated-measures ANOVA. Individual means for BPs and body weights and for serum levels of glucose, insulin, and lipids were compared by *t* test. Statistical significance was defined as $P<0.05$. All procedures involving animals were performed in accordance with institutional guidelines for the use and care of experimental animals.

Results

Genotype results obtained from polymerase chain reaction analysis of 72 widely dispersed polymorphic microsatellite markers confirmed that the SHR.BN-Y consomic strain was genetically identical to the SHR progenitor on all autosomes and the X chromosome. Cytogenetic analysis of the SHR.BN-Y consomic rats confirmed successful transfer of the Y chromosome of the BN strain onto the genetic background of the SHR (Figure 1). Differences in Y-chromosome length and in the number of G-bands between the SHR progenitor strain and the BN donor strain enabled us to clearly distinguish the source of the Y chromosome in the consomic strain. We found the Y chromosome of the BN rat to be significantly longer than that of the SHR. Previous

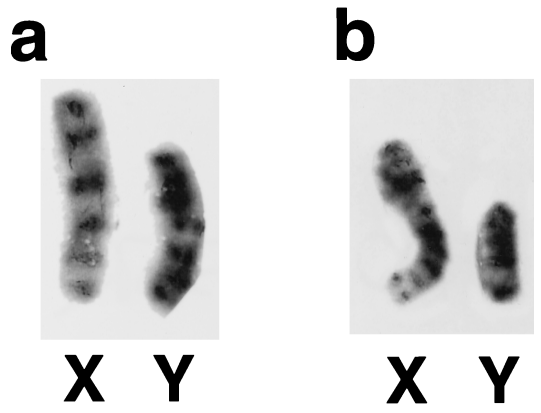

Figure 1. Giemsa-banded X and Y chromosomes from rat bone marrow cells (metaphase). a, Male SHR.BN-Y consomic rat; b, male SHR. SHR.BN-Y consomic rat carries large Y chromosome derived from BN progenitor.[30]

investigators have also noted the Y chromosome of the BN strain to be significantly longer than that of other rat strains.[30] As expected, all 8 of the SHR.BN-Y consomic animals examined were found to carry the conspicuously long Y chromosome of the BN strain.

The weekly averages for daytime and nighttime systolic and diastolic BPs are presented in Figure 2, a and b. Daytime and nighttime systolic and diastolic BPs were significantly decreased in SHR.BN-Y consomic rats compared with the SHR progenitor rats ($P<0.05$). The strain differences in BP were apparent after recovery from surgery and persisted over the entire 8 weeks of the study. Administration of 1% NaCl in the drinking water induced significant increases in BP in both the SHR progenitor and SHR.BN-Y congenic rats. However, the salt-induced increases in BP were similar in magnitude between the two strains.

Daytime and nighttime heart rates tended to be lower in the SHR.BN-Y consomic rats than in SHR rats during the first 4 weeks on the normal salt diet. After this period, there was no significant difference between the two strains (data not shown). The ratio of heart weight to body weight was not different between the strains (data not shown).

There were no significant differences between the SHR progenitor and consomic strains in plasma insulin levels or in insulin/glucose ratios, either on the normal diet or after feeding the high-fructose diet for 15 days (data not shown). In addition, we found no differences between the two strains in glucose and insulin levels after an intraperitoneal glucose load.

On the normal diet, there were no differences in total serum cholesterol or serum triglycerides between the SHR progenitor and consomic strains (Figure 3a). However, transfer of the Y chromosome from the BN strain onto the SHR background induced a significant dyslipidemia after fructose feeding in the SHR.BN-Y consomic strain, characterized by increased serum triglycerides and decreased HDL cholesterol ($P<0.05$) (Figure 3a). Similarly, after dietary challenge in the form of a high-fat diet, SHR.BN-Y rats showed higher levels of triglycerides ($P<0.05$), IDL ($P<0.005$), and LDL ($P<0.005$) as well as total cholesterol ($P<0.005$) and lower levels of HDL_2 ($P<0.05$) versus SHR progenitor rats (Figure 3b).

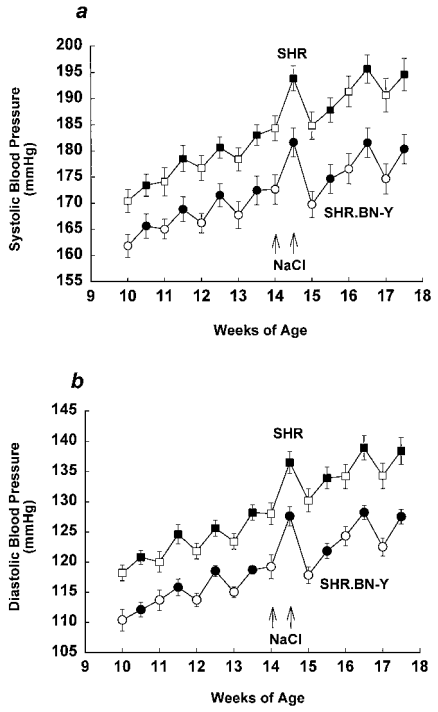

Figure 2. BPs measured by radiotelemetry over period of 8 weeks in SHR progenitor and SHR.BN-Y consomic strains. Each data point represents 12-hour average daytime (open symbols) or nighttime (closed symbols) BP. BPs in SHR progenitor (n=13) (squares) and SHR.BN-Y consomic strains (n=8) (circles) were obtained from weekly averages of ≈1000 daytime and 1000 nighttime measurements in each rat. Arrows indicate week of salt administration (1% NaCl water). a, Systolic BP: average daytime and nighttime systolic BPs in SHR.BN-Y consomic rats were significantly lower than those of SHR ($P<0.05$). b, Diastolic BP: average daytime and nighttime diastolic BPs strain were also significantly lower in SHR.BN-Y rats vs SHR ($P<0.05$).

In our BP study, the body weights of the SHR.BN-Y consomic rats were significantly greater than the body weights of age-matched SHR progenitor rats (17-week-old SHR progenitor rats, 340±7 g; age-matched SHR.BN-Y consomic rats, 382±5 g; $P<0.05$). This weight difference became apparent after the surgery to implant radiotelemetry transducers and persisted throughout the study in rats at 10 to 17 weeks of age. This weight difference was confirmed in additional male SHR (n=7) and SHR.BN-Y consomic rats (n=8) that were housed under identical conditions as the original groups but did not undergo surgery and were fed only standard rat chow ad lib. Thus, the SHR.BN-Y consomic rats have significantly lower BPs than SHR despite having consistently higher body weights.

Discussion

Gender differences in BP have been observed in both humans and experimental animals, in which males usually have higher BPs.[31–33] Although sex-influenced phenotypes such as BP are thought to be affected mainly by sex hormones, studies with the SHR-WKY model have suggested that a regulatory gene or genes with a direct effect on BP may be

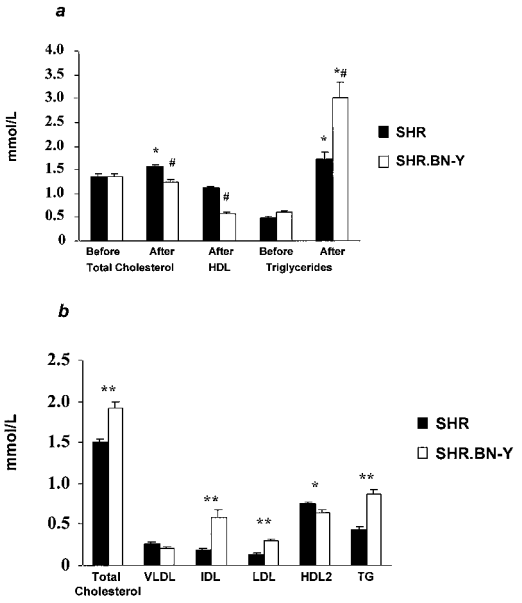

Figure 3. a, Serum lipid profiles measured before and after feeding rats 60% fructose diet for 15 days. Fructose feeding induced significant increases compared with baseline in serum triglycerides in both SHR (solid bars) and SHR.BN-Y rats (open bars) and in total cholesterol in SHR (*$P<0.05$). After fructose feeding, SHR.BN-Y consomic rats had significantly higher serum triglycerides and lower HDL and total cholesterol levels vs SHR progenitor rats (#$P<0.05$). b, Serum cholesterol, triglycerides, and lipoprotein fractions after feeding high-fat diet for 4 weeks. SHR.BN-Y rats (open bars) showed significantly higher levels of total cholesterol, triglycerides (TG), and IDL and LDL cholesterol (all $P<0.005$) and lower levels of HDL_2 cholesterol ($P<0.05$) compared with SHR (solid bars).

located on the Y chromosome.[12,13] In the current study, we developed a new consomic strain derived from SHR and normotensive BN rats in which we replaced the Y chromosome of the SHR with the BN Y chromosome. We found that transfer of the BN Y chromosome into the SHR significantly reduced both systolic and diastolic BPs but induced a significant dyslipidemia after dietary challenge as well as a slight increase in body weight in the SHR.BN-Y consomic strain.

Our BP findings are consistent with the results of previous studies, which showed that the Y chromosomes of the SHR or stroke-prone SHR were associated with increased BP versus the Y chromosomes of normotensive WKY rats.[13,16,34] In the present study, the reductions in BP in the SHR.BN-Y consomic strain approached 15 mm Hg for systolic and 11 mm Hg for diastolic pressures. The SHR.BN-Y consomic rats demonstrated lower BPs despite having higher body weights than the SHR progenitor rats. Because increased body weight is associated with increased BP in both rats and humans,[35,36] the increased body weight of the SHR.BN-Y consomic strain rats may, if anything, tend to reduce the BP difference found between the consomic and progenitor strains. Given that the differences in systolic BP between the SHR and BN progenitor strains is ≈80 mm Hg,[37] the Y chromosome of the SHR.BN-Y consomic strain could account for up to ≈20% of the hypertension in SHR versus BN rats.

In addition to serving as the most widely studied animal model of essential hypertension, the SHR also demonstrates disordered insulin action and lipid metabolism similar to that in patients with the human hypertensive metabolic syndrome.[5,6,38–40] In previous studies, we observed that quantitative trait loci on several chromosome regions that are associated with BP also cosegregate with genes influencing lipid and carbohydrate metabolism phenotypes.[7–9] To test the hypothesis that a gene or genes on chromosome Y might contribute to this clustering of risk factors for cardiovascular disease, we analyzed the effects of chromosome Y transfer on phenotypes for glucose intolerance and dyslipidemia. We found no evidence for an association between chromosome Y and impaired glucose tolerance or insulin resistance, at least in the SHR-BN model under the dietary conditions tested (normal diet and fructose loading), albeit with the use of relatively insensitive screening methods for measuring insulin resistance and glucose tolerance.

In contrast, we found that the SHR.BN-Y rats are significantly dyslipidemic compared with the SHR and have modestly increased body weights despite having lower BPs. Specifically, after feeding a high fructose diet, the consomic strain had significantly increased levels of serum triglycerides and decreased levels of HDL cholesterol compared with the SHR progenitor. After eating a high-fat diet for 4 weeks, SHR.BN-Y consomic rats also developed an adverse lipid profile, including elevated serum triglycerides, and elevated total, IDL, and LDL cholesterol levels and lower HDL_2 cholesterol levels compared with the SHR. Thus, transfer of the Y chromosome from the BN rat onto the SHR background is associated with a significant reduction in BP but an exacerbation of dietary-induced hyperlipidemia.

Our findings of an adverse lipid profile in the SHR.BN-Y consomic strain versus the SHR progenitor after increased dietary intake of fructose or fat agree with previous studies by Bottger et al,[8] who found that the normotensive BN.Lx (BN) progenitor strain developed a significant dyslipidemia compared with the SHR progenitor strain when fed a high-fat diet. Thus, when compared with the SHR.BN-Y consomic or the BN progenitor strains, the SHR does not fit the typical pattern of hypertension combined with disordered lipid metabolism seen in human metabolic syndromes.[1] However, because BP and lipid levels are complex traits influenced by multiple genes, it should not be surprising that both the SHR and BN.Lx strains might carry a mixture of alleles that both promote and ameliorate dyslipidemia and/or hypertension. In the current study, we have succeeded in simplifying the genetic dissection of these complex traits by isolating a single chromosome in the SHR.BN-Y consomic strain and demonstrating that a gene or genes influencing blood pressure and serum lipid levels exist on chromosome Y.

Because of lack of recombination during meiosis along most of the Y chromosome, conventional mapping strategies[41] cannot be used to map genes on the Y chromosome or to further localize Y-chromosome quantitative trait loci influencing BP or lipid levels. However, recent investigation indicates that the nonrecombining region of the Y chromosome contains a limited number of genes that fall into one of two categories: (1) housekeeping genes expressed in a wide variety of tissues and (2) genes associated with traits restricted to males and expressed primarily in the testes.[42,43]

Therefore, the SHR.BN-Y consomic strain will be an ideal model for the application of cDNA microarray technology to identify candidate genes that might be expressed differentially between the SHR consomic and progenitor strains.[10]

Finally, studies in the sons of hypertensive parents suggest a possible role of the human Y chromosome in the determination of BP and body mass index in men.[44] Thus, the relevance of our current findings in the SHR-BN rat model for human hypertension and associated metabolic disturbances could be tested in association studies that use polymorphisms in candidate genes on the Y chromosome to screen human populations.

Note Added in Proof

A recent study by Ellis et al (*Hypertension*. 2000;36:731–733) indicates that a polymorphism on the Y chromosome is associated with diastolic blood pressure in men. These results suggest that genetic variation on the Y chromosome may contribute to differences in male diastolic blood pressure in human populations.

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