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Genetic analysis of the stress-responsive adrenocortical axis

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Solberg, Leah C., Amber E. Baum, Nasim Ahmadiyeh, Kazuhiro Shimomura, Renhua Li, Fred W. Turek, Joseph S. Takahashi, Gary A. Churchill, and Eva E. Redei. Genetic analysis of the stress-responsive adrenocortical axis. *Physiol Genomics* 27: 362–369, 2006. First published August 8, 2006; doi:10.1152/physiolgenomics.00052.2006.—The underlying genetic components contributing to individual variability in functions of the stress-responsive hypothalamic-pituitary-adrenal (HPA) axis are poorly understood. To determine genetic loci mediating three aspects of the adrenocortical function, we conducted a quantitative trait locus (QTL) analysis in the segregating F2 generation of a Wistar Kyoto (WKY) × Fischer 344 (F344) cross, two inbred rat strains that differ in several HPA axis measures. The following three components of adrenocortical function are known to be regulated by different mechanisms that are mediated via suprahypothalamic, hypothalamic, pituitary, and intra-adrenal influences: basal plasma corticosterone (Cort) levels, plasma Cort response to a 10-min restraint stress, and adrenal weight. Genome scans identified a complex genetic architecture for the basal Cort phenotype, including sex and maternal lineage effects. Pairwise interactions were also identified for this trait. We identified three significant and two suggestive QTLs for stress Cort, along with two pairs of interacting loci for this trait. Four highly significant and two suggestive loci were identified for adrenal weight, with no interacting loci. In contrast to basal Cort, no sex- or lineage-dependent QTL were identified for stress Cort or adrenal weight, despite the large sex differences in these phenotypes. We identified three nucleotide alterations in an obvious candidate gene mapped to the most significant QTL for stress Cort, Cort-binding globulin (CBG), one of which is known to alter CBG binding. This analysis confirms that three separate traits regulated by the HPA axis are controlled by multiple, but mainly nonoverlapping, QTLs.

hypothalamic-pituitary-adrenal axis; corticosterone; quantitative trait loci analysis; Wistar Kyoto rat

GENES INVOLVED IN individual variation of hypothalamic-pituitary-adrenal (HPA) axis activity, particularly in regard to disease-causing aspects of chronic stress, are unknown. While acute activation of the HPA axis during stress, "a state of threatened homeostasis," leads to a cascade of physiological and behavioral adaptive responses that increase survival of an organism (e.g., increased arousal, increased respiratory rate, and decreased appetite), chronic stress, which results in constant high levels of circulating glucocorticoids, can lead to pathophysiologies such as depression, diabetes, cardiovascular disease, and hypertension (10). There is a high degree of individual variation in response to both acute and chronic stress, and some individuals will not develop any of the abovementioned diseases when exposed to chronic stress. Family and twin studies have demonstrated that individual variation of HPA axis activity is regulated, at least in part, by genetics (e.g., Refs. 4, 16). Identification of the underlying genetic components, however, is difficult, because HPA axis function is so dependent on the environment.

Although molecular techniques have been used to study the stress response (for a review, see Ref. 37), very little is known about the genetic basis of how stress differentially affects individuals. Quantitative trait locus (QTL) analysis is a method for detecting multiple chromosomal locations involved in complex traits. To date, QTLs have been detected for several physiological and/or behavioral responses to various stressors (e.g., Refs. 12, 14, 21, 24, 40). QTLs for basal corticosterone (Cort) levels have also been identified (20, 25, 27). However, only three studies, each in a different species, have investigated QTL for Cort in response to stress (9, 13, 29), and to date, no QTL studies have been done investigating adrenal gland weight and its relationship to stress.

We have previously reported that three separate measures of HPA function, basal levels of plasma Cort, plasma Cort levels in response to restraint stress, and adrenal weight after repeated behavioral testing, are heritable in the segregating F2 generation of a Wistar Kyoto (WKY) × Fischer 344 (F344) cross (34). Each of these measures is differentially regulated. For example, basal Cort taken at the time of the circadian trough, when plasma Cort levels are normally low, is a trait marker of unactivated HPA activity (17, 46). Stress Cort represents the sensitivity of the HPA axis in response to an acute physical or psychological stress. Stress Cort is regulated by sensitivity of the adrenal cortex to ACTH and indirectly by positive and negative regulators of ACTH stimulation, such as corticotrophin-releasing hormone (CRH) and arginine vasopressin (AVP) from the hypothalamus (10). Adrenal gland size is partially regulated by ACTH stimulation (6) and is a marker of HPA axis activation and adrenal Cort production over time (31, 41, 43). These measures were chosen for their relative ease and their accessibility in human populations as well. In the current study, we have used the abovementioned segregating F2 population to determine QTLs contributing to genetic variation of these HPA activity phenotypes.

MATERIALS AND METHODS

Animals

Four hundred eighty-six F2 generation animals of a WKY × F344 cross were derived as previously described (2). Animals were raised

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in a 14:10-h light-dark cycle (lights on at 0700 and off at 2100; 24-h clock, Central Standard Time) and kept under constant ambient temperature (21 ± 1°C) with food and water available ad libitum. Pups were weaned at 24 days of age, separated by sex, and housed three to five animals per cage. At the time of weaning, tail samples were collected for subsequent DNA isolation. For simultaneous QTL analysis of behavioral traits (1, 2, 35), at 11 wk of age, these animals were administered the open-field, forced swim, and defensive burying behavioral tests over a 4-wk time period. These tests involved the transportation and handling of rats seven times during this period, as well as exposure of them to the stress of novelty in the open-field test, the stress of inescapable swim in the forced swim test, and that of a threat in the form of a small electric stimulus in the defensive burying test. At 15 wk of age, blood samples were taken for determination of plasma Cort levels at the unstimulated state and after restraint stress. The following week, animals were killed, and adrenals were collected and weighed. All animal experimentation was approved by the Northwestern University Animal Care and Use Committee.

Blood Collection for Hormonal Analysis

As previously described (34), the tail cut method was used for blood collection. Blood samples were collected between 1300 and 1500, when WKY males exhibit decreased basal levels of plasma Cort relative to F344 males (34). Basal samples were taken within 2 min of removal from the cage, and stress samples were taken after 10 min of restraint. Samples were collected on ice into EDTA-coated tubes (1 mg/tube), and plasma was stored at −80°C for subsequent determination of Cort by radioimmunoassay.

Radioimmunoassay for Cort

This assay was done in duplicate as described previously (28). Briefly, 1–2 μl of plasma were incubated overnight with the primary Cort antibody raised against corticosterone-3-carboxymethyloxime-bovine serum albumin, with ^{125}I-labeled Cort conjugate as the tracer (ICN Pharmaceuticals, Costa Mesa, CA). The assay sensitivity was 16.7 pg/tube. The intra- and interassay coefficients of variation were 11.6 and 7.5%, respectively.

Genotyping

Genotyping has been described previously (5). Briefly, 108 polymorphic simple sequence-length polymorphism (SSLP) markers were typed on genomic DNA. PCR products of markers with interstrain differences <12 bp were separated on 6% polyacrylamide gels, whereas those >12 bp were separated on agarose gels.

Genome Scan Analysis

Before genetic analysis, data for all three phenotypic traits were log transformed to minimize skew. We carried out standard genome scans using the Pseudomarker (release 1.02) software package (30) (**http://www.jax.org/staff/churchill/labsite/software/**). We included an additive covariate representing all combinations of sex and lineage to account for sex- and lineage-specific differences in the phenotypes. In addition, we carried out scans for QTL-by-sex and QTL-by-lineage effects, as previously described (35). Significance thresholds were established using permutation analysis (11). Significant QTLs were those that exceeded the 0.05 genome-wide adjusted threshold, and suggestive QTLs exceeded or approached the 0.63 genome-wide adjusted threshold (22).

We used a pairwise search strategy (30, 38) to examine all possible locus pairs to search for epistatic interactions between QTLs. We included sex and lineage as additive covariates in the pairwise scans. Significance was determined by permutation analysis (100 permutations). The QTL-by-QTL interaction component of the logarithm of odds (LOD) was assessed by $P < 0.001$, unadjusted. The LOD score reported for interacting loci includes both the main effect and the interaction.

All loci and interactions that were detected by genome scans were entered into a multiple regression model. This multiple regression analysis was carried out using R/qtl software (8) (**http://www.biostat.jhsph.edu/~kbroman/software**). The algorithm used to fit the multiple QTL models uses multiple imputation (30) to account for uncertainty in QTL genotypes and is analogous to the MIM algorithm of Zeng (47). Only those terms that passed the suggestive threshold in the genome-wide scan were entered into the model. For each trait separately, individual terms were dropped in a backward elimination search until all terms remaining in the model were significant at the $P < 0.05$ level for basal Cort and at the $P < 0.01$ level for stress Cort and adrenal weight. Main effects that were included in significant interaction were retained in the model. The result is a list of QTLs with estimated effects that are adjusted for all other QTLs in the model.

Sequencing

We sequenced both mRNA and genomic DNA of Cort-binding globulin (CBG), a candidate gene identified in a stress Cort QTL, in WKY and F344 rats. As described previously (5), markers were designed using the Primer3 program (**http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi**). Purified PCR products were sequenced in both directions by ACGT (Wheeling, IL). Sequences were aligned using the Sequencher program from GeneCodes (Ann Arbor, MI) and compared with mRNA sequences (GenBank; **http://www.ncbi.nlm.nih.gov/**) or genomic sequences (Rat Genome Sequencing Consortium, version 3.1, July 2003; http://genome.ucsc.edu).

RESULTS

HPA measurements in Parent, F1, and F2 Generations

Previously reported data for the HPA axis measures in the parent, F1, and F2 generation rats of a WKY × F344 cross are shown in Table 1. In particular, note the strain × sex interactions for all three measures in the parent generation. As

Table 1. *HPA measures in parent, F1, and F2 generations (adapted from Ref. 34)*

Generation	Basal Cort, ng/ml	Stress Cort, ng/ml	Adrenal Wt, g
WKY male (n = 24)	31.7±4.2[c]	287.5±21.0[a]	43.0±1.2[a]
F344 male (n = 28)	81.0±8.3	354.7±18.9	47.0±0.9
WKY female (n = 20)	113.0±18.9[d]	584.5±33.5[d]	65.8±1.8[cd]
F344 female (n = 17)	94.7±21.2	503.7±32.4[d]	51.5±1.9[d]
F1 male			
F344 mother (n = 36)	122.4±10.8	343.7±16.5	52.1±0.5
WKY mother (n = 38)			
F1 female			
F344 mother (n = 28)	127.7±13.8	705.6±37.7[d]	61.5±1.0[d]
WKY mother (n = 36)			
F2 male			
F344 grandmother (n = 135)	88.4±4.6	305.3±5.3	52.2±0.6
WKY grandmother (n = 128)			53.7±0.6[b]
F2 female			
F344 grandmother (n = 111)	159.7±9.1[e]	517.9±9.1[e]	62.1±0.7
WKY grandmother (n = 112)			63.8±0.8[be]

Values are means ± SE. HPA, hypothalamic-pituitary-adrenal axis; Cort, corticosterone; WKY, Wistar Kyoto rat strain; F344, Fischer 344 rat strain. [a]Significant strain difference of same sex (t-test): $P < 0.05$. [b]Significant lineage difference of same sex (Tukey-Kramer): $P < 0.05$. [c]Significant strain difference of same sex (Tukey-Kramer): $P < 0.01$. [d]Significant sex difference of same strain (Tukey-Kramer): $P < 0.01$. [e]Significant sex difference of same lineage (Tukey-Kramer): $P < 0.001$.

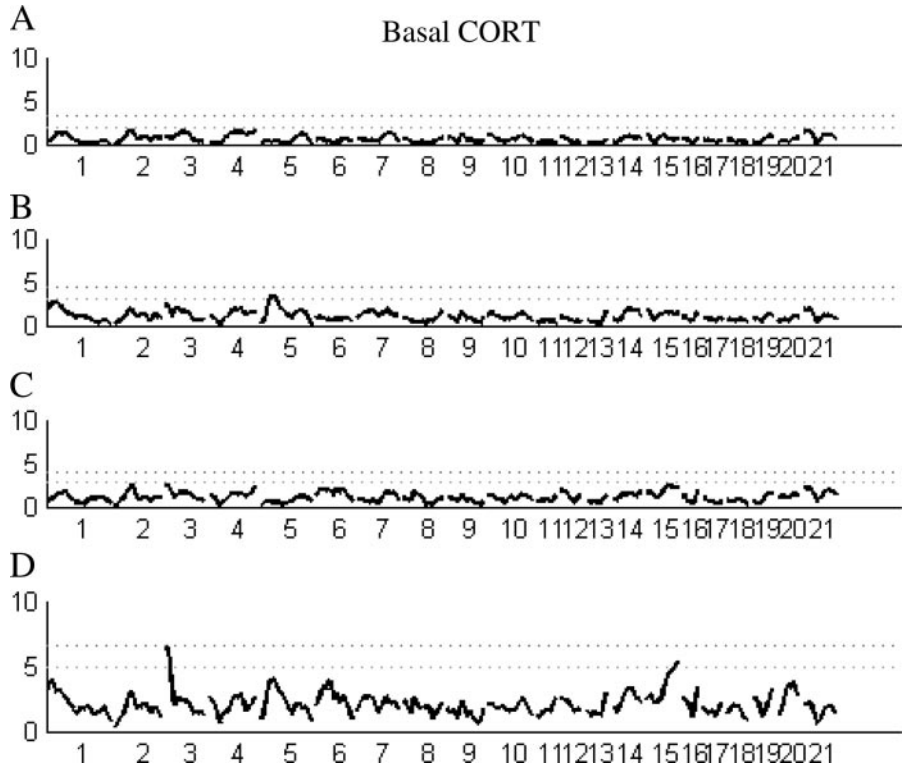

Fig. 1. Logarithm of odds (LOD) plots of genome scan for log(basal Cort). Chromosome location is on the *x*-axis, and LOD score is on the *y*-axis. *Top* dashed line indicates the significant threshold, and *bottom* dashed line indicates the suggestive threshold. Cort, corticosterone. *A*: scan for main effects with sex and lineage as additive covariates. *B*: scan with sex as interactive and lineage as additive covariate. *C*: scan with lineage as interactive and sex as additive covariate. *D*: scan with sex and lineage as interactive covariates.

reported previously, WKY males have significantly lower levels of basal Cort and stress Cort and smaller adrenal gland weights relative to F344 males, while no differences are seen in WKY and F344 females for basal and stress Cort, and WKY females have much larger adrenal glands relative to F344 females (34). Furthermore, note that all HPA axis measures are much higher in females relative to males in the parent, F1, and F2 generations.

We previously found a significant positive correlation between basal Cort and stress Cort in F2 generation males and females (34). A significant correlation was also seen only in males between basal Cort and adrenal weight ($r = 0.22$, $P < 0.05$) and stress Cort and adrenal weight ($r = 0.27$, $P < 0.01$) (34).

Mapping Loci Underlying HPA Function in WKY × F344 F2 Generation Rats

Basal Cort. For basal Cort, there is a main effect of sex ($F_{1, 481} = 207.2$, $P < 0.0001$) and a small effect of lineage ($F_{1, 481} = 5.3$, $P < 0.05$) in the segregating F2 generation of a WKY × F344 cross. We identified two suggestive loci for basal Cort. One [chromosome (Chr)5 at 24 cM; Chr5@24] was identified with sex as an interactive covariate and the other (Chr3@4) with sex and lineage as interactive covariates (see Fig. 1 and Table 2). These loci were named *Srcrtb-1* and *Srcrtb-2* for stress-responsive Cort basal [rat genome database (RGD), **http://www.rgd.mcw.edu**; identification nos. are 1358357 and 1358353, respectively]. Two pairwise interacting loci were also identified for this trait: Chr3@22 × Chr5@82 and Chr5@82 × Chr9@32. Both single loci and interacting loci were retained in the regression model (see Table 3). The total percent variance explained for this trait is 23.9%.

The effect plot for *Srcrtb-1* (Fig. 2*A*) shows that the sex and lineage effects are driven by a difference in the effect of genotype on females from the two lineages. This locus does not seem to affect basal Cort in males or in females from the WKY lineage. Interestingly, whereas females from the WKY lineage exhibit high levels of basal Cort independent of genotype, females from the F344 lineage only exhibit high levels of basal Cort in the presence of a WKY genotype. Because females from the WKY lineage exhibit high basal Cort levels even in animals that are F344 homozygous at this locus, we can conclude that, for this locus, the WKY lineage confers elevated basal Cort in the female offspring. The effect plot for interacting loci Chr3@22 × Chr5@82 is also interesting, as FF/WW and WW/FF (F and W represent F344 and WKY alleles,

Table 2. *Summary of results for single-marker and pairwise genome scans for log(basal Cort)*

Location, cM	Peak Marker (position in Mb)	CI, Mb	LOD	Locus Name
Chr3 at 4	D3Rat55 (10 Mb)	0–17	6.36†	*Srcrtb-1*
Chr5 at 24	D5Rat131 (35 Mb)	24–89	3.48*	*Srcrtb-2*
Chr3 at 22:	D3Rat100 (35 Mb):		6.67‡	
Chr5 at 82	D5Rat40 (169 Mb)			
Chr5 at 82:	D5Rat40 (169 Mb):		6.87‡	
Chr9 at 32	D9Rat26 (39 Mb)			

CI, confidence interval; LOD, logarithm of odds; Chr3 at 4 (and so forth), chromosome 3 at 4 cM (and so forth). Colons indicate pairwise interacting loci. *Sex used as an interactive covariate; suggestive threshold is 3.0, and significant threshold is 4.5. †Sex and lineage used as interactive covariates; suggestive threshold is 4.9, and significant threshold is 6.6. ‡Suggestive threshold for pairwise comparisons is 6.0, and significant threshold at 0.05 is 8.0. Peak marker, marker position of the highest LOD.

respectively) have high basal Cort, whereas animals homozygous for the same allele at both loci have low basal Cort (Fig. 2*B*).

Stress Cort. For stress Cort, there is a main effect of sex ($F_{1, 448} = 21.6$, $P < 0.0001$), as expected, with females showing a greater stress response. We identified five loci with main effects for stress Cort, three of which reached the significance threshold (see Table 4 and Fig. 3). We named these loci *Srcrt-1* through *Srcrt-5* for stress-responsive Cort (RGD identification nos. are 1358356, 1358362, 1358352, 1358355, and 1358354, respectively). We found two interacting loci: Chr4@30 × Chr5@20 and Chr6@75 × Chr10@35. All loci were retained in the regression model. The total percent variance explained for this trait is 61.9%, with none of the loci explaining >5% of the total variance for this phenotype (see Table 5), suggesting that the trait is truly polygenic in nature. Interestingly, despite the large phenotypic difference between males and females for this trait, we did not identify any sex-specific loci.

The effect plots for these loci show phenotypic effects in different directions. For example, whereas WKY homozygosity at *Srcrt-2* and *Srcrt-4* results in decreased plasma levels of stress Cort (WKY male profile) in the F2 generation, the same genotype at *Srcrt-1* and *Srcrt-5* results in increased plasma levels of stress Cort (F344 male profile) regardless of sex (representative effect plots are shown in Fig. 4).

Adrenal weight. We have previously reported that there is no correlation between adrenal weight and body weight in the F2 generation of the WKY × F344 cross (34). To verify this, we ran a genome scan for adrenal weight normalized for body weight. The results from this scan were no different from the scan of adrenal weight alone. As such, results reported here are for adrenal weight alone.

There is a large main effect of sex ($F_{1, 461} = 382.8$, $P < 0.0001$) and no effect of lineage on adrenal weight. We identified six loci with main effects, four of which reached significance (see Table 6 and Fig. 5). We named these loci *Sradr-1* through *Sradr-6* for stress-responsive adrenal weight (RGD identification nos. are 1358359, 1358360, 1358363, 1358364, 1358361, and 1358358, respectively). We found no interacting loci for this trait. All loci were retained in the regression model (see Table 7). The total percent variance explained is 56.8%. As with stress Cort, no sex-specific QTLs were found. With the exception of *Sradr-6*, WKY alleles increased adrenal gland weight (WKY female profile).

Table 3. *Multiple regression model for log(basal Cort)*

Source	% Variance	*F*	*P* Value
Sex	9.8	6.65	0.00000003[c]
Lineage	4.1	3.69	0.001[b]
Chr3 (4 cM)	4.5	3.05	0.002[b]
Chr3 (22 cM)	2.9	2.61	0.02[a]
Chr5 (24 cM)	3.0	4.02	0.003[b]
Chr5 (82 cM)	7.7	4.18	0.00002[c]
Chr9 (32 cM)	3.8	3.40	0.003[b]
Sex × lineage	2.3	4.25	0.006[b]
Sex × Chr3 (4 cM)	2.6	3.54	0.007[b]
Lineage × Chr3 (4 cM)	3.2	4.40	0.002[b]
Chr3 (22 cM):Chr5 (82 cM)	2.7	3.74	0.003[b]
Sex × Chr5 (24 cM)	2.7	7.27	0.0008[c]
Chr5 (82 cM):Chr9 (32 cM)	2.8	3.77	0.005[b]
Sex × lineage × Chr3 (4 cM)	1.8	4.82	0.008[b]

[a] Significant at 0.05. [b]Significant at 0.01. [c]Significant at 0.001.

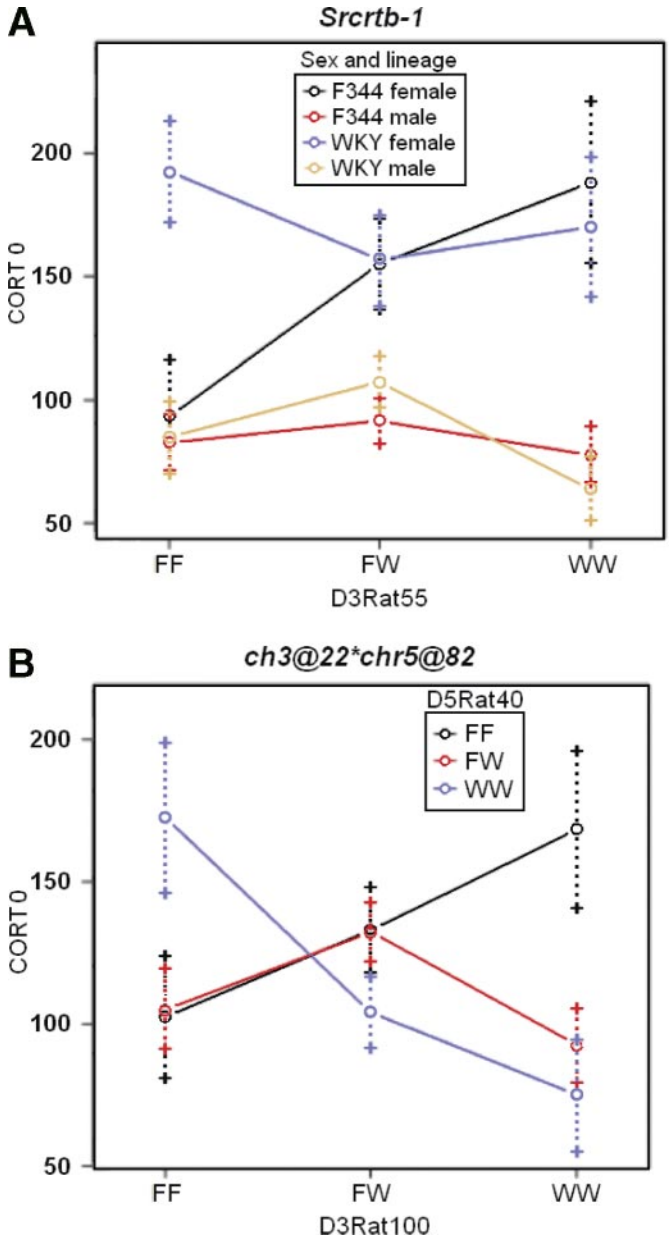

Fig. 2. Plots of allele effects for basal Cort (Cort0) at *Srcrtb-1* × sex (*A*) and chromosome (Chr)3 at 22 cM (Chr3@22) × Chr5@82 (*B*). *x*-Axis represents genotypes. F and W represent Fischer 344 (F344) and Wistar Kyoto (WKY) alleles, respectively. FW represents heterozygote for each allele. *y*-Axis is basal Cort (ng/ml). Error bars are ±1 SE.

Sequence Alterations in CBG

CBG was identified as a candidate gene in *Srcrt-5*, the largest stress Cort QTL. As such, we sequenced the CBG mRNA of WKY. While no differences were found between the WKY sequence and the published Brown Norway sequence, three differences were identified between the WKY and the Wistar rat (33). Two of these differences alter residues 141 and 142 of the mature peptide from threonine and arginine to asparagine and glutamine, respectively. The third is a point

Table 4. *Summary of results for single-marker and pairwise genome scans for log(stress Cort)*

Location, cM	Peak Marker (position in Mb)	CI, Mb	LOD	Locus Name
Chr2 at 86	D2Rat139 (232 Mb)	154–240	3.66*[a]	*Srcrt-1*
Chr3 at 46	D3Rat181 (48 Mb)	35–169	2.78*[a]	*Srcrt-2*
Chr4 at 38	D4Rat128 (79 Mb)	34–126	2.29*[a]	*Srcrt-3*
Chr6 at 72	D6Rat111 (126 Mb)	104–130	6.39*[b]	*Srcrt-4*
Chr15 at 66	D15Rat50 (106 Mb)	67–106	3.38*[a]	*Srcrt-5*
Chr4 at 30:	D4Rat128 (79 Mb)		9.05†[a]	
Chr5 at 20	D5Rat131 (35 Mb)			
Chr6 at 75:	D6Rat111 (126 Mb)		10.19†[a]	
Chr10 at 35	D10Rat104 (42 Mb)			

*No interactive covariate used; suggestive threshold is 1.9, significant threshold at 0.05 is 3.1, and significant threshold at 0.01 is 3.8. †Pairwise comparison thresholds; suggestive is 6.0, and significant is 8.0. [a]Significant at 0.05. [b]Significant at 0.01.

Table 5. *Multiple regression model for log(stress Cort)*

Source	% Variance	*F*	*P* Value
Sex	40.7	436.27	0.0[c]
Chr2 (94 cM)	1.1	5.8	0.003[b]
Chr3 (46 cM)	1.2	6.26	0.002[b]
Chr4 (26 cM)	2.3	4.06	0.0006[c]
Chr4 (38 cM)	1.1	5.96	0.003[b]
Chr5 (18 cM)	2.6	4.60	0.0002[c]
Chr6 (72 cM)	3.9	6.92	0.0000005[c]
Chr10 (38 cM)	1.5	2.71	0.01[a]
Chr15 (66 cM)	1.4	7.39	0.0007[c]
Chr4 (26 cM):Chr5 (18 cM)	2.1	5.74	0.0002[c]
Chr6 (72 cM):Chr10 (38 cM)	1.3	3.58	0.007[b]

[a] Significant at 0.05. [b]Significant at 0.01. [c]Significant at 0.001.

mutation G>A resulting in a methionine-to-isoleucine substitution at residue 276 in the WKY rat CBG sequence (19, 32). There is evidence suggesting that this mutation may result in decreased binding affinity of CBG. To confirm these alterations, the first and third exons of the CBG gene were sequenced from WKY and F344 genomic DNA. Residues 141 and 142 in the first exon were the same in both strains, but residue 276 was confirmed to code for a methionine in F344 and an isoleucine in WKY.

DISCUSSION

Through genome-wide analysis of a segregating F2 population of a F344 × WKY cross, we detected several QTLs involved in three separate elements of the stress-responsive HPA axis. We identified two suggestive main loci and two pairs of interacting loci for basal Cort and five main loci (3 of which are significant) and two pairs of interacting loci for stress Cort, as well as six main loci (4 of which are significant) for adrenal weight. None of the stress Cort or adrenal weight loci interacted with sex or lineage, and only one locus (*Srcrt-3/Sradr-3*) was found for both traits. We identified one promising candidate gene, CBG, in the largest stress Cort QTL on Chr6. We found a sequence variant in both the cDNA and the genomic DNA of CBG in the WKY rat compared with Wistar and F344 strains. This variant results in an amino acid substitution that has been shown to result in a decreased binding affinity of CBG in vitro (32).

Despite the highly significant phenotypic sex-by-strain interactions (decreased adrenocortical activity in WKY males and increased activity in WKY females relative to F344), we identified only two QTLs with sex-specific effects in the current cross, both for basal Cort. Although it is possible that some sex-specific QTLs were below the detection of our

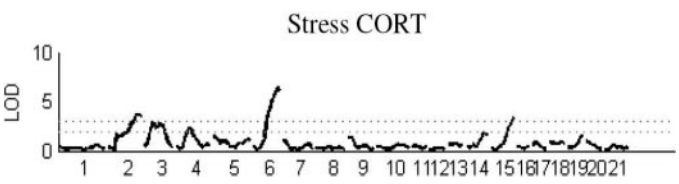

Fig. 3. LOD plots of genome scan for log(stress Cort). Chromosome location is on the *x*-axis, and LOD score is on the *y*-axis. *Top* dashed line indicates significant threshold, and *bottom* dashed line indicates suggestive threshold.

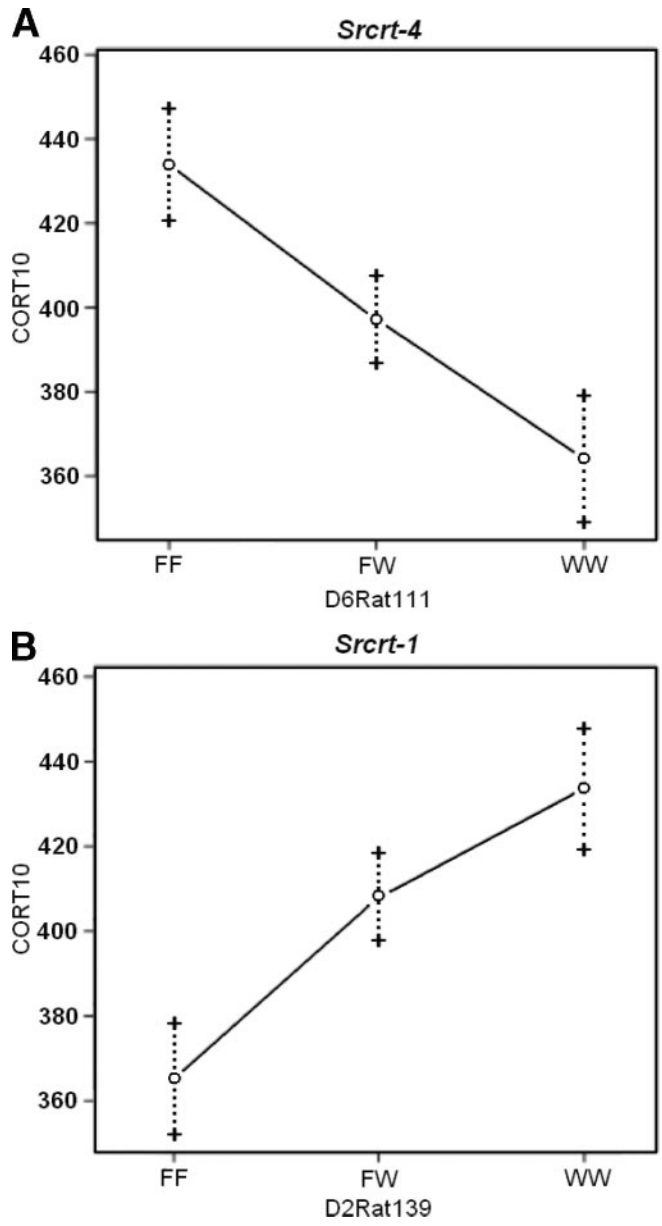

Fig. 4. Representative effect plots for stress Cort (Cort10). *A*: *Srcrt-4* showing decreased stress Cort in WKY homozygous animals. *B*: *Srcrt-1* showing increased stress Cort in WKY homozygous animals. *x*-Axis represents genotypes. F and W represent F344 and WKY alleles, respectively. FW represents heterozygote for each allele. *y*-Axis is stress Cort (ng/ml).

Table 6. *Summary of results for single-marker genome scan for log(adrenal wt)*

Location, cM	Peak Marker (position in Mb)	CI, Mb	LOD	Trait Name
Chr1 at 44	D1Rat24 (76 Mb)	64–169	4.74*[b]	*Sradr-1*
Chr2 at 58	D2Rat220 (142 Mb)	109–169	10.24*[b]	*Sradr-2*
Chr4 at 36	D4Rat128 (79 Mb)		6.19*[b]	*Sradr-3*
Chr4 at 82	D4Rat137 (155 Mb)		Note‡[b]	*Sradr-4*
Chr7 at 50	D7Rat24 (127 Mb)	53–169	5.55*[b]	*Sradr-5*
Chr18 at 16	D18Rat96 (41 Mb)	7–58	2.49*	*Sradr-6*
			5.85†[a]	

*No interactive covariate used; suggestive threshold is 2.0, significant threshold at 0.05 is 3.2, and significant threshold at 0.01 is 4.7. †Sex and lineage used as covariates; suggestive threshold is 4.5, significant threshold at 0.05 is 5.8, and significant threshold at 0.01 is 6.1. ‡Pairscan analysis revealed a significant joint contribution of *Sradr-3* and *Sradr-4* (LOD = 10.82) without interaction. [a]Significant at 0.05. [b]Significant at 0.01.

analysis, our results suggest that similar genetic components contribute to the variation in stress Cort and adrenal weight in males and females, and that the phenotypic differences are a result of other factors such as steroid hormones (42).

Several of our QTLs are in homologous or overlapping regions with previously identified QTLs for glucocorticoid-related phenotypes (see Table 8) as well as QTLs for behavioral responses to stress. For example, *Srcrt-3/Sradr-3* is in an overlapping region with a rat QTL for basal Cort (27) and a rat QTL for expression of a stress-induced heat shock protein (15) and lies in the homologous region for several stress-induced behavioral QTL in mice (24, 40, 45). *Srcrt-1* is in a region homologous to a QTL for human fasted cortisol (25) as well as a QTL previously identified for Cort in response to ethanol consumption in mice (29). Both of these challenges, fasting and ethanol administration, are known to provoke an increase in glucocorticoid production, just like an acute stress response. Furthermore, *Srcrtb-2*, *Srcrt-2*, and *Sradr-2* overlap with three different QTL regions previously identified in this same cross for depressive behavior in the forced swim test (35).

While we identified some potential candidate genes in our QTL regions (neuropeptide Y and CRH receptor-2 map to the *Srcrt-3/Sradr-3* region, and the glucocorticoid receptor maps to the *Sradr-5* region), the most promising candidate gene was CBG in *Srcrt-4*, the largest QTL for stress Cort. CBG is found on rat Chr6 at 127.9 Mb, just over 1 Mb away from the peak marker in *Srcrt-4* (126.6 Mb). A QTL containing CBG has previously been found for both basal and stress Cort in a cross between two pig strains differing in behavioral and neuroendocrine measures (13). On further investigation, this group found that the two pig strains differed significantly in CBG binding capacity (26).

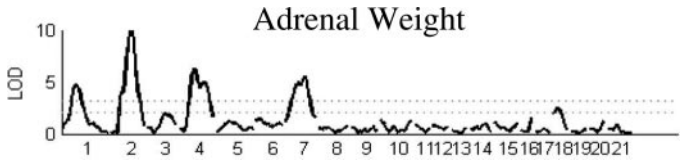

Fig. 5. LOD plots of genome scan for log(adrenal weight). Chromosome location is on the *x*-axis, and LOD score is on the *y*-axis. *Top* dashed line indicates significant threshold, and *bottom* dashed line indicates suggestive threshold.

Table 7. *Multiple regression model for log(adrenal wt)*

Source	% Variance	*F*	*P* Value
Sex	26.5	230.65	0.00[c]
Lineage	1.2	10.67	0.001[b]
Chr1 (44 cM)	3.3	14.5	0.0000008[c]
Chr2 (58 cM)	7.2	31.46	0.00000000000002[c]
Chr4 (36 cM)	1.8	7.73	0.0005[c]
Chr4 (82 cM)	1.5	6.42	0.002[b]
Chr7 (52 cM)	3.1	13.66	0.000002[c]
Chr18 (16 cM)	1.4	6.05	0.003[b]

[b]Significant at 0.01. [c]Significant at 0.001.

CBG, also known as serpina6, is a steroid transport protein that is a member of the serpin superfamily of serine protease inhibitors (18). CBG binds ~90% of Cort in rat plasma and is an important determinant in circulating levels of free (active) Cort (7). CBG has been hypothesized to serve several functions, including *1*) regulation of the bioavailability and clearance of glucocorticoids, *2*) transportation of glucocorticoids to specific tissues, and *3*) partial regulation of the glucocorticoid negative feedback system (7). In addition, the CBG-Cort complex has been shown to activate the intracellular cAMP messenger system, suggesting an independent function of this complex (7). CBG also decreases in response to various stressors (23, 36, 39), which may allow for an increase in the availability of free (active) Cort after the immediate stress response.

The alteration we found in the CBG sequence of WKY is identical to that found in the Wistar-derived BioBreeding rat, a strain that exhibits decreased binding affinity of CBG (32). Furthermore, the binding capacity of Chinese hamster ovary cells transfected with CBG with the G>A mutation is significantly decreased relative to cells transfected with nonmutated CBG (32). It is likely that this variant also leads to decreased binding capacity in the WKY rat strain, which may play a role in the stress Cort phenotype of this strain.

The effect of lineage on basal Cort levels of females as seen at the *Srcrtb-1* locus clearly deserves further study. We have shown that WKY dams have altered and diminished maternal behavior (3), and maternal separation can lead to elevated Cort in the adults, particularly females. But it has also been shown that Cort output depends on both the maternal and the offspring genotype (44). This maternal effect is not unique to Cort production of the offspring, but it is of particular importance in a trait that can affect adaptation. Thus maternal effects can prepare the offspring for the environment in which the mother, and potentially the offspring, has to survive.

Table 8. *Homologous regions with other HPA QTLs*

QTL	Cort/Cortisol QTL
Srcrt-1	mouse: Cort post-EtOH:Chr3, 63 cM (29) human: fasted cortisol:Chr1, 140 cM (25)
Srcrt-3/Sradr-3	rat: afternoon basal Cort (27)
Srcrt-4	pig: basal Cort:Chr7 (13)
Sradr-1	human: fasted cortisol:Chr11, 21 cM (25)

QTL, quantitative trait locus; EtOH, ethanol. Nos. in parentheses are reference nos.

In conclusion, we report several QTLs that may harbor genes contributing to individual variability in three different facets of the stress-responsive adrenocortical function, basal Cort, stress Cort, and adrenal gland weight. Most of these loci are specific to the phenotype in question, with only one locus identified for both stress Cort and adrenal weight. The major question, whether adrenocortical function is a polygenic trait that is regulated by a multitude of relatively small effects, is answered affirmatively by this study. It also seems that the genetic variations in the known essential players of HPA axis regulation (e.g., CRH, CRH receptors, and AVP), if involved in the genetics of the adrenocortical function, may be minor, with their contribution to the overall variance in these phenotypes being low. Very large alterations in these essential regulators would not allow adaptation, but small variations, such as the sequence variant of CBG of the WKY, may play a role in the altered HPA activity of this strain. By further understanding the genetic basis of the stress response, we will gain a greater understanding of stress-related pathophysiologies and how better to treat them.

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