Genetic relationship between placental and fetal weights and markers of the metabolic syndrome in rat recombinant inbred strains

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Genetic relationship between placental and fetal weights and markers of the metabolic syndrome in rat recombinant inbred strains. Physiol Genomics 26: 226–231, 2006; doi:10.1152/physiolgenomics.00056.2006.—Epidemiological studies have shown a clear link between fetal growth retardation and an increased propensity for later cardiovascular disease in adults. It has been hypothesized that such early fetal deprivation “programs” individuals toward a life-long metabolic “thrifty phenotype” that predisposes adults to such diseases. Here we test this hypothesis, and its possible genetic basis, in rat recombinant inbred (RI) strains that uniquely allow the longitudinal studies necessary for its testing. Placental and fetal weights were determined on day 20 of pregnancy in (at least) 6 litters from each of 25 available BXH/HXB RI strains and from their SHR and BN-Lx progenitors and were correlated with metabolic traits determined in adult rats from the same inbred lines. Quantitative trait loci (QTLs) associated with placental and fetal weights were identified by total genome scanning of RI strains using the Map Manager QTX program. Heritabilities of placental and fetal weights were 56% and 62%, respectively, and total genome scanning of RI strains revealed QTLs near the D1Rat266 marker on chromosome 1 and near the D15Rat101 marker on chromosome 15 that were significantly associated with fetal and placental weights respectively. Placental weights correlated with fetal weights (r = 0.60, P = 0.001), while reduced fetal weights correlated with increased insulin concentrations during glucose tolerance test (r = -0.71, P = 0.0001) and with increased serum triglycerides (r = -0.54, P = 0.006) in adult rats. Our results suggest that predisposition toward a thrifty phenotype associated with decreased placental weight and restricted fetal growth is in part genetically determined.

thrifty genotype; genetic and correlation analyses; spontaneously hypertensive rat

According to the diagnostic criteria of either the World Health Organization or the National Cholesterol Education Program, “metabolic syndrome” affects upwards of 15–25% of the adult Western population (17, 18, 26) and is characterized by the clustering of multiple risk factors for diabetes and cardiovascular disease including insulin resistance, dyslipidemia, and increased blood pressure. The current epidemic of metabolic syndrome appears to be a result of both profoundly maladaptive diets and lifestyles, as well as a susceptibility to develop metabolic syndrome and Type 2 diabetes in an appropriately predisposing environment. It is likely that this susceptibility to the development of metabolic syndrome has a strong genetic component, as demonstrated by cross-cultural studies (5). The identification of the genetic determinants responsible for this susceptibility would ultimately help us in understanding the etiology of this disorder as well as improved diagnostic criteria for its detection (15).

Multiple studies have now demonstrated a higher risk of diabetes or impaired glucose tolerance in adults that were born with a low birth weight (reviewed in Refs. 1, 11, 21). The suggestion of a link between adverse intrauterine events and an increased incidence of cardiovascular and diabetic disease at maturity has been supported by numerous animal studies including those wherein offspring of pregnant rats that were exposed to modest dietary insults showed reduced birth weights and impaired glucose tolerance and Type 2 diabetes much later in life (reviewed in Ref. 11). On the basis of these and other findings, Hales and Barker (11) hypothesized that insulin resistance and Type 2 diabetes are a result of fetal metabolic reprogramming that occurs as a result of maternal malnutrition. It is proposed that although such programming allows short-term sparing of fetal brain growth at the expense of somatic growth, it also results in future predisposition to cardiovascular and diabetic disease. Thus, while such metabolic resetting is a successful strategy during times of fetal privation, in the context of excess food availability and consequent fat deposition in adulthood, it is ultimately maladaptive.

It is possible that the level of “thrifty phenotype” exhibited by individuals will be influenced by their respective genotypes. One very powerful way to search for the relationship between fetal phenotypes and metabolic traits at maturity, as well as for genetic determinants that are responsible for these correlations, is the use of linkage analysis in the BXH/HXB recombinant inbred (RI) strains that were derived from the spontaneously hypertensive rat (SHR), a widely used animal model of hypertension and metabolic syndrome. We have successfully used this approach in the past to identify quantitative trait loci (QTLs) that control parameters such as blood pressure or carbohydrate and lipid metabolism (reviewed in Refs. 29, 30). In the current study, we performed genetic and correlation analyses of placental and fetal growth in the BXH/HXB RI strains to test the hypothesis that the thrifty phenotype and its deleterious metabolic consequences later in life have a genetic component.

MATERIALS AND METHODS

Animals. RI strains were produced by brother-sister inbreeding of the F2 offspring resulting from a cross between two highly inbred

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progenitor strains, the Brown Norway (BN-Lx/Cub) and spontaneously hypertensive rat (SHR/Ola) (29, 30). Twenty-five of the available RI strains (BXH and HXB sets) at >F₂ generation were used in the studies detailed below. Rats were housed in an air-conditioned animal facility and allowed free access to standard laboratory chow and water. All experiments were performed in agreement with the Animal Protection Law of the Czech Republic (311/1997) and were approved by the Ethics Committee of the Institute of Physiology, Czech Academy of Sciences, Prague, Czech Republic.

Fetal phenotypes. Fetal and placental weights were determined on day 20 of pregnancy in at least six litters from each of the 25 RI strains and the SHR and BN-Lx progenitors. All female breeders were primiparous and were mated at the age of 3 mo.

Metabolic phenotypes. Metabolic phenotypes were determined in 10-wk-old RI and progenitor males (n = 6–8 per strain) that had been maintained on a high-fructose diet (60% fructose, K4102.0 diet; Hope Farms) for 2 wk.

Oral glucose tolerance test. Oral glucose tolerance testing (OGTT) was performed as previously described (31) and commenced with the administration of a glucose load (300 mg/100 g body wt) to animals that had been fasted for 7 h. Blood was then drawn from the tail without anesthesia immediately before the glucose load (zero time point) and at 30, 60, and 120 min thereafter. A high-fructose diet was used because previous studies have shown that high-fructose diets can accentuate the features of metabolic syndrome in SHR (28).

Skeletal muscle glycogen synthesis. Glycogen synthesis was determined in isolated diaphragm muscle by measuring the incorporation of 14C-U glucose into glycogen as previously described (31, 39). The diaphragm muscles were incubated for 2 h in Krebs-Ringer bicarbonate buffer, pH 7.4, that contained 5.5 mM unlabeled glucose, 0.5 μCi/ml of 14C-U glucose, and 3 mg/ml bovine serum albumin (Armour, fraction V) with or without 250 μU/ml insulin. For measurement of basal and insulin-stimulated incorporation of glucose into glycogen, glycogen was extracted and glucose incorporation into glycogen was determined as previously described (31, 39). For correlation analysis we used the difference between insulin stimulated and basal glycogenesis as an index of insulin sensitivity in muscles.

Hepatic triglyceride measurements. For determination of hepatic triglycerides, liver tissue was powdered under liquid N₂ and extracted for 16 h in chloroform-methanol, after which 2% KH₂PO₄ was added, and the solution was centrifuged. The organic phase was removed and evaporated under N₂. The resulting pellet was dissolved in isopropyl alcohol, and triglyceride content was determined by enzymatic assay (Pliva-Lachema, Brno, Czech Republic) (31).

Biochemical analyses. Blood glucose levels were measured by the glucose oxidase assay (Pliva-Lachema) using tail vein blood drawn into 5% trichloracetic acid and promptly centrifuged. Serum triglyceride concentrations were measured by standard enzymatic methods (Pliva-Lachema). Serum insulin concentrations were determined using a rat insulin ELISA kit (Merckodia, Uppsala, Sweden).

Statistics. Values are expressed as means ± SE. Means of fetal weights from at least six litters and means of metabolic phenotypes at maturity from six to eight males per each RI and progenitor strain were used for these correlation analyses. We tried to adjust individual fetal and placental weights within each strain to the litter size by means of standard analysis of covariance (ANCOVA); however, no significant linear trends were found in the RI strains. In addition, fetal phenotypes used for QTL and correlation analyses were determined as averages from several litters within each RI strain, and therefore the additive part of the mean value of fetal and placental weights in the ANCOVA model (linearly dependent on litter size) trended toward zero. For these reasons we did not “correct” fetal and placental weights for litter size but rather used the raw data for our QTL and correlation analyses. The Map Manager QTX program (version b20) (23) was used to test for single locus associations by regression analysis, and the significance of each potential association was measured using the likelihood ratio statistics (12). The interval regression method of the Map Manager QTX program was used to test for QTLs within marker intervals. The significance thresholds for the genome-wide scans were empirically determined by the Map Manager QTX program permutation test (6) using informative markers and 1,000 permuted data sets. Significant linkage was defined in accordance with the guidelines of Lander and Kruglyak (19) as statistical evidence occurring by chance in the genome scan with a probability of 5% or less. For the genome scanning, >1,000 markers were available (14). The variances explained by the QTL were estimated using the Map Manager QTX program, and the approximate confidence limits of QTL peaks were estimated using the bootstrap method of the QTX program (37).

Heritabilities of the traits were estimated from the variance in mean values between and within the RI strains (27). The additive genetic variance was estimated as 50% of the total variance between the means of the RI strains; the environmental variance was estimated to be the average variance in mean phenotypic values within the RI strains. Narrow heritability was calculated by dividing the additive genetic variance by the sum of the additive genetic variance and the environmental variance.

The statistical strength of the relationship between fetal weights and adult metabolic phenotypes in the RI strains was tested using Pearson’s correlation coefficient (SigmaStat software).

RESULTS

Figure 1A shows the distribution of mean placental weights amongst the RI and progenitor SHR and BN-Lx strains. As can be seen, the distribution of placental weights among RI strains was continuous despite the fact that the progenitor strains exhibit similar placental weights. Such transgressive variation
is not unusual with polygenic traits. The inheritance of placental weights was estimated to be 56%. Figure 1B shows the distribution of mean fetal weights in RI and progenitor strains. Once again, the distribution was continuous, and, as with placental weights, progenitors were found to have similar fetal weights. The heritability of fetal weights was estimated to be 62%.

Because the relatively high heritabilities of both placental and fetal weights suggested that there was a major genetic contribution to both these phenotypes, we decided to search for chromosomal regions (QTL) that might be influencing these contribution to both these phenotypes. We used a total genome scanning approach using >1,000 gene markers available for the BXH/HXB RI strains.

Our genome-wide scan of the RI strains revealed a QTL near the D1Rat266 marker on chromosome 1 that was significantly associated with fetal weights and a QTL near the D15Rat101 marker on chromosome 15 (P<0.005). RI, recombinant inbred; BN, Brown Norway; SHR, spontaneously hypertensive rat; Fw1, fetal weight 1; Pw1, placental weight 1; QTL, quantitative trait loci.

Fig. 2. Interval mapping of quantitative trait loci (QTLs) on chromosomes 1 (A) and 15 (B) that were associated with fetal and placental weights, respectively. Only segments of chromosomes 1 (16–65 cM) and 15 (2–65 cM) are depicted. Criteria for statistical significance as estimated by the Map Manager QTX permutation tests are depicted: the significant likelihood ratio scores (LRS) were estimated to be 12.1 for the QTL on chromosome 1 (A) and 15.0 for the QTL on chromosome 15 (B). Approximate confidence limits of QTL peaks were evaluated by the bootstrap method of the Map Manager QTX program. The height of the gray bars provides a measure of the confidence with which a trait maps to a particular chromosomal region. Fw1, fetal weight 1; Pw1, placental weight 1.

Table 1. Placental and fetal weights of RI strains

<table>
<thead>
<tr>
<th>QTL (marker)</th>
<th>BN Allele, g</th>
<th>SHR Allele, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pw1 (D15Rat101)</td>
<td>0.32±0.02</td>
<td>0.43±0.02*</td>
</tr>
<tr>
<td>Fw1 (D1Rat266)</td>
<td>1.41±0.14</td>
<td>1.88±0.03*</td>
</tr>
</tbody>
</table>

Values are means ± SE. Weights were stratified according to progenitor genotypes at the D1Rat266 marker on chromosome 1 (Fw1 QTL) and D15Rat101 marker on chromosome 15 (Pw1 QTL) (see Figure 2). *P<0.005. RI, recombinant inbred; BN, Brown Norway; SHR, spontaneously hypertensive rat; Fw1, fetal weight 1; Pw1, placental weight 1; QTL, quantitative trait locus.

DISCUSSION

This is the first correlation-based genetic analysis of the thrifty phenotype hypothesis. We found in a panel of BXH/HXB RI strains, significant associations between reduced fetal weights and both increased insulin concentrations during OGTT and increased serum and hepatic triglycerides at maturity. The correlations shown in Figs. 3 and 4 thus support the hypothesis that altered fetal development plays a role in the pathogenesis of maturity-onset dyslipidemia and insulin resistance.
Furthermore, the finding of significant variability in placental and fetal growth amongst the RI strains leads us to conclude that there is a significant genetic component to the control of placental and fetal growth. This suggestion is further supported by the relatively high estimates of heritability for both these traits in the RI animals. This so-called “metabolic programming” of fetuses is, however, also proposed to be strongly dependent on the maternal allocation of nutrients to the developing fetus via the placenta. Thus two broad categories of factors are likely to contribute to fetal underdevelopment through so-called “maternal constraint,” namely, environmental factors such as parity, age, or maternal nutrition, as well as genetically determined factors such as maternal size or density of the placental vasculature (8).

We found a close relationship between placental and fetal growth in the RI strains, supporting the notion that during evolution, natural selection favored the survival of mothers that were able to reduce the allocation of nutrients to fetuses during times of environmental stress (9). Such maternal constraint, while resulting in low birth weights and the fetal programming of what will be a postnatal thrifty phenotype, would however increase the survival rates of both mother and offspring in a nutrient-poor environment. Our results also suggest that if such mechanisms do in fact operate to maximize survival of both mother and offspring via alterations in feto-placental functioning, then there is a significant genetic component to the control of such mechanisms.

According to the thrifty genotype hypothesis proposed by Neel (25), it is possible that some individuals are genetically predisposed to a greater rate of fat accumulation in a nutrient-rich environment than others. However, while such a thrifty genotype may have been an evolutionary advantage in the more impoverished environments of the evolutionary past, it may prove to be metabolically maladaptive in times of nutrient excess. A variant of the thrifty genotype hypothesis proposed by Reaven (32) suggests that “insulin-resistance genes” were in fact positively selected for during evolution to allow the maintenance of glucose delivery to the brain at the expense of catabolized muscle tissue. On the other hand, Hales and Barker (11) believe that thrifty phenotype is induced mainly by environmental insults, not by genetic factors. Whether we are dealing here with a thrifty phenotype (11) or “thrifty genotype” (25, 32) is more than a semantic issue. The question raised is whether environmental as opposed to genetic factors underlie the relationship between early growth and later disease. Contrary to the original hypothesis of Hales and Barker (11), results of the current study strongly suggest that thrifty phenotype has a significant genetic component.

On the basis of our findings we propose that the current epidemic of metabolic syndrome (so-called diseases of plenty) might be attributable to a genetically predetermined placental insufficiency that programs offspring to exhibit a thrifty pheno-
notype acting in conjunction with the overconsumption of energy-rich foodstuffs readily available to Western communities. In support of our suggestion are the findings that heritability estimates for human birth weight is in the range of 25–40% and that these genetic effects are largely of maternal origin acting via uteroplacental factors (2, 4, 7, 13, 22, 33, 38). The finding of a substantial variability in birth weights in Western populations, despite the fact that most mothers receive more than sufficient nutrition, strongly supports this notion.

Although the thrifty phenotype/genotype in humans is hypothesized to have arisen from the evolutionary pressures provided by a paucity of nutrients, it should be borne in mind that this process has occurred in human communities over a long period of time. Thus, in a genetic sense, humans have been “domesticated” for a much longer period than the rat, which has only been domesticated in comparatively recent times. However, this does not mean that genetic findings in rats have no relevance for the human situation. We believe that both humans and animals developed thrifty phenotype/genotype during their evolutionary histories since this was necessary for their survival in impoverished environments. However, although extrapolation of findings in animal models to complex human diseases must always be done with caution, we believe that our present study might shed light on genetic factors that contribute to the current epidemic of metabolic syndrome and Type 2 diabetes.

We have identified a number of candidate genes for both the Fw1 and Pw1 QTLs that could conceivably give rise to factors that control fetal and placental development. For example, near the fetal growth QTL (Fw1), we note the presence of the genes coding for cyclin E1, growth arrest-specific 2, and insulin-like growth factor receptor 1 (Ccnel, Gas2, and Igr1r, respectively). Cyclin E1 is an important regulator of both the cell cycle and cellular differentiation (40), whereas Gas2 has been shown to regulate somatic development of the embryo (20). Mutations in the Igr1r gene have been found to be associated with the intrauterine growth retardation of children with short stature (16). Thus it is possible that one of these genes, as well as the hormonal factor it encodes, could play a role in the variation seen in fetal weight amongst the RI strains. As such, they are deserving of further investigation. In addition, the Fw1 QTL overlaps with QTLs previously found to be associated with features of metabolic syndrome such as altered blood pressure (24, 41), urine albumin (34), and serum cholesterol (36).

Near the QTL we identified as controlling placental weight (Pw1), we find the genes for gonadotropin releasing hormone 1 (Gnrh1) and transforming growth factor-β1 (Tgfβ1). Gonadotropins are the central regulators of the hypothalamic-pituitary axis and, as such, are intimately involved in both normal and abnormal placental function (35) as well as fetal development itself (3). We thus also propose these as candidate genes worthy of further investigation as possible programmers of the thrifty phenotype. It should also be noted that the Pw1 QTL overlaps with a previously reported QTL found to be associated with noninsulin-dependent diabetes mellitus (42).

In summary, our genetic and correlational analyses of the fetal and placental weights and adult metabolic indexes of BXH/HXB RI strains provide evidence for an important role for genetic factors in the mechanism by which maternal constraint induces a thrifty phenotype in offspring and, subse- quently, dyslipidemia and insulin resistance. Identification of the precise causal genes mediating these effects, however, awaits further investigation.

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