Rat model of familial combined hyperlipidemia as a result of comparative mapping

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THE METABOLIC SYNDROME is one of the potential targets of comparative mapping

Rat model of familial combined hyperlipidemia as a result of comparative mapping. Physiol Genomics 17: 38–47, 2004. First published January 6, 2004; 10.1152/physiolgenomics.00043.2003.—Total genome scan was carried out in 266 F2 intercrosses from the Prague hypertriglyceridemic (HTG) rat that shares several clinical characteristics with human metabolic syndrome. Two loci for plasma triglycerides (TG) were localized on chromosome 2 (Chr 2) (LOD 4.4, 3.2). The first locus overlapped with the rat syntenic region of the human locus for the metabolic syndrome and for small, dense LDL, while the second overlapped with the syntenic region of another locus for small, dense LDL in humans by the comparative mapping approach. Loci for TG on rat Chr 13 (LOD 3.3) and Chr 1 (LOD 2.7) overlapped with the syntenic region of loci for human familial combined hyperlipidemia (FCHL) in Finnish and Dutch populations, respectively. The concordances of loci for TG localized in this study with previously reported loci for FCHL and its related phenotypes are underlying the generalized importance of these loci in dyslipidemia. These data suggest the close relationship between dyslipidemia in HTG rats and human FCHL, establishing a novel animal model for exploration of pathophysiology and therapy based on genomic determinants.

Prague hypertriglyceridemic rat; quantitative trait locus; triglycerides; cholesterol; metabolic syndrome

THE METABOLIC SYNDROME is one of the potential targets of therapy in the primary prevention of coronary heart disease characterized by abdominal obesity, atherogenic dyslipidemia, elevated blood pressure, and insulin resistance (17, 50) present in more than 20% of the US adult population (40). Although the metabolic syndrome is a broad category, it may include several distinct genetic diseases that share similar clinical features.

Familial combined hyperlipidemia (FCHL) was originally described in the early 1970s (19, 52). The affected members of FCHL families present different lipid phenotypes: hypercholesterolemia, hypertriglyceridemia, or combined hyperlipidemia and high serum apolipoprotein B. FCHL is also known to share features of the metabolic syndrome in higher prevalence compared with the general population, which include insulin resistance, obesity, and hypertension (2, 25, 48). Therefore, individuals with FCHL seem to form, at least partially, a subset of the metabolic syndrome. The metabolic defect in lipoprotein metabolism is associated with a predominance of small, dense LDL particles (6, 23, 24) and appears to be a consequence of hepatic apolipoprotein B-100 overproduction (60). FCHL is the most common of the dyslipidemias, with 1% to 2% prevalence in the general population and in up to 20% of patients with premature coronary heart disease (4).

Genetic studies of FCHL have been complicated by uncertain phenotype definition, genetic heterogeneity, and unknown modes of inheritance. In Finnish FCHL families, genome-wide scan revealed a significant locus for the FCHL trait on chromosome 1q21–23 (Chr 1q21–23), between but not including the flanking apolipoprotein A-II gene and the P, L, and E selectin genes (38, 39). In Dutch FCHL families, a significant locus for FCHL on Chr 11p was determined by a two-step genome scan approach (5). Candidate gene studies have provided evidence that common variations of many genes, including lipoprotein lipase (7, 66), protein AI-III-AIV cluster (63–65), fatty acid binding protein 2 (45), hormone-sensitive lipase (47), hepatic lipase (43), β3-adrenergic receptor and uncoupling protein 1 (46), as well as peroxisome proliferator-activated receptor-γ (PPARγ) (44), can influence lipid levels in affected individuals, but none of these genes has been found to be a primary determinant. Numerous factors, such as a lack of unequivocal diagnostic criteria, impact of the environment, and age dependence of the lipid phenotype, are increasing the heterogeneity of affected subjects (21, 22, 41).

Physiological genomics approaches to cardiovascular disease include single and complex genetic manipulations (18). One of the most productive approaches to the study of FCHL is the development of animal models that closely resemble both the clinical and genetic features of this disease. Currently, there are three animal models of FCHL: the Hyplip1 mutant mouse (8, 37), the LDLR1/APOC3/CETP combined transgenic mouse (35), and the St. Thomas Hospital rabbit (14). However, these models have not been reported to share genetic abnormalities with human FCHL. The Prague hypertriglyceridemic (HTG) rat is a novel strain that develops features of human FCHL, such as hypertriglyceridemia, age-dependent changes of plasma total cholesterol, and overproduction of triglyceride-rich lipoproteins (31, 55, 56, 61, 62), as well as of human metabolic syndrome and hypertension. The use of rodent models for complex disorders has considerably advanced our understanding of polygenic diseases (32, 58). Until recently, the utility of the comparative genomics approach was limited by the lack of rodent and human dense, gene-based maps. However, with progress in human, mouse, and rat genome projects, dense maps now exist that allow the construction of...
comparative maps for these models and the human genome. The purpose of this study was to investigate the relationship of the genetic backgrounds of the HTG strain and human FCHL using comparative genomics.

METHODS

Animal procedure. HTG were originally derived from a colony of Wistar rats (61), and their characteristics have been described previously (55). This rat strain develops several major features of the metabolic syndrome, such as hypertension, hypertriglyceridemia, hyperinsulinemia, and impaired glucose tolerance (56, 62).

Lewis and HTG rats were reciprocally mated to produce F1 hybrids. Females and males of the F1 generation were randomly mated to provide F2 cohorts (31).

All the animals used in this study were housed under standard laboratory conditions (23 ± 1°C, 12-h light-dark cycle) and fed standard laboratory chow as well as tap water ad libitum. Under light ether anesthesia, polyethylene catheters were inserted into the left carotid artery and jugular vein and exteriorized in the interscapular region. Blood pressure was recorded in conscious animals after 24-h recovery. The procedures and experimental protocols were approved by the local animal Ethics Committee of the Institute of Physiology, Academy of Sciences of the Czech Republic. Body weight, plasma triglycerides, plasma cholesterol, and systolic and diastolic blood pressure traits of the progenitors and 266 (137 male, 129 female) F2 hybrids were measured at the age of 5–6 mo.

Genotyping and mapping. A two-step genome-wide scan was performed using markers based on simple sequence length polymorphisms (SSLP). The aim of the first step was to create a genetic map in the HTG × Lewis cross and to identify chromosomal regions of interest, while the second step comprised detailed mapping of these regions as used previously for blood pressure determinants (58). The first step was carried out as follows: 135 SSLP markers covering 21 chromosomes were selected to provide a genomic map with an intermarker average distance of ∼20 cM. This distance was expected to limit the number of false-negative results, as previous theoretical computations have suggested (3, 13). To increase the efficacy of the first map, an approach was adopted to select animals with extreme phenotypes; 46 F2 rats, representing extreme values for plasma triglycerides, total cholesterol, and mean arterial pressure, were chosen. This approach maximized genetic contrast and potential linkage data. Complete genome-wide study allowed us to build a genetic linkage map for our cross and provided preliminary mapping information with greater efficiency than scanning in all F2 rats (11, 12).

The second step was performed as follows: chromosomal regions of interest were identified on the basis of a logarithm of likelihood (LOD) >1.0. For these regions, the density of the markers was augmented to reach an intermarker distance of less than 10 cM. The number of genotyped animals was also increased to comprise all 266 F2 hybrids for 137 of 191 markers, including all markers on Chr 1, 2, 5, 8, 12, 13, and 16. Finally, 191 markers covered the genetic map length of 1,634.9 cM, and the averaged genetic distance between adjacent markers was 10.5 cM. The average distance in the region of interest was 6.1 cM.

SSLP marker information and mapping data were taken from the Whitehead Institute/Massachusetts Institute of Technology Rat Database (Cambridge, MA; http://www.ratmap.gen.su), Mouse Genome Informatics/Jackson Laboratory (Bar Harbor, ME; http://www.informatics.jax.org), the Wellcome Trust Centre for Human Genetics (Oxford, UK; http://www.well.ox.ac.uk), the Rat Genome Database (RGD)/Medical College of Wisconsin (Milwaukee, WI; http://www.rgd.mcw.edu), the National Center for Biotechnology Information (NCBI)/National Library of Medicine (Bethesda, MD; http://www.ncbi.nlm.nih.gov), and the Journal of Clinical Investigation (16).

Statistical analysis. The normality of all phenotypes was examined by application of the Kolmogorov-Smirnov test. Phenotypes that did not pass the normality test were then corrected by log transformation. The significance of differences within and between groups was determined by one-way analysis of variance (ANOVA), followed by Tukey multiple comparison tests. Construction of linkage maps and quantitative trait locus (QTL) mapping were achieved with the Map Manager QT program (Version 3.0b; Ref. 34). The significance of each potential association was measured by likelihood ratio statistics (LRS). Then, LRS were converted to conventional base-10 LOD scores by division with 4.61. Each of the traits reported here was evaluated as “model free.” A permutation test, randomly assigning phenotypes relative to genotypes in 10,000 replicated tests, was used to determine the threshold of significance.

RESULTS

Clinical phenotypes in parental and F2 populations. As summarized in Table 1, plasma triglyceride levels were significantly higher in HTG rats than in Lewis rats for both sexes. Plasma total cholesterol levels were lower in HTG than in Lewis rats but significantly lower only in females. Plasma triglycerides and total cholesterol levels were higher in HTG males than in HTG females, but these differences did not reach statistical significance. Plasma triglycerides and total cholesterol levels were significantly lower in Lewis males than in Lewis females. HTG rats showed significantly higher systolic blood pressure compared with Lewis rats for both sexes and diastolic blood pressure in males. However, in the F2 population, plasma triglycerides and blood pressure levels were not significantly different between males and females.

QTL on rat Chr 2. In the whole rat F2 population, the most significant QTL for contributing to plasma triglycerides with significant linkage by the permutation test was located on Chr 2 between D2Rat182 and D2Min8 (LOD 4.4) (Fig. 1A). At the nearest marker from this peak (D2Rat210), the homozygous HTG genotype (HH) was associated with significantly higher plasma triglycerides than the homozygous Lewis genotype (LL) and the heterozygous genotype (HL) (ANOVA P = 0.0002).}

| Table 1. Plasma lipids, blood pressures, and body weight in HTG and Lewis progenitors and F2 hybrids |

<table>
<thead>
<tr>
<th>HTG Male Rats (n = 10)</th>
<th>HTG Female Rats (n = 9)</th>
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</thead>
<tbody>
<tr>
<td>TG, mmol/l</td>
<td>2.69 ± 0.39*</td>
</tr>
<tr>
<td>TC, mmol/l</td>
<td>1.93 ± 0.11</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>156.4 ± 2.1*</td>
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<tr>
<td>DPP, mmHg</td>
<td>103.2 ± 1.2*†</td>
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<tr>
<td>BW, g</td>
<td>309.3 ± 15.9†</td>
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<th>Lewis Male Rats (n = 9)</th>
<th>Lewis Female Rats (n = 12)</th>
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<td>TG, mmol/l</td>
<td>0.81 ± 0.08†</td>
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<tr>
<td>TC, mmol/l</td>
<td>2.11 ± 0.13†</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>124.3 ± 3.9†</td>
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<tr>
<td>DPP, mmHg</td>
<td>80.3 ± 2.9†</td>
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<td>BW, g</td>
<td>300.2 ± 8.0†</td>
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<th>F2 Male Rats (n = 137)</th>
<th>F2 Female Rats (n = 129)</th>
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<td>TG, mmol/l</td>
<td>1.76 ± 0.61†</td>
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<tr>
<td>TC, mmol/l</td>
<td>2.04 ± 0.37</td>
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<tr>
<td>SBP, mmHg</td>
<td>133.8 ± 9.5</td>
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<td>DPP, mmHg</td>
<td>92.8 ± 9.7</td>
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<tr>
<td>BW, g</td>
<td>363.0 ± 39.2†</td>
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</table>

Data are means ± SE. *P < 0.05 compared with Lewis rats. †P < 0.05 compared with female rats. TG, triglycerides; TC, total cholesterol; SBP and DPP, systolic and diastolic blood pressure; BW, body weight.
0.0001, Tukey/Kramer P < 0.05) (Table 2). Another QTL for plasma triglycerides was located on Chr 2, between D2Rat55 and D2Rat111 (LOD 3.1) (Fig. 1A). At this locus, rats with the HH genotype also showed significantly higher plasma triglyceride levels than rats with LL and HL genotypes (ANOVA P = 0.0035, Tukey/Kramer P < 0.05) (Table 2). The region between D2Rat136 and D2Mgh9 presented suggestive linkage for body weight in the whole F2 population (LOD 3.2) (Fig. 1A). At D2Rat40, rats with HH and HL genotypes had significantly greater body weight than rats with the LL genotype (ANOVA P = 0.0013, Tukey/Kramer P < 0.05) (Table 2). All three loci on Chr 2 were sex-specific, and we could localize them in the whole F2 and female populations.

These two loci for plasma triglycerides on rat Chr 2 are novel loci and did not overlap with any previously reported rat loci for lipid phenotypes. No significant or suggestive loci were localized for plasma total cholesterol on this Chr. To examine the relationship between the located QTL of this novel strain of experimental rats and previously located loci for human metabolic syndrome, FCHL, and their related phenotypes, we constructed an interspecies map between rat, mouse, and human chromosomes based on our QTL. Figure 1B shows this interspecies map of identified loci for plasma triglycerides on rat Chr 2. In these regions, many genes were mapped on the RGD radiation hybrid (RH) map. They were also mapped on the Mouse Genome Database (MGD) RH map on mouse Chr 13 or 3 and on the NCBI human gene sequence map on Chr 5, 8, 3, 1, or 4. The region between D2Rat19 and D2Rat36 showed above significant or suggestive linkage with plasma triglycerides and body weight in the whole F2 and female populations. The syntenic region of this locus on the human Chr overlapped with previously reported loci for human metabolic syndrome (24) and for cholesterol levels of small, dense LDL on human Chr 3 (49). The human syntenic region of this locus overlapped with a previously reported human FCHL locus in the Dutch population (5).

**QTL on rat Chr 1.** Suggestive linkage for plasma triglycerides was localized only in the male population on Chr 1 (between D1Rat64 and D1Rat71: LOD 2.7). This locus also showed suggestive linkage with plasma total cholesterol only in the male population (LOD 2.5). Another locus for plasma total cholesterol was determined to have significant linkage in the male population between D1Rat137 and D1Rat35 (LOD 4.6). This locus showed suggestive linkage with plasma total cholesterol in the whole F2 population (LOD 3.8). The region be-

- **QTL on rat Chr 5 and 12.** For plasma total cholesterol, two significant QTL were localized on Chr 5 (between D5Rat147 and D5Rat49: LOD 5.6) and on Chr 12 (between D12Rat12 and D12Rat40: LOD 3.8) in the whole F2 population. At these loci, plasma total cholesterol levels in rats with the HH genotype were significantly lower than in those with HL or LL genotypes. These negatively contributing loci were also sex-specific, as the locus on Chr 5 was significant in the whole F2 and female populations while the locus on Chr 12 was relevant on the whole F2 and male populations (Table 2). Another locus on Chr 5 suggestively linked with plasma triglyceride levels, and rats with the HH genotype at this locus showed significantly higher plasma triglyceride levels than rats with LL or HL genotypes (ANOVA P = 0.0035, Tukey/Kramer P < 0.05) (Table 2). Another QTL for plasma triglycerides was located on Chr 5 (between D1Rat64 and D1Rat71: LOD 2.7). This locus also showed suggestive linkage with plasma total cholesterol only in the male population (LOD 2.5). Another locus for plasma total cholesterol was determined to have significant linkage in the male population between D1Rat137 and D1Rat35 (LOD 4.6). This locus showed suggestive linkage with plasma total cholesterol in the whole F2 population (LOD 3.8). The region between

- **QTL on rat Chr 1.** Suggestive linkage for plasma triglycerides was localized only in the male population on Chr 1 (between D1Rat64 and D1Rat71: LOD 2.7). This locus also showed suggestive linkage with plasma total cholesterol only in the male population (LOD 2.5). Another locus for plasma total cholesterol was determined to have significant linkage in the male population between D1Rat137 and D1Rat35 (LOD 4.6). This locus showed suggestive linkage with plasma total cholesterol in the whole F2 population (LOD 3.8). The region between...
RAT MODEL OF FAMILIAL COMBINED HYPERLIPIDEMIA

Table 2. Mean values of phenotypes in each genotype at the nearest marker from peak LOD. All F2 rats (n = 266)

<table>
<thead>
<tr>
<th></th>
<th>LOD</th>
<th>% Explained</th>
<th>HTG/HTG</th>
<th>HTG/Lew</th>
<th>Lew/Lew</th>
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<tr>
<td>D1Rat71</td>
<td>1.7</td>
<td>+2</td>
<td>1.89±0.08†</td>
<td>1.76±0.07</td>
<td>1.54±0.09</td>
<td>0.0367</td>
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<td>D2Rat61</td>
<td>3.1*</td>
<td>+5</td>
<td>2.03±0.09‡</td>
<td>1.69±0.06</td>
<td>1.61±0.10</td>
<td>0.0035</td>
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<td>D2Rat20</td>
<td>4.4*</td>
<td>+6</td>
<td>2.07±0.10‡</td>
<td>1.60±0.06</td>
<td>1.72±0.08</td>
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</tr>
<tr>
<td>D5Mco21</td>
<td>2.4†</td>
<td>+3</td>
<td>1.78±0.10‡</td>
<td>1.87±0.06†</td>
<td>1.57±0.09</td>
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<tr>
<td>D13Rat34</td>
<td>3.3*</td>
<td>+5</td>
<td>1.84±0.12‡</td>
<td>1.93±0.07†</td>
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<td>D16Mco7</td>
<td>2.6†</td>
<td>+4</td>
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<td>D8Rat59</td>
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<td>D12Rat20</td>
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<td>-5</td>
<td>2.10±0.08‡</td>
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<tr>
<td>D2Rat40</td>
<td>3.2†</td>
<td>+5</td>
<td>314.9±8.0†</td>
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<td>278.8±7.8</td>
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<tr>
<td>D1Rat71</td>
<td>2.7†</td>
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<td>1.53±0.10</td>
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<td>TC, mmol/l</td>
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<tr>
<td>D1Rat27</td>
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<td>+13</td>
<td>2.29±0.06§</td>
<td>1.96±0.04</td>
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<td>-6</td>
<td>1.89±0.07†</td>
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<td>-9</td>
<td>1.84±0.05§</td>
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<td>342.2±5.8§</td>
<td>370.6±4.8</td>
<td>364.7±6.2</td>
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<td>Female Rats (n = 129)</td>
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<tr>
<td>D2Rat61</td>
<td>2.8†</td>
<td>+7</td>
<td>2.11±0.14§</td>
<td>1.63±0.11</td>
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<td>2.33±0.20§</td>
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<td>1.69±0.13</td>
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<td>D5Mco21</td>
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<td>+7</td>
<td>1.77±0.16‡</td>
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<td>D2Mgb9</td>
<td>5.0*</td>
<td>+15</td>
<td>252.5±3.5§</td>
<td>235.1±2.6</td>
<td>232.4±2.7</td>
<td>&lt;0.0001</td>
</tr>
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</table>

Values of phenotypes are means ± SE. The significance of each potential association is measured by logarithm of likelihood (LOD). *Significant linkage and †suggestive linkage of the QTL for each phenotype by the permutation test. ‡P < 0.05, compared with the LL genotype and §P < 0.05, compared with the HL genotype by the Tukey multiple comparison test.

Kramer P < 0.05). This locus presented suggestive linkage in the whole F2 population and in females (Table 2).

All the cosegregation results tested by one-way ANOVA were confirmatory to the effects of genotypes on each phenotypic trait as obtained by Map Manager (Table 2).

DISCUSSION

Dense gene maps were established recently for the rat by the RGD and for the mouse by MGD. The outcome of the human genome project has enabled construction of the human genome map, currently available from the NCBI. These maps are applicable as translations to compare the results of quantitative genetics on rat chromosomes with previously established loci on human chromosomes. Recently, Stoll et al. (57) reported a strategy to apply data from rat quantitative trait genetics to the human genome, using a genomic-systems biology map for cardiovascular functions. Identification of the overlapping regions of loci for similar traits between humans and other species by comparative mapping should empower us to find animal models for human complex trait diseases as well as to narrow down target regions during steps of the positional cloning of candidate genes. Such is the case in the present study.

The metabolic syndrome, characterized by abdominal obesity, atherogenic dyslipidemia, heightened blood pressure, insulin resistance, and prothrombotic and proinflammatory states, has been recognized as an important target of risk-reduction therapy of coronary artery disease (CAD) (20). Human FCHL is also known to increase the risk for CAD, expression of diverse lipid abnormalities in the same family (19, 36, 52), and sometimes, to associate insulin resistance and hypertension (51). Because of these similarities with clinical features, at least a part of FCHL patients are diagnosed as having metabolic syndrome. The search for causal genes of the metabolic syndrome in human studies has been very difficult because of its complex, heterogeneous, and multifactorial nature resulting from the interplay of genetic and environmental factors (15, 54). Several strains of animals have been reported as models for human metabolic syndrome or FCHL, and several loci for metabolic phenotypes have been proposed (1, 2, 8, 14, 28).

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The HTG rat is a novel strain expressing hereditary hypertriglyceridemia and overproduction of lipoprotein as in human FCHL. It also associates hyperinsulinemia and hypertension, which are known to be highly prevalent in individuals with FCHL (31, 51, 61). In a previous study using a pharmacogenetic approach, we have determined genetic components of major blood pressure controlling systems in this strain (59). In this study, we successfully identified loci for plasma triglycerides, and the majority of them did not overlap with previously reported rat loci for plasma lipid with the exception of the suggestive locus on Chr 1, which overlapped with the previously reported region for plasma triglycerides in a diabetic rat strain (28). QTL for plasma triglycerides located on rat Chr 2 are novel and syntenic to previously reported human loci for the increased cholesterol concentration of small, dense LDL on Chr 3 and 4, one of the clinical characteristics of human FCHL (49). The human syntenic region for the other locus for plasma triglycerides on rat Chr 2 also overlapped with the previously reported locus for the metabolic syndrome phenotypes, such as body mass index, waist, hip, and plasma insulin (26) and the locus for small, dense LDL on human Chr 3 (49). In this region, Kissebah et al. (26) predicted solute carrier family member 2 (GLUT2) and phosphoinositide-3-kinase as candidate genes involved in glucose metabolism. Moreover, the phospholipase D1 gene can also be an attractive candidate gene involved in lipid metabolism. The second locus for plasma triglycerides on rat Chr 2 overlaps with the locus for small, dense LDL, including microsomal triglyceride transfer protein (MTP) and intestinal fatty acid binding protein (FABP2) genes as positional candidates. MTP is known to have an important role in lipoprotein production by the liver, and FABP2 is thought to have a significant function in lipoprotein production by the small intestine. The locus for plasma triglycerides localized on rat Chr 13 overlaps with the human locus for FCHL on Chr 1 reported in Finnish, German, Chinese, and American populations (10, 38, 39, 42). Pei et al. (42) predicted

**Fig. 2.** A: QTL plots for plasma triglycerides on rat Chr 13. The significance of each potential association is measured by LOD. *Significant linkage and †suggestive linkage of the QTL for each phenotype after the permutation test. B: comparative map between rat Chr 13, mouse Chr 1, and human Chr 1. The bar for rat QTL in the HTG × Lewis map shows a region with above suggestive level of LOD by the permutation test for each phenotype. Scale bar = 10 cM in the HTG × Lew map. See legend to Fig. 1 for the sources of the established maps. Rxrg, retinoid X receptor; γ; Selp, P selectin; Cd3z, CD3 antigen, ζ-polypeptide; Rgs4, regulator of G protein signaling 4; Ddr2, discoidin domain receptor family, member 2; Pigm, phosphatidylinositol glycan, class M; Fcer1a, Fc receptor, IgE, high-affinity I, α-polypeptide; Sele, E selectin; Sell, I selectin; Pou2f1, POU domain, class 2, transcription factor 1; Pbx1, pre-β cell leukemia transcription factor 1; Apoa2, apolipoprotein AI; Dfy (fy), Duffy blood group; Cpg, C reactive protein; NR1I3, nuclear receptor subfamily 1, group J, member 9.
the retinoid X receptor-γ gene as a possible candidate gene at this region. The suggestive locus for plasma triglycerides on rat Chr 1 overlaps with the human FCHL region in the Dutch population (5) containing the oxysterol-binding protein (OSBP) gene. OSBP is believed to transport sterols from lysosomes to the nucleus where LDL receptor and 3-hydroxy-3-methylglutaryl-CoA synthase downregulation occur. Our data indicate that the plasma triglyceride level of this rat strain was controlled for up to 30% of its variance in females by the same gene loci as lipids in human FCHL patients, and these loci include relevant candidate genes involved in lipid and insulin-glucose metabolism. Overlapping of loci for plasma triglycerides in hypertensive, hyperlipidemic, hyperinsulinemic animals with loci for plasma triglycerides or metabolic phenotypes in human subjects with hyperlipidemia or metabolic syndrome clearly suggests the importance of these loci.
for plasma lipid determination in metabolic abnormalities. Although several strains of animals share clinical characteristics with human metabolic syndrome or FCHL, and many loci have been localized as QTL for traits related to these diseases, concordance of the results of genetic studies in animals with those in humans has not been reported. We used here a novel strain of HTG rats as a first animal model providing evidence of sharing its clinical and genetic basis with human complex trait diseases.

As the HTG rat strain is hypertensive as well as hyperlipidemic, like human subjects with metabolic syndrome, loci affecting both blood pressure and plasma triglycerides were our targets of interest. We have determined loci linked with blood pressure phenotypes during pharmacological interventions in this F2 population, and significant linkage with blood pressure phenotypes has been observed at loci on Chr 1, 3, 5, and 8 (59). However, the located region for plasma triglycerides in this study did not overlap with these loci for blood pressure, except for one suggestive, male sex-specific locus for triglycerides on rat Chr 1. This observation was consistent with the results of Kovacs et al. (30) on the dissection of loci for plasma lipids and blood pressure in hypertensive hypertriglyceridemic Wistar-Ottawa-Karlsburg rats with the RT1bm haplotype. Although the association of hypertriglyceridemia and hypertension in the HTG rat cannot be explained by one or more causative genes affecting both phenotypes, it may be subject to gene-gene interaction or epigenetic factors that affect both hypertensive and dyslipidemic phenotypes.

QTL mapping showed obvious sex differences for all of the loci determined in this study while both sexes of rats had the same genomic DNA sequences for each allele at every QTL. Sex differences of QTL for factors of the metabolic syndrome have been reported by Kloting et al. (27), but these loci did not include the locus for plasma triglycerides. Although sexual specificity with the QTL effects can be explained by a sex chromosome action, hormonal interaction at the transcripional and posttranscriptional levels provides an alternative possibility that needs to be addressed in future studies. Moreover, changing of the hyperlipidemic phenotype is frequently observed in affected patients from FCHL families, and it is one of the clinical features of this disease. Kovacs et al. (29) reported age-dependent changes of the QTL effect for lipid loci on rat chromosomes. Their locus for triglycerides had maximal impact at 20 wk of age but disappeared at age 32 wk. As we phenotyped at almost the same rat age, we were evaluating phenotypes only at one point in life for each rat. The different sets of QTL in both sexes may be reflecting the effect of QTL at different time points of their life.

On Chr 5 and 12, we located significant negatively linked loci for plasma total cholesterol, and both loci also displayed sex differences. A significantly lower plasma total cholesterol level in the HTG progenitor strain can be explained by these loci in both sexes. In contrast to humans, the major cholesterol-containing lipoprotein in rodents is HDL rather than LDL and VLDL. The low plasma total cholesterol level induced by the HTG allele may reflect the decline of the amount of HDL particles as well as of β-lipoprotein particles, and low HDL cholesterol is known to be one of the common features of human FCHL. However, this observation is limited by the fact that in the rat, LDL and HDL particles are of similar size, and therefore separation is incomplete as we have verified by additional experiments (T. Ueno, unpublished observations). Sequential ultracentrifugation has been used to isolate lipoprotein fractions in “poled” rat serum (33), which makes it currently inaccessible in F2 hybrid studies where individual samples have to be analyzed in the entire set of rats.

This novel strain of rat is a relevant model of FCHL and metabolic syndrome, available for the assessment of the effect of agents affecting lipoprotein metabolism and insulin resistance. Chvojkova et al. (9) reported the plasma triglyceride-lowering effect of PPARγ activators in a strain of the same origin as the one used in this investigation, yet bred for several generations under different dietary and environmental conditions. Moreover, it could be interesting to perform a pharmacogenetical study by administration of PPARα, PPARγ, or other agents affecting lipid or glucose metabolism using F2 intercross from this strain, as demonstrated to be useful in other models of metabolic syndrome (O. Seda, L. Kazdova, D. Krenova, and V. Kren, unpublished observations).

In this study, we successfully showed by comparative mapping that the HTG rat is a model of FCHL. This is the first animal model that both clinically and genetically confirmed the sharing of characteristics with human complex trait disorders. Comparative genomics, using the interspecies map of rat, mouse, and human chromosomes, is a powerful tool to compare the results of genetic analysis of animal disease models and of human complex trait diseases. It also narrows down the target region derived from quantitative genetic study during the positional cloning of disease-causing genes. Concordance of loci for lipids in animal models and in human subjects by
comparative mapping unraveled the importance of these loci for the determination of plasma lipid levels in metabolic syndrome.

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