Resting metabolic rate and respiratory quotient: results from a genome-wide scan in the Quebec Family Study

Peter Jacobson, Tuomo Rankinen, Angelo Tremblay, Louis Pérusse, Yvon C Chagnon, and Claude Bouchard

ABSTRACT
Background: Genes influencing resting metabolic rate (RMR) and respiratory quotient (RQ) represent candidate genes for obesity, type 2 diabetes, and the metabolic syndrome because of the involvement of these traits in energy balance and substrate oxidation.

Objective: We conducted a genome-wide scan for quantitative trait loci (QTL) contributing to the variability in RMR and RQ.

Design: Regression-based and variance components–based genome-wide autosomal scans on RMR and RQ phenotypes, obtained from indirect calorimetry, were performed in 169 families ascertained via an obese proband or from the general population.

Results: We found evidence for linkage to RMR on chromosomes 3q26.1 (lod = 2.74), 1q21.2 (2.44), and 22q12.3 (1.33). QTL influencing RQ were found on chromosomes 12q13 (1.65) and 14q22 (1.83) when the analyses were performed in all families. Considerable locus heterogeneity within this population was suggested because most of the families were unrelated to any one quantitative trait locus. Significant associations between traits and linked microsatellites were detected within the linked, informative subsets.

Conclusions: We found several new QTL for energy metabolism, but the QTL on 1q may be a replication of the one reported in Pima Indians. All 3 RMR linkages overlapped regions previously linked to the metabolic syndrome or its components, and the significant association between RMR and the metabolic syndrome in the present cohort reinforces this relation. We conclude that considerable locus heterogeneity exists even within populations, which should be taken into account when considering candidate gene studies of energy metabolism phenotypes and other complex traits. Am J Clin Nutr 2006;84:1527–33.

KEY WORDS Resting metabolic rate, respiratory quotient, Quebec Family Study, linkage, locus heterogeneity, candidate genes

INTRODUCTION
Resting metabolic rate (RMR) constitutes ≈70% of total energy expenditure (EE) in sedentary persons. The factors that determine energy balance, at a given level of energy intake and physical activity, vary between persons and are to some extent determined by genes (1, 2). These factors may involve nutrient partitioning and the relative proportions of lipid and carbohydrate substrates that are oxidized to meet energy needs. The implications of the dominance of autonomic factors in EE are 2-fold. First, variation in this trait is mainly attributable to genes. Second, genetic influence will be stronger still, given a sedentary lifestyle.

The literature on metabolic rate phenotypes as predictors of weight gain is somewhat inconclusive. High respiratory quotient (RQ) values predicted body weight gain in some studies (3–5), but not in others (6). Ideally, a high RQ indicates that lipids are stored rather than metabolized. Alternatively, a high RQ could result from noncompliance toward fasting instructions. Likewise, low RMR predicted weight gain in some studies (7–10), but not in others (4, 6, 11).

Quantitative trait loci (QTL), or genes associated with energy metabolism, have been uncovered on chromosomal regions 1p31, 2q11, 7p21, 11q13, 11q23, 16q22, and 18q11 for EE and on 1p31-21, 11q13, 17q25, 18q22, 20q11, and 20q13 for RQ. These regions were reported from 2 genome-wide scans and several candidate gene studies from independent populations. With the exception of 11q13, which harbors the UCP2 and UCP3 genes, none of these results were replicated among the study populations (12). Here we report suggestive evidence for 4 novel QTL, 2 of which are linked to RMR and 2 to RQ. In addition, a QTL found linked to RMR may represent the second replicated region linked to EE.

SUBJECTS AND METHODS
Subjects
All subjects were of French descent and lived in the greater Quebec City area. The design of the Quebec Family Study was described previously (13). Recruitment took place in 2 phases through advertisements in the media. Phase 1 comprised families recruited from the general population (n = 96), whereas families included during phase 2 (n = 73) were ascertained through an

obese proband with a body mass index (BMI; in kg/m²) of ≥32. The sample included 426 siblings (372 sibpairs) from 169 families. The Institutional Review Board of Laval University, Quebec, Canada, approved the study, and all subjects gave informed consent.

Phenotype and covariate measurements

RMR and RQ were measured by indirect calorimetry in a ventilated-hood system. Measurements were made in the morning after an overnight fast while the subjects sat quietly in a semireclined position. RMR and RQ were calculated from respiratory exchange data obtained during the final 10 min of the 30-min data collection period. Gas samples were assayed with a zirconia cell oxygen analyzer (Amatek CD-3A; Thermox Instruments Division, Pittsburgh, PA) and an infrared carbon dioxide analyzer (Amatek S-3A). The instruments were calibrated before each sampling with the use of standard gases.

Percentage body fat was measured by hydrodensitometry, and total fat mass (FM; in kg) was derived from the equation of Siri as described previously (14, 15). Fat-free mass (FFM) was obtained by subtracting FM from body mass. Helium dilution techniques were used to estimate pulmonary residual volume (16). Body mass (in kg) was measured on calibrated scales to the nearest 0.1 kg. Body height was measured to the nearest centimeter.

Metabolic rates were adjusted for the effects of age, age², age³, stature, FM, and FFM, retained at α < 0.1. Parameters for computing standardized residuals were obtained by applying regression models on 4 sex-by-age groups with an age cutoff of 40 y and considering only the randomly ascertained persons with trait values within 3 SD of the mean.

Molecular and linkage analyses

Details on genomic DNA preparation, polymerase chain reaction conditions, and genotyping were described in detail elsewhere (17). Markers (n = 388) selected from different sources, but mainly from the Marshfield panel version 8a, were used. Genotypes for each marker were typed with the use of automatic DNA sequencers from LI-COR (Lincoln, NE) and the computer software SAGA (LI-COR). The genotypes were exported in a local dBase IV database (GENEMARK), and <10% was retyped completely due to Mendelian incompatibilities. Allele frequencies were derived from parents.

Tests for linkage were performed by using nonparametric sibpair linkage analyses implemented in the SIBPAL program of the S.A.G.E. package, version 4.6 (Internet: http://genepi.cwru.edu). The recommended W4 option in SIBPAL uses a modification of the Haseman-Elston (HE) linear regression, in which the weighted combination of the squared trait difference and squared mean-corrected trait sum is regressed on the estimated proportion of alleles shared identical by descent (IBD) at each marker (18). Linkage analyses were performed based on multipoint IBD information, as estimated by the GENIBD program in S.A.G.E. To confirm pointwise significance, linkages with nominal P < 0.01 were recalculated 10 000 times, simulating IBD sharing within sibships ≥2 and across sibships of 2 members.

We also used a complementary method for QT analysis based on variance components (VCs) and implemented in the computer package MERLIN (19). VC analysis partitions the variance into components attributable to an additive major gene, an additive polygenic effect, and nonshared environmental effects at genomic positions where multipoint IBD sharing has been estimated. To evaluate genome-wide significance, 500 data sets with identical genotype, phenotype, and pedigree structure were simulated and analyzed. Empirical genome-wide significance was given by the proportion of independent regions with lod scores exceeding the ones obtained from the real data. QTL were considered if the HE and the VC methods showed empirical P values <0.01 and lod scores >1.17, respectively.

Subsets of families contributing to linkages were identified by assessing the sibling trait covariances, conditional on the estimated proportion of alleles shared IBD (from GENIBD) at linkage maxima, ie, families in which most sibpairs fitted the HE regression were considered informative.

Family-based orthogonal tests for additive or dominant association between trait and linked markers were performed by using the variance-components based QTDT program (20). This test is robust in the presence of stratification in heterogeneous populations. Multiple comparison was minimized by using the multiallelic option, which tests marker alleles globally rather than one-by-one.

RESULTS

Subject characteristics are shown in Table 1. The major determinants of RMR were FFM and FM, which accounted for 23–61% of the trait variance (R²) across sex-by-age groups, with additional effects of stature and age terms yielding 28–67% for the full models. As expected, RQ was less dependent on body composition and body size, as indicated by R² = 0–11%. Estimates of maximal heritabilities, computed by the maximum-likelihood program SEGPATH, were found to be 47% for RMR and 36% for RQ.

Resting metabolic rate

We performed multipoint sibpair analyses using HE and VC methods. Suggestive evidence for linkage (1.18 ≤ lodHE ≤ 1.74/0.01 ≤ PHE ≤ 0.0023) to RMR by both methods was seen on 2 chromosomal regions: 3q26.1 and 22q12.3 (Figure 1A). The strongest QTL evidence was on chromosome 3q26.1, close to the marker D3S1763, with PHE = 0.002 and lodVC = 2.74, and with corresponding lod-1 support intervals (SI) SIHE = 168.2–190.2 centiMorgan (cM) and SIVC = 171.1–184.6 cM. This peak was

<table>
<thead>
<tr>
<th>Variable</th>
<th>Male sibpairs (n = 183)</th>
<th>Female sibpairs (n = 243)</th>
</tr>
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<tbody>
<tr>
<td>Age (y)</td>
<td>30.0 ± 12.0²</td>
<td>31.6 ± 12.6</td>
</tr>
<tr>
<td>Body height (cm)</td>
<td>174.4 ± 7.1</td>
<td>161.6 ± 6.4</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>79.6 ± 21.1</td>
<td>69.2 ± 20.8</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.1 ± 6.5</td>
<td>26.5 ± 7.8</td>
</tr>
<tr>
<td>FM (kg)</td>
<td>14.1 (12.6, 15.5)</td>
<td>19.1 (17.8, 20.4)</td>
</tr>
<tr>
<td>FFM (kg)</td>
<td>61.9 ± 9.9</td>
<td>46.4 ± 7.1</td>
</tr>
<tr>
<td>RMR (kJ/min)</td>
<td>1.23 ± 0.22</td>
<td>0.98 ± 0.17</td>
</tr>
<tr>
<td>RQ</td>
<td>0.79 ± 0.05</td>
<td>0.79 ± 0.05</td>
</tr>
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² FM, fat mass; FFM, fat-free mass; RMR, resting metabolic rate; RQ, respiratory quotient.

3 Geographic igit; 95% CI in parentheses (all such values).
further analyzed with regard to the number of families linked to the region. The purpose was to establish whether the peak emanated from a large number of families, each with a small linkage contribution, or whether there was a smaller subset of families more strongly linked to the region. This QTL was found to be wholly accounted for by a subset of 197 sibpairs from 75 families, with $P_{HE} = 10^{-10}$ (SI$_{HE}$ = 174.3–184.3 cM) and lod$_{VC} = 4.71$ (SI$_{VC}$ = 169.1–180.8 cM; Figure 1B). Note that the $P_{HE}$ and lod$_{VC}$ from the subgroup analysis were obtained post hoc and are shown solely for the purpose of indicating that the definition of linkage informativeness was successful.

Because most families were unlinked to this region, the genome scan was repeated after temporary removal of the subset linked to chromosome 3. This unveiled a broad peak on chromosome 1p21.1–q21.2, with minimal $P_{HE} = 0.0002$ and maximal lod$_{VC} = 2.44$. A subanalysis showed that this peak, close to D1S2222, was brought about by a rather small subset of 119 sibpairs from 56 families (lod$_{VC} = 5.65$, $P_{HE} = 10^{-5}$), with SI$_{HE}$ = 136.5–146.6 cM and SI$_{VC}$ = 129.1–153.3 cM (Figure 1C). The region on chromosome 22q12 (lod$_{VC} = 1.33$, $P_{HE} = 0.0023$) peaked at marker D22S1685 and originated from 56 families (lod$_{VC} = 6.11$, $P_{HE} = 1.60 \times 10^{-5}$, SI$_{VC}$ = 31.2–34.9 cM, SI$_{HE}$ = 30.0–36.1 cM).

One quarter of the families were not linked to any of the regions on chromosome 1, 3, or 22, and they did not exhibit significant linkage to any other chromosomal region when analyzed separately. The proportions of families linked to 1, 2, or all 3 QTL were 45%, 25%, and 5%, respectively.

Tests for associations between RMR and linked microsatellites were positive for marker D3S2427 ($F = 3.82$, $P = 0.0024$) among the families linked to chromosome 3 region, but not across the whole sample or the subset of unlinked families. This pattern was observed also in chromosomes 1 and 22, where only linked subsamples showed evidence for association at marker D1S2222 ($F = 3.52$, $P = 0.016$) and marginally at marker D22S685 ($F = 1.81$, $P = 0.11$), respectively.

**FIGURE 1.** A: Linkage results from the Haseman-Elston (HE) linear regression (upper section) and variance components (VC; lower section) for resting metabolic rate (RMR) for all 22 autosomes. B: RMR quantitative trait locus (QTL) at chromosome 3. The gray line represents the total sample, the solid black line represents the informative subset, and the broken black line represents the results after exclusion of the informative subset. C: RMR QTL at chromosome 1. The gray line represents the total sample after exclusion of the families linked to chromosome 3, the solid black line represents the informative subset, and the broken black line represents the results after exclusion of the informative subset. Note that the linkage results from the subgroup analyses were obtained post hoc and are shown solely to indicate that the definition of linkage informativeness was successful. cM, centiMorgan.
Respiratory quotient

In the genome scan for RQ, chromosomes 12q13 and 14q22 showed evidence for linkage by both QTL detection methods, (ie, \( P_{HE} < 0.01 \) and \( \text{lod}_{VC} > 1.17 \)) (Figure 2). At chromosome 12, the peak was at marker D12S1712; \( \text{lod}_{VC} = 1.65, P_{HE} = 0.005 \). Ninety-eight sibpairs from 51 families were responsible for this peak (\( \text{lod}_{VC} = 4.99, P_{HE} = 2.2 \times 10^{-6}, \text{Sl}_{VC} = 54.1–59.3 \text{ cM}, \text{Sl}_{HE} = 54.1–63.5 \text{ cM} \)). Again, only the informative subgroup showed significant association between linked marker D12S1712 and RQ (\( F = 3.24, P = 0.023 \)). This region harbors the gene encoding muscular phosphofructokinase (PFK). A study of identical twins showed a positive association between baseline PFK concentration and an increase in metabolic rates in response to overfeeding (19). In the present study, a multiallelic marker at the muscular PFK locus was unassociated with RQ.

The linkage at 14q22.2 (\( \text{lod}_{VC} = 1.83, P_{HE} = 0.0043 \)) was close to D14S587 and was carried by 72 sibpairs from 44 families (\( \text{lod}_{VC} = 11.35, P_{HE} = 1.60 \times 10^{-6}, \text{Sl}_{VC} = 46.0–51.0 \text{ cM}, \text{Sl}_{HE} = 44.3–52.8 \text{ cM} \)). No associations with markers in this region were found in the subset.

Consistent with a previous candidate gene study of the present cohort (21), markers near the Na,K-ATPase \( \alpha 2 \) gene on chromosome 1 were linked to RQ (\( \text{lod}_{VC} = 1.38, P_{HE} = 0.0006 \)). However, this was true only for unadjusted RQ (data not shown).

Forty-four percent of the families were linked to either QTL, and 9% were linked to both RQ QTL. Exclusion of either or both informative subgroups did not uncover linkages elsewhere, even though 47% of the families were unlinked to 12q13 and 14q22.

DISCUSSION

This study represents the first genome-wide scan of metabolic rates in the Quebec Family Study cohort, and the third ever published, after 2 scans performed in Pima Indians (22) and Nigerians (23). We found evidence for 5 QTL influencing the variabilities in either RMR or RQ, and the trait effect of each QTL appeared to be confined to certain subsets of families.

The RMR linkage on 1q21 is amid 2 regions that showed marginal linkages to sleeping metabolic rate (1p21.1) and 24-h EE (1q23.2-1q24.2) in the genome scan performed in Pima Indians (22) and may represent the second replicated QTL for metabolic rates, beside 11q13. Moreover, in view of recent findings linking energy metabolism to glucose homeostasis (24, 25), the linkage on 1q21 is particularly interesting. This gene-dense region (1q21-23) has been linked to type 2 diabetes in several populations (26–33), to BMI (34), to lipid metabolism (35–39), and to other traits related to the metabolic syndrome concept (40–42).

Likewise, the region on 3q26-27 has been linked to type 2 diabetes (30, 43, 44), lipoprotein phenotypes (45), the metabolic syndrome (46), and coronary heart disease (47), whereas the 22q11-13 region has been linked to components of the metabolic syndrome in several populations (41, 44, 48–52).

Given these QTL colocalizations, the relation between RMR and the metabolic syndrome was investigated in the present cohort. A \( t \) test showed that the mean adjusted RMR in 74 siblings who fulfilled the National Cholesterol Education Program Adult Treatment Panel III definition of the metabolic syndrome (53) was 10% higher (\( P = 0.007 \)) than in metabolically healthier siblings, and this finding was confirmed in the parental generation. Thus, RMR may be linked to metabolic aberrations via unifying mechanisms such as genetic pleiotropy or afferent signals from the autonomic nervous system. No relation between adjusted RQ and the metabolic syndrome was found in the present cohort.

When considering possible candidate genes, we focused on those directly related to energy metabolism. The 1q21 region includes the interleukin 6 receptor (IL6R). A knockout of its natural ligand, IL-6, was found to cause obesity in mice, and central administration of IL-6 partially reversed obesity and increased EE (54). Furthermore, variants of \( IL6R \) were associated with human obesity and type 2 diabetes (55–57). Two genes whose products are involved in oxidative phosphorylation are located at 1q23: 1) NADH dehydrogenase (ubiquinone) Fe-S protein 2 (\( NDUF2 \)), a subunit of human complex I, and 2) succinate dehydrogenase subunit C (\( SDHC \)) of complex II.

The region on chromosome 3 that showed evidence for linkage with RMR harbors several candidates. Glucose transporter, type...
2 (GLUT2) facilitates transport of glucosamine in addition to glucose (58). In rats, exogenous glucosamine down-regulates several nuclear-encoded mitochondrial genes involved in oxidative phosphorylation and fatty acid oxidation (59). Ghrelin—the growth hormone secretagogue—was shown to be linked to energy metabolism (60). The gene encoding its receptor, GHSR, maps to 3q26. A candidate located at 3q27 is the adiponectin gene. Serum adiponectin concentration was associated with EE in 2 studies (61, 62). Similar to the RMR linkage peak at chromosome 1, the 3q26 region harbors a gene involved in electron transport within complex I, NADH dehydrogenase (ubiquinone) 1 beta subcomplex 5 (NDUFB5). No obvious candidate genes with a known effect on energy metabolism were identified near the QTL on chromosomes 22, 12, and 14.

The HE and VC methods have limitations and strengths that make them mutually complimentary, VC, which is more computationally demanding, has greater statistical power than HE, but it is more sensitive for violations of the assumption of multivariate normality. This may be of consequence also for quantitative traits, which are normally distributed in the general population, because ascertainment schemes often entail selective sampling, e.g., for extreme trait values.

Similar to most previous studies (12), none of the described linkages met the proposed requirements for genome-wide significance (63), except when subsets of informative families were considered. Simulated thresholds corresponding to one false positive QTL per 20 genome scans were lod = 2.98 and lod = 3.07 for RQ and RMR, respectively. The range of lod scores reported here (1.33–2.74) equaled 2 false positives per scan to one false positive per 9 scans. It is thus possible that one or more of the proposed QTL represents a false positive. However, the presence of marker-trait association within subgroups informative for linkage suggests that several QTL were true positives. Linkage and association analyses yield complementary information because they are conceptually different. The study of genetic linkage focuses on the transmission of chromosomal segments within pedigrees, and one utilizes the marker information solely to estimate IBD sharing among relatives. The marker alleles do not need to have an historical relation (ie, to form a haplotype) with a functional mutation to be useful in linkage analysis. Association analysis, by contrast, focuses on the relation between particular marker alleles and a trait, typically among seemingly unrelated persons. Association depends on linkage disequilibrium, ie, the marker polymorphism was present on the ancestral chromosome where the functional mutation originally occurred. A close interlocus proximity has made the haplotype withstand the stochastic forces of recombination such that the haplotype prevails in an appreciable proportion of the population despite numerous historical meioses. In this study, we used multiallelic microsatellites to test for association. It should be noted that microsatellites may be less suitable than single-nucleotide polymorphisms for association analyses because of the greater likelihood of multiple founders for a particular microsatellite allele. The evolution of microsatellite variants entails expansion of repetitive sequence, which is far more likely to yield identical alleles in multiple founders, compared with multiple occurrences of a point mutation at a particular base position resulting in the same base substitution.

Although this study included the largest number of families of the 3 genome scans undertaken to date, it is still relatively small compared with many studies of more easily obtained phenotypes. Speaking against insufficient statistical power due to study size alone are previous QTL scans performed on this cohort in which stronger linkages were detected for other complex phenotypes, including eating behavior (64), lipid and lipoprotein concentrations (65), physical activity level (66), LDL peak particle diameter (67), abdominal fat (68), and blood pressure (50). However, it is not unlikely that the study size together with the apparent locus heterogeneity caused attenuation of statistical power, which might explain the relative modesty of the linkage results. Moreover, if epistasis (gene-by-gene interactions) constitutes a substantial component of the trait variance, it will increase the risk of type II error.

Many genomic regions have shown association with energy metabolism, but only 2 have been replicated in distinct populations. This finding indicates genetic heterogeneity across populations, which is rather the norm for complex traits, such as metabolic rates. Indeed, results from the subgroup analyses used in the present study imply that considerable locus heterogeneity also exists within a population, which should be taken into account when candidate gene studies are conducted.

The paucity of genome scans for energy metabolism phenotypes may reflect the inconsistencies among previous attempts to link metabolic rates to obesity. However, the emerging picture, where energy metabolism appears closely related to other components of the metabolic syndrome, provides more incentive to undertake genome screens in other populations. Whereas replication is much needed, it will be difficult because energy metabolism phenotypes are not easily obtained in large numbers of persons.

AT, LP, YCC, and CB were involved in the study design. PJ reviewed the relevant literature, performed the statistical analyses, interpreted the results, and drafted the manuscript. TR, AT, LP, YCC, and CB collected the data. All authors reviewed the manuscript. The authors declared no conflicts of interest.

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