Original Article

Bardet-Biedl Syndrome Gene Variants Are Associated With Both Childhood and Adult Common Obesity in French Caucasians

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Bardet-Biedl syndrome (BBS) is a rare developmental disorder with the cardinal features of abdominal obesity, retinopathy, polydactyly, cognitive impairment, renal and cardiac anomalies, hypertension, and diabetes. BBS is genetically heterogeneous, with nine genes identified to date and evidence for additional loci. In this study, we performed mutation analysis of the coding and conserved regions of *BBS1*, *BBS2*, *BBS4*, and *BBS6* in 48 French Caucasian individuals. Among the 36 variants identified, 12 were selected and genotyped in 1,943 French-Caucasian case subjects and 1,299 French-Caucasian nonobese nondiabetic control subjects. Variants in *BBS2*, *BBS4*, and *BBS6* showed evidence of association with common obesity in an age-dependent manner, the *BBS2* single nucleotide polymorphism (SNP) being associated with common adult obesity ($P = 0.0005$) and the *BBS4* and *BBS6* SNPs being associated with common early-onset childhood obesity ($P = 0.0003$) and common adult morbid obesity ($0.0003 < P < 0.007$). The association of the *BBS4* rs7178130 variant was found to be supported by transmission disequilibrium testing ($P = 0.006$). The *BBS6* variants also showed nominal evidence of association with quantitative components of the metabolic syndrome (e.g., dyslipidemia, hyperglycemia), a complication previously described in BBS patients. In summary, our preliminary data suggest that variations at *BBS* genes are associated with risk of common obesity.
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BBS, Bardet-Biedl syndrome; MAF, minor allele frequency; SNP, single nucleotide polymorphism; TDT, transmission disequilibrium test.

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Common obesity is caused by the interaction of many genes and the environment, with each gene variant producing only a moderate effect. Despite some success using positional cloning strategies (1–3), identifying new susceptibility genes involved in common obesity remains a difficult task. Bardet-Biedl syndrome (BBS; OMIM 209900) is a rare developmental disorder that segregates in families as both a classical autosomal recessive and a digenic trait. BBS exhibits significant clinical heterogeneity of the six main traits that characterize it (4,5), which are progressive (obesity, retinal dystrophy, and learning disabilities) or structural (polydactyly, renal and cardiac malformations, and hypogenitalism) (6,7). The genetic heterogeneity is clear from the identification of pathogenic mutations in nine *BBS* genes, with *BBS1–6* (8–14) and *BBS9* (15) identified through genetic linkage studies and/or comparative genomic analysis and *BBS7* (16) and *BBS8* (17) identified based on their homology to previously identified *BBS* genes. Although the cellular mechanisms that underlie BBS remain unclear, it is now evident that all of the known BBS proteins are components of the centrosome and/or basal body and have an impact on ciliary transport (18). Interestingly, other established centrosomal disorders, such as Alstrom syndrome (19), or suspected centrosomal disorders, such as Cohen syndrome, share many phenotypic aspects of BBS and obesity in particular, suggesting a role for these proteins and organelles in the pathogenesis of obesity.

Investigation of *BBS* gene variants could potentially be useful in determining whether they also contribute to common obesity because early-onset obesity is a typical symptom of this syndrome. It has been shown that obligate carriers of *BBS* heterozygous mutations are more obese than noncarriers, without displaying the developmental pleiotropic features characteristic of the syndrome (20). Based on this hypothesis, one group had previously investigated the contribution of *BBS6* to polygenic obesity in a Danish population, but they failed to identify a role of this gene with common obesity (21).

Murine models have recently brought new insights into the origin of obesity in BBS patients. Recent studies have demonstrated that *Bbs2*$^{-/-}$ (22), *Bbs4*$^{-/-}$ (23), and *Bbs6*$^{-/-}$ (15) mice have features of the human disorder and develop obesity associated with increased food consumption. Elevated leptin levels have been observed in *Bbs6*$^{-/-}$ mice before and after the onset of obesity, suggesting that the

obesity phenotype may be dependent on the leptin signaling pathway (15).

In this study, we tested the hypothesis that polymorphisms in *BBS1*, *BBS2*, *BBS4*, and *BBS6* are involved in common obesity. To identify a possible role of BBS gene variants in polygenic obesity, we screened the four genes in 48 French Caucasians (24 obese case subjects and 24 nonobese nondiabetic control subjects). The common frequent single nucleotide polymorphisms (SNPs), i.e., minor allele frequency (MAF) >5%, were genotyped in obese French Caucasians consisting of 627 obese adults, 694 morbidly obese adults, and 622 obese children. For case-control analysis, the allele, genotype, and haplotype frequencies were compared with the frequencies in 1,299 French-Caucasian nonobese nondiabetic control subjects.

RESEARCH DESIGN AND METHODS

Subjects were all French Caucasian and were recruited using a multimedia campaign run by the Centre National de la Recherche Scientifique (CNRS), the Department of Nutrition of the Paris Hôtel-Dieu Hospital, and the Institut Pasteur de Lille and in the Department of Pediatric Endocrinology of Jeanne de Flandres Hospital, as well as in the Toulouse Children's Hospital. For this study, a cohort of 627 unrelated class 1 and 2 obese adults ($40 > BMI > 30$ kg/m^2), 694 unrelated class 3 obese adults (BMI >40 kg/m^2), and 615 unrelated obese children (age <18 years with a BMI >97th percentile for age and sex) were recruited, giving a total of 1,936 case subjects (online appendix Table 1 [available at http://diabetes.diabetesjournals.org]). All subjects had been previously screened for MC4R mutations. Additionally, a total of 1,299 nonobese nondiabetic adult control subjects were recruited for genotyping for the association study. The control subjects were unrelated adult nonobese nondiabetic French Caucasians pooled from three separate studies: a set of 266 individuals (mean BMI 23.09 ± 2.16 kg/m^2, mean age 42.52 ± 4.48 years, 105 men, 161 women) and a set of 297 control subjects (BMI 22.93 ± 2.30 kg/m^2, age 60.70 ± 11.51 years, 123 men, 174 women) were recruited at the CNRS Lille and through the Fleurbaix-Laventie Ville Santé study (24). The third set was 736 individuals (BMI 3.79 ± 1.80 kg/m^2, age 53.47 ± 5.65 years, 293 men, 443 women) selected from the Data from an Epidemiologic Study on the Insulin Resistance Syndrome (DESIR) study. For the transmission disequilibrium test (TDT), we analyzed 638 French trios (two parents and one obese child) for childhood obesity (affected children: mean BMI 4.58 ± 0.18 kg/m^2, mean age 11.28 ± 3.16 years), 435 French trios with adult obesity (BMI 39.25 ± 7.97 kg/m^2, age 46.65 ± 14.94 years), and 428 French trios with unaffected children (BMI 21.33 ± 6.64 kg/m^2, age 28.84 ± 10.18 years). The genetic study was approved by the ethical committee of Hotel-Dieu in Paris and CHRU (Centre Hospitalier Régional Universitaire) in Lille.

Phenotypes. Weight was measured in a nonpostprandial state and with an empty bladder, and it was determined to the nearest 0.1 kg on a standard physician's beam scale with the subject dressed only in light underwear and without shoes. Height was measured to the nearest 0.5 cm on a standard height board, again without shoes. BMI was calculated as weight in kilograms divided by the square of the height in meters. The *z* score of BMI was obtained according to Cole's method (25).

DNA isolation. Genomic DNA was extracted from peripheral blood cells using a Pure-Gene D50K DNA isolation kit (Gentra Systems) according to the manufacturer's instructions.

Sequencing. For the sequencing of *BBS1*, *BBS2*, *BBS4*, and *BBS6*, a random subset of 24 unrelated French obese adults from the morbid adult obesity set described above and a subset of 24 unrelated French nonobese normoglycemic control subjects were utilized. A total of 92 overlapping PCR fragments were designed to cover the *BBS1*, *BBS2*, *BBS4*, and *BBS6* genes, including the exons, a plausible promoter region 1 kb upstream from the start of exon 1, and a region 1 kb downstream from the end of the last exon. The fragments were sequenced in a forward and reverse direction. Primers were designed for these fragments, using Primer3 (www-genome.wi.mit.edu/genome_software/other/primer3.html). The genes were sequenced using an automated ABI Prism 3700 DNA sequencer in combination with a Big Dye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Foster City, CA). SNPs found in one primer direction were confirmed by viewing the opposite strand. SNP allele frequencies were calculated from this sequencing data.

Genotyping. Among the 36 variants identified through the sequencing analysis, 12 SNPs were selected based on the SNP tagging approach described below and genotyped in the case-control groups and in the obesity pedigrees, using TaqMan or SNPlex (Applied Biosystems) assays. We genotyped 104 obese case subjects and 88 nonobese nondiabetic control subjects (6%), using both the TaqMan and the SNPlex assays, to assess the concordance between the different genotyping methods, and we found no discrepancies.

Genotyping for markers was considered successful if >85% of the genotypes could be called. No genotyping was discarded because the expected allele frequencies significantly diverged from Hardy-Weinberg equilibrium ($P < 0.05$). For the trios, there were no Mendelian errors.

Statistical analysis. We compared allele frequencies between case and control subjects using the χ^2 test and computed the *P* value empirically with the program CLUMP. Genotype frequencies were then analyzed using dominant and recessive models. The dominant model compared the combined group of the heterozygotes and homozygotes for the rare allele with the homozygotes for the common allele, and the recessive model compared the homozygote rare allele group versus the rest. To confirm that there was no difference between the three groups that comprised the control subjects, allele and genotype frequencies for each group were also compared by the χ^2 test (Table 1).

We determined haplotype frequencies and compared them between groups, using UNPHASED software. We evaluated the effect of haplotypes on qualitative or quantitative trait variation, using the subprograms cocaphase and qt-phase of UNPHASED. We tested independence of association with the software THESIAS (26). THESIAS also implements an expectation-maximization algorithm and allows for likelihood testing of models of haplotype effect in a linear framework. We used this program to test whether the effect of each SNP on obesity status was independent from the effect of the significantly associated variants alone.

Transmissions of alleles were analyzed using the Tdtphase subprogram of UNPHASED, which assesses allele transmission rates in trios and tests for deviation from the expected 50% transmission. The power for the TDT analysis of the four associated variants has been estimated using the program Quanto (27) (Table 2). A sex effect on the four associated SNPs was studied and not found in the families.

Linkage disequilibrium was estimated using the expectation-maximization algorithm as implemented in GOLD (28). "Haplotype-tagging" SNPs were selected using the D′ and the Δ^2. When two SNPs had a $D' > 0.9$ and a $\Delta^2 > 0.7$, only one SNP was retained and identified as a haplotype-tagging SNP. With a minimum r^2 value of 0.7, the 12 genotyped SNPs were found to capture the tag SNPs identified, using Tagger with the HapMap Phase II database.

Statistical analyses of SNPs (corrections and comparisons of means) were performed using SPSS software (version 12; SPSS, Chicago, IL). Quantitative trait analyses for *z* score of BMI, rebound, obesity onset, and lipid- and lipoprotein-related traits were performed using Student's *t* test, unless the sample size in one group fell below 30 individuals, in which case a Mann-Whitney *U* test was used. A univariate general linear model taking into account sex and gestational age was performed for birth weight and ponderal index phenotype analyses. A univariate general linear model taking into account sex, age, puberty stage, and BMI was performed for insulin/glucose parameters.

Potential interactions between *BBS* variants were addressed in the entire population, using logistic regression for the obesity status, taking into account sex. Linear regression analyzed the interaction of quantitative traits between *BBS* variants. First, we compared the slope between two genotyped groups for each SNP of *BBS1*, *BBS2*, *BBS4*, and *BBS6* by predicting a selected phenotype for each variant. Second, we looked at the prediction improvement of the regression model with and without the interaction parameter.

Given that the genotyped SNPs within each gene are in strong linkage disequilibrium with each other and that each SNP test is not completely independent, the uncorrected *P* values are presented in Table 1 and the main text. Similarly, in view of the strong correlation that exists between the different quantitative traits (e.g., between the *z* score of BMI and the *z* score of height or the apolipoprotein B, fasting triglyceride, and HDL-to-total cholesterol ratio), the uncorrected *P* values are presented in Table 3 and the main text. In consideration of the number of statistical tests carried out, a simple conservative Bonferroni correction was applied, and the remaining significant corrected *P* values ($P < 0.05$ after correction) were identified within the tables. In Table 3, no *P* values survived the conservative Bonferroni correction.

RESULTS

Identifying SNPs for genotyping. The sequencing of *BBS1*, *BBS2*, *BBS4*, and *BBS6* genes in obese and lean individuals ($n = 48$) identified 36 SNPs. The locations of these SNPs in the genes are summarized in Fig. 1, and their description is in online appendix Table 2.

TABLE 1
Genotypic and allelic distribution of the *BBS* SNPs associated with childhood and/or adult obesity

Cohorts	Genotype number (frequency) 1	2	3	Allelic *P*	Odds ratio (95% CI)
BBS2_rs4784675	GG	GC	CC		
Control subjects	971 (0.76)	277 (0.22)	23 (0.02)	—	—
Class 1 and 2 adult obese	428 (0.69)	173 (0.28)	18 (0.03)	0.0005*	1.39 (1.15–1.69)
Class 3 adult obese	483 (0.71)	176 (0.26)	20 (0.03)	0.006	1.30 (1.08–1.57)
Obese children	438 (0.72)	156 (0.26)	11 (0.02)	0.09	1.18 (0.97–1.44)
BBS4_rs7178130	GG	GA	AA		
Control subjects	488 (0.40)	552 (0.45)	178 (0.15)	—	—
Class 1 and 2 adult obese	266 (0.44)	277 (0.45)	68 (0.11)	0.039	0.86 (0.74–0.99)
Class 3 adult obese	292 (0.46)	262 (0.41)	80 (0.13)	0.01	0.84 (0.73–0.97)
Obese children	259 (0.46)	256 (0.46)	46 (0.08)	0.0003*	0.76 (0.65–0.88)
BBS6_rs6108572	AA	AT	TT		
Control subjects	458 (0.37)	578 (0.46)	217 (0.17)	—	—
Class 1 and 2 adult obese	221 (0.36)	287 (0.46)	110 (0.18)	0.71	1.03 (0.89–1.18)
Class 3 adult obese	225 (0.33)	325 (0.48)	130 (0.19)	0.11	1.11 (0.97–1.27)
Obese children	190 (0.32)	278 (0.46)	131 (0.22)	0.007	1.21 (1.05–1.39)
BBS6_rs221667	CC	CG	GG		
Control subjects	806 (0.63)	415 (0.33)	50 (0.04)	—	—
Class 1 and 2 adult obese	374 (0.61)	211 (0.34)	28 (0.05)	0.28	1.10 (0.93–1.29)
Class 3 adult obese	394 (0.58)	250 (0.37)	37 (0.05)	0.01	1.23 (1.05–1.44)
Obese children	337 (0.57)	216 (0.36)	42 (0.07)	0.0007*	1.33 (1.13–1.56)

*Significant *P* values after simple Bonferroni correction for 36 tests (3 populations × 12 SNPs).

BBS1. Two SNPs were identified in the upstream sequence and five in intron/exon junctions, two were synonymous mutations, and three were identified in the 3′ untranslated region sequence. Among these 12 polymorphisms, 11 had an MAF >5%. Linkage disequilibrium analysis (D′) of the common (MAF >5%) SNPs revealed that all of the identified SNPs were in strong linkage disequilibrium (D′ >0.8) (online appendix Figs. 1 and 2). Based on the Δ^2 value, two haplotype-tagging SNPs (rs2298806 and rs1791686) were selected and typed in the whole set of samples.

BBS2. Three SNPs were identified in the upstream sequence and five in intron/exon junctions, two were missense mutations, and one was identified in the downstream sequence. Among these 11 polymorphisms, 10 had an MAF >5%. A set of five haplotype-tagging SNPs were selected and typed in the whole set of samples.

BBS4. One SNP was identified in the upstream sequence and two in intron/exon junctions, one was a missense mutation, and one was identified in the downstream sequence. Among these five polymorphisms, all had an MAF >5%. A set of two haplotype-tagging SNPs (rs730180 and rs7167076) were selected and typed in the whole set of samples.

BBS6. One SNP was identified in the upstream sequence and one in intron/exon junctions, two were missense mutations, two were synonymous mutations, and two were identified in the downstream sequence. Among these eight polymorphisms, all had an MAF >5%. A set of three haplotype-tagging SNPs were selected and typed in the whole set of samples.

TABLE 2
Familial association of obesity in 638 obesity trios (two parents and one affected child) and 435 adult obesity trios

SNPs and alleles	Obese children pedigrees: Transmitted alleles (*n*)	Untransmitted alleles (*n*)	Transmitted alleles (%)	*P*	% Power	Obese adult pedigrees: Transmitted alleles (*n*)	Untransmitted alleles (*n*)	Transmitted alleles (%)	*P*	% Power
rs4784675	—	—	—	0.5	36	—	—	—	0.4	77
G	942	957	49.6	—	—	343	351	49.4	—	—
C	143	130	52.5	—	—	58	51	53.4	—	—
rs7178130	—	—	—	0.006*	90				0.11	42
G	704	641	52.4	—	—	294	278	51.4	—	—
A	330	409	45.1	—	—	100	122	45.4	—	—
rs6108572	—	—	—	0.6	67				0.5	20
A	577	589	49.5	—	—	218	226	49.1	—	—
T	471	459	50.6	—	—	146	138	51.4	—	—
rs221667	—	—	—	0.19	74				0.5	45
C	820	842	49.3	—	—	334	341	49.5	—	—
G	255	225	53.4	—	—	74	67	52.5	—	—

*Significant *P* values after simple Bonferroni correction for eight tests (two populations × four SNPs).

TABLE 3
Quantitative trait studies of the rs4784675, rs7178130, rs6108572, and variant rs221667 in 622 French-Caucasian obese children

	z score of BMI	Rebound mean age	*z* score of height	Fasting insulin	Insulin at $t = 120$	Fasting glucose	Glucose at $t = 120$	Apolipoprotein B	Fasting triglyceride	HDL-to-total cholesterol ratio
BBS2 rs4784675	0.91	0.05	0.87	0.45	0.56	0.68	0.39	0.02	0.9	0.5
GG/GC	4.08 ± 1.2	2.38 ± 1.5	1.93 ± 1.4	13.85 ± 10.2	46.6 ± 40.1	4.91 ± 0.4	5.55 ± 0.9	0.81 ± 0.2	1.02 ± 0.5	0.29 ± 0.1
CC	4.12 ± 1.1	1.28 ± 1.5	1.87 ± 0.9	12.6 ± 6.7	41.2 ± 41.1	4.85 ± 0.5	5.27 ± 0.9	0.92 ± 0.2	1.01 ± 0.7	0.27 ± 0.1
BBS4 rs7178130	0.96	0.55	0.23	0.93	0.42	0.15	0.77	0.97	0.23	0.89
GG/GA	4.07 ± 1.2	2.34 ± 1.5	1.96 ± 1.4	13.74 ± 10.9	48.1 ± 40.1	4.9 ± 0.4	5.54 ± 0.9	0.82 ± 0.2	1.04 ± 0.5	0.29 ± 0.1
AA	4.08 ± 1.6	2.53 ± 1.5	1.71 ± 1.4	13.6 ± 8.7	45.8 ± 39.1	5.01 ± 0.4	5.6 ± 1.0	0.82 ± 0.2	0.94 ± 0.4	0.29 ± 0.01
BBS6 rs6108572	0.1	0.73	0.21	0.08	0.72	0.1	0.9	0.03	0.02	0.16
AA/AT	4.12 ± 1.2	2.35 ± 1.5	1.96 ± 1.4	14.07 ± 11.6	46.8 ± 40.6	4.93 ± 0.4	5.55 ± 0.9	0.81 ± 0.2	1.01 ± 0.5	0.29 ± 0.4
TT	3.88 ± 1.2	2.41 ± 1.7	1.8 ± 1.2	12.5 ± 7.4	46.1 ± 39.7	4.82 ± 0.5	5.51 ± 0.9	0.85 ± 0.2	1.07 ± 0.5	0.28 ± 0.7
BBS6 rs221667	0.27	0.008	0.005	0.57	0.21	0.08	0.006	0.02	0.004	0.05
CC/CG	4.1 ± 1.2	2.39 ± 1.5	1.96 ± 1.4	13.95 ± 10.5	45.8 ± 40.4	4.92 ± 0.4	5.5 ± 0.9	0.82 ± 0.2	1.01 ± 0.5	0.29 ± 0.1
GG	3.82 ± 1.1	1.57 ± 1.5	1.64 ± 1.3	12.23 ± 7.2	53.5 ± 34.5	4.77 ± 0.5	5.95 ± 0.7	0.89 ± 0.3	1.24 ± 0.6	0.27 ± 0.1

Data are means ± SE or *P* values. No *P* values survived simple Bonferroni correction for 40 tests (10 phenotypes × 4 SNPs).

Genetic association with obesity. The allelic and genotypic analysis of the associated variants with childhood and/or adult obesity is described in Table 1. Online appendix Table 3 describes the variants not found to be associated with obesity in our population. In *BBS1*, the two haplotype-tagging SNPs rs2298806 and rs1791686 were not found to be associated with obesity in our cohort (online appendix Table 3).

In *BBS2*, one intronic SNP, rs4784675, was found to be significantly different in allele frequency in both class 1 and 2 obese adults (odds ratio [OR] 1.39 [95% CI 1.15–1.69], $P = 0.0005$) and in class 3 obese adults (1.30 [1.08–1.57], $P = 0.006$) compared with control subjects. SNP rs4784675 showed no significant association with childhood obesity.

In *BBS4*, an association was observed for one of the two genotyped SNPs, rs7178130, with obese children (OR 0.76 [95% CI 0.65–0.88], $P = 0.0003$), class 1 and 2 obese adults (0.86 [0.74–0.99], $P = 0.04$), and class 3 obese adults (0.84 [0.73–0.97], $P = 0.02$).

In *BBS6*, two SNPs were found to be associated with childhood obesity: rs6108572 (OR 1.21 [95% CI 1.05–1.39], $P = 0.007$) and rs221667 (1.33 [1.13–1.56], $P = 0.0007$). SNP rs221667 was also found to be significantly different in allele frequency in the severely obese adults (1.23 [1.05–1.44], $P = 0.01$).

Major haplotypes (MAF >5%) were identified in each gene using Phase. None showed stronger association with obesity compared with the single SNP analysis. We then genotyped the four associated variants in 638 French trios with childhood obesity. Using TDTs, we found that the frequent G-allele of rs7178130 was significantly overtransmitted to obese offspring ($P = 0.006$), supporting the results of the case-control analysis (Table 2). Nonsignificant overtransmission of the at-risk allele with adult obesity was also observed in 435 French trios. Transmission distortion was excluded in a set of 428 French trios with unaffected children (Table 2).

Effect of *BBS6* SNPs rs221667 and rs6108572, *BBS2* SNP rs4784675, and *BBS4* SNP rs7178130 on obesity and obesity-related traits. The potential effect of the four significantly associated SNPs on quantitative obesity-related phenotypes (*z* score of BMI, rebound age, *z* score of height, fasting insulin, glucose, lipids, and lipoproteins) was analyzed in the 622 French-Caucasian obese children (Table 3).

In these children, an association was observed for the *BBS6* SNP rs221667 with age of rebound of adiposity ($P = 0.008$) and height (*z* score of height, $P = 0.005$) but not with *z* score of BMI ($P > 0.05$). Children homozygous for the rare G-allele are shorter and show a 10-month earlier second rise of childhood adiposity. The obese children who are homozygous for the variant rs221667 have significantly higher postprandial glycemia (glycemia 2 h post–glucose load, $P = 0.006$). The effect of the variant on lipids and lipoproteins was then investigated. Children homozygous for the variant have a significant increase in fasting triglycerides ($P = 0.004$), a significant decrease in the HDL-to-total cholesterol ratio ($P = 0.05$), and a significant increase in apolipoprotein B ($P = 0.02$). Analysis of *BBS6* rs6108572 also revealed increased levels of fasting triglycerides ($P = 0.02$) and of apolipoprotein B ($P = 0.03$) in the children homozygous for the variant.

Children homozygous for the at-risk C-allele of *BBS2* rs4784675 showed a significant trend toward earlier second rise of childhood adiposity ($P = 0.05$). Analysis of the effect of the variant on lipids and lipoproteins showed no significant association with fasting triglycerides, HDL, or HDL-to-total cholesterol ratio, but it revealed a significant increase in apolipoprotein B ($P = 0.02$) in the CC carriers.

Analysis of the obesity-related phenotypes in 1,009 French-Caucasian obese adults also revealed a significant increased prevalence of arterial hypertension in the rare CC carriers of *BBS6* rs221667 ($P = 0.006$), in the rare TT carriers of *BBS6* rs6108572 ($P = 0.03$), and in the frequent AA carriers of *BBS4* rs7178130 ($P = 0.04$). Although the quantitative traits are known to be strongly correlated, when a simple Bonferroni correction was applied, none of the associations remained statistically significant.

DISCUSSION

Significant advances in the understanding of BBS have been made in recent years. Although identification of the nine causative genes has revealed that cilia function and intraflagellar transport may be key elements in BBS pathogenesis, it remains to be seen what role these proteins play in the obesity subphenotype.

We report four SNPs in three *BBS* genes showing evidence of association with common obesity in a French-Caucasian population. Although *BBS1* common variants showed no association with obesity, *BBS4* and *BBS6* were found to be associated with both childhood and adult obesity, and *BBS2* was only associated with adult obesity.

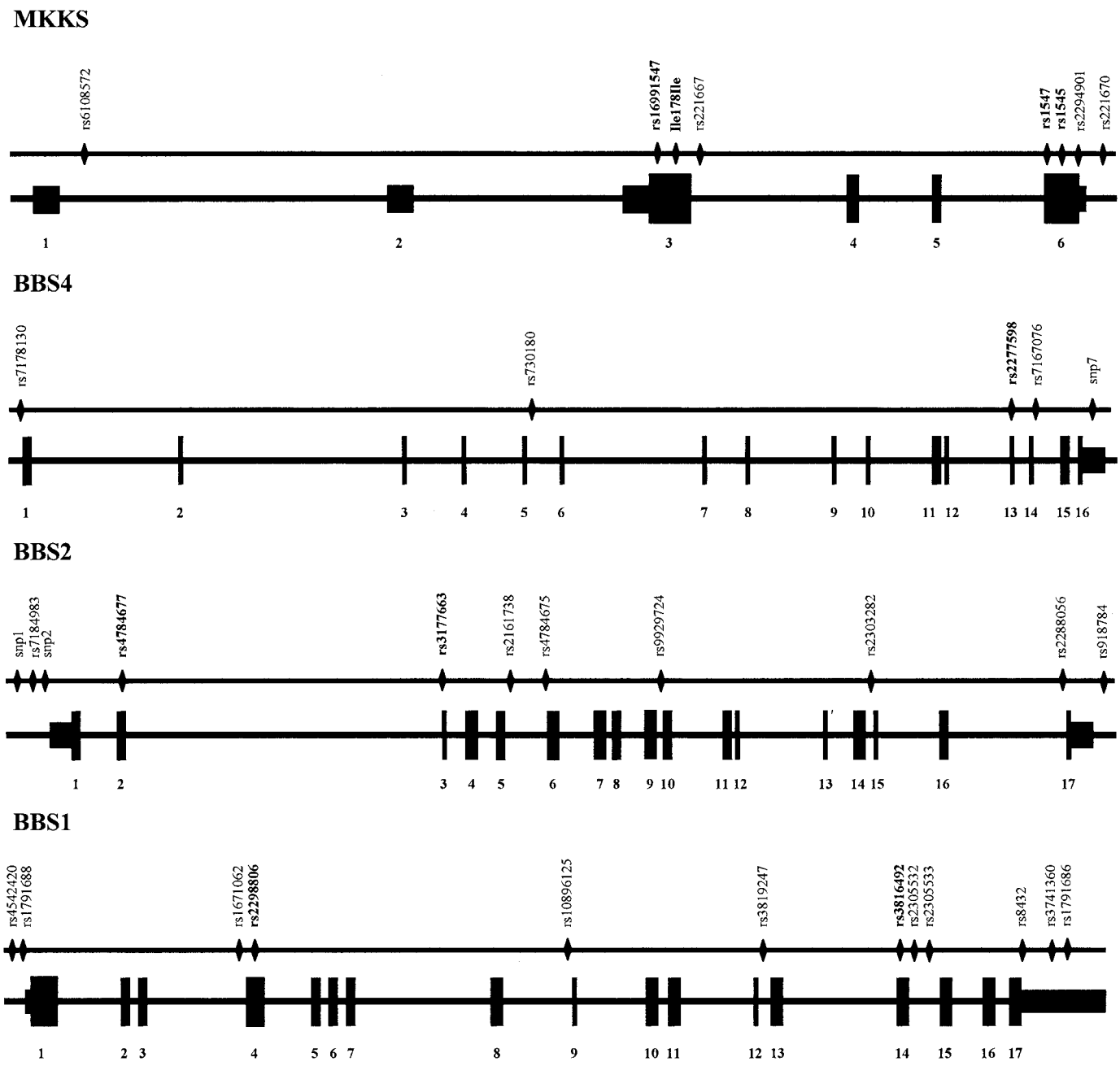

FIG. 1. Structure of the *BBS1*, *BBS2*, *BBS4*, and *BBS6* genes and location of 36 common polymorphisms identified in 24 obese subjects and 24 nonobese nondiabetic subjects. Exonic SNPs are shown in bold.

The association of the *BBS4* variant was also supported by TDT analysis in childhood obesity ($P = 0.006$).

Although our study suggests that *BBS* mutations might increase the risk of obesity in non-BBS individuals, a study of *BBS6* coding variants in a Danish obese population failed to identify a role for this gene in common obesity (21). Based on the hypothesis that less severe variation of the *BBS6* genes might be involved in the pathogenesis of common obesity, this group performed mutation analysis of the *BBS6* coding regions. A possible role in complex traits of less severe variants of genes responsible for monogenic forms of obesity had previously been described in type 2 diabetes with the monogenic MODY1 (maturity-onset diabetes of the young 1) hepatocyte nuclear factor-α (*HNF4A*) gene involved in susceptibility for the polygenic disease (2,29). The application of this hypothesis to BBS was further supported by the finding that obligate carriers of *BBS* heterozygous mutations are more obese than noncarriers, without displaying other phenotypes associated with BBS (20). However, the prevalent haplotype represented by the Arg517Cys (rs1547) variant was not found to be more prevalent in Danish obese subjects compared with lean individuals. This result was replicated in our cohorts in which no association was found for the *BBS1/2/4/6* coding polymorphisms, whereas four noncoding variants were found to be significantly more prevalent in obese compared with lean individuals. The effect of the noncoding sequence variations remains largely unpredictable and difficult to discern (30), and they could be seen as the less deleterious polymorphisms causative of the BBS subphenotype's obesity. The associated variants may also be in linkage disequilibrium with

true functional variants possibly upstream from the sequenced region.

A group that studied the phenotypic differences between three different loci among BBS patients argued that *BBS* genes vary in their effect on obesity with regard to extent and, possibly, progression (31). *BBS4* was described as having a major effect on weight, starting early in life and progressing to morbid obesity, a result consistent with our finding, with the association of *BBS4* (and *BBS6*) variants being stronger in childhood obesity than in adult obesity. The effect of a disease-causing variant in *BBS2* was mild excess weight during childhood and adolescent age and increasing weight in adulthood (31). This result is concordant with our results that describe the *BBS2* variant to be only associated with adult obesity, the strongest association having been identified in the moderately obese adults.

Analysis of the quantitative traits allowed us to investigate more subtle differences in the clinical manifestations of the various BBS types. Uncorrected *P* values are discussed because of the known strong correlation between the analyzed traits and the continuing debate about the validity of the Bonferroni correction (32).

Body fatness that normally declines at 5–6 years of age at a point called the adiposity rebound was found to occur significantly earlier in children homozygous for the rare allele of both the *BBS2* rs4784675 and *BBS6* rs221667 variants ($P = 0.05$ and $P = 0.008$, respectively). Early adiposity rebound was associated with an increased risk of both childhood (33) and adult obesity (34), independent of both parent obesity and BMI at adiposity rebound. *BBS6* rs221667 was also significantly associated with height (*z* score of height, $P = 0.005$), a trait previously described as influenced by *BBS* genes (35), the children homozygous for the mutant allele being shorter than the A-allele carriers.

Children homozygous for both *BBS6* variants showed significant association with factors characteristic of atherogenic dyslipidemia (e.g., increased fasting triglycerides, increased apolipoprotein B). Obese children who were homozygous for the *BBS6* variant rs221667 also had significantly higher postprandial glycemia. Dyslipidemia and hyperglycemia have been described as major risk factors for the metabolic syndrome, a previously described component and associated complication of BBS (36).

The analysis of the obesity-associated variants in obese adults revealed an increased prevalence of arterial hypertension in the rare CC carriers of *BBS6* rs221667, in the rare TT carriers of *BBS6* rs6108572, and in the frequent AA carriers of *BBS4* rs7178130. This finding is consistent with the increased risk of raised blood pressure observed in BBS patients and $Bbs4^{-/-}$ and $Bbs6^{-/-}$ mice (15).

It has been reported that mutations in *BBS1* can interact genetically with mutations at *BBS2*, *BBS4*, *BBS6*, and *BBS7*, as well as at unknown loci, to cause the phenotype (37). We attempted to test the hypothesis that mutations in one *BBS* gene can interact genetically with mutations at another *BBS* loci to increase the obesity incidence or to cause more deficient phenotypes. No apparent interaction between *BBS* variants was identified (data not shown), although this is most likely to be caused by the limited number of subjects with a combination of two at-risk alleles.

In conclusion, *BBS2*, *BBS4*, and *BBS6* genes contribute to common forms of obesity in an age-dependent manner, *BBS2* being associated with common adult-onset obesity and *BBS4* and *BBS6* being associated with common early-onset obesity that progresses to common adult morbid obesity. *BBS6* also showed nominal evidence of association with a phenotype previously described in BBS patients (36), the metabolic syndrome. Further work is needed for the identification of the true functional SNPs and/or the potential effect of the associated noncoding variants.

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APPENDIX

Electronic database information: dbSNP (www.ncbi.nlm.nih.gov/SNP), Ensemble (www.ensembl.org), National Centre for Biotechnology Information (www.ncbi.nlm.nih.gov), PhredPhrap software (www.genome.washington.edu), Primer3 (www-genome.wi.mit.edu/genome_software/other/primer3.html), and UCSC (University of California Santa Cruz) genome browser (http://genome.ucsc.edu/).

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