Brief Genetics Report

Genetic Variation in the Small Heterodimer Partner Gene and Young-Onset Type 2 Diabetes, Obesity, and Birth Weight in U.K. Subjects

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The orphan receptor small heterodimer partner (SHP, NR0B2) modulates the transcription activity of the MODY1 gene *HNF4a*. Mutations in *SHP* were found in 7% of Japanese obese young-onset type 2 diabetic patients and were associated with moderate obesity and increased birth weight. We investigated *SHP* in 1927 U.K. subjects, examining relationships with type 2 diabetes, obesity, and birth weight. Sequencing of the coding region of *SHP* in 122 obese, young-onset type 2 diabetic patients detected the polymorphism G171A. The polymorphism was not associated with diabetes in case control or familial association studies. The A allele (frequency 0.07) was not associated with obesity in type 2 diabetic subjects (n = 348), their parents (n = 272), or young nondiabetic adults (n = 925). However, the rare (<1%) AA homozygotes had a raised BMI in each cohort; this was significant when all cohorts were combined (Z score = 0.67 AA vs. −0.05 G/x, P = 0.02). There was no association with corrected birth weight in 382 normal babies, but the only AA baby was 4,069 g. Our study suggests that genetic variation in *SHP* is unlikely to be common in the predisposition to diabetes, obesity, or increased birth weight in U.K. Caucasians. *Diabetes* 52:1276–1279, 2003

The protein small heterodimer partner (SHP, also called NR0B2) is an atypical orphan receptor that lacks a conserved DNA binding domain. SHP is expressed in the liver where it is involved in a nuclear receptor cascade involved in the regulation of cholesterol catabolism (1,2). Recent work has shown that SHP is also expressed in the pancreas and inhibits the transcriptional activity of hepatocyte nuclear factor (HNF)-4α (3). HNF4α is a key member of a regulatory network of maturity-onset diabetes of the young (MODY)-related transcription factors that are required for normal β-cell function (4).

The regulation of HNF4α led Nishigori et al. (3) to investigate the role of the *SHP* gene in Japanese subjects with young-onset type 2 diabetes. They found five heterozygous mutations in 6 of 173 subjects (3.5%) who had reduced activity in functional studies. Further analysis showed that all subjects were moderately obese (BMI >25 kg/m^2 is the Japanese criterion for obesity [5]), and within families, the mutations cosegregated with obesity (logarithm of odds [LOD] score 2.31) and not diabetes (LOD score ∞). *SHP* mutations were also found in 5.9% (6 of 101) of nondiabetic young subjects with obesity (BMI >25 kg/m^2) but in none (0 of 116) of the nonobese control subjects. Birth weight was also shown to be markedly higher in mutation carriers (3,899 vs. 2,707 g, P = 0.008). These data suggested that genetic variation in *SHP* was the most common monogenic determinant of obesity and birth weight in Japanese subjects.

The work of Nishigori et al. was also important as it suggested that increased pancreatic insulin secretion might be a pathway in the etiology of obesity. Mutations reducing activity of SHP would result in reduced inhibition of HNF4α and, hence, could potentially increase insulin secretion. The finding of birth weight >1-kg higher than non–mutation carriers supports increased insulin secretion in utero; the birth weights were similar to that found in activating mutations of the sulfonylurea receptor (6). Replication of this initial finding is important, but no further studies of SHP have been reported.

The aim of our study was to investigate the role of SHP in type 2 diabetes, obesity, and birth weight in large U.K. Caucasian cohorts. As mutations had been found initially in obese, young-onset type 2 diabetic patients in Japan, we sequenced the whole coding region and intron/exon boundaries for *SHP* in 122 U.K. subjects with young-onset (diagnosis before 45 years) type 2 diabetes with a BMI >30 kg/m^2 (Table 1). The only sequence variation detected was an amino-acid substituting polymorphism (G171A) in exon 1 in codon 171, where GGG encoding G was replaced by GCG encoding A. This polymorphism was present in 16 individuals (13%) and was in Hardy-Weinberg equilibrium

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HNF, hepatocyte nuclear factor; LOD, logarithm of odds; MODY, maturity-onset diabetes of the young; SHP, small heterodimer partner; TDT, transmission disequilibrium test.

TABLE 1
Clinical details of diabetic and nondiabetic subjects studied

	Case subjects (young-onset type 2 diabetes)	Control subjects (population controls)	Parents of case subjects
n	348	925	272 (124 diabetic)
Male (%)	58	49	50
Age at diagnosis (years)*	40.5 (18.0–58.0)	32 (17–61)	68 (38–87)
BMI	31.7 (18.1–59.6)	26.6 (17.0–47.1)	27.2 (16.0–45.0)
Treatment, D/OHA/I (%)	13/28/59	†	17/34/14
WHR male	0.98 (0.80–1.16)	0.88 (0.70–1.11)	1.0 (0.5–1.3)
WHR female	0.90 (0.70–1.20)	‡	0.9 (0.7–1.2)

Continuous data are given as median (range). *Age at diagnosis for case subjects, age at study for control subjects; †control subjects were not on treatment; ‡there are no data for WHR for females in the population controls as females were pregnant at the time of study. D/OHA/I, diet/oral hypoglycemic agents/insulin; WHR, waist-to-hip ratio.

with two subjects being homozygous for the rare A allele. This polymorphism had not been described in Japanese subjects (3).

To assess the role of the G171A polymorphism in type 2 diabetes, we performed case control and familial association studies. The case control study consisted of 348 young type 2 diabetic cases diagnosed before 59 years (median 40.5 years) and 925 young adult nondiabetic control subjects (Table 1). There was no association with G171A alleles or genotypes and diabetes (Table 2). To assess familial association, we used a subset of the young-onset subjects on whom both parents were available ($n = 132$). There were 34 heterozygous parents and 12 transmitted the A allele to their diabetic offspring (transmission disequilibrium test, $P = 0.12$).

The role of the G171A polymorphism in the predisposition to obesity was assessed in both nondiabetic ($n = 1,073$) and diabetic ($n = 472$) subjects from the cohorts used to assess association with type 2 diabetes (Table 1). There was no association with the A allele with BMI in young adult nondiabetic males, pregnant mothers at 28 weeks' pregnancy, young-onset type 2 diabetic subjects, and parents of young-onset type 2 diabetic patients (Table 3). The BMI of the two common genotypes G/G or G/A were similar in all groups and when combined (G/G $Z = -0.054$, G/A $Z = 0.004$, $P = 0.68$). In all groups the small number (<1%) of subjects homozygous for the rare A allele were more obese than the subjects with the other two genotypes. This trend was not significant in any individual cohort but was significant when all the cohorts were combined: the median *Z* score, derived from within each separate cohort, for BMI was 0.672 in the AA homozygotes compared with −0.05 in the other genotypes ($P = 0.02$).

TABLE 2
Genotype and allele frequencies in type 2 diabetic subjects and population control subjects

	Case subjects (young-onset type 2 diabetic subjects) ($n = 348$)	Control subjects (population controls) ($n = 925$)
Genotypes		
G/G	295 (0.85)	795 (0.86)
G/A	50 (0.14)	126 (0.14)
A/A	3 (0.009)	4 (0.004)
P value		0.61
Alleles		
G	640 (0.92)	1,716 (0.93)
A	56 (0.08)	134 (0.07)
OR (95% CI)		1.12 (0.81–1.55)
P value		0.49

Values in parentheses represent genotype or allelic frequency. OR = odds ratio for diabetes with 95% CIs.

As a considerable increase in birth weight (1,193 g) had been reported in the Japanese study (3) when comparing mutation carriers with nonmutation carriers, we looked at birth weight corrected for gestational age in 382 U.K. Caucasian children (Table 4). There was no association with birth weight with the A allele, although the single case (0.3%) homozygous for the AA allele had a corrected birth weight of 4,069 g, which is consistent with macrosomia seen in the Japanese subjects heterozygous for *SHP* mutations with severe functional implications.

Our results suggest that mutations in *SHP* are less common in U.K. subjects with obese young-onset diabetes than in Japanese subjects (0% [0 of 122] vs. 7.0% [6 of 86], $P = 0.003$). As we have not identified any *SHP* mutations in these U.K. populations, we cannot test directly whether they result in moderate obesity and macrosomia. The U.K. subjects we tested were more obese than the Japanese subjects in absolute terms but were similarly obese compared with nationally agreed levels of BMI for obesity (U.K. = 30 kg/m^2, Japan = 25 kg/m^2).

Although no mutations were detected, there was a common coding polymorphism G171A in exon 1 in U.K. subjects that had not been found in the Japanese study. The A allele of this polymorphism had an allele frequency of 7.2% (from control data) and was potentially significant, as G is conserved at this position in mouse and rat. However, our results showed no association of diabetes, obesity, or birth weight with the G171A allele or the heterozygous G/A genotype.

Subjects homozygous for the A allele of the G171A polymorphism are consistently more obese, although this observation was not significant in any single dataset. However, this suggests the G171A polymorphism may have a similar role as the heterozygous severe mutations seen in the Japanese. Due to small numbers of homozygotes, this is only significant if all cohorts are combined using *Z* scores. Given the multiple phenotypes analyzed and the modest association observed, this result should not be emphasized. If this result was replicated in further large cohorts, it would suggest this polymorphism was only altering function of SHP when it was homozygous and

TABLE 3
Association of SHP G171A genotype with BMI in all cohorts stratified by sex and in all three cohorts combined using *Z* scores from log-transformed data

	Population control subjects		Young-onset type 2 diabetic subjects		Young-onset type 2 diabetic parents		Combined
Genotype	Fathers (n = 457)	Mothers (28 weeks pregnant) (n = 468)	Men (n = 201)	Women (n = 147)	Fathers (n = 137)	Mothers (n = 135)	*Z* scores (n = 1,545)
G/G	26.3 (17.0–40.0) (n = 391)	26.9 (20.1–47.1) (n = 404)	30.1 (18.8–44.6) (n = 169)	33.2 (18.1–50.5) (n = 126)	26.8 (16.3–40.5) (n = 119)	28.3 (17.3–45.0) (n = 114)	−0.054 (n = 1323)
G/A	26.5 (20.2–34.9) (n = 64)	27.0 (20.9–41.1) (n = 62)	30.3 (19.7–46.4) (n = 30)	34.0 (21.1–59.6) (n = 20)	27.1 (21.3–41.2) (n = 16)	26.4 (17.4–40.9) (n = 21)	0.004 (n = 213)
A/A	31.0 (26.3–35.7) (n = 2)	29.4 (25.9–32.9) (n = 2)	34.2 (32.9–35.5) (n = 2)	36.0 (n = 1)	30.4 (28.6–32.2) (n = 2)	— (n = 0)	0.672 (n = 9)
P value							
A/G vs. G/G	0.61	0.72	0.89	0.56	0.75	0.06	0.68
A/A vs. G/*	0.25	0.52	0.23	—	0.12	—	0.02

Data are median (range).

would also support the work of Nishigori et al., which suggested a role for SHP in the regulation of obesity. It is of interest that the only child homozygous for the A allele had a birth weight >4 kg. Further large association studies involving cohorts of over 3,000 subjects and functional studies are required to further assess the relationship with obesity and birth weight in subjects homozygous for the A allele.

In conclusion, we have shown that mutations in *SHP* are less common in U.K. obese type 2 diabetic subjects than in Japanese obese type 2 subjects. We have identified a common G171A coding polymorphism that is present in 14.1% of U.K. subjects. The A allele or G/A genotype is not associated with obesity or increased birth weight. Our preliminary results suggest subjects who are homozygous for the rare A allele may be predisposed to moderate obesity and possibly increased birth weight, but further studies are required to confirm this.

RESEARCH DESIGN AND METHODS

Subjects. Table 1 gives details of the subjects who were genotyped. Informed consent was obtained from all subjects. The young-onset type 2 diabetic subjects were unrelated, were Caucasian, and had diabetes defined either by World Health Organization criteria (7) or by being treated with medication for diabetes. Known genetic subtypes were excluded by clinical criteria and/or genetic testing. Patients were excluded if they had a first-degree relative with type 1 diabetes or an elevated titer of GAD antibodies. The young-onset type 2 subjects were all diagnosed before the age of 59 years (median 40.5 years) and were recruited from two sources: *1*) parent-offspring trios with type 2 diabetes from the Diabetes U.K. Warren 2 repository (8) and *2*) an additional collection of young onset (≤45 years of age at diagnosis) type 2 diabetic subjects (9). For the familial association study, the parents of the trios were also studied to allow a transmission disequilibrium test (TDT) analysis (8). The population control subjects were U.K. Caucasian and were parents from a consecutive birth cohort (the Exeter Family Study of Childhood Growth) with normal (<6.0 mmol/l) fasting glucose and normal HbA_{1c} levels. The mothers were recruited when 28 weeks pregnant. Birth weight was studied in the offspring of these parents. We also looked at obesity in the parents of the Diabetes U.K. Warren 2 Parent offspring trios; 46% of these parents had diabetes.

Genotyping. We assessed variation in the *SHP* coding region by sequencing both exons in 122 young-onset type 2 diabetic subjects (BMI >30 kg/m^2) (Table 1). The *SHP* exons were PCR amplified in three amplicons from genomic DNA using the following primer pairs: forward 5′-CAGAACACA GAGCCAGAGAG-3′ and reverse 5′-CTCAAAGGTCACAGCATCTT-3′; forward 5′-CAAGACAGTGGCCTTCCT-3′ and reverse 5′-GAGGACCCAATGAGATAA CA-3′; and forward 5′-GCCAGTCTTGTCCTTTGG-3′ and reverse 5′-CTCTGC CCACCTGATCTC-3′. PCR was performed in a 50-μl volume containing, in addition to the standard reagents, 1 mol/l betaine, 5% DMSO, deaza GTP, and 0.5 units AmpliTaq Gold (Applied Biosystems). PCR cycling conditions were denaturation at 95°C for 12 min followed by 40 cycles of denaturation at 95°C for 1 min and annealing at 55°C for 1 min and extension at 72°C for 2 min, with a final 10 min extension at 72°C. PCR products were purified using the QIAquick PCR purification kit (QIAgen) before both strands were sequenced using an ABI 377 DNA sequencer according to the manufacturer's instructions (Applied Biosystems).

We used tetra-primer ARMS-PCR (10) to screen for the G171A polymorphism in our populations using the following primers: forward inner 5′-CCAAGG AATATGCCTGCCTGAATGC-3′; reverse inner 5′-ACCGGGGTTGAAGAGGAT GGACC-3′; forward outer 5′-CTATGTGCACCTCATCGCACCTGC-3′; and reverse outer 5′-CTGGGTGACAGAGTGAGACTCTGTCTCAGA-3′.

PCR was performed in a 10-μl volume containing the standard reagents and 0.1 pmol/μl of each outer primer, 1 pmol/μl of forward inner primer, and 2 pmol/μl of reverse inner primer. PCR cycling conditions were denaturation at 95°C for 10 min followed by 35 cycles of denaturation at 94°C for 45 s and annealing at 65°C for 45 s and extension at 72°C for 45 s, with a final 10 min extension at 72°C. We resolved the tetra-primer ARMS-PCR products on 2% agarose gel stained with ethidium bromide.

Statistical methods. We assessed Hardy-Weinberg equilibrium of the G171A polymorphism in our cohorts by χ^2 comparisons of observed genotype frequencies with expected genotype frequencies inferred from observed allele frequencies. All cohorts were in Hardy-Weinberg equilibrium.

The significance of allele and genotype frequency differences were calculated using χ^2 analysis, with overall allele numbers used to calculate odds ratios and 95% confidence intervals using 2×2 contingency tables. TDT in parent offspring trios for individual variants was performed using TDT/S-TDT program (11).

To assess the effect of the polymorphism in our populations as a whole, we

TABLE 4
Association of SHP G171A genotype with birth weight.

	Birth weight (g)		
Genotype	Boys* (n = 193)	Girls* (n = 189)	Combined† (n = 382)
G/G	3650 (3585–3715)	3495 (3429–3564)	3573 (3527–3620)
n	167	159	326
G/A	3531 (3366–3695)	3454 (3298–3611)	3496 (3383–3609)
n	26	29	55
A/A	—	4013	4069
n	0	1	1
P value (G/G vs. G/A)	0.19	0.64	0.21

Data are mean (interquartile range). *Birth weight corrected for gestational age; †birth weight corrected for gestational age and sex.

log-transformed BMI data to make it normally distributed and then generated cohort-, sex-, and generation-specific *Z* scores. We combined the *Z* scores from each cohort and stratified these scores by genotype. We assessed the significance of the effect of SHP171 genotype on BMI by using a Mann-Whitney *U* test.

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