The cocaine- and amphetamine-regulated transcript (CART) peptide is a recently characterized neuropeptide implicated in the control of appetite. We hypothesized that genetic variation in CART may contribute to human obesity. The entire coding region of CART was determined by nucleotide sequencing in 91 unrelated subjects with severe early-onset obesity. A novel amino acid change, Ser667hr, was found in 2 probands and in 0 of 100 control subjects but did not cosegregate with obesity in family studies. Two common polymorphisms were found in the 3′-untranslated region (A1475G and ΔA1457). An effect of these polymorphisms on body composition and intermediate phenotypes related to obesity was examined in a large Caucasian population in the U.K. Neither polymorphism showed any significant relationship with obesity; however, men heterozygous for the A1475G variant had significantly lower waist-to-hip ratio (WHR), fasting plasma insulin, and fasting triglycerides. Regression analysis indicated that the effects on insulin and triglycerides were likely to be secondary to the effects on WHR. Thus, we have conducted the first systematic study of the CART gene in human obesity, and although no clear association with obesity was found, the data suggest that genetic variation in the CART locus might influence fat distribution and variables related to syndrome X. Diabetes 49:872–875, 2000

Data from twin, adoption, and family studies indicate that genetic factors play a major role in the determination of the interindividual variation in fat mass within human populations (1,2). Until recently, however, little precise information regarding genetic causes of human obesity has been available. In the past 3 years, 5 monogenic defects causing human morbid obesity have been identified, namely, mutations in the genes encoding leptin (3,4), leptin receptor (5), prohormone convertase (PC)-1 (6), pro-opiomelanocortin (POMC) (7), and the melanocortin-4 receptor (MC4R) (8,9). In all of these syndromes, hyperphagia results from dysfunction of hypothalamic pathways controlling satiety. Genetic defects in these molecules are responsible for only a small fraction of human morbid obesity, and it is likely that the detailed elucidation of the molecular mechanisms of appetite control will provide further candidate genes that may be implicated in human obesity. One such molecule is cocaine- and amphetamine-regulated transcript (CART), a recently discovered hypothalamic neuropeptide (10).

CART was originally identified by polymerase chain reaction (PCR) differential display as a novel mRNA, the expression of which was induced in rat striatum after acute administration of psychomotor stimulants (10). Subsequently, CART was found to be one of the most abundant mRNAs in the hypothalamus (11). Considerable evidence implicates CART in the control of mammalian feeding behavior. CART is highly expressed in the arcuate and paraventricular nuclei of the hypothalamus, areas known to be involved in the control of appetite (10,12). CART neurons show fos-immunoreactivity after intravenous injections of leptin in rats (13), and conversely, hypothalamic CART mRNA levels are decreased in hypoleptinemic states, such as in the ob/ob mouse, and after prolonged fasting. In these conditions, CART expression is restored by leptin administration (14). Intracerebroventricular injection of recombinant CART into rats and mice inhibits normal and starvation-induced feeding and blocks the feeding response induced by neuropeptide Y (14). Conversely, after central administration of anti-serum against CART in rats, the feeding response was increased (14). These data strongly implicate CART as an endogenous satiety factor.

To establish whether genetic defects in CART might contribute to human obesity, we used direct nucleotide sequencing to examine the coding regions of the CART gene in 91 unrelated subjects with severe early-onset obesity. Having detected common sequence variants within CART in this cohort, we have subsequently examined the relationships between these polymorphisms and obesity-related phenotypes in an independent, large, ethnically homogeneous Caucasian population.

We studied 91 severely obese children in whom no evidence for a recognized clinical syndrome or a structural hypothalamic cause for their obesity has been found. The possibility of...
of previously described monogenic defects contributing to the
obesities has been excluded through biochemical assays
(leptin, PC-1), clinical descriptions (POMC deficiency), and
mutational screening (MC4R). In all cases, obesity was man-
ifest before the age of 10 years and BMI was >4 SD above the
mean for their age. The human CART gene is encoded in a
2.5-kb segment of genomic DNA on chromosome 5 (15). The
900-nucleotide CART transcript is contained within 3 exons
and encodes a mature peptide 89 amino acids in length (15).
A 1.5-kb amplicon, including the entire coding sequence of
CART and all intron/exon boundaries, was amplified by PCR.
To determine the nucleotide sequence of CART, sequencing
primers were designed from intronic sequence flanking the
3 exons, thus allowing the entire coding region, including all
intron/exon boundaries, to be sequenced.

One subject was found to be heterozygous for a missense
amino acid substitution, Ser66Thr, in exon 2 of CART (Gen-
Bank accession number U20325). Although little informa-
tion is as yet available on the structure/function relation-
ships in CART, serine is conserved in this position in the
human and rat sequences. The proband was a Caucasian
female from a nonconsanguineous family who was first
noted to be severely obese at age 4 and currently weighs
95 kg at age 14 years. Of 11 other family members available
for study, 4 had a history of early-onset obesity and 7 were
nonobese. The mutation did not clearly cosegregate with the
obese phenotype, with 3 of 4 obese and 3 of 7 nonobese rel-
atives being carriers of the mutation (data not shown). The
same variant (Ser66Thr) was found in another severely obese
subject and also in his lean father. The variant was not
detected in 100 unrelated adult Caucasian control subjects.

In addition to the rare missense mutation, 2 common sin-
gle nucleotide polymorphisms were identified within the
3'-untranslated region (3'-UTR) of exon 3. A heterozygous
variant (A1475G) occurring 39 base pairs 3' from the stop
codon was found in 18 (19.8%) subjects. A second variant was
also found, involving the deletion of an adenine at position
1457 (ΔA1457), 21 nucleotides 3' from the stop codon. For the
ΔA1457 variant, among the 91 morbidly obese subjects, 80
(87.9%) were wild-type, 10 (11.0%) were heterozygous, and 1
(1.1%) was homozygous. It is possible that these common vari-
ants in the 3'-UTR might have an influence on CART expres-
sion, either directly through influences on mRNA stability or
indirectly through linkage disequilibrium with other func-
tional variants in or near the CART gene. We therefore exam-
ined the association between these variants and obesity-
related phenotypes in a large, ethnically homogenous, well-
characterized Caucasian population.

The Isle of Ely Study (16,17) is a prospective population-
based cohort study of the etiology and pathogenesis of type 2
diabetes and related metabolic disorders. Anthropometric
and biochemical data were available in this study both at
baseline and after a 4.5-year follow-up period. Subjects, all
Caucasians from the U.K., were aged between 40 and 65
years at baseline. Responses for successive time periods
were analyzed with repeated measures design using the Sta-
tistical Analysis System procedure MIXED for unbalanced
repeated data, with and without adjustment (for age or waist-
to-hip ratio [WHR]). We examined 412 subjects for the
ΔA1457 variant using direct sequencing and 811 for the
A1475G variant using a combination of direct sequencing
and mutagenically separated PCR (MS-PCR). In the latter
 technique, mutagenic bases are incorporated within primer
sequences to deliberately introduce additional differences
into the allelic PCR products and prevent cross-reactions in
subsequent cycles. This selective mutagenesis separates the
amplification reactions, allowing for allele-specific screening
to occur in the same reaction.

Allele frequencies of the A1475G and ΔA1457 polymor-
phisms in the Isle of Ely cohort were 0.076 and 0.087, respec-
tively. Neither polymorphism deviated significantly from
Hardy-Weinberg predictions, and the 2 variants were in link-
age disequilibrium (P = 0.0072; n = 406). The relationships
between these polymorphisms and obesity-related pheno-
types were examined using a mixed model analysis, incor-
porating phenotypic data from baseline and 4.5-year follow-
up and adjusted for age, with men and women analyzed inde-
pendently. Phenotypic variables examined were BMI, WHR,
fasting plasma glucose, fasting plasma insulin (FPI), fasting
glucose, triglycerides (FT), and fasting nonesterified fatty acids.
Neither variant showed any significant association with BMI in
men or women. There were no significant associations
between any obesity-associated phenotype and the ΔA1457
variant in either men or women (data not shown). In contrast,
men heterozygous for the A1475G variant had significantly
lower WHR, FPI, and FT (Table 1). There were no significant
genotype- or gene-WHR interactions for any of the responses.

Table 1 also shows the results adjusted for WHR, which
demonstrate that the effect of the gene on triglycerides and
fasting insulin is not independent of WHR, suggesting that the
observed univariate effects are likely to be mediated through
central obesity. There were no significant associations
observed in women (data not shown). Combined analysis of
the A1475G and ΔA1457 polymorphisms did not strengthen
or significantly alter the associations found (data not shown).

To our knowledge, this is the first systematic analysis of the
CART gene in human obesity. We have identified 3 novel
gene variants, a rare missense mutation (Ser66Thr) and 2
common variants (A1475G, and ΔA1457), within the 3'-UTR
of the gene. The amino acid substitution did not clearly
cosegregate with obesity in the 2 pedigrees. However, a con-
tributory role of this variant to the severe obesity seen in 3 car-
diers in the first pedigree cannot be excluded. Neither of the
common 3'-UTR polymorphisms showed any association
with BMI. Of note, however, the A1475G variant was associ-
ated with a lower WHR in male A/G heterozygotes. Although
the association was seen in men only and was of modest sta-
tistical significance, an effect of hypothalamic neuropeptide
expression on the manifestation of features of syndrome X is
biologically plausible.
TABLE 1
Association studies of the A1475G sequence variant with obesity-related phenotypes in men from the Isle of Ely cohort

<table>
<thead>
<tr>
<th>Response</th>
<th>A/A (wild-type)</th>
<th>A/G (heterozygous)</th>
<th>Significance Before adjustment</th>
<th>Adjusted for age</th>
<th>Adjusted for WHR</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>522</td>
<td>90</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.69 (26.40–26.98)</td>
<td>26.04 (25.36–26.72)</td>
<td>0.0965</td>
<td>0.0900</td>
<td>—</td>
</tr>
<tr>
<td>WHR</td>
<td>0.94 (0.93–0.95)</td>
<td>0.91 (0.90–0.93)</td>
<td>0.0046</td>
<td>0.0021</td>
<td>—</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/l)*</td>
<td>5.47 (5.40–5.54)</td>
<td>5.55 (5.40–5.69)</td>
<td>0.4198</td>
<td>0.4722</td>
<td>0.4131</td>
</tr>
<tr>
<td>FPI (pmol/l)*</td>
<td>43.01 (40.71–45.43)</td>
<td>37.38 (33.86–41.26)</td>
<td>0.0482</td>
<td>0.0465</td>
<td>0.3526</td>
</tr>
<tr>
<td>FT (mmol/l)*</td>
<td>1.40 (1.34–1.46)</td>
<td>1.22 (1.11–1.35)</td>
<td>0.0218</td>
<td>0.0239</td>
<td>0.1185</td>
</tr>
<tr>
<td>Fasting nonesterified fatty acids (mmol/l)</td>
<td>0.45 (0.43–0.47)</td>
<td>0.42 (0.38–0.47)</td>
<td>0.2994</td>
<td>0.2941</td>
<td>0.4186</td>
</tr>
</tbody>
</table>

Data are arithmetic means (95%CI) and *geometric means (95%CI). Significance was determined with mixed model analysis. n, the number of total observations in the 2 visits.

In conclusion, mutations in CART are unlikely to be a major cause of early-onset morbid obesity in Caucasians. In this study, however, the regulatory elements of the CART gene were not screened, and thus pathogenic mutations occurring within regulatory regions cannot be excluded. However, the possibility is raised that inherited variation in the CART gene may influence the development of some metabolic features, a hypothesis that provides a potentially novel link between the central nervous system and syndrome X.

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REFERENCES

