Human Hormone-Sensitive Lipase

Genetic Mapping, Identification of a New Dinucleotide Repeat, and Association With Obesity and NIDDM

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NIDDM and obesity are complex metabolic disorders with a significant genetic component (1). The two conditions are frequently associated and share many metabolic abnormalities suggesting that they may also share some susceptibility genes. However, their multifactorial nature makes it difficult to dissect the genetic determinants. Despite numerous studies, only a few candidate genes accounting for a minor part of the genetic background have been implicated, usually in particular populations.

Hormone-sensitive lipase (HSL), which plays a critical role in the control of energy homeostasis by catalyzing the hydrolysis of adipose tissue triglycerides into free fatty acids, appears as a good candidate gene. Indeed, this enzyme may be involved in the loss of insulin sensitivity since there is increasing evidence that free fatty acids are involved in the development of insulin resistance (2). The adipose tissue isoform of HSL is encoded by a single gene composed of nine exons (3). The gene locus symbolized LIPE has been assigned to chromosome 19q13.1-13.2 (4). However, no genetic mapping studies have been carried out for LIPE to date.

This report describes the characterization of a novel polymorphism in the HSL gene coupled with its genetic mapping. Moreover, we report association studies of this novel microsatellite with NIDDM and obesity within a Caucasian population.

First, we characterized a $(CA)_{21}$ repeat, named HSLi6[CA]$_n$, found within HSL intron six after sequencing of genomic clone hHSL-III1 (3). Polymorphism of this repeat was detected by performing polymerase chain reaction (PCR) with oligonucleotides flanking the repetitive sequence (upstream primer: 5′-CTCAGCAGGGAAACAGGACTG-3′; downstream primer: 5′-GTTTGAGCCACTGCACTCAGC-3′). PCR amplification of HSLi6[CA]$_n$ produced 228–246 bp DNA fragments. Eight Centre d'Étude Polymorphisme Humain (CEPH) reference families (102, 884, 1331, 1332, 1347, 1362, 1413, and 1416) and French Caucasian unrelated individuals were genotyped for HSLi6[CA]$_n$ following a multiplexing procedure (5). Eleven different alleles were found among 80 unrelated individuals with a heterozygosity (HET) of 0.75. Mendelian inheritance was confirmed in the eight CEPH families studied.

To map LIPE, a two-point linkage analysis was performed between HSLi6[CA]$_n$ and markers from Généthon (6) using version 5.2 of the LINKAGE package of computer programs (7). Based on segregation analysis in the eight CEPH pedigrees, LIPE was mapped on chromosome 19 in the interval between AFM238wf10 and AFMa131wf5 with recombination fractions (θ) of 0.043 and 0.016, and logarithm of odds (LOD) scores of 24.76 and 29.57, respectively. Multipoint analysis allowed us to determine the location of LIPE, relative to close microsatellites in the following order:

cen-AFM238wf10(θ = 0.013)-AFMa197xa5(θ = 0.006)—LIPE—(θ = 0.004)AFM126zc1-(θ = 0.001)AFMa131wf5-(θ = 0.001)AFM326xh9-tel.

This most likely order was supported by odds of 1,000:1 against any permutation of two adjacent markers. Therefore, LIPE is located within the 1-cM interval flanked by AFMa197xa5 (D19S872) and AFM126zc1 (D19S211) at a distance of 0.6 and 0.4 cM, respectively.

Haplotype analysis was performed on grandparents or parents in the CEPH families with HSLi6[CA]$_n$ and the previously reported dinucleotide repeat located in the seventh intron (4), these polymorphisms being separated by a physical distance of ~2,800 bp. We identified nine alleles for HSLi6[CA]$_n$ (HET = 0.78, polymorphism information content [PIC] of 0.75) and six alleles (HET = 0.71, PIC = 0.66) for the marker described by Levitt et al. (4). Our results indicate that the observed occurrence of the most frequent haplotypes is in good agreement with expected values. Furthermore, there are no specific alleles occurring together with a frequency different from that expected, i.e., there is no indication for linkage disequilibrium.

To study the possible implication of HSL in obesity and/or diabetes, we analyzed the distribution of genotypes for HSLi6[CA]$_n$ in unrelated French Caucasian individuals.

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CEPH, Centre d'Étude Polymorphisme Humain; df, degrees of freedom; IGT, impaired glucose tolerance; HET, heterozygosity; HSL, hormone-sensitive lipase; PCR, polymerase chain reaction; PIC, polymorphism information content.

TABLE 1
Distribution of HSLi6[CA]n alleles in unrelated French Caucasian subjects

		Control lean	Obese	IGT	NIDDM	NIDDM-IGT	NIDDM-IGT lean	NIDDM-IGT obese
n		150	94	98	240	338	130	136
Allele	Size (bp)							
A1	246	0	1.1	0	0.8	0.6	1.5	0
A1bis	245	0	0	0	0.4	0.3	0	0.7
A2	244	2	0	1	4.2	3.3	2.3	3.7
A2bis	243	0	0	0	0.8	0.6	0.8	0.7
A3	242	12	12.8	7.1	7.5	7.4	8.5	6.6
A3bis	241	0	0	1	0.4	0.6	0	1.5
A4	240	15.3	5.3	10.2	9.6	9.8	10	6.6
A5	**238**	**41.3**	**57.4†**	**56.1***	**52.1***	**53.3†**	**53***	**54.4***
A6	236	8.7	8.5	9.2	7.1	7.7	7	8
A6bis	235	0	0	0	1.3	0.9	0.8	0.7
A7	234	14.7	6.4	6.1	10.8	9.5	10	9.6
A8	232	0	1.1	1	0.4	0.6	1.5	0
A9	230	6	4.3	6.1	3.8	4.4	3	6.6
A10	228	0	2.1	2	0.4	0.9	0.8	1.5
A11	226	0	1.1	0	0.4	0.3	0.8	0
χ^2 (*P* with 6 df)			14 ($P = 0.03$)	11 ($P = 0.08$)	16.6 ($P = 0.07$)	17.5 ($P = 0.005$)	12.3 ($P = 0.05$)	16.6 ($P = 0.01$)

Data are percentages of alleles. Alleles are listed in order of size (alleles-bis are rare size-intermediates). NIDDM-IGT group corresponds to the pool of patients with diabetes or IGT. *n* indicates the number of chromosomes sampled. For the comparison with the lean controls, the rare alleles with frequencies <5% (1, 1bis, 2, 2bis, 3bis, 6bis, 8, 10, and 11) were pooled into a single category; thus the χ^2 for the overall distribution have 6 df. Because of the significant differences observed, the role of allele 5 in excess was evaluated by χ^2 with 1 df (*$P < 0.05$, †$P < 0.02$).

NIDDM and IGT were diagnosed by World Health Organization criteria. Lean and obese subjects were classified according to a BMI ≤25 and >27 kg/m^2, respectively. We studied 75 healthy lean control subjects with no history of diabetes (38 women, 38 men; age range 26–69 years; mean age 44 years; BMI 22 ± 2 kg/m^2) and patients affected with obesity and normal glucose tolerance (23 women, 24 men; age range 23–69 years, mean age 43 years; BMI 32 ± 4 kg/m^2), with impaired glucose tolerance (IGT; 15 women, 34 men; age range 29–77 years; mean age 49 years; BMI 29 ± 6 kg/m^2), or with NIDDM (49 women, 71 men; age range 35–80 years; mean age 56 years; BMI 27 ± 5 kg/m^2).

Comparison of control and patient groups was performed using χ^2 tests. The nine rare alleles, i.e., with frequencies <5% (alleles 1, 1bis, 2, 2bis, 3bis, 6bis, 8, 10, and 11), were pooled into a single category to ensure the validity of the χ^2 test, thus resulting in a marker of seven alleles. The allele distribution differed between the patient groups and the control group (Table 1). The association with obesity that was observed with the obese normoglycemic group was strengthened when the obese patients, either diabetic or not, were pooled and compared with lean control subjects ($\chi^2 = 20.4$, $P = 0.002$ with 6 df; data not shown). Also, when the IGT and NIDDM groups that presented similar allelic distributions were pooled, the difference from the control group was more significant ($P = 0.005$, Table 1). Therefore, the HSLi6[CA]$_n$ allelic distribution was altered in obesity and diabetes.

The differences observed in the allelic distribution were essentially due to a significant increase in the frequency of allele 5 in the obese and NIDDM-IGT groups as compared with the control subjects (obese nondiabetic patients $\chi^2 = 6$, $P < 0.02$; NIDDM-IGT patients $\chi^2 = 5.9$, $P < 0.02$ with 1 df). The relative risk conferred by the presence of allele 5, estimated by the odds ratio, was 1.92 (95% CI 1.4–2.44) for obese patients and 1.62 (95% CI 1.23–2) for NIDDM-IGT patients. Interestingly, the prevalence of homozygotes for allele 5 was increased in patient groups since we found 37% of obese subjects homozygous for this allele as compared with 12% of lean subjects ($\chi^2 = 10.5$, $P = 0.001$). An increased frequency of homozygotes was observed in the group of NIDDM-IGT patients either obese (31%, $\chi^2 = 7.7$, $P < 0.01$) or lean (26%, $\chi^2 = 4.6$, $P < 0.05$), indicating that the association of allele 5 with diabetes occurs independently of obesity.

When allele 5 was removed, the distribution of the remaining alleles was still altered in the NIDDM-IGT group ($\chi^2 = 10.8$, $P < 0.03$), while not in the obese group. This was mainly due to an increased frequency of the rare alleles. However, due to their low frequency, their individual contribution could not be evaluated in our population.

Our data show that the HSLi6[CA]$_n$ microsatellite is associated with obesity and with NIDDM, suggesting that HSL could represent a susceptibility gene for both disorders. Although studies in other populations are required, our data suggest that variants of the HSL gene may present some altered function or expression. The characterization of a novel polymorphism in the HSL gene coupled with its localization relative to other highly polymorphic flanking markers should prove useful for familial genetic analysis in obesity, NIDDM, and related disorders.

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REFERENCES

1. McCarthy MI, Froguel P, Hitman GA: The genetics of non-insulin-dependent diabetes mellitus: tools and aims. *Diabetologia* 37:959–968, 1994
2. Boden G: Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. *Diabetes* 46:3–10, 1997
3. Langin D, Laurell H, Stenson Holst L, Belfrage P, Holm C: Gene organization and primary structure of human hormone-sensitive lipase: possible significance of a sequence homology with a lipase of Moraxella TA144, an antarctic bacterium. *Proc Natl Acad Sci USA* 90:4897–4901, 1993
4. Levitt RC, Liu Z, Nouri N, Meyers DA, Brandriff B, Mohrenweiser HM: Mapping of the gene for hormone sensitive lipase (LIPE) to chromosome 19q13.1 kq13.2. *Cytogenet Cell Genet* 69:211–214, 1995
5. Vignal A, Gyapay G, Hazan J, Nguyen S, Dupraz C, Cheron N, Becuwe N, Tranchant M, Weissenbach J: A non radioactive multiplex procedure for genotyping of microsatellite markers. In *Gene and Chromosome Analysis, part A*. Adolph KW, Ed. San Diego, CA, Academic 1:211–221, 1993
6. Dib C, Fauré S, Fizames C, Samson D, Drouot N, Vignal A, Millasseau P, Marc S, Hazan J, Seboun E, Lathrop M, Gyapay G, Morissette J, Weissenbach J: A comprehensive genetic map of the human genome based on 5,264 microsatellites. *Nature* 380:152–154, 1996
7. Lathrop GM, Lalouel JM: Easy calculation of lod scores and genetic risk on small computers. *Am J Hum Genet* 36:460–465, 1984