No Coding Mutations Are Detected in the Peroxisome Proliferator-Activated Receptor-γ Gene in Japanese Patients with Lipoatrophic Diabetes

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Lipoatrophic diabetes is a rare disease characterized by generalized lipodystrophy, severe insulin resistance, hyperlipidemia, hepatomegaly, and a lack of ketoacidosis (1). In this disease, a high incidence of parental consanguinity or family antecedents with diabetes has been reported (2,3), therefore several candidate genes that might contribute to the etiology of lipoatrophic diabetes have been investigated and genetic linkage analysis has been performed (3–5). Nevertheless, the lipoatrophic diabetes locus has not been identified and the mechanism of disease pathogenesis remains unknown.

Peroxisome proliferator-activated receptor-γ (PPAR-γ) is an adipocyte-specific nuclear receptor that appears to be a key regulator of adipogenesis (6). The demonstration that PPAR-γ is the high-affinity receptor for the thiazolidinedione class of insulin-sensitizing drugs also suggests that this protein is important in systemic insulin action (7). Thus, functional defects in PPAR-γ might be expected to result in impaired adipogenesis and insulin resistance, conditions typical of lipoatrophic diabetes. The human PPAR-γ gene was therefore cloned and screened for mutations in individuals with lipoatrophic diabetes with the use of polymerase chain reaction and single-strand conformation polymorphism (PCR-SSCP) analysis.

PPAR-γ exists as two isoforms, PPAR-γ1 and PPAR-γ2, differing only in their N-terminal amino acids (8). Both PPAR-γ isoforms are derived from the same gene by alternative promoter usage and splicing (9). PPAR-γ2 expression is restricted to adipose tissue, while PPAR-γ1 shows a wide tissue distribution (6,8).

Reverse transcription and PCR with total RNA from 3T3-L1 adipocytes and specific primers yielded the entire coding region of mouse PPAR-γ2 cDNA, the nucleotide sequence of which was identical to that previously described (8). With the ^{32}P-labeled mouse PPAR-γ2 cDNA as a probe, we screened ~1 × 10^6 clones of a human genomic DNA library. Genomic DNA from the 23 positive clones was isolated and subjected to Southern blot analysis with the same mouse probe. The resulting six positive fragments were subcloned, subjected to DNA sequencing, and found to encompass the entire coding regions of both the human PPAR-γ1 and PPAR-γ2 genes and a part of 5′ flanking region of the human PPAR-γ2 gene (GenBank accession number:AB005520–AB005526; Fig. A1 shown on *Diabetes* website).

The open reading frames of the human PPAR-γ1 and PPAR-γ2 genes are contained in six and seven exons, respectively. The predicted human PPAR-γ2 protein contains an additional 28 amino acids at the amino terminus compared with human PPAR-γ1 (10). These additional amino acids are encoded by a single exon, designated exon γ2. The six downstream exons, shared with the PPAR-γ1 and PPAR-γ2 genes, are designated exons 1 to 6. The exon-intron boundaries were identified with the use of the human PPAR-γ1 and PPAR-γ2 cDNA sequences (10). The sequence of the protein-coding region of the PPAR-γ gene was identical to that of the corresponding human cDNA (10). As in the mouse PPAR-γ gene (9), the DNA-binding domain of human PPAR-γ is encoded by exons 2 and 3, and the ligand-binding domain is encoded by exons 5 and 6. The nucleotide sequence of the partial 5′ upstream region of the human PPAR-γ2 gene was also determined (GenBank accession number: AB005520; Fig. A1 shown on *Diabetes* website). The first TATA-like element (TATAAA) is located 714 bp upstream from the initiating ATG codon. This 5′ upstream region also contains the consensus binding sequence of transcription factors of C/EBPs, the CCAAT box, the CAGCTG sequence, and cap boxes.

Twelve Japanese individuals with lipoatrophic diabetes were studied (Table 1). Subjects LD2 and LD3 were first cousins, and LD10 and LD11 were siblings from the same kinship; the other eight subjects were unrelated. All subjects except LD5, LD6, LD7, and LD12 were the offspring of consanguineous parents or had family antecedents with diabetes or lipoatrophy. All subjects except LD5 and LD12 also exhibited acanthosis nigricans. Hepatomegaly was present in all subjects except LD9. All 12 subjects showed hyperinsulinemia and abnormalities in lipid metabolism.

Genomic DNA was extracted from peripheral blood leukocytes of each subject. All participating individuals were informed of the aim of the study and gave their informed consent. The investigation was conducted according to the guidelines expressed in the Declaration of Helsinki.

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Additional information can be found in the on-line appendix at www.diabetes.org/diabetes/appendix.htm.

PCR, polymerase chain reaction; PPAR-γ, peroxisome proliferator-activated receptor-γ; SSCP, single-strand conformation polymorphism.

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TABLE 1
Clinical profiles of study subjects

Subjects	Age (years)	Sex	Parental consanguinity	Relatives with diabetes or lipoatrophy	Onset of total lipodystrophy* (years)	Onset of diabetes* (years)	Height (cm)/ body mass (kg)	Fasting IRI (μU/ml)†
LD1	30	M	Yes	No	Birth	2	156/48	95
LD2	37	M	No	Yes	Birth	12	166/60	113
LD3	32	M	No	Yes	Birth	5	158/62	73
LD4	41	F	No	Yes	Birth	25	157/53	31
LD5	5	F	No	No	Birth	2	115/23	32
LD6	7	F	No	No	Birth	Birth	129/27	49
LD7	24	F	No	No	2	15	151/47	46
LD8	32	F	Yes	No	3	13	159/44	50
LD9	18	F	No	Yes	4	12	151/33	70
LD10	16	F	No	Yes	1	6	143/43	35
LD11	11	M	No	Yes	2	8	148/40	15
LD12	36	M	No	No	8	10	166/42	192

*Age of onset refers to the first clinical manifestation of lipoatrophy or diabetes. †Normal range for fasting immunoreactive insulin is 6.3 ± 2.2 μU/ml.

A total of 15 specific oligonucleotide primer sets were used for PCR-SSCP analysis (Table A1; shown on *Diabetes* website). The entire protein-coding regions of both the PPAR-γ1 and PPAR-γ2 genes including exon-intron boundaries and the partial 5′ upstream region of PPAR-γ2 gene were amplified successfully with these primer sets. We divided each of exons 1 to 6 and exon γ2 with the partial 5′ upstream region into two and three partially overlapping sequences, respectively, for PCR amplification in an attempt to increase the sensitivity of SSCP analysis. PCR products that showed abnormal migration on SSCP analysis were subjected to direct sequencing.

We detected a silent CAC (His) → CAT (His) mutation at codon 447 in exon 6 of subject LD12, and an intronic T → C transition 22 bp upstream of exon 2 in subject LD7 and LD10. However, neither significant mutations in the protein-coding region of the PPAR-γ gene nor polymorphisms in the partial 5′ upstream region of PPAR-γ2 gene were detected. The exonic silent mutation was also detected in 9 of 27 normal control subjects, indicating that it is not relevant to the pathogenesis of lipoatrophic diabetes. The intronic T → C transition did not affect a splicing site.

In the result, no mutations that affect the amino acid sequence of PPAR-γ were detected in any of the 12 subjects, indicating that such mutations do not appear to be a major cause of lipoatrophic diabetes. Although it was shown that C/EBPβ is induced early phase in adipogenesis and increases the expression of PPAR-γ (11), there were no polymorphisms in the partial 5′ upstream region of PPAR-γ2 gene that contains the consensus binding sequence of C/EBP. However, we did not analyze the entire PPAR-γ gene; it is thus possible that mutations in the farther 5′ upstream region or in intron-exon–bordering regions of introns impair gene transcription or result in abnormal RNA splicing and that such mutations might be the pathogenesis of lipoatrophic diabetes. At present, the targeted disruption of the PPAR-γ gene has not been reported, so the pathological conditions caused by gene deletion are not known. However, our results indicate that at least the coding sequences of the PPAR-γ gene are normal in individuals with lipoatrophic diabetes. It is therefore possible that abnormalities in the endogenous ligand for PPAR-γ are responsible for this disease.

Finally, our results suggest that thiazolidinedione drugs might be effective for the treatment of insulin resistance and lipodystrophy in patients with lipoatrophic diabetes, given that PPAR-γ, the receptor for these agents, appears to be intact in such individuals.

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