Human leptin receptor gene in obese Japanese subjects: evidence against either obesity-causing mutations or association of sequence variants with obesity

N. Matsuoka¹, Y. Ogawa¹, K. Hosoda^{1,2}, J. Matsuda¹, H. Masuzaki¹, T. Miyawaki¹, N. Azuma¹, K. Natsui¹, H. Nishimura¹, Y. Yoshimasa¹, S. Nishi¹, D. B. Thompson³, K. Nakao¹

¹ Department of Medicine and Clinical Science, Kyoto University Graduate School of Medicine, Kyoto, Japan

² Kyoto University Graduate School of Human and Environmental Studies, Kyoto, Japan

³ Clinical Diabetes and Nutrition Section, National Institute Diabetes and Digestive and Kidney Diseases, National Institute of Health, Phoenix, Arizona, USA

Summary Leptin is an adipocyte-derived blood-borne satiety factor that acts on its cognate leptin receptor (Ob-R) in the hypothalamus, thereby regulating food intake and energy expenditure. To explore whether mutations in the Ob-R gene cause obesity in humans, we have searched for mutations in the gene for Ob-Rb, a biologically active receptor isoform, in obese Japanese subjects. We have also examined associations between such mutants and obesity in the Japanese. Genomic DNAs were used as templates in polymerase chain reaction (PCR) with primers selected to amplify exons 2 to 20 of the human Ob-Rb gene. Direct sequence analysis of the PCR products revealed 7 nucleotide sequence variants (Lys109Arg, Gln223Arg, Ser343Ser, Ser492Thr, Lys656Asn, Ala976Asp, and Pro1019Pro) in the Ob-Rb coding region from 17 obese Japanese subjects with a family history of obesity (BMI 39.3 ± 8.4 kg/m²). No

missense and nonsense mutations were found such as those in Zucker fatty (*fa/fa*) rats and Koletsky (*fa^k/fa^k*) rats. Nucleotide substitutions occurred at relatively high frequencies at codons 109, 223, 976, and 1019 (79, 91, 100, and 85 %, respectively). Allele frequency of each variant determined by PCR-RFLP and PCR-single strand conformation polymorphism analyses showed no significant differences between 47 obese (BMI 35.1 ± 6.5 kg/m²) and 68 non-obese (BMI 21.6 ± 2.2 kg/m²) subjects. The present study represents the first report of sequence variants of the Ob-Rb gene in the Japanese and provides evidence against either obesity-causing mutations or association of sequence variants with obesity in obese Japanese subjects. [Diabetologia (1997) 40: 1204–1210]

Keywords Leptin, leptin receptor, Ob-R, obesity, sequence variant.

Obesity arises from complex interactions between genetic and environmental factors, conferring a higher risk of cardiovascular and metabolic disorders such as hypertension, hyperlipidaemia, and non-insulin-dependent diabetes mellitus (NIDDM) [1]. The

molecular pathogenesis of obesity is, however, largely unknown. The discovery of leptin, the *obese* (*ob*) gene product, has provided a useful molecular tool to elucidate the pathophysiology of obesity and obesity-associated diabetes [2–7]. It has been recognized that leptin is an adipocyte-derived blood-borne satiety factor that is involved in the regulation of food intake and energy expenditure [8]. The leptin receptor (Ob-R), the *diabetes* (*db*) gene product, is a single transmembrane protein that belongs to the gp130 family of cytokine receptor superfamily [9–12]. The receptor has several alternatively spliced isoforms, one of which, a biologically active Ob-Rb isoform, is expressed most abundantly in the hypothalamus [9, 11, 13–15]. Leptin has been shown to activate signal transducers and activators of transcription (STATs) in the hypothalamus, suggesting that the

Received: 12 March 1997 and in revised form: 19 May 1997

Corresponding author: Y. Ogawa M. D., Ph. D., Department of Medicine and Clinical Science, Kyoto University Graduate School of Medicine, 54 Shogoin Kawahara-cho, Sakyo-ku, Kyoto 606, Japan

Abbreviations: Ob-R, leptin receptor; PCR, polymerase chain reaction; *db*, *diabetes*; *fa*, *fatty*; *fa^k*, *fatty Koletsky*; *ob*, *obese*; PCR-SSCP, PCR-single strand conformation polymorphism; STAT, signal transducer and activator of transcription; JAK, Janus activated kinase; NIDDM, non-insulin-dependent diabetes mellitus.

Table 1. Clinical profiles and polymorphic nucleotides in the Ob-Rb gene from 17 obese subjects

Sex	Age (years)	BMI ^a (kg/m ²)	Complications	Lys109Arg <u>AAG/AGG</u>	Gln223Arg <u>CAG/CGG</u>	Ser343Ser <u>AGT/AGC</u>	Ser492Thr <u>AGT/ACT</u>	Lys656Asn <u>AAG/AAC</u>	Ala976Asp <u>GCC/GAC</u>	Pro1019Pro <u>CCG/CCA</u>
F	36	32.2	—	A/G	G/G	T/T	G/G	G/G	A/A	G/A
F	22	34.7	HT	A/G	G/G	T/C	G/G	G/G	A/A	G/G
F	30	55.3	NIDDM	G/G	G/G	T/T	G/G	G/G	A/A	A/A
M	25	38.1	NIDDM	G/G	G/G	T/T	G/G	G/G	A/A	A/A
F	55	29.9	NIDDM	G/G	G/G	T/T	G/G	G/G	A/A	A/A
F	55	27.5	—	G/G	A/G	T/T	G/G	G/G	A/A	G/A
F	28	34.7	—	A/G	G/G	T/C	G/G	G/G	A/A	A/A
F	46	36.8	Graves' disease, HL	G/G	G/G	T/T	G/G	G/G	A/A	A/A
F	20	33.2	IGT	A/G	G/G	T/T	G/C	G/G	A/A	A/A
M	50	42.1	—	G/G	G/G	T/T	G/G	G/G	A/A	A/A
M	32	35.6	HL	G/G	G/G	T/T	G/G	G/G	A/A	A/A
M	17	39.1	—	A/G	G/G	T/T	G/G	G/G	A/A	A/A
F	16	39.7	HT	G/G	G/G	T/T	G/G	G/G	A/A	A/A
M	25	51.1	hypothyroidism hypogonadism, CHF	A/G	A/A	T/C	G/G	G/C	A/A	G/A
M	15	45.1	—	A/G	G/G	T/T	G/G	G/G	A/A	A/A
F	43	57.5	IGT, HT, HL	G/G	G/G	T/T	G/G	G/G	A/A	A/A
F	32	36.1	eating disorders	G/G	G/G	T/T	G/G	G/C	A/A	A/A

Polymorphic nucleotides are underlined. IGT, impaired glucose tolerance; HT, hypertension; HL, hyperlipidaemia; CHF, congestive heart failure; —, no complications. ^a BMI values are determined at the time of genotyping

hypothalamus is a major target site of leptin [16, 17]. Indeed, we and others have recently demonstrated that leptin exerts its satiety effect by acting directly on the hypothalamus [14, 16, 18, 19]. These findings suggest that leptin and Ob-R constitute an important feedback system linking the hypothalamus and adiposity, thus regulating energy homeostasis.

Synthesis and secretion of leptin are increased in several models of rodent obesity and in human obesity in proportion to disease severity [20–23]. These observations suggest that augmented production of leptin represents one of the deterrent mechanisms for the development of obesity. In some models of rodent obesity or human obesity, however, plasma leptin levels exceed those expected for the degree of adiposity or BMI [21, 22]. Since leptin causes a reduction in food intake and an increase in energy expenditure, thereby resulting in weight loss, it is speculated that obese animals and humans are resistant to endogenous leptin (a condition called leptin resistance) [4, 8, 24].

Genetic evidence has indicated that *ob/ob* mice develop marked obesity and obesity-related diabetes because of a failure to produce bioactive leptin due to a nonsense mutation of the *ob* gene [2]. No such mutations of the *ob* gene were, however, found in human obesity from several ethnic groups (the Caucasian, Pima Indians, and Japanese) [25–28]. It has been shown that Ob-Rb is missing in *db/db* mice due to a point mutation leading to its abnormal transcript [10, 11]. Recently, we and others have demonstrated that Ob-R is also mutated in genetically obese Zucker fatty (*fa/fa*) rats and obese spontaneously hypertensive Kolesky (*fa^k/fa^k*) rats [13, 29–31]. For analysis of the Ob-R gene in humans, previous studies revealed several

sequence polymorphisms in the Ob-Rb gene with no obesity-causing mutations in black African-Americans, Caucasians and Pima Indians [32, 33].

The present study was undertaken to search for mutations in the Ob-Rb gene in obese Japanese subjects and to assess whether such mutations are associated with obesity in the Japanese.

Subjects and methods

Subjects: The study groups consisted of 47 obese subjects (maximum BMI > 30, mean ± SEM, 35.1 ± 6.5 kg/m²; age 41.9 ± 16.6 years; NIDDM *n* = 13, impaired glucose tolerance *n* = 3, hypertension *n* = 6, hyperlipidaemia *n* = 6, osteoarthritis *n* = 4, hypothyroidism *n* = 2, eating disorders *n* = 2) and 68 non-obese subjects without any endocrine and metabolic disorders (maximum BMI < 25, 21.6 ± 2.2 kg/m²; age 45.9 ± 8.0 years). The present study was conducted with informed consent and approved by the ethical committee on human research of Kyoto University Graduate School of Medicine.

Polymerase chain reaction (PCR)-direct sequence analysis: Genomic DNA was extracted from peripheral blood leukocytes according to the standard procedure. Genomic DNAs from 17 obese Japanese subjects (Table 1) with a family history of obesity (maximum BMI > 30, 39.3 ± 8.4 kg/m²; age 32.6 ± 1.3 years) were used as templates in PCR with upstream and downstream primers selected to amplify sequences corresponding to exons 2 to 20 of the human Ob-Rb gene according to the GENBANK databank (U59247–U59263) (Fig. 1 and Table 2) [33]. The reaction profiles were as follows; denaturation at 94°C for 45 s, annealing at 55°C for 45 s, and extension at 72°C for 90 s, for 30 cycles. All PCR products were analysed by agarose gel electrophoresis, purified, and sequenced using automated protocols of fluorescent detection of dideoxy termination products of a model 373B DNA autosequencing system (Applied Biosystems Inc., Foster City, Calif., USA).

Fig 1. Schematic representation of the Ob-Rb gene (upper panel) and its protein structure (lower panel). Genomic organization of the Ob-Rb gene is based on the GENBANK database data (U59247-U59263) [33]. The extracellular, transmembrane, and intracellular regions of the receptor are indicated by shaded, open, and hatched areas, respectively. Locations of two cytokine motifs (GXWSXWS) and Box 1, Box 2, and Box 3 consensus sequences are also depicted. Seven sequence variants are indicated above the Ob-Rb gene structure. The *db*, *fa*, and *fa^k* mutations found in genetically obese rodents [10–13, 29–31] are marked by *, †, § respectively

PCR-RFLP analysis: PCR-RFLP analysis was performed for genotyping at codons 223, 492, 976, and 1019 (Fig.2). The PCR primers were sequence-specific and/or mutated for convenience (Fig.2 and Table 3). The PCR products were digested with *Msp* I, *Pst* I, *Hae* III, and *Hinc* II at codons 223, 492, 976, and 1019, respectively, and were analysed as described [13].

PCR-single strand conformation polymorphism (PCR-SSCP) analysis: PCR-SSCP analysis was used for genotyping at codons 109, 343, and 656 (Fig.3). Fragments encompassing the polymorphic sites were amplified by PCR with sequence-specific primers (Table 3). After amplification, 3 µl of each PCR reaction plus 15 µl of 95 % formamide 2 mmol/l EDTA loading solution was heat denatured at 85 °C for 10 min. Denatured samples were loaded on 10 % nondenaturing polyacrylamide gels with or without 5 % glycerol. Electrophoresis was done at room temperature or 4 °C at a constant current of 10–20 mA for 10–16 h. The gels were stained with SYBR Green II (FMC BioProducts, Rockland, Me., USA) according to the manufacturer's recommendations.

Statistical analysis: Values are expressed as the mean ± SEM. The statistical differences between groups in allele frequencies were assessed by Fishers's exact test. The *p* values less than 0.05 were considered significant. All statistical procedures were performed with a statistical software package (STAT VIEW J4.02; Abacus Concepts, Inc., Berkeley, Calif., USA).

Table 2. Sequences of primer pairs for PCR-direct sequence

	Upstream primers	Downstream primers
Exons		
2	5'-AGAAGGTTATGCAGCCATNCTACTATC-3'	5'-CCACAAAGGGACTGTGTTTCATAAACTG-3'
3	5'-TAAATTNAGAGACTTATCTATAATCCC-3'	5'-TAACTAGAAATAGGAAATCTGTAGC-3'
4	5'-CACATTGTACAATGGAAGCACAAAGTT-3'	5'-TGTTAAAATCATAGCCATAAGACATCT-3'
5	5'-TTTTTTTTTAATTCAGATGCAAACTGGA-3'	5'-TGCAAGAGGTTTATAATCTACTTTCCGT-3'
6	5'-ACCCTTTAAGCTGGGTGTCCAAATAG-3'	5'-AGCTAGCAAATATTTTGTAAAGCAATT-3'
7	5'-TGACTTTATTTTATTCAGCTATAATTG-3'	5'-GTAATTGCTATGGGACTTAAAGAGGGTC-3'
8	5'-TTTGTAGTAACGGTTCACATCAACTTG-3'	5'-CAAGTTCAGGAAATATATTTCCCTCCC-3'
9–10	5'-TCATTGAATTTTTGGAGATTTTATGC-3'	5'-CAGGAAGCAAATAATCTGTAAGAC-3'
11	5'-GACTGCTGTTTTAAACAACAAATCAG-3'	5'-CTGCATACAAATCTGCTAACACAAATG-3'
12	5'-TGAAAATATAACAAATGTTTTAGGC-3'	5'-TTTATGCCAATAAAATTAATCTAATGC-3'
13–14	5'-GGTTTAAATAAAATGTACTTCAGGGC-3'	5'-TGGACCATGAAGTCTTTTAAAGAGTA-3'
15	5'-GAGACTGTGGCAGAGGCAAACATATC-3'	5'-ATTGCAGGCTGCTTGAAAGATAATTTA-3'
16	5'-TTCCCTTAGTAGGTTATAAGTTCCTC-3'	5'-TTTTTGAAGTTTTCATTAAGTGGCTAT-3'
17	5'-TTTGGAACCTCCCTTGATAATTTAATC-3'	5'-CCTCACCATGAAAAATCTACGAAGAC-3'
18	5'-AGAGATTGTGATGAATTCAGAAAATG-3'	5'-TTAAATCAGGGTTGAATACGCGTAAG-3'
19	5'-CGGAGGCATAGTTGATCTGGTGGCTAA-3'	5'-GCGCTTGAAATTTGTTTCTCTGATT-3'
20	5'-CAAACCTCCATTTTCTGCCAGTATGAC-3'	5'-CATTGGTAGGCTTATGAAGGCTTTTAC-3'

Fig. 2A–D. Genotyping of the polymorphic alleles in the Ob-Rb gene by PCR-RFLP at codons 223 (nucleotide 668) (A), 492 (1475) (B), 976 (2927) (C), and 1019 (3057) (D), where A to G, G to C, C to A, and G to A transversions occurred, respectively. (Left) Schematic representation of wild and polymorphic alleles. PCR-RFLP analyses were performed using upstream and downstream sequence-specific primers (for Gln223Arg and Ala976Asp) (A and C) or upstream sequence-specific and downstream mutated primers (for Ser492Thr and Pro1019Pro) (B and D) as described in Subjects and methods. The restriction sites created (*Msp* I (A) and *Pst* I (B)) and those abolished (*Hae* III (C) and *Hinc* II (D)) in polymorphic alleles were depicted. Polymorphic nucleotides are shown in bold. (Right) Gel electrophoresis of the PCR products after digestion with restriction enzymes. The cytosine allele reported at codon 976 [9] was not observed in the present study (Table 4). Genotypes are indicated by N/N (N = A, C, G). M; DNA size marker ϕ X174-*Hae* III digest. U; uncut PCR products. bp; basepair

Results

Identification of sequence variants in obese subjects. Direct sequence analysis of the PCR products revealed 7 nucleotide sequence variants in the Ob-Rb coding region from 17 obese Japanese subjects. In the present study, there were no such mutations as

those found in Zucker fatty (*fa/fa*) rats and Koletsky (*fa^k/fa^k*) rats (Gln269Pro and Tyr763Stop, respectively) (Fig. 1) [13, 29–31]. At nucleotide 326 (codon 109) in exon 4, an A to G transversion resulted in Lys109Arg substitution. Another A to G transversion was found at nucleotide 668 (codon 223) in exon 6, producing Gln223Arg, which was reported previously [32, 33]. This substitution created a new *Msp* I restriction site (Fig. 2A). In all 17 obese subjects examined at codon 976 in exon 20, Ala was changed to Asp (Ala976Asp) due to a transversion from C to A at nucleotide 2927, which abolished an *Hae* III site (Fig. 2C). At nucleotide 3057 (codon 1019), a G to A transversion was found, which caused no amino acid substitution. These 4 polymorphic alleles occurred at high frequencies in 17 obese subjects examined (79, 91, 100, and 85% for Lys109Arg, Gln223Arg, Ala976Asp, and Pro1019Pro, respectively). Three other substitutions, Ser343Ser (T to C at nucleotide 1029), Ser492Thr (G to C at nucleotide 1475), and Lys656Asn (G to C at nucleotide 1968) were observed at relatively low frequencies (1, 2, and 3 heterozygotes out of 17 subjects, respectively). No other sequence variants were found in the Ob-Rb coding region compared to the human Ob-Rb cDNA sequences reported previously [9].

Allele frequencies in obese and non-obese subjects. To investigate whether the Ob-Rb gene polymorphisms identified are related to obesity in the Japanese, we examined allele frequencies of sequence variants in 47 obese subjects compared to those in 68 non-obese subjects (Table 4). Genotypes of polymorphic sites were determined by PCR-RFLP at codons 223, 492, 976, and 1019 (Fig. 2) and by PCR-SSCP at codons 109, 343, and 656 (Fig. 3). In 47 obese subjects (BMI 35.1 ± 6.5 kg/m²), allele frequencies of polymorphic sites at codons 109, 223, and 1019 (Gly109Arg, Gln223Arg, and Pro1019Pro) were 82, 87, and 88% respectively. In 68 non-obese subjects (BMI 21.6 ± 2.2 kg/m²), they were 77, 84, and 88%, respectively. No significant differences in frequency of each allele were observed between obese and non-obese groups ($p = 0.41$, 0.57 , and > 0.99 , respectively). Allele frequencies in sequence variants at codons 109, 223, and 1019 in all the subjects examined were 79, 85, and 88% respectively. The sequence variants were also reported in Pima Indians (58, 75, and 85%, respectively) and Caucasians (28, 44, and 37%, respectively) [33]. The Ala976Asp variant was observed in all the subjects analysed (Table 4); no one carried the previously reported Ala allele [9]. Other polymorphisms (Ser343Ser, Ser492Thr, Lys656Asn) were detected with no significant differences in allele frequency between obese and non-obese groups (Ser343Ser 11 vs 9%, $p = 0.66$; Ser492Thr 1 vs 0%, $p = 0.41$; Lys656Asn 10 vs 14%, $p = 0.41$). In the present study, sequence variants at codons 109,

Fig 3A-C. Genotyping of polymorphic sites in the Ob-Rb gene by PCR-SSCP at codons 109 (nucleotide 326) (**A**), 343 (1029) (**B**), and 656 (1968) (**C**), where G to A, T to C, and G to C transversions occurred, respectively. Genotypes are indicated by N/N (N = A, C, G, T). M; undegraded DNA size marker ϕ X174-*Hae* III digest

223, 656, 976, and 1019 were found to be homozygous in both obese and non-obese subjects. Sequence variants at codons 343 and 492 were found to be only heterozygous.

Discussion

We have searched here for mutations in the Ob-Rb gene from 17 obese Japanese subjects and found 7 sequence variants in its coding region. In the present study, 4 amino acid substitutions (Lys109Arg, Gln223Arg, Ser492Thr, and Lys656Asn) were located in the extracellular region of Ob-Rb. An amino acid substitution (Ala976Asp) was found in the intracellular region. Ob-Rb contains several functionally important domains; it contains 2 cytokine motifs (GXWSXWS) in the extracellular region (Fig. 1), which are implicated in the ligand binding [9]. In the intracellular region, it contains Box 1, Box 2, and Box 3 consensus sequences, which are involved in interactions with Janus activated kinase (JAK) and STATs [9, 34]. No sequence variants were, however, located in such functionally defined domains (Fig. 1). Genetic evidence showed that *ob*, *db*, *fa*, and *fa^k*, all

of which code for leptin or Ob-R, are autosomal recessive mutations in rodents [2, 10–13, 29–31]. In the present study, 4 amino acid substitutions (Lys109Arg, Gln223Arg, Lys656Asn, and Ala976Asp) were found to be homozygous but were distributed in both obese and non-obese subjects. The amino acid change at codon 492 (Ser492Thr) was found to be only heterozygous. These observations, taken together, suggest that, unlike genetically obese animals, sequence variants identified in the present study are not obesity-causing mutations. The discussion above, however, does not rule out the possibility of a compound heterozygotic mechanism [35], in which a missense mutation in one allele may cause obesity in combination with an as yet unidentified mutation in the other allele (possibly to be found in the promoter and/or intron sequences).

The present study revealed no significant differences in allele frequency of each of 7 sequence variants between obese and non-obese subjects. These results are consistent with a recent report that sequence variants in the Ob-Rb coding region are not linked with obesity in Pima Indians and Caucasians [33]. The findings, taken together, suggest that sequence variants in the Ob-Rb coding region are not strongly associated with obesity. In this regard, the sequence variants identified may not be used as markers for other mutations involved in the development of obesity.

It was reported that *db/db* mice carry a point mutation in intron 19 of the Ob-Rb gene (Fig. 1), which results in an abnormal splice acceptor site, thereby leading to the absence of Ob-Rb in these animals [10, 11]. Considine et al. [32] previously showed in 15 African-Americans no mutations such as those found in *db/db* mice. Furthermore, the authors found no correlation between the amount of hypothalamic Ob-Rb mRNA and BMI. In the present study, we have mainly examined the human Ob-Rb coding region and have not analysed the promoter and intron sequences of the Ob-Rb gene. Whether mutations occur in the promoter and/or intron sequences of the Ob-Rb gene from obese Japanese subjects must await further investigations.

Table 3. Sequences of primer pairs for PCR-RFLP and PCR-SSCP

	Upstream primers	Downstream primers
PCR-RFLP		
Gln223Arg	5'-ACCCTTTAAGCTGGGTGTCCCAAATAG-3'	5'-AGCTAGCAAATATTTTTGTAAGCAATT-3'
Ser492Thr	5'-GTATACTAATTGACTATTTTTGTATCT-3'	5'-GGCTGGAAAATGCATTCATAAAACCTGCA-3' ^a
Ala976Asp	5'-ACTGTGGTCTCTCTACTTTC-3'	5'-TTTGCTAGAGAAGCACTTGG-3'
Pro1019Pro	5'-GAAACTGGTGAAGAACAAGGGCTTATAAAT-3'	5'-CCATGAGCTATTAGAGAAAGAATCCGCTCAA-3' ^a
PCR-SSCP		
Lys109Arg	5'-CCTGCTGGACTCTCAAAGAA-3'	5'-TGTTAAATCATAGCCATAAGACATCT-3'
Ser343Ser	5'-TCATTGAATTTTTGGAGATTTTATGC-3'	5'-TGCTAACATGATCACTCACA-3'
Lys656Asn	5'-ATACACAACCTGTTCATTTGCAGT-3'	5'-TGGACCATGAAGTCTTTTAAAGAGTA-3'

^a To make restriction sites, these primers are synthesized with mutations (underlined)

Table 4. Allele frequencies of polymorphic sites

Codons	109	223	343	492	656	976	1019
Obese (<i>n</i> = 47)	A 0.181 G 0.819	A 0.128 G 0.872	T 0.894 C 0.106	G 0.989 C 0.011	G 0.904 C 0.096	C 0.00 A 1.00	G 0.117 A 0.883
Non-obese (<i>n</i> = 68)	A 0.235 G 0.765	A 0.162 G 0.838	T 0.912 C 0.088	G 1.00 C 0.00	G 0.860 C 0.140	C 0.00 A 1.00	G 0.118 A 0.882
<i>p</i> value	0.41	0.57	0.66	0.41	0.41	> 0.99	> 0.99

Nucleotides reported [9] and those identified in the present study are in upper and lower portions, respectively

In conclusion, we have identified 7 sequence variants in the human Ob-Rb coding region and provided evidence against either obesity-causing mutations or association of sequence variants with obesity in obese Japanese subjects. The present study represents the first report of sequence variants in the Ob-Rb gene in the Japanese, thereby providing a genetic basis for the Ob-R gene research in humans.

Acknowledgements. We would like to thank Dr. T. Shirakawa of the Lung Research Unit, Churchill Hospital, University of Oxford for valuable advice and critical reading of this manuscript, Dr. T. Matsuo and Dr. A. Sugawara of Osaka Saiseikai Nakatsu Hospital, Dr. M. Imura of Yaizu Municipal General Hospital, Dr. Y. Hasegawa of Tokyo Metropolitan Kiyose Children's Hospital, Dr. S. Nakaishi of Osaka Red Cross Hospital, Y. Harano of the National Cardiovascular Center, Dr. Y. Toda of Kansai Medical College, and Dr. M. Okubo of Toranomon Hospital for collecting genomic DNA samples. The authors also thank Ms. K. Hiramatsu and Ms. C. Kawahara for their secretarial assistance. This work was supported in part by research grants from the Japanese Ministry of Education, Science and Culture, the Japanese Ministry of Health and Welfare, Yamanouchi Foundation for Research on Metabolic Disorders, Uehara Memorial Foundation, and a grant for diabetes research for Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan.

References

1. Björntorp P, Brodoff BN (1992) Obesity. JB Lippincott Company, Philadelphia
2. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM (1994) Positional cloning of the mouse *obese* gene and its human homologue. *Nature* 372: 425–432
3. Spiegelman BM, Flier JS (1996) Adipogenesis and obesity: rounding out the big picture. *Cell* 87: 377–389
4. Caro JF, Sinha MK, Kolaczynski JW, Zhang PL, Considine RV (1996) Leptin: The tale of an obesity gene. *Diabetes* 45: 1455–1462
5. Ogawa Y, Masuzaki H, Isse N et al. (1995) Molecular cloning of rat *obese* cDNA and augmented gene expression in genetically obese Zucker fatty (*fa/fa*) rats. *J Clin Invest* 96: 1647–1652
6. Masuzaki H, Ogawa Y, Isse N et al. (1995) Human *obese* gene expression: Adipocyte-specific expression and regional differences in the adipose tissue. *Diabetes* 44: 855–858
7. Taylor SI, Barr V, Reitman M (1996) Does leptin contribute to diabetes caused by obesity? *Science* 274: 1151–1152
8. Campfield LA, Smith FJ, Guisez Y, Devos R, Burn P (1995) Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. *Science* 269: 546–549
9. Tartaglia LA, Dembski M, Weng X et al. (1995) Identification and expression cloning of a leptin receptor, OB-R. *Cell* 83: 1263–1271
10. Chen H, Charlat O, Tartaglia LA et al. (1996) Evidence that the diabetes gene encodes the leptin receptor: identification of a mutation in the leptin receptor gene in *db/db* mice. *Cell* 84: 491–495
11. Lee GH, Proenca R, Montez JM et al. (1996) Abnormal splicing of the leptin receptor in *diabetic* mice. *Nature* 379: 632–635
12. Chua SC Jr, Chung WK, Wu-Peng XS et al. (1996) Phenotypes of mouse *diabetes* and rat *fatty* due to mutations in the OB (leptin) receptor. *Science* 271: 994–996
13. Takaya K, Ogawa Y, Isse N et al. (1996) Molecular cloning of rat leptin receptor isoform complementary DNAs – identification of a missense mutation in Zucker fatty (*fa/fa*) rats. *Biochem Biophys Res Commun* 225: 75–83
14. Woods AJ, Stock MJ (1996) Leptin activation in hypothalamus. *Nature* 381: 745
15. Mercer JG, Hoggard N, Williams LM, Lawrence CB, Hannah LT, Trayhurn P (1996) Localization of leptin receptor mRNA and the long form splice variant (Ob-Rb) in mouse hypothalamus and adjacent brain regions by in situ hybridization. *FEBS Lett* 387: 113–116
16. Vaisse C, Halaas JL, Horvath CM, Darnell JE Jr, Stoffel M, Friedman JM (1996) Leptin activation of Stat 3 in the hypothalamus of wildtype and *ob/ob* mice but not *db/db* mice. *Nature Genet* 14: 95–97
17. Chikardi N, Ziegler S, Wiestner A, Stoffel R, Heim MH, Skoda RC (1996) Defective STAT signaling by the leptin receptor in *diabetic* mice. *Proc Natl Acad Sci USA* 93: 6231–6235
18. Satoh N, Ogawa Y, Katsuura G et al. (1997) The arcuate nucleus as a primary site of satiety effect of leptin in rats. *Neurosci Lett* 224: 149–152
19. Jacob RJ, Dziura J, Medwick MB et al. (1997) The effect of leptin is enhanced by microinjection into the ventromedial hypothalamus. *Diabetes* 46: 150–152
20. Considine RV, Sinha MK, Heinman ML et al. (1996) Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med* 334: 292–295
21. Hosoda K, Masuzaki H, Ogawa Y et al. (1996) Development of radioimmunoassay for human leptin. *Biochem Biophys Res Commun* 221: 234–239
22. Hiraoka J, Hosoda K, Ogawa Y et al. (1997) Augmentation of *obese* (*ob*) gene expression and leptin secretion in obese spontaneously hypertensive rats (obese SHR or Koletsky rats). *Biochem Biophys Res Commun* 231: 582–585

23. Maffei M, Halaas J, Ravussin E et al. (1995) Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. *Nature Med* 1: 1155–1161
24. Frederich RC, Hamann A, Anderson S, Lollmann B, Lowell BB, Flier JS (1995) Leptin levels reflect body lipid content in mice: evidence for diet-induced resistance to leptin action. *Nature Med* 1: 1311–1314
25. Maffei M, Stoffel M, Barone M et al. (1996) Absence of mutations in the human *OB* gene in obese/diabetic subjects. *Diabetes* 45: 679–682
26. Considine RV, Considine EL, Williams CJ et al. (1995) Evidence against either a premature stop codon or the absence of obese gene mRNA in human obesity. *J Clin Invest* 95:2986–2988
27. Norman RA, Leibel RL, Devoto M et al. (1996) Linkage of obesity and energy metabolism to markers flanking homologous of rodent obesity genes is not evident in Pima Indians. *Diabetes* 45: 1299–1232
28. Niki T, Mori H, Tamori Y et al. (1996) Human obese gene molecular screening in Japanese and Asian Indian NIDDM patients associated with obesity. *Diabetes* 45: 675–678
29. Chua SC Jr, White D, Wu-Peng X et al. (1996) Phenotype of *fatty* due to Gln269Pro mutation in the leptin receptor (*Lepr*). *Diabetes* 45: 1141–1143
30. Phillips MS, Liu Q, Hammond HA et al. (1996) Leptin receptor missense mutation in the *fatty* Zucker rat. *Nature Genet* 13: 18–19
31. Takaya K, Ogawa Y, Hiraoka J et al. (1996) Nonsense mutation of leptin receptor in the obese spontaneously hypertensive Koletsky rat. *Nature Genet* 14: 130–131
32. Considine RV, Considine EL, Williams CJ, Hyde TM, Caro JF (1996) The hypothalamic leptin receptor in humans: identification of incidental sequence polymorphisms and absence of the *db/db* mouse and *fa/fa* rat mutations. *Diabetes* 45: 992–994
33. Thompson DB, Ravussin E, Bennett PH, Bogardus C (1997) Structure and sequence variation at the human leptin receptor gene in lean and obese Pima Indians. *Hum Mol Genet* 6: 975–679
34. Kishimoto T, Akira S, Taga T (1994) Cytokine signal transduction. *Cell* 76: 253–262
35. McConkey EH (1993) Human genetics: the molecular revolution, Jones and Bartlett Publishers Inc., Boston, London