Diabetologia (1993) 36: 414–422

Diabetologia

A mutation ($Trp^{1193} \rightarrow Leu^{1193}$) in the tyrosine kinase domain of the insulin receptor associated with type A syndrome of insulin resistance

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Summary. We evaluated a 35-year-old diabetic male patient with type A insulin resistance, showing acanthosis nigricans. Insulin binding to the patient's Epstein-Barr-virus transformed lymphocytes was mildly reduced. The maximal insulin-stimulated autophosphorylation of the insulin receptor from the patient's transformed lymphocytes was decreased to 45 % of that from the control subjects. On examination, the biological activities of insulin and insulin-like growth factor I in the patient's cultured fibroblasts, insulin sensitivity of amino isobutyric acid uptake and thymidine incorporation was decreased, but insulin-like growth factor I action was normal. The sequence analysis of amplified genomic DNA revealed that the patient was heterozygous for a mutation substituting Leu for Trp at codon 1193 in exon 20 of the insulin receptor gene. The patient's mother and sister were also heterozygous for a mutation in the insulin receptor gene that substituted Leu for Trp^{1193} in the β subunit of the receptor. Therefore, the mutation causes insulin resistance in a dominant fashion. They were less hyperglycaemic and more hyperinsulinaemic than the proband after glucose loading. The mother had diabetes mellitus but did not show acanthosis nigricans, while the sister did not have diabetes and showed acanthosis nigricans. These results suggest that this mutation causes defective tyrosine kinase activity of the insulin receptor, which results in insulin resistance. Insulin action and phenotypic appearance may be mediated by different factors.

Key words: Insulin receptor, mutation, tyrosine kinase activity.

The insulin receptor is a heterotetrameric membrane protein made up of two α and two β subunits. When insulin binds to the α-subunits, this activates the intrinsic tyrosine kinase of the β-subunit to phosphorylate tyrosine residues in the receptor and also to phosphorylate cellular protein substrates. Activation of the protein tyrosine kinase in the β-subunit of the insulin receptor is thought to be responsible for mediating insulin action [1].

Various mutations have been identified in the insulin receptor genes of the patients with genetic syndromes associated with extreme insulin resistance [1]. These studies have contributed to our understanding of the insulin receptor structure-function relationship.

We reported the first mutation ($Arg^{735} \rightarrow Ser$) to decrease the affinity of insulin binding in the patient with type A extreme insulin resistance [2, 3]. The mutation changed the structure of the cleavage site from Arg-Lys-Arg-Arg to Arg-Lys-Arg-Ser resulting in unprocessed insulin receptor, for which insulin had a decreased affinity.

In this report, we have characterized a new case of insulin receptor disease due to a defect in tyrosine kinase activities of the insulin receptor. We have demonstrated that the patient is heterozygous for a mutation in the insulin receptor gene that substitutes Leu for Trp^{1193} in the kinase domain of the receptor, which is inherited in a dominant fashion.

Subjects, materials and methods

Clinical characteristics of the patient

The patient was a product of a first cousin-consanguineous marriage and was in good health until he was 35 years old, when glycosuria was found on a routine health examination. His weight was 54 kg and his height was 158 cm. His insulin levels in the fasting state and after glucose loading were 58 μU/ml and 500 μU/ml, respectively and fasting blood glucose was 8.4 mmol/l (Table 1). He had acanthosis nigricans in the axillae, cubital fossa, back and inguinal regions. Other endocrine tests showed no abnormalities. Neither anti-insulin antibody nor anti-insulin receptor antibody was detected. His mother and sister were insulin resistant, and their fasting insulin levels were 59 μU/ml and 55 μU/ml, respectively. His mother had diabetes mellitus but did not show acanthosis nigricans, while his sister did not have diabetes but showed acanthosis nigricans. They were more hyperinsulinaemic and less hyperglycaemic than the proband after

Table 1. Clinical characteristics of the family with insulin resistance studied

Family member	Age (years)	Glucose (mmol/l)		Insulin (μU/ml)	
		Fasting	1 h/2 h	Fasting	1 h/2 h
Proband	35	8.4	13.8/11.6	58	250/500
Sister	33	5.6	5.2/5.5	55	600/700
Father	65	7.7		11	
Mother	63	5.8	11.1/11.6	59	700/1000
Normal range		< 6.1	< 11.1/ < 7.7	< 20	

Glucose tolerance tests were performed using a standard oral glucose load (75 g). Plasma glucose and serum insulin concentration were measured after an overnight (12 h) fast and after glucose ingestion

glucose loading. His father who had died before the study, had Type 2 (non-insulin-dependent) diabetes with fasting insulin levels within normal range and the normal insulin binding to erythrocytes (Table 1). Informed consent was obtained from all study participants and the study protocol was approved by the Ethical Committee of the Toyama Medical and Pharmaceutical University.

Materials

Purified porcine insulin was a gift from Shimizu Pharmaceutical Co. (Shizuoka, Japan). Na [^{125}I], [gamma-^{32}P] ATP (1000–3000 Ci/mmol), α-[methyl-^{3}H] aminoisobutyric acid (AIB, 10–25 Ci/mmol), and [6-^{3}H] thymidine (> 15 Ci/mmol) were purchased from New England Nuclear (Boston, Mass., USA). RPMI 1640 medium, fetal bovine serum (FBS), and trypsin were purchased from Gibco Biological Co. (Grand Island, NY, USA). Fatty acid free bovine serum albumin (BSA) and polyethylene glycol (M 8000) were purchased from Sigma Chemical Co. (St. Louis, Mo., USA). Disuccinimidyl suberate (DSS) was purchased from Calbiochem-Behring Co. (La Jolla, Calif., USA). Wheat germ agglutinin (WGA) agarose and Reverse Transcriptase (M-MuLV) were purchased from Pharmacia PL Biochemical Co. (Uppsala, Sweden). Primer, Taq polymerase and T4 polynucletotide kinase were purchased from Takara Shuzo Co. (Kyoto, Japan). The Sequenase kit was purchased from Toyobo Co. (Osaka, Japan), and The Gene Clean II kit was from Funakoshi Co. (Tokyo, Japan). Human insulin receptor cDNA (HIR-cDNA) was kindly supplied by Dr G. I. Bell of the University of Chicago (Chicago, Ill., USA).

Methods

Transformed lymphocyte culture. Epstein-Barr virus (EBV) transformed lymphocyte cell lines were established from peripheral blood lymphocytes of the patients and 11 normal subjects who were 20–50-year-old male medical staff in the university, as described previously [4].

Skin fibroblast culture. Fibroblasts from the type A patient and the normal subjects were obtained by punch biopsies of the thigh and forearm, respectively. They were grown in Eagle's minimum essential medium (MEM) supplemented with 80 μg/ml penicillin, 80 μg/ml streptomycin, and 10 % FBS.

Insulin binding to transformed lymphocytes. The method of ^{125}I-insulin binding to transformed lymphocytes has been described elsewhere [5]. The binding study was carried out at 15 °C for 3.5 h.

Insulin binding to cultured fibroblast monolayers. The method of insulin binding to cultured fibroblast monolayers has been described previously [6]. The monolayer cells were incubated with ^{125}I-insulin at 4 °C for 16 h in the presence of various concentrations of unlabelled insulin.

Preparation of partially purified insulin receptor. The method of purifying insulin receptors, using WGA agarose column has been described previously [5].

Insulin binding to solubilized receptors. Lectin-purified insulin receptors were incubated with ^{125}I-insulin at 4 °C for 16 h in the presence of various concentrations of unlabelled insulin.

Affinity cross-linking study. Cross-linking of [^{125}I] insulin to transformed lymphocytes was performed as previously described [7].

Autophosphorylation of insulin receptor. The lectin-purified extracts were preincubated with various concentrations of insulin at 4 °C for 16 h. The phosphorylation was initiated by adding a solution composed of 20 mmol/l Mn acetate, 5 mmol/l CTP, 20 μmol/l ATP, and 30 μCi [γ-^{32}P] ATP. After incubation at 4 °C for 10 min, the reaction was terminated by adding stopping solution containing 0.2 % Triton X-100, 10 mmol/l EDTA, 100 mmol/l NaF, 20 mmol/l sodium pyrophosphate, 20 mmol/l ATP, and 20 mmol/l Hepes (pH 7.6) as previously described [5].

AIB uptake. AIB transport was measured by the method of Knight et al. [8]. After 3 h at 37 °C in the absence of CO_2, a solution of 0.5 μCi/ml [^{3}H] AIB and 8 μmol/l unlabelled AIB was added. After 12 min, AIB uptake was terminated by washing rapidly three times with ice-cold PBS and the radioactivity was determined.

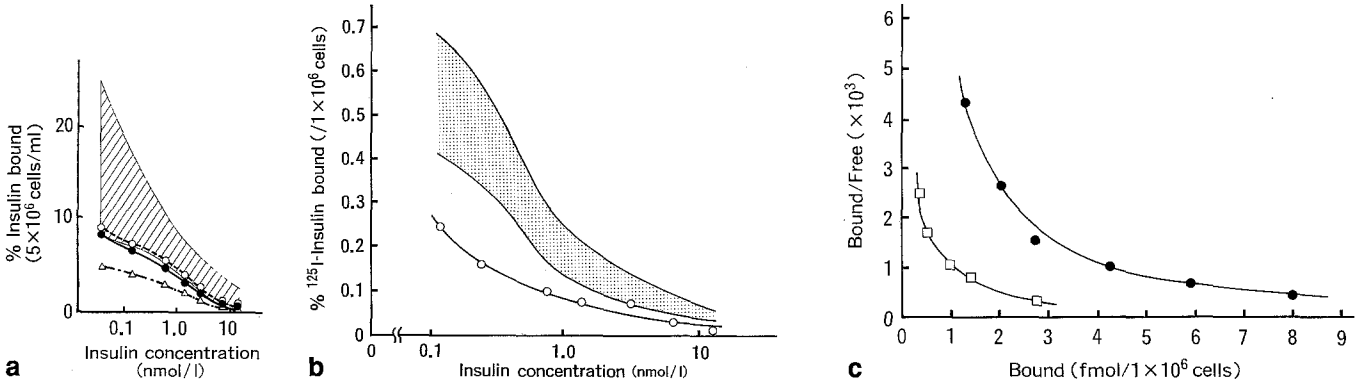

Fig. 1 (a–c). Insulin binding studies. **a** Insulin binding to Epstein-Barr virus-transformed lymphocytes from the patient (○), his mother (●) and his sister (△), plotted as the mean of five separate experiments. The shaded area represents the mean ± 2SD range of the binding study in 11 EBV-transformed lymphocyte cell lines derived from normal subjects. **b** Insulin binding to fibroblast monolayers. Specific binding of insulin to fibroblasts from the patient (○) is plotted as the means of five separate experiments. The shaded area represents the mean ± 2SD range of the binding study in seven control subjects. **c** Scatchard analysis of insulin binding to fibroblasts. ●: control subject, □: patient

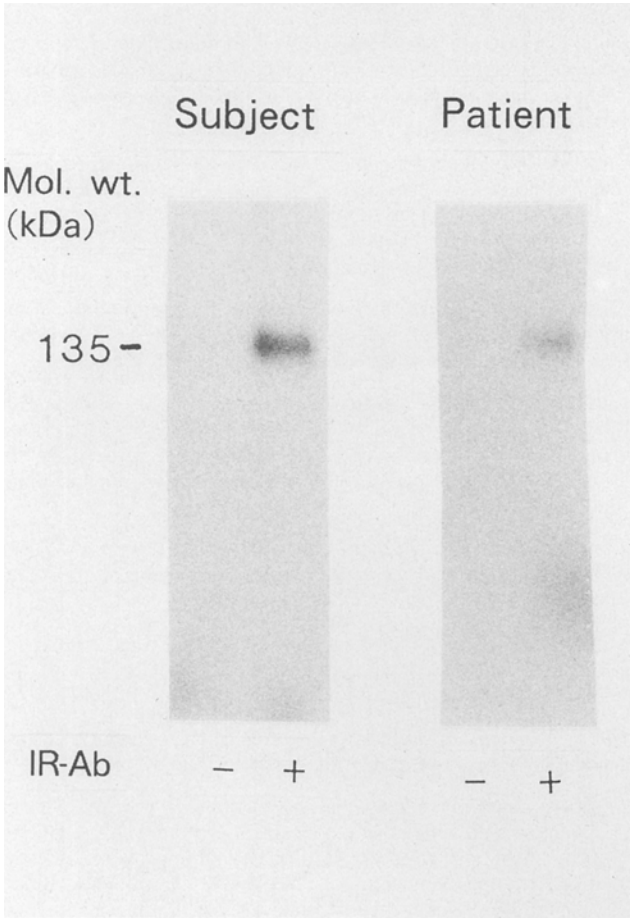

Fig. 2. Affinity cross-linking study in transformed lymphocytes. The transformed lymphocytes were incubated with ^{125}I-labelled insulin for 16 h at 4 °C and cross-linked with 1 mmol/l disuccinimidyl suberate. After immunoprecipitation with (+) or without (−) anti-insulin receptor antibody (IR-Ab) and Pansorbin, the sample was mixed with Laemmli buffer containing 100 mmol/l dithiothreitol and applied to SDS-PAGE

Thymidine incorporation. The method of Flier et al. [9] was used with some modifications. After various concentrations of insulin or insulin-like growth factor I (IGF-I) were incubated for 4 h, the fibroblasts were washed twice with PBS, twice with 10% trichloroacetic acid and 95% ethanol, the radioactivity was determined.

Northern blot analysis. Total RNA was extracted from transformed lymphocytes by the acid guanidium phenolchloroform method [10] and electrophoresed after denaturation with 0.5 mmol/l glyoxal. The RNA was blotted into nylon and hybridized by standard methods [11]. The probes were a 3.8-kilobase (kb) Sac I fragment of HIR-cDNA and an 0.9-kb Hpa II fragment of β-actin cDNA.

Isolation of genomic DNA and cDNA synthesis. Genomic DNA was isolated from peripheral blood leucocytes using standard techniques [12]. Total cellular RNA was from transformed lymphocytes isolated using the guanidinium thiocyanate-CsCl. cDNA was synthesized by the reverse transcriptase (RT) from total cellular RNA and randomized primer [13].

Direct sequencing of amplified genomic DNA. Exons of the insulin receptor gene were amplified by the polymerase chain reaction (PCR) catalysed by Taq DNA polymerase, using the patient's genomic DNA as template and primers chosen in the introns flanking each exon [14]. Amplified double-stranded DNA was analysed by electrophoresis through a 2% agarose gel. The DNA was denatured for 10 min at 100 °C and was then sequenced using Sequenase version 2.0. Amino acids of the insulin receptor are numbered according to the definition of Ebina et al. [15].

Allele-specific oligonucleotide hybridization. Genomic DNA including exon 20 of the insulin receptor gene was amplified from a normal control subject individual and the proband. The amplified double-stranded DNA (200 ng) was analysed by electrophoresis through a 2% agarose gel and transferred to nylon membranes. The DNA blots were hybridized with ^{32}P-labelled synthetic oligonucleotides corresponding to either the normal probe 5′-CTGA-CATGTGGTGAGTTG-3′ or mutant probe 5′-CTGA-CATGTTGTGAGTTG-3′ [16]. Fragments of cDNA were amplified from total cellular RNA by RT-PCR with sense primer, 5′-GGCACCGGAGTCCCTGAAG-3′ and antisense primer, 5′-AACGACACCTCTGGAAAGC-3′ to amplify the region spanning codon 1193. The DNA blots were hybridized with ^{32}P-labelled synthetic oligonucleotides corresponding to either the normal probe 5′-CTGACATGTGGTCCTTTG-3′ or mutant probe 5′-CTGACATGTTGTCCTTTG-3′ [16].

Results

Insulin binding to transformed lymphocytes and fibroblast monolayers

Insulin binding to transformed lymphocytes from the patient was 60% of the mean value of the normal control subjects, but was still within the normal range. Insulin binding to transformed lymphocytes from the mother and sister was decreased to 60% and 33% of that of the normal control subjects, respectively (Fig. 1 a). Insulin binding to fibroblast monolayers from the patient was also decreased to 50% of that of the normal control subjects (Fig. 1 b). Scatchard analysis revealed that low insulin binding in the patient was due to decreased receptor number without a change in affinity (Fig. 1 c).

Affinity cross linking study

By affinity labelling, we identified a ^{125}I-labelled band with a molecular weight of approximately 135 kDa, representing the α-subunit of the insulin receptor. The density of the patient's band was less when compared with the control band, and this suggested that the patient had a decreased receptor number in the cell surface (Fig. 2).

Autophosphorylation study

The maximal insulin-stimulated autophosphorylation of insulin receptor from the patient's transformed lymphocytes was decreased to 45% of that from the control subject (Fig. 3). Similarly, the maximal insulin stimulated autophosphorylation of insulin receptor from the mother and sister's transformed lymphocytes was also decreased to 45% of that from the control subject (data not shown).

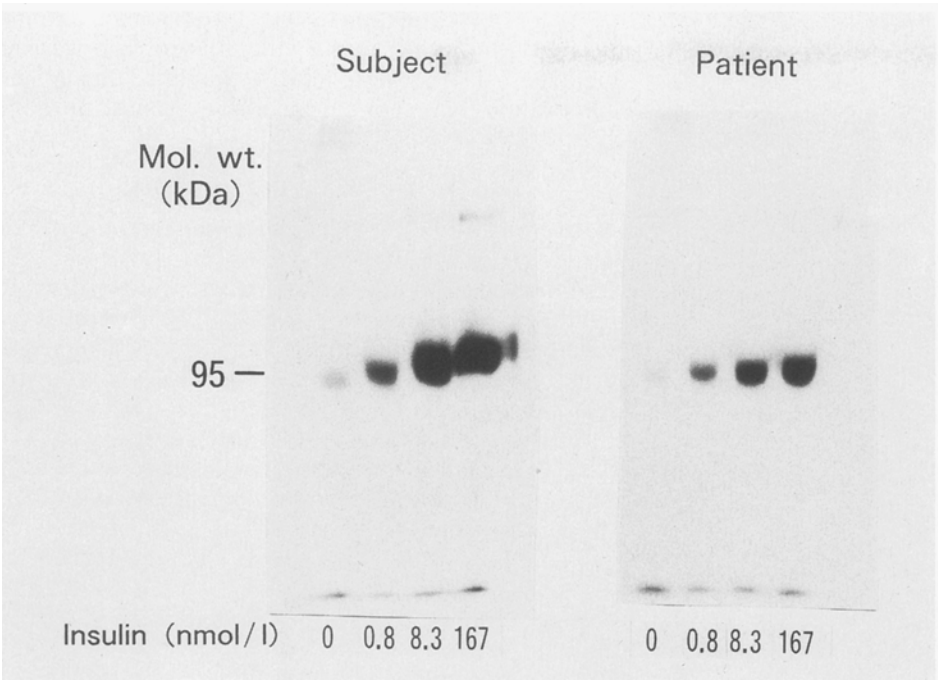

Fig. 3. Autoradiograph of insulin-stimulated phosphorylation of β-subunit of the insulin receptor from Epstein-Barr virus transformed lymphocytes in a cell-free system. Solubilized insulin receptors from the lymphocytes of the control subject and the patient were adjusted for insulin binding capacity, and pre-incubated with various amounts of insulin. Phosphorylation of the β-subunit of the insulin receptor was performed as described in Subjects, materials and methods

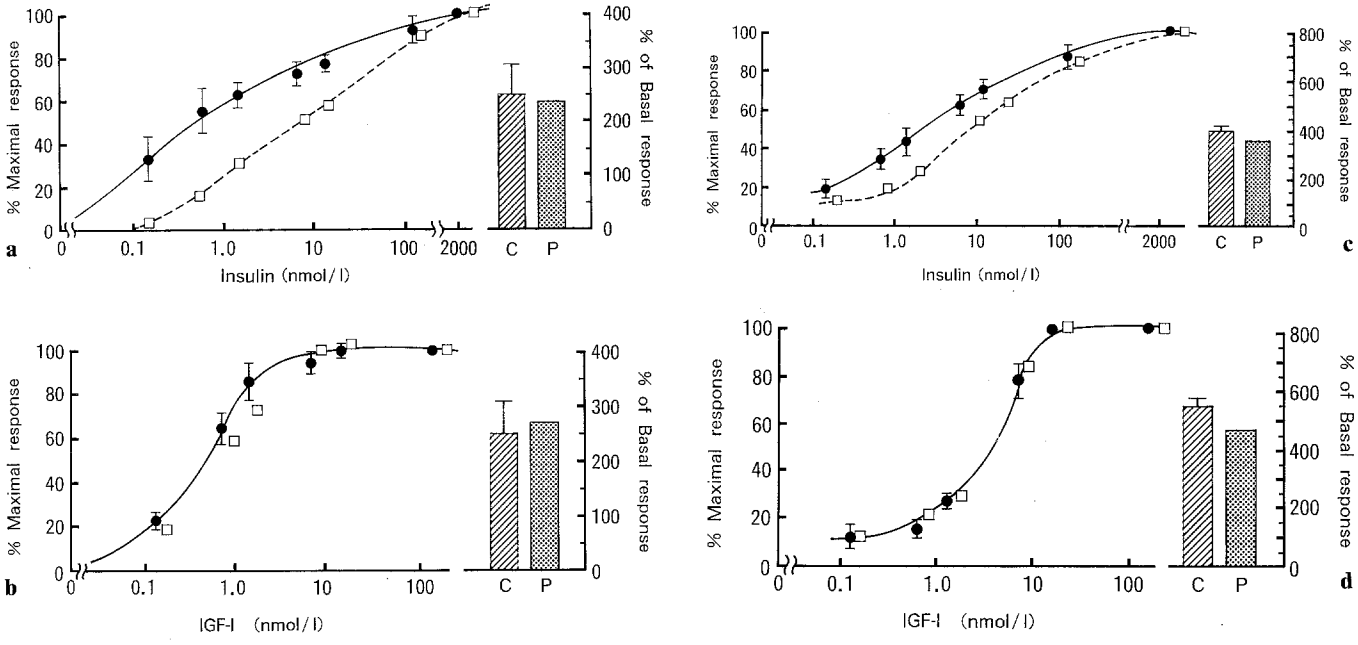

Fig. 4 (a–d). Biological activities of insulin and insulin-like growth factor I (IGF-I) in the cultured fibroblasts. **a** Insulin stimulated α-aminoisobutyric acid (AIB) uptake in cultured fibroblasts. Dose-response curves plotted as percent maximal response (left panel) and percent basal response (right panel) are shown. Results are mean ± SEM of five experiments for control subjects and three experiments for the patient. **b** IGF-I stimulated AIB uptake study in cultured fibroblasts. Results are means ± SEM of five experiments for control subjects and three experiments for the patient. **c** Insulin-stimulated thymidine incorporation in cultured fibroblasts. Dose-response curves plotted as percent maximal response (left panel) and percent basal response (right panel) are shown. Results are mean ± SEM of ten experiments for control subjects, three experiments for the patient. **d** IGF-I stimulated thymidine incorporation study in cultured fibroblasts. Results are mean ± SEM of five experiments for control subjects and three experiments for the patient. ●: control subject (C), □: patient (P)

AIB uptake

The maximal stimulation of AIB uptake by insulin was comparable in the two groups, and the values described as percent over basal were 253 ± 65% in control subjects, and 240% in the patient. The insulin concentration for ED_{50} in control subjects and the patient was 0.6 nmol/l and 8 nmol/l, respectively. Therefore, the dose-response curve of the patient shifted to the right for AIB uptake (Fig. 4a).

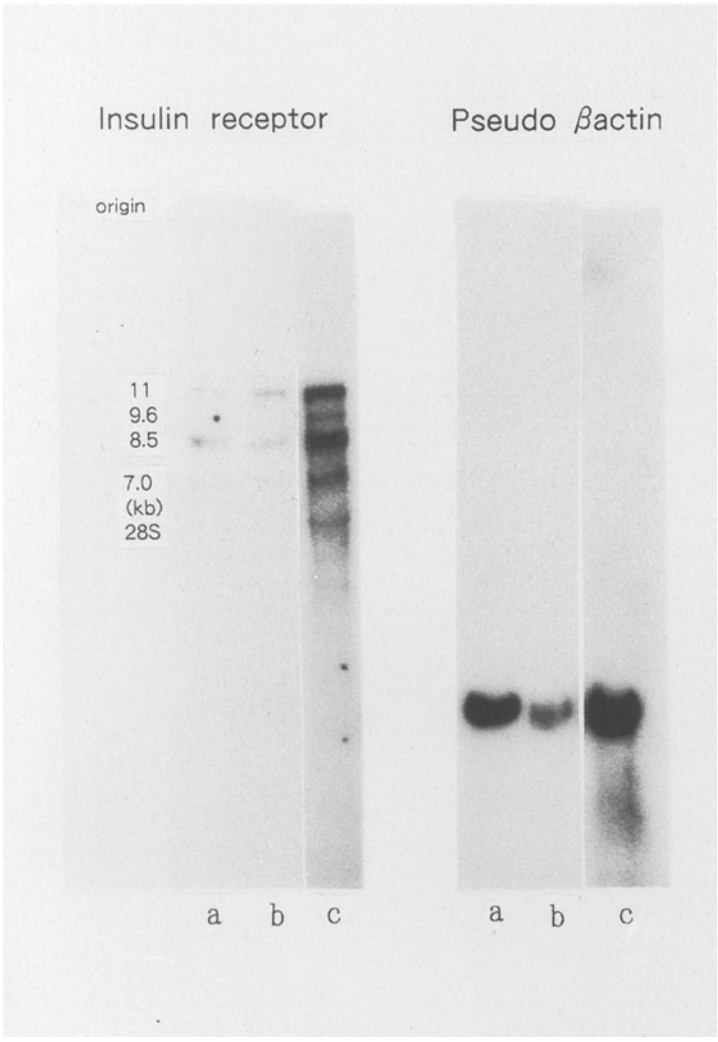

Fig. 5. Northern blot hybridization of poly-adenylated RNA from the patient's transformed lymphocytes to define HIR-cDNA. Ten μg of glyoxal-denatured polyadenylated RNA samples were run on 1% agarose gel and were electrophoretically blotted to a nylon membrane filter and baked. The filter was hybridized to HIR-cDNA (left three lanes), erased, and was rehybridized to human pseudo β-actin gene DNA (right three lanes). Lane a and b indicate normal subjects, and lane c indicates the proband

As for the stimulation by IGF-I, the maximal stimulation over basal in control subjects and the patient was 255 ± 61 and 260%, respectively. The value of ED_{50} was comparable between control subjects and the patient. These data suggested that IGF-I stimulated AIB uptake was normal in the patient's fibroblasts (Fig. 4b).

Thymidine incorporation

The maximal insulin stimulation of thymidine incorporation was 389 ± 22% over the basal in control subjects, and 350% in the patient. The insulin concentration for ED_{50} in control subjects and the patients was 2.0 nmol/l and 9.0 nmol/l, respectively. Therefore, the dose-response curve of the patient shifted to the right for thymidine incorporation (Fig. 4c).

As for the stimulation by IGF-I, both maximal stimulation and ED_{50} values in the patient' fibroblasts were normal. These data suggested IGF-I stimulated thymidine incorporation was normal in the patient's fibroblasts (Fig. 4d).

Northern blot analysis of human insulin receptor mRNA

As reported for other human tissues [15], Northern blot hybridization analysis of poly(A)$^+$ RNA from the cytosolic fraction of transformed lymphocytes detected five principal species of insulin receptor mRNA with apparent sizes ranging from 4.7–11 kb in length.

The major bands were two mRNA species (11 and 8.5 kb). The mRNA expression of the patient's cells when rehybridized with β-actin was comparable, with that of the control subject (Fig. 5).

Sequence analysis of the insulin receptor gene

By the sequence analysis of amplified genomic DNA, we demonstrated that this patient was heterozygous for a mutation substituting Leu for Trp at codon 1193 in exon 20. We could not find any other missense or nonsense mutation in 22 exons of the insulin receptor gene (Fig. 6).

Allele-specific oligonucleotide hybridization

The oligonucleotide, specific for the wild type sequences, hybridized to the genomic DNA and cDNA from both the control subjects and the patient. The oligonucleotide specific for missense mutation hybridized to DNA from the patient but did not hybridize to genomic DNA and cDNA from a control subject. These results confirmed that the proband was heterozygous for a missense mutation at codon 1193 in insulin receptor genomic DNA and mRNA, and that there was no deletion or splicing abnormalities (Fig. 7).

Family studies of the mutant alleles

The patient's mother and sister are also heterozygous for a mutation substituting Leu for Trp codon 1193 in exon 20 of the insulin receptor (Fig. 8). These findings were in accordance with the results of the autophosphorylation study.

Discussion

Mutations have been identified in the insulin receptor genes of patients with genetic syndromes of insulin resistance. They can be placed into five categories according to the classification by Taylor [1]. These are class 1: impaired receptor biosynthesis as shown in Trp^{133}→stop [17], Arg^{1000}→stop [17]; class 2: impaired transport of receptors to the cell as shown in His^{209}→Arg [18], Leu^{233}→Pro [19]; class 3: decreased affinity of insulin binding as shown in

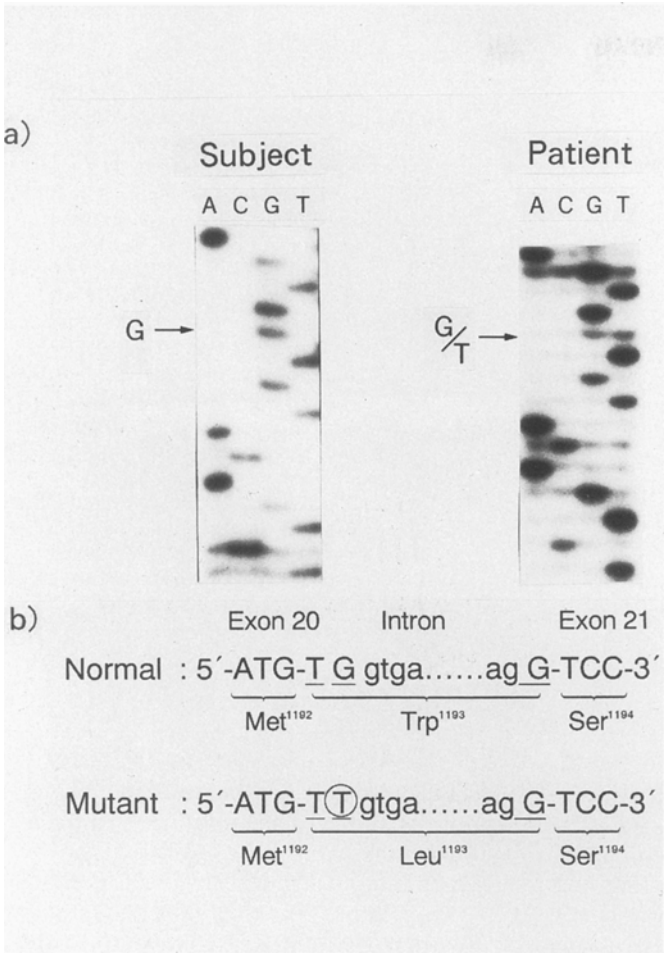

Fig. 6 (a, b). Partial nucleotide sequence of mutant alleles. **a** Fragments of genomic DNA including exon 20 from control subjects and patient were amplified by polymerase chain reaction. Next the sense strands of DNA were amplified and the nucleotide sequences were determined. The portion of the sequencing ladder spanning the mutations at codon 1193 is shown. For comparison, sequencing ladders are presented with amplified DNA from control subjects who were homozygous for the wild-type sequences at codon 1193. The mutation was located at the terminal base in exon 20 and the third base (G) in the codon 1193 was the first one in exon 21 of the insulin receptor gene, which we confirmed had no mutation (data not shown). **b** The sequence of the normal and mutant alleles from codon 1192 to codon 1194 is depicted

Arg^{735}→Ser [2, 3]; class 4: accelerated receptor degradation as shown in Lys^{460}→Glu [20], Asn^{462}→Ser [17]; class 5: impaired tyrosine kinase activity. The reported class 5 abnormality includes two mutations (Arg^{993}→Gln [21], Gly^{1008}→Val [22]) in the region of the ATP binding domain which inhibits ATP binding and three mutations (Ala^{1134}→Thr [23], Met^{1153}→Ile [24], Trp^{1200}→Ser [25]) which cluster near the autophosphorylation sites (Tyr^{1158}, Tyr^{1162}, Tyr^{1163}).

Trp^{1193} is in the region of kinase domain and is almost perfectly conserved in all known protein tyrosine kinases [26]. It appeared to be likely that Trp^{1193} is important for normal function of the kinase domain of the entire family of membrane tyrosine kinases. Therefore, Leu^{1193} mutant receptor may alter the conformation of the molecule in this region, resulting in decreased tyrosine kinase. Transfection study in COS 7 cells revealed that Leu^{1193} mutant receptor showed markedly impaired insulin-stimulated autophosphorylation (unpublished observations). Since the patient is a heterozygote, and the $\alpha_2 \beta_2$ tetrameric insulin receptor is a dimer formed between two $\alpha\beta$ species, three different tetrameric formations are possible: mutant-mutant, mutant-normal, and normal-normal in a ratio of 1:2:1. It has been reported [27] that the kinase-defective receptor may exert a dominant negative effect and inhibit normal receptor function, thus, mutant-mutant and mutant-normal receptors may have defective tyrosine kinase. These effects may cause insulin resistance in this patient with a heterozygous mutation in the tyrosine kinase domain.

Insulin binding to the transformed lymphocytes, and cultured fibroblasts from the patient was mildly reduced. Similar alterations in insulin binding were also observed in the patient's erythrocytes. Insulin binding to erythrocytes was decreased to 28% of the control subjects (data not shown). Scatchard analysis and an affinity cross-linking study revealed that low insulin binding in the patient was due to decreased receptor number. These data suggest a mild reduction in insulin receptor number which may contribute to exacerbation of insulin resistance in addition to decreased tyrosine kinase. It has been shown that mutations (Gly^{1008}→Val [22], Ala^{1134}→Thr [23], Met^{1153}→Ile [24]) in the tyrosine kinase domain do not influence insulin binding. On the other hand, Moller et al. [28] reported that insulin binding to circulating monocytes in a patient with Trp^{1200}→Ser mutation was reduced to 46% of that of the control, although the reason for decreased insulin binding was not clear. The mRNA expression of the patient's cells was comparable with that of the control, when it was normalized with β-actin. Thus, change in expression of insulin receptor gene did not appear to contribute to decreased insulin binding. The reason for decreased insulin binding remains to be clarified, but the possibility may be abnormal processing, or impairment of receptor recycling system. These abnormalities should be evaluated by examination of receptor synthesis and the processing pathway in permanent cell lines transfected by the mutant gene. This study is in progress.

We examined AIB uptake and thymidine incorporation using cultured fibroblasts to study insulin action. Insulin sensitivity in AIB uptake and thymidine incorporation was impaired in this patient. These data suggest that the studies on insulin action in cultured fibroblasts reflect insulin resistance in vivo, and Leu^{1193} mutant receptor fails to mediate insulin signals, including amino acid uptake and mitogenesis. On the other hand, some previous in vitro studies failed to show insulin resistance in Chinese hamster ovary cells transfected by kinase defective mutant receptor (Trp^{1200}→Ser [25]) cDNA. Both mutant receptors (Thr^{1134}, Ser^{1200}) expressed in Chinese hamster ovary cells demonstrated severely impaired insulin-stimulated autophosphorylation and kinase activity toward endogenous and exogenous substrates. However, cells expressing Ser^{1200} receptors

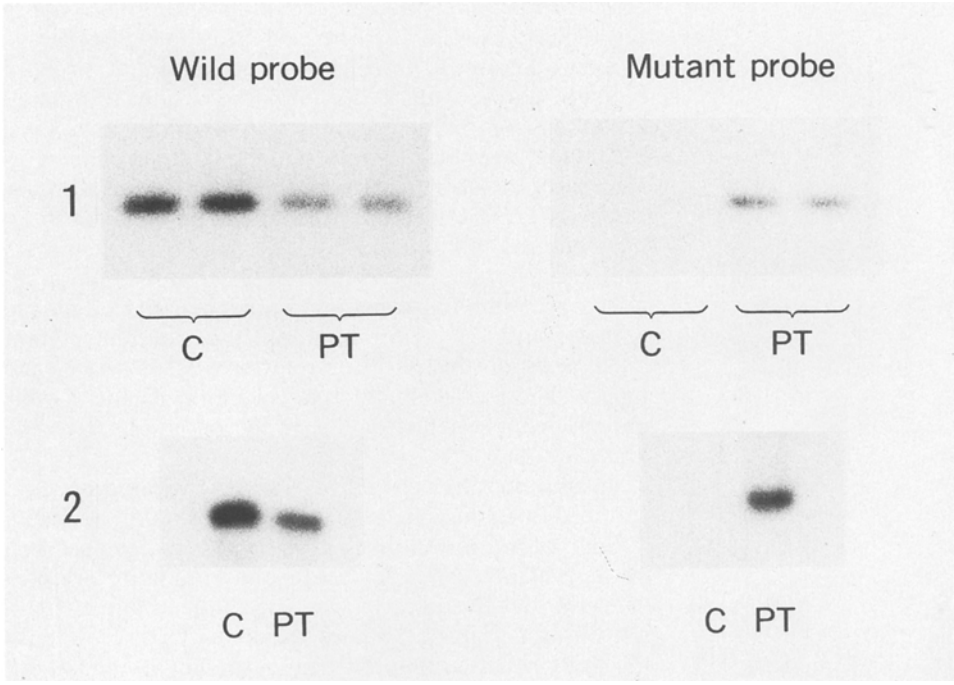

Fig. 7. Allele-specific oligonucleotide hybridization of amplified genomic DNA and cDNA from the proband. 1. Genomic DNA including exon 20 of the insulin receptor gene was amplified from a normal individual (C) and the proband (PT). About 200 ng of the amplified double-stranded DNA was analysed by electrophoresis through a 2% agarose gel and transferred to nylon membranes. The DNA blots were hybridized with ^{32}P-labelled synthetic oligonucleotides corresponding to either the normal or mutant sequences. 2. Fragments of cDNA were amplified from total cellular RNA by reverse transcriptase-polymerase chain reaction with appropriate oligonucleotide primers, as described in Subjects, materials and methods to amplify the region spanning codon 1193

showed increased insulin-stimulated 2-deoxyglucose uptake compared with cells expressing normal receptors, whereas Thr^{1134} receptors failed to transmit the insulin signal normally in 2-deoxyglucose uptake, glucose incorporation, and thymidine uptake [23, 25]. Cama et al. [24] reported that the Ile^{1153} mutant receptors expressed in NIH-3T3 cells showed impaired receptor tyrosine kinase activity and also inhibited insulin-stimulated 2-deoxyglucose uptake and thymidine incorporation. In the Ser^{1200} mutant receptor study, 2-deoxyglucose uptake in cells expressing the mutant receptor contrasted with in vivo glucose intolerance. However, the study of insulin action on Thr^{1134} and Ile^{1153} mutant receptor was consistent with the hypothesis that all of these insulin actions require intact receptor tyrosine kinase.

IGF-I stimulated AIB uptake and thymidine incorporation was normal in the patient's fibroblasts. Recently Cama et al. [24] reported tyrosine kinase activity of IGF-I receptor and IGF-I stimulated thymidine uptake was defective in NIH-3T3 cells transfected by kinase defective mutant insulin receptor [Met^{1153}→Ile] cDNA. Thus, the Ile^{1153} mutant insulin receptor might exert an inhibitory effect upon the function of IGF-I receptor. The Leu^{1193} mutant receptor might also exert an inhibitory effect upon the function of IGF-I receptor similar to the Ile^{1153} mutant receptor, but such an effect may be minor based on the current data of IGF-I action in the patient's fibroblasts.

The patient's mother and sister are insulin-resistant, hyperinsulinaemic and showed defective tyrosine kinase activity of the insulin receptor to a degree similar to the proband. They are also heterozygous for a mutation in the insulin receptor gene that substitutes Leu for Trp^{1193} in the β-subunit of the receptor. It is plausible that this mutation causes insulin resistance in a dominant fashion. However, their clinical courses and phenotypic appearance such as acanthosis nigricans were different, and glucose intolerance was more severe in the proband than the other two family members. Although the exact mechanism is not yet known, it is possible that either genetic or environmental factors may additively contribute to the pathogenesis of glucose intolerance in the proband. Based on the fact that the patient's father had Type 2 diabetes, the patient's genetic background may predispose him to be more diabetogenic. His insulin levels at 1 h and 2 h after glucose loading were 250 μU/ml and 500 μU/ml, respectively. The mother and sister's insulin secretion after glucose loading was greater than that of the proband, although the fasting insulin levels were comparable in these patients. Therefore, a conserved insulin secretory capacity may be an important factor in maintaining the normal glucose tolerance of the mother and the sister.

We identified the same mutation (Trp^{1193}→Leu) in another family with type A insulin resistance syndrome (unpublished observations). Insulin binding to the transformed lymphocytes from another family member was severely decreased, but autophosphorylation of the purified insulin receptors was normal. Therefore, there is a significant difference in the characteristics of insulin receptors between the two families. To determine whether the two families are related, we are now examining the silent polymorphisms of the insulin receptor gene in both families.

In conclusion, these results suggest that a novel naturally occuring mutation (Trp^{1193}→Leu^{1193}) at the insulin receptor β-subunit results in severe impairment of tyrosine kinase function, which may be the cause of insulin resistance in this family. Although the receptor mutation was inherited in a dominant fashion and resulted in insulin re-

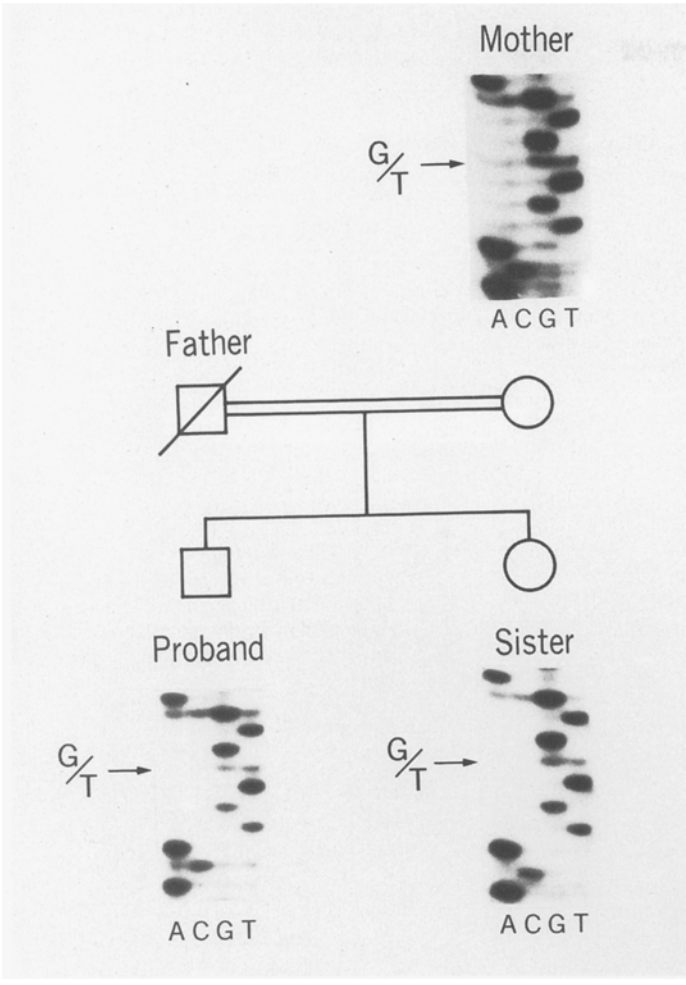

Fig. 8. Simplified pedigree of the proband's family together with partial nucleotide sequence of exon 20 of the insulin receptor gene. The proband, his sister, and his mother are heterozygous for a mutation substituting Leu for Trp at codon 1193 in exon 20 of the insulin receptor gene

sistance, the phenotypic appearance such as acanthosis nigricans was different among the family members with the same mutation. These results suggest that insulin action and phenotypic appearance may be mediated by different factors.

Acknowledgements. This study was supported in part by a research grant for intractable disease from the Ministry of Health and Welfare, a Grant-in-Aid from the Ministry of Education, Science, and Culture, and a grant for diabetes research from Otsuka Pharmaceutical Co, Ltd, Japan.

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Received: 21 September 1992
and in final revised form: 17 December 1992

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