Familial Hyperproinsulinemia Associated With NIDDM

A case study

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OBJECTIVE — To report studies on an elderly patient with moderate NIDDM associated with marked fasting hyperinsulinemia.

RESEARCH DESIGN AND METHODS — The propositus and several family members were studied by a combination of clinical, biochemical, and molecular genetic approaches to define the underlying genetic defect.

RESULTS — Fasting levels of contrainsulin hormones were normal, and resistance to exogenous insulin was absent. Gel filtration and reverse-phase high-performance liquid chromatography revealed elevated amounts of a structurally abnormal proinsulin intermediate (AC proinsulin). A study of the family of the propositus showed the same abnormality in 4 of 5 members in 3 successive generations. Genetic analysis revealed a point mutation affecting residue 65 of human proinsulin (Arg→His) in one allele of the insulin gene in the propositus, a defect similar to that described previously in 3 other apparently unrelated lineages.

CONCLUSIONS — This family exhibits a clear-cut relationship between increasing age and metabolic decompensation in all the hyperproinsulinemic members, suggesting that (inherited) metabolic stress and age both contribute to development of diabetes mellitus.

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NIDDM, non-insulin-dependent diabetes mellitus; PLM, proinsulin-like material; AC proinsulin, des 31,32 proinsulin; BC proinsulin, des 64,65 proinsulin; IRI, insulin immunoreactivity; HPLC, high-performance liquid chromatography; BMI, body mass index; PAGE, polyacrylamide gel electrophoresis; RIA, radioimmunoassay; PCR, polymerase chain reaction; bp, base pair; GTT, glucose tolerance test.

Proinsulin, the precursor of insulin, is normally processed at Arg^{31}-Arg^{32} and Lys^{64}-Arg^{65} to release insulin and C-peptide. Structural abnormalities in these regions of the proinsulin molecule can impair this cleavage, leading to the accumulation of PLM (1). Such defects show an autosomal dominant inheritance and are the main cause of the syndrome of familial hyperproinsulinemia. Up to now, 4 pedigrees of familial hyperproinsulinemia of this type have been described (2–5). All of these result from a mutation in Arg^{65} and thus give rise to a proinsulin intermediate having an uncleaved C-peptide–A-chain junction (AC proinsulin). This material reacts well with insulin antibodies and some anti–C-peptide antibodies (6). Normally proinsulin-like forms account for <20% of total IRI in the circulation, whereas in familial proinsulinemia >80% of IRI may be in the form of PLM. Its accumulation is thought to be caused by its relatively slow clearance rate relative to insulin. HPLC is useful to define the nature of the PLM (7), i.e., whether intact or partly cleaved intermediate forms are present.

In this study we describe an elderly patient with diabetes, who had elevated IRI associated with normal insulin sensitivity. HPLC analysis revealed the presence of large amounts of PLM corresponding to AC proinsulin. Members of 3 successive generations of this family were found to have hyperproinsulinemia accompanied by increasing degrees of glucose intolerance ranging from normal to definitely diabetic according to their increasing age. Analysis of both alleles of the insulin gene of the propositus revealed a point mutation in the codon for Arg^{65} changing it to His in 1 allele.

RESEARCH DESIGN AND METHODS

Case report

A 68-yr-old, nonobese Japanese woman was referred for treatment of diabetes

mellitus and insulin allergy. Diabetes was originally detected by chance when her eldest son was diagnosed as diabetic, and she underwent a health-care check (61 yr of age) resulting in the diagnosis of diabetes. On diet alone, she was free of symptoms for the next 5 yr. However, general fatigue developed and continued for 2 yr, then she began treatment with oral hypoglycemic agents. No improvement was observed on diet and oral agent therapy over a period of 5 mo. Lente insulin (Novo, Denmark) was then administered for 2 wk (16 U/day), but she developed an allergic reaction at the insulin injection site. Changing the source of insulin to Ultralente and then to Monotard Porcine monocomponent (24 U/day) was ineffective, the allergic reaction worsened, and she was referred to our hospital. At 50 yr of age she had been treated for a duodenal ulcer. A benign rectal polyp was removed 9 mo before admission. Her eldest son and daughter were both diabetic and being treated with diet therapy.

Physical examination of the patient was entirely normal except for urticaria of the body, trunk, and extremities. No signs of acanthosis nigricans, liver disease, or renal dysfunction were noted. Her body weight was 48 kg and BMI was 20.2 kg/m^2. Diabetic retinopathy was absent. Routine laboratory findings were normal except for fasting hyperglycemia (187 mg/dl [10.4 mM]) and high levels of IRI (142 μU/ml [0.99 pmol/ml]) and C-peptide (38.0 ng/ml [12.6 pmol/ml]) with high levels of circulating insulin antibodies (bound insulin = 67.0%). The presence of subcutaneous allergy to insulin (human, porcine, and bovine) was also confirmed. Despite the presence of insulin antibodies, the elevated insulin and C-peptide levels were of such magnitude to warrant investigation of their possible origins. The patient was admitted to the Diabetes Center, Tokyo Women's Medical College, Tokyo, Japan, and strict diet therapy was started. Insulin was changed to human Monotard/Actrapid (Novo, Denmark), and the insulin dose was gradually reduced. Insulin therapy was discontinued 1 mo after admission because of good serum glucose control. All studies were explained to the patient, and written informed consent was obtained beforehand.

Clinical studies

After an overnight fast, venous blood was drawn for determination of serum glucose, serum insulin, and serum C-peptide levels before and at 30, 60, 90, and 120 min after oral administration of 75 g glucose.

Insulin sensitivity in the fasting state was assessed by the intravenous injection of human Actrapid insulin (0.1 U/kg body weight; Novo). Venous serum glucose levels were determined before and at 15, 30, 45, 60, and 90 min after the injection.

Laboratory studies

Assay techniques. Plasma glucose was measured by the glucose oxidase method, (Glucose Analyzer II, Beckman, Fullerton, CA). A site-specific RIA for human proinsulin and its intermediates was applied as described previously (8). Serum growth hormone was measured by Growth Hormone RIA Kit (Eiken, Japan), plasma cortisol and serum T4 by Amerlex RIA Kit (Amersham International, Amersham, England), serum T3 by T3 Kit (Eiken), and serum glucagon by Glucagon Riakit (Dainabot, Japan). Circulating insulin antibodies and C-peptide antibodies were measured by competitive-binding assay. Insulin receptors on red blood cells were measured as described previously (9).

Gel filtration studies. Samples were fractionated by Biogel P-30 (fine) gel chromatography. We applied 0.2 ml of fasting sample to a 1 × 60-cm column and eluted with borate-albumin (0.25%) buffer or 3N acetic acid-albumin (0.25% BSA). Aliquots of the fractions were lyophilized and analyzed for IRI (using a pork insulin standard) and immunoreactive C-peptide (using a synthetic human C-peptide standard).

Reverse-phase HPLC. HPLC separations of immunopurified PLM were conducted on an Altex C-18 ultrasphere ion-pair column (0.46 × 25 cm) using a Perkin-Elmer Series 4 liquid chromatograph (Perkin-Elmer, Norwalk, CT) equipped with an ISS-100 automatic injector. Elution of samples proceeded by a series of three-stepped isocratic mixtures of the aqueous and organic phases. Aliquots of the fractions were lyophilized and analyzed for IRI (7). Biosynthetic human proinsulin, human insulin, and two intermediate forms of human proinsulin (des Lys^{64}, Arg^{65} proinsulin [BC proinsulin] and des Arg^{31}, Arg^{32} proinsulin [AC proinsulin]) prepared by in vitro enzymatic cleavage (6) were used as reference standards.

Sequencing insulin gene exon 3 by PCR. Genomic DNA was isolated from the propositus' blood leukocytes, and insulin gene exon 3 was amplified by PCR (10). The sense primer E3–1 was 5'GCT GTTCCGGAACCTGCTCT, and the antisense primer E3-rC1 was 5'ACAGCAGG GCTGGTTCAAG. PCR was performed with 3 μg genomic DNA for 30 cycles with each cycle = 94° × 1 min, 50° × 2 min, and 65° × 3 min. The 266-bp fragment was purified by PAGE, subcloned into pGEM4Z (*Sma* I site), and sequenced by the dideoxy method (11).

RESULTS

Screening for insulin resistance

The propositus showed a high level of insulin antibodies (binding of [^{125}I]insulin, 67%), because of insulin treatment for 4 wk before admission. After insulin injections ceased, the patient's insulin antibody titers decreased and disappeared after 8 wk. Other causes of insulin resistance could not be demonstrated. Fasting levels of contrainsulin hormones were normal (glucagon 76.8 pg/ml, growth hormone 1.6 ng/ml, cortisol 9.7 μg/ml, ACTH 48 pg/ml, T3 112 ng/ml, and T4 10.5 μg/ml). Insulin receptor an-

Table 1—*Results of GGTs*

	Insulin antibody (Bound %)	Time (min)					
		0	30	60	90	120	Σ
Study 1 (6 August 1981)	5.7						
Plasma glucose (mm)		6.5	11.8	13.9	12.9	10.8	55.9
IRI (pmol/ml)		0.35	1.24	1.39	2.58	1.85	7.40
Study 2 (10 December 1983)	41.4						
Plasma glucose (mm)		6.9	12.4	17.3	16.9	15.9	69.5
Free IRI (pmol/ml)		0.27	0.44	0.62	0.85	1.00	3.19
Free C-peptide reactivity (pmol/ml)		0.47	0.60	1.07	1.67	1.87	5.67
Molar ratio (free C–peptide reactivity/free IRI)		1.70	1.35	1.73	1.95	1.86	1.78
Study 3 (7 October 1984)	3.4						
Plasma glucose (mm)		9.2	11.9	12.6	12.2	12.2	58.0
IRI (pmol/ml)		0.33	1.81	0.44	0.45	0.55	3.59
C-peptide reactivity (pmol/ml)		0.80	4.40	1.13	0.93	1.23	8.50
Molar ratio (C–peptide reactivity/IRI)		2.45	2.43	2.55	2.07	2.23	2.37
Study 4 (13 October 1985)	3.3						
Plasma glucose (mm)		11.1	16.6	17.9	18.8	19.2	83.7
IRI (pmol/ml)		0.27	0.38	0.50	0.61	0.65	2.42
C-peptide reactivity (pmol/ml)		0.73	0.90	1.37	1.43	1.83	6.27
Molar ratio (C–peptide reactivity/IRI)		2.70	2.34	2.72	2.35	2.84	2.59

tibodies were negative (12) and binding of [^{125}I]insulin to receptors on the patient's erythrocytes was not decreased compared with control values (data not shown), suggesting that the elevated insulin-like material was biologically ineffective.

GTTs and insulin tolerance tests

The results of a series of oral GTTs are shown in Table 1. Before treatment with insulin (Table 1, study 1) and after disappearance of the insulin antibodies (Table 1, studies 3 and 4), all studies showed abnormal glucose tolerance with elevated levels of fasting and postglucose IRI. The fasting molar ratio of C-peptide to IRI remained lower than 3.0 (>4.0 for healthy control subjects).

The propositus exhibited a normal response to exogenous insulin infused intravenously without signs of any allergic reaction. The serum glucose levels fell from 6.7 (baseline) to 1.7 mM (at 45 min after infusion) at a rate of 0.11 mM/min. In response to the hypoglycemia, endogenous insulin secretion, assessed by C-peptide levels, was suppressed (data not shown).

Biogel P-30 column chromatography

Gel filtration chromatography profiles of the propositus' serum on Biogel P-30 columns revealed that 88% (0.29 pmol/ml fasting serum) of total IRI eluted at the position of biosynthetic human proinsulin. Of total C-peptide reactivity, 40% (0.32 pmol/ml fasting serum) eluted in the same fractions. The molar ratio of C-peptide reactivity to IRI eluting at the position of human proinsulin was 1.1:1 in the fasting state, and the ratio did not change during glucose stimulation. The presence of PLM at the expected elution volumes indicated the likelihood of the familial hyperproinsulinemia syndrome and ruled out hyperinsulinemia caused by the secretion of an abnormal insulin (14).

The propositus' son's serum (Case no. 4) was also examined by means of gel chromatography and the site-specific RIA for proinsulin and intermediates, as shown in Fig. 1. Antiserum 18D (recognizing the B-chain–C-peptide junction) showed no response, whereas antiserum 11E (recognizing the C-peptide–A-chain junction) revealed a single large peak, which coincided with the IRI peak and the human proinsulin marker. These results indicated that AC proinsulin intermediate was the predominant circulating component.

Reverse-phase HPLC

Immunopurified PLM of the propositus was separated on HPLC, and the elution fractions measured in terms of IRI (Fig. 2). One major peak and three small peaks were present. The major peak eluted at the position of an AC proinsulin standard and the small peaks coincided with insulin, BC proinsulin, and the proinsulin marker, respectively.

The kindred

Because a diagnosis of familial hyperproinsulinemia was under consideration, family studies were undertaken to define the hereditary trait. As shown in Table 2 and Fig. 3, 4 family members in 3 successive generations showed high levels of IRI (range 0.28–0.90 pmol/ml in fasting serum samples), with relatively high C-peptide immunoreactivity (range 0.6–2.07 pmol/ml). The fasting molar ratio of C-peptide reactivity to IRI ranged between 2.4 and 2.70. The molar ratio was lower than in healthy control subjects, but was relatively higher than in familial hyperinsulinemia (14).

Analysis of the mutation

We used PCR to sequence the propositus' allelic insulin genes by amplifying and cloning a 266-bp fragment, which contained the entire exon 3 region from the patient's blood leukocyte DNA. Five plasmid clones were isolated and se-

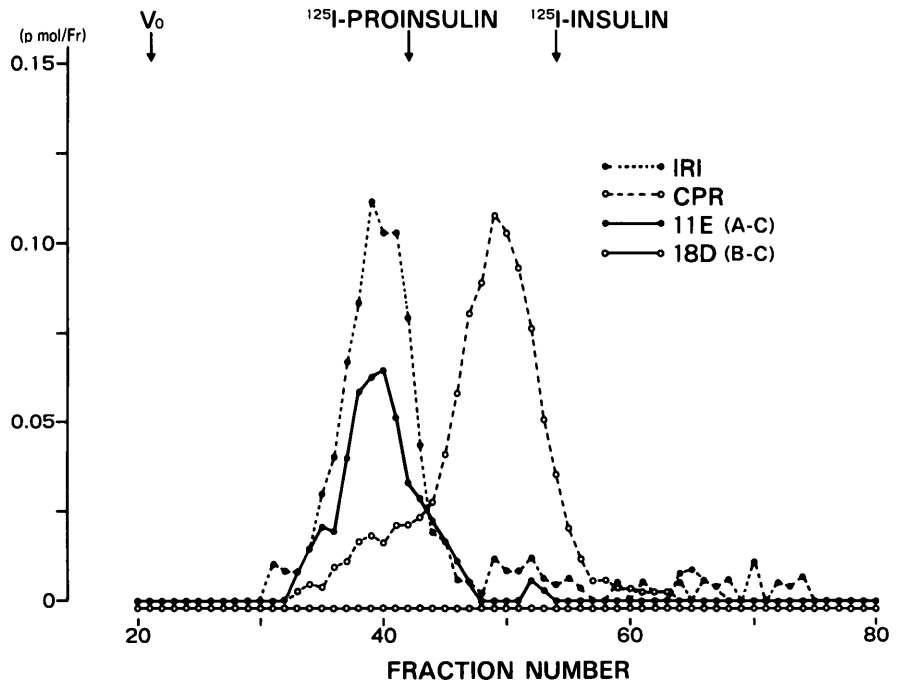

Figure 1—*Column elution profile (Biogel P-30; see METHODS) of the serum from the propositus' son. Antiserum 11E (AC) recognizes the C-peptide–A-chain junction and cross-reacts with AC proinsulin predominantly. Antiserum 18D recognizes the B-chain–C-peptide junction and cross-reacts with intact human proinsulin and BC proinsulin. The arrows show the peak position of each standard marker.*

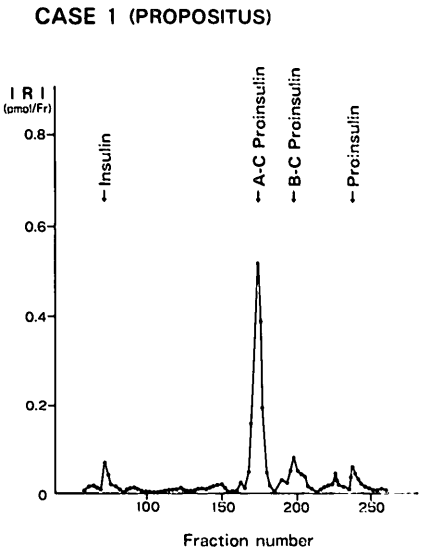

Figure 2—*Reverse-phase HPLC profile of the immunopurified PLM from the propositus. The arrows show the peak position of each standard marker.*

quenced. Two clones contained exon 3 in exact agreement with the normal human insulin gene (15). In 3 clones, however, a single G→A mutation was observed that changed the codon of Arg^{65} to His^{65}. These results demonstrate that the patient is heterozygous in the mutant insulin gene and localized the mutation to the dibasic C-peptide–A-chain cleavage site of the proinsulin.

Glucose tolerance and aging

As shown in Table 1, the glucose tolerance and insulin secretory capacity of the propositus tended to decrease as the 68-yr-old, nonobese Japanese woman aged. Representative patterns of glucose tolerance for various family members affected by hyperproinsulinemia are indicated in Fig. 4. The youngest individual showed normal glucose tolerance with a magnified, but normal, pattern of insulin secretion. On the other hand, older individuals showed increasing glucose intolerance and declining insulin secretory response. For each family member the insulin secretory responses during oral GTTs were expressed (Fig. 5) as the sum of IRI levels at fasting, 30, 60, 90, and 120 min after glucose loading (Σ IRI). In the same way, degree of glucose intolerance was expressed as the sum of plasma glucose levels at these times (Σ plasma glucose). Nonobese, healthy control subjects and diabetic subjects showing no definite insulin resistance are indicated by the small dots. The affected family members were arranged linearly from upper left corner to lower right corner according to their age at the time of examination. The linear regression model is $Y = 12.60 - 0.12X$, and the correlation coefficient is -0.99 (X = Σ plasma glucose [mM], Y = Σ IRI [pmol/ml]). This scat-

Table 2—*Comparison of values for fasting glucose, insulin, and C-peptide for all family members studied*

Case no.	Subject	Age (yr)	Fasting plasma glucose	IRI (pmol/ml)	C-peptide reactivity	C-peptide reactivity/IRI molar ratio
1	SK (propositus)	68	11.1	0.27	0.73	2.70
2	SK	43	8.4	0.90	2.07	2.29
3	TK	41	3.6	0.05	0.33	6.15
4	KK	38	3.3	0.28	0.60	2.14
5	UK	17	3.5	0.34	0.80	2.36

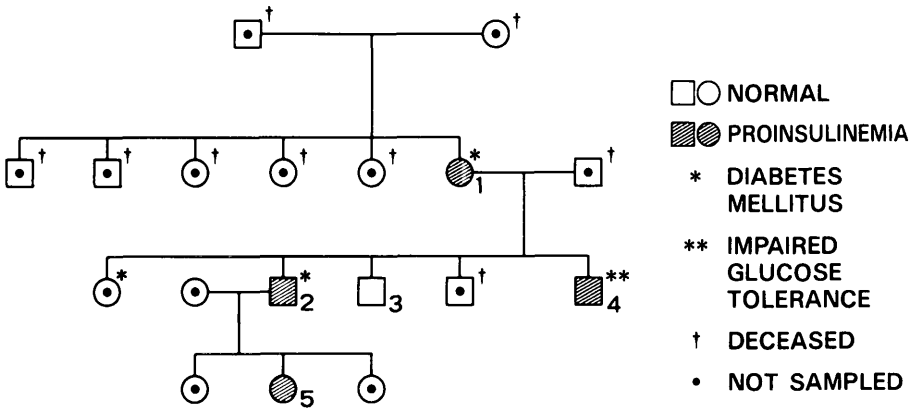

Figure 3—*Pedigree of the propositus (case no. 1) and her family members. Numbers refer to cases. Members with hyperproinsulinemia and unaffected members are indicated. Diabetes, impaired glucose tolerance, deceased, and not sampled are flagged.*

tergram illustrates the in vivo biological activity of endogenously secreted insulin and/or PLM measured as IRI. It shows that to maintain the same degree of glucose control during oral glucose ingestion, the affected subjects require 5- to 10-fold higher levels of Σ IRI than do the nonaffected control subjects.

Discussion

The propositus in this family characteristically exhibited diabetes mellitus, high levels of IRI, and a reduced ratio of C-peptide to IRI. Though insulin antibodies were present transiently, no other cause of insulin resistance, such as obesity, high levels of contrainsulin hormones, insulin receptor antibodies, or abnormality of insulin receptor binding could be demonstrated. Resistance to exogenous insulin was absent, as evidenced by the hypoglycemic response to an insulin infusion. On the other hand, the reduced biological effectiveness of the endogenous IRI of the propositus is demonstrated by the scattergram of Σ IRI and Σ plasma glucose (Fig. 5).

Gel chromatography of the propositus' serum revealed that most of the IRI eluted with a human proinsulin standard. A similar abnormally elevated level of IRI in the Japanese woman's son's serum was shown to consist of antibody 11E reacting material, corresponding to the AC proinsulin intermediate.

These findings suggested the possibility that the elevated circulating IRI in affected family members consists mainly of PLM, especially the partially processed AC proinsulin intermediate. This was confirmed by HPLC analysis; PLM in Case no. 2 was identified as AC proinsulin, as in the propositus. As shown in Fig. 3, 4 of 5 family members in 3 successive generations show the same abnormality of C-peptide/IRI molar ratio, Σ IRI compared with Σ plasma glucose and an altered gel filtration profile.

Four pedigrees of familial hyperproinsulinemia (1,3,4,5,7) associated with a circulating AC intermediate have been reported previously. Thus, including this report, 5 pedigrees have been identified as having the AC type of familial hyperproinsulinemia. The second pedigree was found in Tokyo, Japan (3), and was attributable to the substitution of His^{65} for Arg^{65} caused by a point mutation in 1 allele of the insulin gene (16). A similar basis for the earlier case of Gabbay et al. and an additional patient from Denver has been reported (4), whereas the patient of Yano et al. (5) has Leu^{65} substituting Arg^{65}. Two pedigrees of AC hyperproinsulinemia caused by substitution of His^{65} at position 65 have now been found in the same area of Japan. Because the mutation in both families is the same, the possibility of consanguinity becomes an issue that will need to be resolved. The analysis of a highly polymorphic region of variable length 5′ to the insulin gene may prove helpful in elucidating the genetic origin of the point mutation (17). Recently, an additional pedigree of hyperproinsuline-

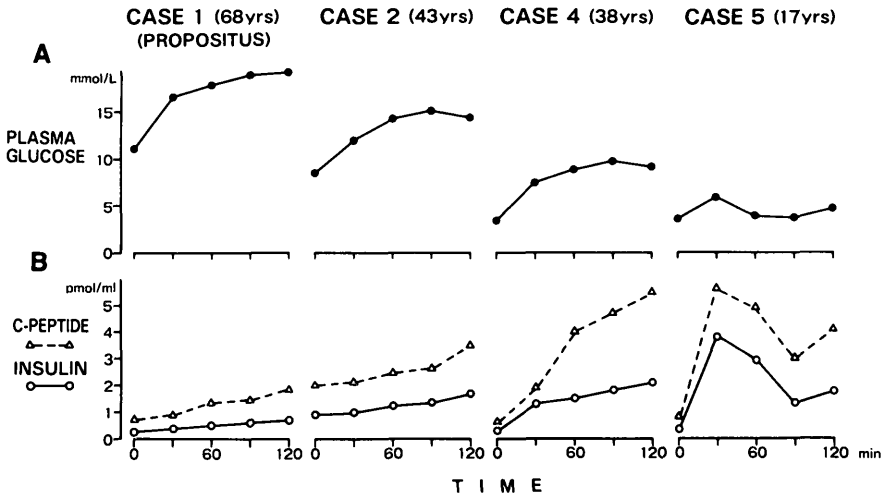

Figure 4—*Representative patterns of repeated GTTs in the family members. Indicated are changes in the levels of glucose (A), immunoreactive insulin, and C-peptide immunoreactivity (B) after the administration of 75 g of glucose.*

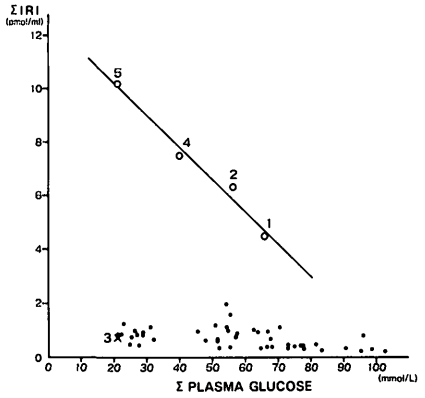

Figure 5—*Correlation of insulin secretory response and glucose intolerance defined by oral GTTs. Insulin secretory response (Σ IRI) is expressed as the sum of IRI levels at 5 time points (fasting, 30, 60, 90, and 120 min). (○), Affected family members; (×), nonaffected member; (○), control subjects without insulin resistance. Suffix shows the case number.*

mia, caused by the elevation of an apparently intact proinsulin molecule, has been found to be attributable to the substitution of Asp^{B10} for His^{B10} caused by a single nucleotide transversion in 1 allele of the insulin gene (18–20). This proinsulin is secreted in increased amounts via an alternative, or unregulated, secretory pathway (20).

On the other hand, 6 pedigrees of abnormal insulinemia (Leu^{B25}:Insulin Chicago, Ser^{B24}:Insulin Los Angeles, Leu^{A3}:Insulin Wakayama) have been described (14,21–27). Out of 5 pedigrees of abnormal insulinemia, 3 are Japanese and have been determined to be Insulin Wakayama. Although 2 of the families with Insulin Wakayama are living in the same district, analysis of above mentioned polymorphism, suggests that the genetic distance between the 2 pedigrees is probably different, with both having separate origins (28). Similarly to familial hyperproinsulinemia, analysis of their insulin genes reveals that nearly all patients with abnormal insulinemia have 1 abnormal and 1 normal insulin allele (14).

CONCLUSIONS — Both abnormal insulinemia and familial hyperproinsulinemia, so-called mutant proinsulin syndromes (14), show various grades of glucose intolerance. In this syndrome, some subjects are diabetic, with a relative deficiency of PLM and some are nondiabetic with excessive secretion of PLM. No clinical parameter predicting the grade of glucose intolerance has been reported. In this study, the subject's age is a good predictor of the development of diabetes. As shown in Fig. 5, affected subjects can be arranged linearly from the upper left hand corner to lower right hand corner in order of their ages. This suggests that proinsulin secretory capacity decreases and glucose intolerance is worsened as a function of age; this tendency has also been observed to some extent in other cases of mutant proinsulin syndrome (2,3,23,24,28). However, note that the members of this family all live together and ingest a similar diet. Presumably because their life-styles and habits are very similar, the correlation between aging and glucose intolerance was more evident. In this syndrome, a progressive lack of β-cell responsiveness to glucose appears as an important factor in the development of hyperglycemia with aging. Thus, subjects with inherited defects in the proinsulin molecule may be asymptomatic in their youth, but develop diabetes when increased β-cell function is no longer able to compensate for the reduced biological activity of the secretory product. The specific nature of the process leading to β-cell failure, however, remains unclear, but may be genetically determined. A prospective follow-up study on the members of this family is now underway.

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