Individuals with only one allele for a functional insulin receptor have a tendency to hyperinsulinaemia but not to hyperglycaemia

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Summary. Recently, we described a leprechaun patient with a genetically transmitted severe insulin resistance due to the absence of functional insulin receptors as inferred from the loss of insulin binding to the patients' fibroblasts and the impaired autophosphorylation of the β -chain of the receptor. This patient was homozygous for the genetic defect which was recently found to be a leucine to proline mutation at position 233 in the α -chain of the insulin receptor. In the present study we have examined insulin receptor functions in relatives of this patient. Some of these individuals are heterozygous for the genetic defect and have only one allele coding for a functional insulin receptor. Insulin binding to cultured fibroblasts from the heterozygous individuals is only 20–40% of control values indicating a Mendelian mode of inheritance of the binding defect. In contrast, insulin stimulated autophosphorylation of the β -chain of the insulin receptor shows normal values, indicating compensation mechanisms operating on this process. The stimulation of the basal level of 2-deoxyglucose uptake by

insulin in fibroblasts from the homozygous patient is 1.2 fold whereas the heterozygous and control individuals show stimulation values of approximately 1.65 fold. Basal levels of 2-deoxyglucose uptake are similar in these fibroblasts. Oral glucose tolerance tests on the heterozygous individuals indicate an increased requirement for insulin of the target tissues as concluded from the tendency towards hyperinsulinaemia with no observed hyperglycaemia. The results show that individuals with a genetic lesion in the insulin receptor which leads to decreased insulin binding, have mild abnormalities in their glucose tolerance tests but do not seem to develop hyperglycaemia as seen in Type 2 (non-insulin-dependent) diabetes mellitus.

Key words: Leprechaunism, insulin receptor, insulin resistance, autophosphorylation, insulin binding, glucose tolerance tests.

A variety of genetic defects may result in an impaired glucose homeostasis due to insulin resistance of the target tissues for insulin such as the liver, muscle and adipose tissue. The inherited syndromes of acanthosis nigricans type A and leprechaunism, which are characterized by an extreme resistance to insulin, are the result of mutations in the gene for the insulin receptor as indicated by recent data on molecular cloning of the insulin receptor DNA from these patients [1, 2]. Type 2 (non-insulin-dependent) diabetes mellitus, being a disease with a strong genetic component, is also characterized by insulin resistance of the target tissues. The genetic components involved in the predisposition to Type 2 diabetes are unknown and it seems possible that the disease is polygenic in nature, complicating the identification of the genes involved in the pathogenesis of the disease.

To investigate the effect of lesions in the insulin receptor gene on glucose homeostasis, we have examined insulin receptor functions and glucose tolerance in rela-

tives from a leprechaun patient. This leprechaun patient is homozygous for a genetic defect in the insulin receptor which was recently found to be a leucine to proline mutation at position 233 in the receptor α -chain [3]. This defect is expressed as an almost complete absence of insulin binding to cultured fibroblasts and a severe decrease of insulin stimulated autophosphorylation of the β -subunit of the insulin receptor [4]. The latter activity is thought to reflect the process of signal transduction [5, 6].

Relatives from this patient, who are heterozygous for this mutation, have only one allele coding for a functional insulin receptor, whereas the other allele codes for a non-functional receptor. Our data show that such a genetic pattern results in a marked decrease of insulin binding to cultured fibroblasts and a tendency of these individuals towards hyperinsulinaemia. None of these individuals showed hyperglycaemia, which is a characteristic of Type 2 diabetes mellitus.

Table 1. Characteristics of the subjects who underwent an oral glucose tolerance test

Subjects	Sex	Age years	Weight (kg)	Weight/Height (kg/m ²)	HbA _{1c} (%)
327	F	60	64	22.1	5.1
328	F	69	60	21.3	5.1
329	M	29	78	25.5	5.3

Subjects, materials and methods

The patient (leprechaun Geldermalsen) has been described previously [4].

Subject data are given in Table 1. Subject numbers refer to the individuals given in the pedigree in Figure 1. Informed consent was obtained. The Ethical Committee of the Faculty of Medicine of the University of Leiden has approved these studies.

Fibroblast culture

Fibroblasts were developed from explants of skin biopsies (forearm) from the leprechaun patient (470), his parents (329, 330) and three grandparents (326, 327, 328). Control fibroblasts were grown from six subjects (four males, two females), their ages ranging from 0 to 40 years. Cells were grown in F10 medium supplemented with 10% heat inactivated fetal calf serum in a 2.5% CO₂ atmosphere.

Insulin binding, uptake of 2-deoxyglucose (2-DOG) and insulin stimulated autophosphorylation of the receptor

These assays were performed as described previously [4].

Oral glucose tolerance test (OGTT)

After an overnight fast, blood was drawn for the determination of insulin and glucose concentrations. Subsequently, a 75 g oral glucose load was given and blood was drawn 30, 60, 90, 120, 150 and 180 min later for measurement of plasma glucose and insulin concentrations. Glucose was measured in whole blood using the continuous flow hexokinase method and insulin was measured by radioimmunoassay, as described previously [7].

Statistical analysis

Results are expressed as mean values $\pm$ SD for at least two independently performed experiments. Each of these experiments was performed in duplicate. Differences were evaluated by Student's *t*-test.

Results

The pedigree of the Geldermalsen family is shown in Figure 1, indicating the consanguinity. The consanguinity suggests that the leprechaun patient (470) had a homozygous defect. This was recently confirmed by our finding of a homozygous leucine to proline mutation in the α -chain of the receptor. The parents of the patient (329 and 330) and the grandparents from the consan-

Fig. 1. Pedigree of the family of leprechaun Geldermalsen. Specific binding of ¹²⁵I-insulin to fibroblasts from the members of this family are indicated as percentage of specific binding to control fibroblasts (see also Fig. 2). Numbers represent specific fibroblast codes from these individuals used in Figures 2, 3, 4 and 5. Circles represent females, squares represent males, a diagonal line through the symbol means deceased

Fig. 2. Levels of insulin binding to fibroblasts from members of the Geldermalsen family and the averaged binding to the fibroblasts from six control subjects (C). Open bars represent total binding of ¹²⁵I-insulin, filled bars non-specific binding obtained by the addition of 10⁻⁶ mol/l non-radioactive insulin. Insulin binding was performed using 10⁶ fibroblasts and a ¹²⁵I-insulin concentration of 3 · 10⁻¹¹ mol/l. Standard deviations are indicated. Numbers represent the individuals in the pedigree of Figure 1

guineous line (327 and 328) were found to be heterozygous for the mutation. The grandfather (326) who maps outside the consanguineous line and 21 control individuals did not have this mutation [3]. The levels of ¹²⁵I-insulin binding to fibroblasts from the members of this family are given in Figure 2. Averaged binding data of insulin to fibroblasts from 6 healthy control subjects are also shown (C). The data indicate that the heterozygous carriers of the disease within this family (329, 330, 327, 328) have a severe reduction of insulin binding, whereas the non-carrier grandfather (326) has normal binding. Fibroblasts from the leprechaun patient lack a significant level of specific binding of insulin. As these binding data were obtained at low insulin concentrations (approximately 3 · 10⁻¹¹ mol/l), the results indicate that the number of high affinity insulin binding sites on the cell surface is decreased. The binding data on parents

Fig. 3. Maximum autophosphorylation levels of the insulin receptor β chain in glycoprotein from fibroblasts from the members of the Geldermalsen family and the averaged level from fibroblasts of six control subjects. Autophosphorylation experiments were performed on identical amounts of glycoproteins in the presence of 10^{-6} mol/l insulin. The level of ^{32}P incorporation in the β chain was measured by densitometry. Standard deviations are indicated. For number assignment see the pedigree in Figure 1. Maximal labelling corresponds to 10^{-16} mol ^{32}P incorporated/10 μg of glycoprotein

Fig. 4. Insulin stimulated uptake of 2-deoxyglucose (2-DOG) in fibroblasts from the members of the Geldermalsen family using 10^{-6} mol/l insulin. Standard deviations are indicated. For cell number assignment see Figure 1. Unstimulated uptake is taken at a relative value of 1 and corresponds to 0.25 nmol of 2-DOG/ 10^6 fibroblasts

and grandparents are consistent with a Mendelian mode of inheritance of the binding defect.

We have previously described [4] that an additional defect in the insulin receptor from this patient is a decrease in insulin stimulated autophosphorylation of the β -chain of the insulin receptor. In view of this, we have also investigated this activity of the insulin receptor from the family members. Figure 3 shows the maximum levels of autophosphorylation at 10^{-6} mol/l insulin. Except for the patient, no significantly decreased activity of insulin stimulated autophosphorylation has been observed in the members of this family. It is unlikely that the normalization of β -chain autophosphorylation is the result of an increased receptor number in the heterozygous carriers as mRNA levels for the insulin receptor are invariant in fibroblasts from these individuals. Also, cross-linking of ^{125}I -insulin to the receptor using glycoprotein fractions from these fibroblasts gives similar yields of labelled α -chain (data not shown).

Another indicator of the functionality of the insulin receptor is the stimulation of 2-DOG uptake in fibroblasts by insulin (Fig. 4). The insulin stimulated 2-DOG uptake by fibroblasts from the homozygous leprechaun patient (470) is 1.2 fold, whereas the heterozygous and control individuals show stimulation values of approximately 1.65 fold. The absolute levels of unstimulated 2-DOG uptake in these fibroblasts are given in the legend to Figure 4. These data indicate that fibroblasts from the heterozygous individuals have no detectable decrease in insulin stimulated uptake of 2-DOG.

Besides in vitro investigation of the function of the insulin receptor, OGTT's were performed to obtain an impression about insulin action in vivo. Three heterozygous non-obese members of this family (327, 328, 329, ages 60, 69 and 29 years, respectively) were studied. Some characteristics of these individuals are given in Table 1. Insulin and blood glucose levels were

Fig. 5 A and B. Values of (A) insulin (mU/l) and (B) plasma glucose (mmol/l) before (0 min) and after a 75 g glucose load. The values from grandmother (327, ●—●), grandmother (328, +—+) and father (329, ■—■) of the leprechaun patient are shown together with the data from healthy, non-obese control subjects [taken from 8–10 and 16]. The shaded area reflects the range in glucose and insulin levels of these control individuals. The diagnosis diabetes mellitus is given when glucose values rise above the glucose levels indicated in this figure by (*—*) [11]

measured over a period of 180 min and the values were compared with data from a healthy non-obese control group [8–11, 16]. The results are summarized in Figure 5. A slightly disturbed OGTT is observed in these heterozygous individuals as indicated by a tendency toward hyperinsulinaemia with no observed hyperglycaemia. On the contrary, blood glucose level tended toward low-normal values.

Discussion

In this study we have investigated insulin receptor functions *in vitro* and insulin action *in vivo* in relatives from a previously described leprechaun patient [4]. This leprechaun patient has a severe insulin resistance which is caused by an almost complete absence of insulin binding to cultured fibroblasts and an impaired autophosphorylation of the β -chain of the receptor. Because this patient is born from a consanguineous marriage it seemed likely that the genetic defect associated with the insulin resistance is homozygous in the patient and that the father, the mother and paternal as well as the maternal grandmother are heterozygous for the same mutation. We have recently characterized this mutation and shown that the patient is homozygous for a leucine to proline mutation at position 233 in the α -chain of the insulin receptor, whereas these family members are heterozygous for this mutation. Insulin binding was performed on fibroblasts from relatives of this patient and control subjects. The heterozygous relatives show insulin binding of only 20–40% of control values indicating a Mendelian mode of inheritance for the binding defect. The observation that individuals from the paternal and maternal line both show the insulin binding defect supports the homozygous nature of the defect in this patient. Studies on fibroblasts from other leprechaun patients, who are born from non-consanguineous relations and who are compound heterozygotes, also show an insulin binding defect [12, 13]. These studies indicate however, that a different type of binding defect can be inherited from each parent. These observations correlate with the finding that leprechaun Geldermalsen is indeed homozygous for the defect in insulin receptor function and that the heterozygous individuals from this family have only one allele coding for a functional receptor.

The autophosphorylation of the insulin receptor at tyrosine residues is considered to reflect the receptors' activity for signal transduction which results in enhanced glucose uptake [5, 6]. Quantitating the autophosphorylation activity in heterozygous carriers is another way of investigating the mode of inheritance of the receptor malfunction found in the patient. Within this family none of the individuals showed a significantly decreased autophosphorylation of the receptor after insulin stimulation. No decrease of insulin stimulated 2-DOG uptake was detected in cultured fibroblasts from

this family either, confirming the role of autophosphorylation in the insulin stimulated glucose uptake. The autophosphorylation activity apparently overcomes the binding defect.

It should be emphasized, however, that the 2-DOG assay using fibroblasts is not sensitive enough to detect small variations in hexose uptake due to the large SD's. Thus, it is also possible that subtle differences in glucose uptake are not detected in our tests. Because the heterozygous individuals show a reduced insulin binding on their cultured fibroblasts we suppose that compensation mechanisms acting at the level of autophosphorylation are involved in maintaining a more or less normal glucose uptake. The insulin stimulated autophosphorylation of the insulin receptor is regulated by different factors, such as calcium-activated and phospholipid-dependent protein kinase C [15]. Consequently, there may exist a population of receptors active in autophosphorylation and an inactive receptor population and the ratio between these populations may vary depending on regulatory feed-back processes. The normalisation of the insulin stimulated receptor autophosphorylation seems unable to fully compensate for the insulin binding defect in the heterozygous individuals as the OGTT tests indicate a tendency toward hyperinsulinaemia. The resulting glucose levels, however, are almost normal. These data agree with a situation in which the target tissues, as a result of a decreased number of functional insulin receptors, require elevated insulin concentrations for glucose disposal. On the other hand, the pancreas responds in such a way to the glucose level that a more or less normal glucose homeostasis can be maintained.

Genetic factors involved in the genesis of milder forms of insulin resistance such as Type 2 diabetes mellitus are unknown. The OGTT data on the heterozygous individuals indicate that a genetic make-up which leads to a decrease in the number of functional insulin receptors is not sufficient for the development of Type 2 diabetes mellitus. Two of these individuals are 60 and 69 years old. At that age a genetic predisposition for Type 2 diabetes is likely to manifest itself by high blood glucose levels during OGTT [11]. None of the three tested heterozygous relatives showed such an abnormality, in contrast blood glucose levels tended toward low values, with elevated insulin levels. As a binding defect alone seems insufficient to develop Type 2 diabetes, other or additional factors must be involved in the development of Type 2 diabetes mellitus. One of these factors may be the tyrosine kinase activity of the insulin receptor. An acquired decrease in this activity is often observed in Type 2 diabetes [8, 14]. In the heterozygous individuals studied in this paper, no indication for a decreased activity is observed. In contrast, the *in vitro* experiments indicate that this activity even seems to compensate for the decreased level of insulin binding. Other studies have suggested that in addition to insulin resistance of the periphery an insufficient in-

sulin production by the pancreas is involved in the development of Type 2 diabetes mellitus [17]. The pancreases of the individuals studied here seemed quite responsive to an increase in blood glucose levels.

Also, variations in the glucose transporter function may be an additional factor that is involved in this disease [18]. Studies of other patients with extreme insulin resistance may help to identify additional genetic factors involved in the pathogenesis of the several forms of insulin resistance. This may give insight into those factors specifically involved in the genesis of Type 2 diabetes mellitus.

Acknowledgements. This work was supported by the Netherlands Organization for Advancement of Pure Research (NWO) through the Foundation for Medical Research (MEDIGON) and the Diabetes Fonds Nederland. We wish to thank Dr. J. K. Radder for helpful discussions, Dr. M. Fröhlich for assistance with the clinical chemistry and Dr. W. Kleijer for providing the fibroblasts.

References

1. Yoshimasa Y, Seino S, Whittaker J, Kakehi T, Kosaki A, Kuzuya H, Imura H, Bell GI, Steiner DF (1988) Insulin-resistant diabetes due to a point mutation that prevents insulin-proreceptor processing. *Science* 240: 784-787
2. Kadowaki T, Bevins CL, Cama A, Ojamaa K, Marcus-Samuels B, Kadowaki H, Beitz L, McKeon C, Taylor SI (1988) Two mutant alleles of the insulin receptor gene in a patient with extreme insulin resistance. *Science* 240: 787-790
3. Klinkhamer MP, Groen NA, van der Zon GCM, Lindhout D, Krans HMJ, Möller W, Maassen JA (1989) A leucine to proline mutation in the insulin receptor in a family with insulin resistance. *EMBO J* 8: 2503-2507
4. Maassen JA, Klinkhamer MP, van der Zon GCM, Sips H, Möller W, Krans HMJ, Lindhout D, Beemer FA (1988) Fibroblasts from a leprechaun patient have defects in insulin binding and insulin receptor autophosphorylation. *Diabetologia* 31: 612-617
5. Chou CK, Dull TJ, Russell DS, Gerzi R, Lebwohl D, Ullrich A, Rosen OM (1987) Human insulin receptors mutated at the ATP binding site lack protein tyrosine kinase activity and fail to mediate post receptor effects of insulin. *J Biol Chem* 262: 1842-1847
6. White MF, Kahn CR (1984) The insulin receptor and tyrosine phosphorylation. *The enzymes*, Vol XVII. Academic Press, New York London, pp 247-309
7. Nijs HGT, Radder JK, Fröhlich M, Krans HMJ (1988) Insulin action is normalized in newly diagnosed type I diabetic patients after three months of insulin treatment. *Metabolism* 37: 473-478
8. Freidenberg GR, Henry RR, Klein HH, Reichart DR, Olefsky JM (1987) Decreased kinase activity of insulin receptors from adipocytes of non-insulin-dependent diabetic subjects. *J Clin Invest* 79: 240-250
9. Mohan V, Snehalatha C, Ramachandran A, Viswanathan R (1985) C-peptide responses to glucose load in maturity onset diabetes of the young. *Diabetes Care* 8: 69-72
10. Itoh M, Hirooka Y, Nihei N (1983) Response of plasma somatostatin-like immunoreactivity (SLI) to a 75 g oral glucose tolerance test in normal subject and patients with impaired glucose tolerance. *Acta Endocrinol* 104: 468-474
11. National Diabetes Data Group (1979) Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. *Diabetes* 28: 1039-1057
12. Endo F, Nagata N, Priest JH, Longo N, Elsas II LJ (1987) Structural analysis of normal and mutant insulin receptors in fibroblasts cultured from families with leprechaunism. *Am J Hum Genet* 41: 402-417
13. Maassen JA, Klinkhamer MP, Odink RJH, Sips H, van der Zon GCM, Wieringa T, Krans HMJ, Möller W (1988) Improper expression of insulin receptors on fibroblasts from a leprechaun patient. *Eur J Biochem* 172: 725-729
14. Caro JF, Ittoop O, Pories WJ, Meelheim D, Flickinger EG, Thomas F, Jenquin M, Silverman JF, Khazanie PG, Sinh MK (1986) Studies on the mechanism of insulin resistance in the liver from humans with non-insulin dependent diabetes mellitus. Insulin action and binding in isolated hepatocytes, insulin receptor structure and kinase activity. *J Clin Invest* 78: 249-258
15. Takayama S, White MF, Lauris V, Kahn CR (1984) Phorbol esters modulate insulin receptor phosphorylation and insulin actions in cultured hepatoma cells. *Proc Natl Acad Sci USA* 81: 7797-7801
16. Hollenbeck C, Reaven GM (1987) Variations in insulin-stimulated glucose uptake with normal glucose tolerance. *J Clin Endocrinol Metab* 64: 1169-1173
17. Reaven GM (1988) Role of insulin resistance in human disease. *Diabetes* 37: 1595-1607
18. Garvey WT, Huecksteadt TP, Matthaei S, Olefsky JM (1988) Role of glucose transporters in the cellular insulin resistance of type II non-insulin-dependent diabetes mellitus. *J Clin Invest* 81: 1528-1536

Received: 27 January 1989
and in final revised form: 2 July 1989

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