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Homozygosity for a new mutation (Ile^{119}→Met) in the insulin receptor gene in five sibs with familial insulin resistance

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Abstract

Mutations in the insulin receptor gene can cause genetic syndromes such as leprechaunism that are associated with extreme insulin resistance. We have investigated a patient with leprechaunism born of a consanguineous marriage. All 22 exons of the insulin receptor gene were screened for mutations using denaturing gradient gel electrophoresis. Thereafter, the nucleotide sequences of selected exons were determined directly. The patient was homozygous for a point mutation in exon 2 of the insulin receptor gene which results in the substitution of methionine for isoleucine at codon 119. Thus, the mutant allele encodes a receptor that has a mutation in the putative insulin binding domain. Accordingly, the mutant receptor would be predicted not to transduce the insulin signal effectively. In spite of a homozygous abnormality of the insulin receptor gene and many of the clinical features of severe insulin resistance, the proband's clinical syndrome was noticeably different from previously described patients with leprechaunism who usually die within the first six months of life. There are a total of nine children in the family, five of whom are homozygous for the Ile^{119}→Met mutation in the insulin receptor gene, and are clinically affected with varying degrees of severity. Four unaffected sibs are clinically normal; two are heterozygous carriers of the mutant allele, one is homozygous for the normal allele, and one unaffected sib was not available for molecular studies.

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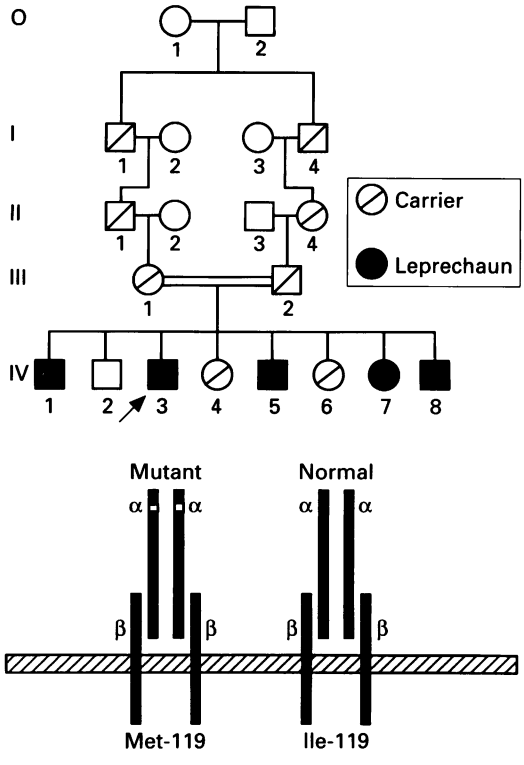
Mutations in the insulin receptor (IR) gene can impair insulin action in vivo. Approximately 50 mutations have been identified in the IR gene in patients with several distinct clinical syndromes associated with insulin resistance, including leprechaunism, the Rabson-Mendenhall syndrome, type A extreme insulin resistance, and also in a few patients with non- insulin dependent diabetes mellitus.[1,2] Recently, we have investigated a large consanguineous pedigree in which five of nine children had a syndrome with features of both leprechaunism and the Rabson-Mendenhall syndrome.[3] As discussed more extensively in the previous publication,[3] the patients in this family have multiple clinical features that are frequently associated with extreme insulin resistance, including acanthosis nigricans, growth retardation, and diabetes mellitus with hyperinsulinaemia. We now report that the disease in this family results from a missense mutation in exon 2 of the insulin receptor gene substituting Met for Ile^{119} in the α subunit of the insulin receptor.

Methods and results

We used denaturing gradient gel electrophoresis (DGGE) to screen all 22 exons of the insulin receptor gene.[4] Because the parallel DGGE for exon 2 was equivocal, we carried out perpendicular DGGE, which was abnormal. Direct sequencing of exon 2 showed that ATG^{Met} was substituted for the normal ATC^{Ile} at codon 119. Since the mutation creates a new recognition site for the restriction endonuclease *Nla*III, restriction analysis was used to confirm the presence of the mutation, as well as to screen the seven sibs for whom genetic material was available. Both parents were heterozygous and all five affected children were homozygous for this mutation. Two of the four clinically unaffected children were heterozygous for the mutation (figure). All five clinically affected children were hyperinsulinaemic during oral glucose tolerance testing (table). They had acanthosis nigricans, and symptomatic hypoglycaemia. The father was obese and had fasting hyperinsulinaemia, suggesting that he was moderately insulin resistant. The healthy child who was homozygous for the normal allele had normal insulin sensitivity. A more complete clinical description has been reported previously.[3]

We calculated a lod score for this consanguineous pedigree using the method of Lander and Botstein.[5-7] The calculated lod score ranges from 3·4 to 4·6 in favour of the conclusion that the disease is caused by the allele from the Ile^{119}→Met mutation. The calculated value of the lod score depends upon the estimated prevalence of the mutant allele with high values of lod corresponding to low prevalences, and low values of lod corresponding to high prevalences of the mutant allele in the general population.[5,6] Inasmuch as this mutation has never been detected previously, it is likely that the higher values of lod are more appropriate for this pedigree.



Pedigree of the Lep-UAE family (upper panel). The proband is IV·3. The parents of the affected children, III·1 and III·2, are second cousins. Clinically affected persons (homozygotes) are indicated by solid symbols. Heterozygotes are indicated by a diagonal line in the symbol. The pedigree shows eight children; samples from the ninth child were not available for molecular genetic studies. A diagram depicting the Met[119] mutation in the α subunit of the insulin receptor is shown in the lower panel.

Oral glucose tolerance test in family of Lep UAE-1. Glucose tolerance testing performed at the Tawam Hospital in the United Arab Emirates. Glucose values are in mmol/l. Insulin values are in mU/l, with a normal range of 4-24 mU/l fasting, and 20-300 mU/l after 1·75 g/kg oral glucose load

Patient	Parameter	Time			
		Fasting	*30 min*	*60 min*	*120 min*
Father	Glucose	5·8	11·0	15·7	11·4
	Insulin	31·4	64·4	141	102
Mother	Glucose	6·3	8·8	11·3	8·8
	Insulin	15·2	82·3	54·2	108·4
IV·1	Glucose	3·6	8·5	13	15·0
	Insulin	44	420	656	1590
IV·2 (normal)	Glucose	5·3	6·3	ND	5·0
	Insulin	ND	78	ND	19·5
IV·3	Glucose	2·6	12·2	14·4	13·2
	Insulin	48	576	227	495
IV·5	Glucose	2·6	4·1	6·1	9·0
	Insulin	21	112	129	656
IV·7	Glucose	1·5	2·6	6·0	9·4
	Insulin	63	141	372	640
IV·8	Glucose	2·6	5·7	5·4	9·8
	Insulin	55	161	240	268

ND signifies not done.

Discussion

We have identified a consanguineous pedigree containing five children who have a syndrome with features of both leprechaunism and the Rabson-Mendenhall syndrome resulting from a homozygous mutation in codon 119 of exon 2 of the insulin receptor gene. Ile[119] is conserved in insulin receptors of several species (human, monkey, rat, and mouse) and in the human insulin-like growth factor-1 receptor. In the insulin receptor related receptor gene, there is a conservative substitution of Val for Ile. Previous IR mutations in this general area have been shown to result in defective intracellular transport and post-translational processing of the receptor.[1 2] In addition, this region of the insulin receptor is believed to be important in insulin binding.[1] Transfection experiments are under way to investigate the functional properties of the Met[119] mutant receptor.

The observations reported in this study strongly support the hypothesis that the Ile[119]→Met mutation is responsible for causing insulin resistance in this family. Furthermore, the mutation appears to cause the syndrome of leprechaunism in a recessive fashion. As is typically observed in leprechaunism, there appear to be two metabolic defects in these patients: fasting hypoglycaemia and abnormal glucose tolerance (table). It is likely that the glucose intolerance is the result of insulin resistance caused by the mutation in the insulin receptor gene. At least two explanations have been proposed to explain hypoglycaemia. Longo *et al*[8] have suggested that although some mutations may decrease the number of receptors, they may also increase the basal activity of the receptors. The decrease in the number of receptors could cause insulin resistance, while the increased basal activity could cause fasting hypoglycaemia. However, this explanation could not account for hypoglycaemia in all patients, for example, patients who do not express mutant receptors on the cell surface.[1 2 9] On the other hand, Bier *et al*[10] provided evidence that hypoglycaemia in leprechaunism was the result of metabolic changes that resembled "accelerated starvation". According to this hypothesis, starvation and leprechaunism share in common a marked diminution of insulin action. In starvation, this is because of physiological suppression of insulin secretion; in leprechaunism, it is because of the pathological impairment in the ability of the insulin receptor to mediate insulin action. The identification of mutations causing leprechaunism will facilitate further studies into the pathogenetic mechanisms in this syndrome.

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