CLINICAL STUDY

Comparison of the impact of the I27L polymorphism of the hepatocyte nuclear factor-1alpha on estimated and measured beta cell indices

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Abstract

Objective: We investigated the impact of the I27L polymorphism of the hepatocyte nuclear factor-1alpha gene on measured and estimated beta cell indices. We also examined the conservation of this amino acid among different species.

Design and methods: Estimated first and second phase insulin responses (1stPH_S and 2ndPH_S) were estimated from oral glucose tolerance tests in 78 glucose tolerant subjects. Among these subjects, first and second phase insulin responses (1stIR and 2ndIR) were measured in 60 subjects. The I27L genotypes were determined from genomic DNA. We also reviewed the published peptide sequence data on this polymorphism.

Results: The estimated beta cell indices correlated well with the measured indices. Although the impact of this polymorphism was noted in the measured indices ($P < 0.01$ for 1stIR and $P = 0.04$ for 2ndIR) from 60 subjects, the differences in the estimated indices were only noted in the extended sample set with 78 subjects ($P = 0.05$ for 1stPH_S and $P = 0.04$ for 2ndPH_S). This polymorphism occurs in the dimerization domain, which is completely conserved within human, rat, mouse and hamster. This amino acid is also conserved in chicken and zebrafish, but not in the frog. This conservation suggests a possible biological importance of this amino acid.

Conclusions: By increasing the sample size, we demonstrated the role of the I27L polymorphism in the pathogenesis of type 2 diabetes by using estimated beta cell indices. The conservation among species suggests a possible biological importance of this amino acid. Analysis of the published data confirms a modest role of this polymorphism in type 2 diabetes.

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Introduction

Type 2 diabetes is the most common metabolic disorder, affecting at least 5% of the world population and is a result of complex interactions between genetic and non-genetic (including environmental) factors. Although most epidemiological studies have demonstrated the role of insulin resistance in the pathogenesis of type 2 diabetes, it is now well accepted that the failure of beta cells to compensate for prevailing insulin resistance is also required for the development of overt diabetes. Although diabetes and its related traits have been shown to cluster within families, their transmissions do not follow a Mendelian fashion, except for some rare syndromes such as maturity-onset diabetes of the young (MODY), which accounts for 2–5% of patients with type 2 diabetes. Beta cell dysfunction has been demonstrated in all subtypes of MODY

identified. Compared with the other types of MODY, MODY3 is the most common form in most populations, except in France where it is the second most common form, after MODY2 (1). The defect of insulin secretion is a hallmark sign of MODY3 patients and defective beta cell function is evident before the onset of diabetes (2). Mutations in the hepatocyte nuclear factor-1alpha (HNF1alpha) gene have been identified in MODY3 patients from various ethnic backgrounds (1). Studies of HNF1alpha knockout mice have demonstrated defective beta cell function with hyperglycemia (3). Therefore, the MODY3 locus is a superb candidate region for type 2 diabetes, especially for beta cell dysfunction.

This region has been implicated as playing a role in type 2 diabetes. A study of 26 families from a Swedish-speaking population isolated in western Finland revealed a suggestive linkage of type 2 diabetes

to this region (4). Further analysis of six families with the lowest insulin levels at 30 min confirmed the linkage at this locus (4). Another study of 53 affected sib pairs from 21 Caucasian families examined for nephropathy also revealed the linkage at this locus (5). It is most intriguing that nephropathy is a common complication of MODY3 (6) and renal dysfunction is a feature of HNF1alpha knockout mice (7). Linkage analysis of a three-generation family of 25 members from Australia demonstrated a linkage of diabetes at this region (8). Furthermore, suggestive linkage of diabetes at this locus was also found in Caucasian- and African-Americans (9). Very recently, a private single nucleotide polymorphism within HNF1alpha was found to affect the age of onset in Oji-Cree Indians (10). These studies indicate that the MODY3 region could play a role in some patients with type 2 diabetes, raising an intriguing possibility of different mutations at a single gene, in which severe mutations cause MODY3 with profound beta cell dysfunction and mild mutations lead to type 2 diabetes with a low beta cell response (4).

Although beta cell dysfunction is a well-recognized phenomenon in type 2 diabetes, the measurement of beta cell function is a time-consuming and labor-intensive process. Two methods have been proposed to estimate beta cell function from fasting samples and/or oral glucose tolerance tests (OGTTs). Based on the homeostasis model assessment (HOMA), beta cell function is calculated from fasting plasma glucose and insulin concentrations (FPG and FPI), and expressed as %B (11). Recently, new formulae have been developed for both the estimated first and second phase insulin responses (1stPH_S and 2ndPH_S) based on the result of the OGTT (12).

To elucidate the role of the MODY3 locus in beta cell function, we examined the impact of the I27L polymorphism of the HNF1alpha gene on the estimated beta cell indices. To explore the potential importance of the amino acid at this location, we investigated the amino acid conservation among species around the I27L polymorphism.

Subjects and methods

Study subjects

Only healthy subjects who were receiving no regular medical treatments were invited to undergo a screening test as outpatients after an overnight fast. This included a standard OGTT with 75 g glucose and a brief physical examination as previously described (13). To exclude the secondary influence of beta cell function from abnormal glucose tolerance (14), only those who were noted to be glucose tolerant (FPG < 6.1 mmol/l, interval plasma glucose < 11.1 mmol/l and 2 h plasma glucose < 7.7 mmol/l) were invited back for the assessment of beta cell function using a hyperglycemic clamp technique. To minimize the effect of smoking, they were asked to refrain from smoking for at least 12 h before the study. Briefly, after fasting overnight and resting in the General Clinical Research Center of this institution, participants received a bolus of 50% dextrose solution based on their body surface area (11.4 g/m²) at $T = 0$ min. Continuous infusion of 30% dextrose solution commenced at $T = 15$ min at variable rates which were adjusted every 5 min based on the prevailing plasma glucose levels to maintain a plasma glucose level around 10 mmol/l toward $T = 180$ min using the negative feedback principle (15). The coefficient of variation for steady-state plasma glucose levels was $5.8 \pm 0.3\%$. The first phase insulin response (1stIR) was the sum of plasma insulin levels during the first 10 min ($T = 2.5, 5, 7.5$ and 10 min) and the second phase insulin response (2ndIR) was the average of plasma insulin levels at $T = 130, 140, 150, 160, 170$ and 180 min. FPG and FPI were the averages of three samples before the OGTT. Plasma glucose, insulin and lipid concentrations were assayed as previously described (16). The demographic features of the studied subjects are shown in Table 1. The study was approved by the Human Subject Protection Committee of this institution. Written informed consent was obtained from all participants before entering

Table 1 Clinical characteristics of the study subjects.

	Mean $\pm$ s.d.	Minimum	Maximum
<i>n</i>	78	—	—
Age (years)	27 $\pm$ 6	19	39
Gender (female)	41 (53%)	—	—
Body mass index (kg/m ²)	24.14 $\pm$ 3.78	17.58	34.26
Waist to hip ratio (cm/cm)	0.800 $\pm$ 0.082	0.649	1.033
Systolic blood pressure (mmHg)	115 $\pm$ 10	94	137
Diastolic blood pressure (mmHg)	67 $\pm$ 7	48	89
Fasting plasma glucose (mmol/l)	4.69 $\pm$ 0.38	3.88	5.56
Plasma glucose at 30 min (mmol/l)	7.43 $\pm$ 1.25	5.07	10.10
Plasma glucose at 60 min (mmol/l)	7.02 $\pm$ 1.46	4.44	10.20
Plasma glucose at 90 min (mmol/l)	6.14 $\pm$ 1.30	3.62	9.69
Plasma glucose at 120 min (mmol/l)	5.59 $\pm$ 1.02	3.38	7.60

the study. We confirm that the study has complied with the recommendations of the Declaration of Helsinki.

Estimation of beta cell function

The estimated beta cell function based on the HOMA (11) %B, was calculated based on the formula: $\%B = 20 \times \text{FPI} / (\text{FPG} - 3.5)$ (17). Plasma glucose concentration was in mmol/l and plasma insulin concentration was in $\mu\text{U/ml}$ for this estimate.

The estimates of beta cell function proposed by Stumvoll *et al.* (12) were calculated using the two formulae: $1\text{stPH}_S = 1283 + (1.829 \times \text{plasma insulin concentration at 30 min}) - (138.7 \times \text{plasma glucose concentration at 30 min}) + (3.772 \times \text{FPI})$ and $2\text{ndPH}_S = 287 + (0.4164 \times \text{plasma insulin concentration at 30 min}) - (26.07 \times \text{plasma glucose concentration at 30 min}) + (0.9226 \times \text{FPI})$. These estimations were based on plasma glucose concentrations in mmol/l and plasma insulin concentrations in pmol/l (12).

Search and retrieval for the HNF1alpha protein

The protein database at the National Center for Biotechnology Information (NCBI, <http://www.ncbi.nlm.nih.gov/blast/>) was searched by the Protein BLAST function with 'Human HNF1 α ' (A36749). All the potential full-length peptides containing the I27L polymorphism were reviewed. The longest peptides of each species were compared.

Statistical analysis

The continuous variables that failed the normality test were logarithmically transformed before analysis. The variables transformed were age, body mass index (BMI), waist to hip ratio (WHR), plasma insulin levels, 1stIR, 2ndIR, %B, 1stPH_S and 2ndPH_S. Differences in continuous variables between groups of subjects were tested with either one-way ANOVA or Student's *t*-test when appropriate. Differences in proportions were evaluated by a chi-square test. The relationships between variables were determined by using a simple regression analysis. SYSTAT 10.0 for Windows package from SPSS, Inc. (Chicago, IL, USA) was used for the statistical analysis. Data are presented as arithmetic means with 95% confidence intervals, unless otherwise specified. $P < 0.05$ (two-tailed) was considered significant.

Results

This study included 78 healthy and normotensive Caucasian subjects with normal glucose tolerance as shown in Table 1. Among them, 60 subjects underwent hyperglycemic clamps for the assessment of 1stIR and

2ndIR with highly similar clinical characteristics (33 females; age 27 ± 6 years old; BMI $24.17 \pm 3.86 \text{ kg/m}^2$; WHR $0.798 \pm 0.082 \text{ cm/cm}$; systolic blood pressure $116 \pm 10 \text{ mmHg}$; diastolic blood pressure $68 \pm 8 \text{ mmHg}$; FPG $4.65 \pm 0.37 \text{ mmol/l}$). In spite of normal glucose tolerance, their 1stIR and 2ndIR were widely distributed with a range of 412–4569 pmol/l for 1stIR and 104–1567 pmol/l for 2ndIR. The measured beta cell indices (1stIR and 2ndIR) were well correlated with the estimated beta cell indices (1stPH_S and 2ndPH_S respectively) as shown in Fig. 1. However, only %B correlated with 2ndIR ($P = 0.04$, $r^2 = 0.07$).

The impact of the I27L polymorphism on beta cell function was first examined in 1stIR and 2ndIR. We found differences in both 1stIR ($P < 0.01$) and 2ndIR ($P = 0.04$) among three genotypes in 60 subjects who underwent the hyperglycemic clamps (Table 2 and Fig. 2). Although no differences in either 1stPH_S or 2ndPH_S were noted among the three genotypes in 60 clamped subjects, the differences in 1stPH_S ($P = 0.05$) and 2ndPH_S ($P = 0.04$) were observed in the extended 78 subjects. However, no difference was noted in %B in either sample set.

By blasting the GenBank database with human HNF1alpha (A36749), we identified HNF1alpha from rat (*Rattus norvegicus*) (P15257), mouse (*Mus musculus*) (P22361), hamster (*Cricetinae* species) (A46149), chicken (*Gallus gallus*) (S35574), frog (*Xenopus laevis*) (JT0766) and zebrafish (*Danio rerio*) (AAL48219). A comparison with the human HNF1alpha showed highly conserved amino acid identities in rats, mice and hamsters, up to 81%. The I27L polymorphism occurs in a completely conserved region among human, rats, mice and hamsters. This amino acid remains conserved even in zebrafish, which had the lowest identity (52%) among these six species (Fig. 3). However, frogs carry lysine instead of isoleucine at this position. High conservation among species at this position suggests a possible biological importance of this amino acid.

Discussion

An impact of the I27L polymorphism at the HNF1alpha gene locus was observed in both measured and estimated beta cell indices. Although differences were not observed in estimated beta cell indices (1stPH_S and 2ndPH_S) from the subset of 60 subjects who underwent hyperglycemic clamps, an impact on 1stPH_S and 2ndPH_S was observed in 78 subjects. Therefore, the I27L polymorphism is associated with beta cell dysfunction.

Despite the correlation between 1stPH_S and 1stIR and between 2ndPH_S and 2ndIR, 1stPH_S only explained 30% of the variation in 1stIR and 2ndPH_S only explained 22% of the variation in 2ndIR. Therefore,

Figure 1 Regression analyses of estimated and measured beta cell indices. (A) The relationship between estimated first phase insulin response (1stPH_s) and measured first phase insulin response (1stIR, $r^2 = 0.30$, $P < 0.01$). (B) The relationship between estimated second phase insulin response (2ndPH_s) and measured second phase insulin response (2ndIR, $r^2 = 0.22$, $P < 0.01$). All data are plotted on a logarithmic scale. Solid lines represent the regression lines and dashed lines the 95% confidence interval.

the impact of the I27L polymorphism on 1stPH_s and 2ndPH_s was not observed in the subset of 60 subjects in whom the differences in 1stIR and 2ndIR were observed. The differences in 1stPH_s and 2ndPH_s were only observed in the whole sample. These observations suggest that although the estimated beta cell indices are not perfectly correlated with the measured beta

cell indices, they can be used as surrogates. Less than perfect correlation can be overcome by increasing sample size, as observed in 1stPH_s and 2ndPH_s.

The conservation among species provides indirect evidence of the importance of amino acid(s) at certain position(s). The dimerization domain of HNF1alpha consists of the first 33 amino acids (18). Comparing

Table 2 Comparison of clinical characteristics, estimated and measured beta cell indices by the I27L polymorphism: means (95% confidence intervals).

	II	IL	LL	P value			
				II vs IL	IL vs LL	II vs LL	ANOVA
<i>n</i>	29	37	12	—	—	—	—
Age (years)	27 (25–30)	26 (25–28)	28 (25–32)	NS	NS	NS	NS
Gender (female)	14 (48%)	20 (54%)	6 (58%)	NS	NS	NS	NS
Body mass index ^a (kg/m ²)	24.54 (23.07–26.10)	23.88 (22.69–25.13)	22.28 (20.90–23.75)	NS	NS	NS	NS
Waist to hip ratio ^a (cm/cm)	0.811 (0.781–0.842)	0.798 (0.774–0.823)	0.751 (0.697–0.811)	NS	NS	0.04	NS
Systolic blood pressure (mmHg)	115 (111–119)	115 (112–119)	114 (107–120)	NS	NS	NS	NS
Diastolic blood pressure (mmHg)	67 (64–70)	66 (64–69)	69 (64–74)	NS	NS	NS	NS
Estimated beta cell indices							
%B ^a	167 (145–193)	141 (124–159)	129 (100–167)	NS	0.07	0.06	NS
1stPH _s ^a	1216 (1056–1400)	1085 (1003–1175)	958 (829–1107)	NS	NS	0.05	0.05
2ndPH _s ^a	319 (283–361)	288 (269–308)	258 (227–292)	NS	NS	0.04	0.04
Measured beta cell indices							
<i>n</i>	22	30	8	—	—	—	—
1stIR ^a (pmol/l)	1623 (1299–2029)	1324 (1114–1575)	827 (550–1244)	NS	0.02	<0.01	<0.01
2ndIR ^a (pmol/l)	375 (295–476)	359 (315–410)	237 (156–360)	NS	0.01	0.04	0.04

^a Geometric means (95% confidence intervals).

NS, $P > 0.10$.

Figure 2 Measured beta cell function by genotypes. (A) The measured first phase insulin response by the I27L polymorphisms. (B) The measured second phase insulin response by the I27L polymorphisms. Data are medians with 75 percentiles.

peptide sequences among human, mouse, rat and hamster, we found complete conservation of the first 37 amino acids, which extends beyond the dimerization domain. The next functional region is the POU A domain, which consists of amino acids 100–184 (18). The only non-conserved amino acid within the POU A domain is amino acid 106, threonine in human and serine in mouse, rat and hamster. In contrast, more variations are observed in the interval peptide sequence (amino acids 34–99) between the dimerization and POU A domains and non-conservations are noted in amino acids 38, 39, 41, 42, 53, 58, 67, 69, 73, 82 and 89. These observations indicate the importance of functional domains and conservation within the functional domains. Conservation of the dimerization domain is less in the comparison of human with chicken, frog and zebrafish. However,

isoleucine at this position is conserved among species, except frog. Since the I27L polymorphism occurs within the dimerization domain and on a highly conserved amino acid, this substitution of isoleucine with leucine could have a potential biological consequence, which remains to be demonstrated.

To further elucidate the role of I27L polymorphism, we searched the published association studies of this polymorphism. A computer search in the PubMed database of the NCBI was performed on 1 July 2002 using a combination of either 'HNF1 AND diabetes' or 'TCF1 AND diabetes'. Abstracts of identified articles, if available on-line, were reviewed. For those without on-line abstracts, identified articles were reviewed. The population association studies of this gene with type 2 diabetes were identified. Systematic search and critical appraisal of the identified articles were performed

Species	Accession	Identity	Amino acid sequence
Homo sapiens	A36749		LESGLSKEALI <u>Q</u> ALGEPGPYL
Rattus norvegicus	P15257	81%	LESGLSKEALI <u>Q</u> ALGEPGPYL
Mus musculus	P22361	81%	LESGLSKEALI <u>Q</u> ALGEPGPYL
Cricetinae gen. sp.	A46149	81%	LESGLSKEALI <u>Q</u> ALGEPGPYL
Gallus gallus	S35574	66%	LESGLtKETLlkgLsEaePYv
Xenopus laevis	JT0766	61%	LESGvtKEALkkALadgdnYa
Danio rerio	AAL48219	52%	LgSGLSKElLIQAmGdlerer

Figure 3 Comparison of peptide sequence among difference species. GenBank accession number for each species are provided. Identity for each species was compared with the human peptide sequence. The amino acids shown corresponded to amino acids 16–37. The underlined amino acids are those corresponding to the wild type amino acid at position 27 of the human peptide sequence.

Table 3 Analysis of the published association data on the I27L polymorphism.

Author	Ethnicity	Control group		Diabetic group		P
		n	L allele (%)	n	L allele (%)	
Urhammer <i>et al.</i> (24)	Caucasian	242	26.30	254	31.50	0.07
Behn <i>et al.</i> (20)	Caucasian	124	45.16	109	47.24	0.65
Rissanen <i>et al.</i> (23)	Caucasian	82	40.00	140	46.00	0.23
Lehto <i>et al.</i> (21)	Caucasian	92	34.24	115	35.65	0.76
Nishigori <i>et al.</i> (22)	Japanese	100	42.00	60	44.00	0.70
Babaya <i>et al.</i> (19)	Japanese	88	48.30	57	52.63	0.47
Rissanen <i>et al.</i> (23)	Chinese	51	39.00	105	50.00	0.07
Total		779	37.01	840	41.17	0.02

with special attention to the validity of genotyping and design of the studies. The articles were reviewed and allelic frequencies were extracted for analysis. As shown in Table 3, we identified seven population association studies for this polymorphism (19–24). By pooling all the studies, the frequency of the L allele was 41.17% in the diabetic group and 37.01% in the control group ($P = 0.02$).

Estimated beta cell indices (1stPH_S and 2ndPH_S) from OGTTs are well correlated with measured beta cell indices (1stIR and 2ndIR) by hyperglycemic clamps. Although the impact of the I27L polymorphism was noted in both measured and estimated beta cell indices, a much larger sample size was required for estimated beta cell indices. This polymorphism accounted for 7.5% of the variation in 1stPH_S, 8.1% in 2ndPH_S, 16.8% in 1stIR and 10.4% in 2ndIR. Analysis of the published association studies confirmed a modest risk of 1.19 (95% confidence interval 1.03–1.37) of the L allele for type 2 diabetes. The conservation of this polymorphism among species suggests the importance of this amino acid in dimerization. Further studies are required to demonstrate the biological consequence of this polymorphism.

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