

ORIGINAL ARTICLE

## Association of His1085His *INSR* Gene Polymorphism with Type 2 Diabetes in South Indians

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### Abstract

**Background and Objective:** The *INSR* gene, which encodes the insulin receptor, is a candidate gene for type 2 diabetes (T2D). The objective of the present study was to sequence some of the crucial exons in the *INSR* gene such as exon 2, which encodes the insulin-binding domain of the *INSR* protein, and exons 17–21, which encode the protein tyrosine kinase domain for mutations/polymorphisms, and to study their association with T2D in the South Indian population.

**Subjects and Methods:** The *INSR* gene was sequenced in 25 normal glucose-tolerant (NGT) and 25 T2D subjects, and the variant found was genotyped by polymerase chain reaction–restriction fragment length polymorphism in 1,016 NGT and 1,010 T2D subjects, randomly selected from the Chennai Urban Rural Epidemiology Study.

**Results:** Only one previously reported polymorphism, His1085His [rs1799817, (C→T)], in exon 17 was detected by sequencing. The frequency of the “T” allele of the His1085His polymorphism was significantly lower in the T2D subjects (31%) compared with the NGT subjects (35%) and showed significant protection against diabetes (odds ratio 0.85, 95% confidence interval 0.75–0.97,  $P=0.019$ ). Regression analysis according to a recessive model taking the CC+CT genotype as the reference showed that the TT genotype was protective against diabetes (odds ratio 0.71, 95% confidence interval 0.50–0.99,  $P=0.048$ ). Adjusting this  $P$  value by the number of competing models (three) using Bonferroni’s correction, we found that the association finding did not remain significant.

**Conclusions:** The “T” allele of the His1085His polymorphism in the *INSR* gene shows significant protection against diabetes. This study gains importance because there are no data available to date on the role of *INSR* variants in T2D in the Indian population.

### Introduction

THE HUMAN INSULIN RECEPTOR (*INSR*) is an integral membrane-spanning glycoprotein on the surface of all cells and a family member of tyrosine kinase receptors.<sup>1</sup> The human *INSR* gene spans a region about 120,000 basepairs on the short arm of chromosome 19 consisting of 22 exons and 21 introns. Mature human *INSR* is a heterotetramer of two  $\alpha$ -subunits and two  $\beta$ -subunits. Exons 1–11 encode the  $\alpha$ -subunit of the receptor, and exons 12–22 encode the  $\beta$ -subunit of the receptor.<sup>2</sup> Exon 2 encodes the insulin-binding domain of the *INSR* protein, and exons 17–21 encode the protein tyrosine kinase domain whose activity is required for insulin action.<sup>3</sup> *INSR* mediates the action of insulin on target cells. Any defect of *INSR* in number or function might decrease the action of insulin and cause insulin resistance,<sup>1</sup> leading to type 2 diabetes (T2D).

Upon insulin interaction, the *INSR* undergoes autophosphorylation on multiple tyrosine residues. This results in activation of its intrinsic tyrosine kinase, which leads to tyrosine phosphorylation of a variety of docking proteins, including *INSR* substrate. Once phosphorylated, *INSR* substrate proteins bind and activate several Src homology 2 domain proteins, leading to the activation of multiple downstream effectors that mediate metabolic responses, cell survival, growth, and differentiation. In the light of its key role, the *INSR* gene has been considered a plausible candidate gene for T2D.

The role of the *INSR* gene in type 2 diabetes has not been studied in India, which currently has 62.4 million people with diabetes, and over 95% have T2D.<sup>4</sup> It has been reported that Asian Indians have greater insulin resistance,<sup>5</sup> increased susceptibility to T2D, and a strong genetic background<sup>6,7</sup>

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compared with Europeans. Hence, the objective of the present study was to screen some of the crucial exons in the *INSR* gene such as exon 2 and exons 17–21 for mutations/polymorphisms and to study their association with T2D in an Asian Indian population in southern India.

## Research Design and Methods

### Subjects

In total, 2,026 unrelated subjects were chosen from the Chennai Urban Rural Epidemiology Study (CURES). The methodology of the study has been published elsewhere.<sup>8</sup> In Phase 1 of CURES, 26,001 subjects were recruited based on a systematic random sampling technique. Self-reported diabetes subjects were classified as “known diabetes subjects.” In Phase 2 of CURES, all known diabetes subjects ( $n=1,529$ ) were invited to our center for detailed studies, of whom 1,382 responded. Diabetes was confirmed if the diagnosis was made by a physician, if they were on drug treatment for diabetes, and if they had a fasting plasma glucose level of 7 mmol/L (126 mg/dL) or higher. In Phase 3 of CURES, every 10<sup>th</sup> individual from Phase 1 ( $n=2,600$ ) was invited to undergo an oral glucose tolerance test using a 75-g oral glucose load (dissolved in 250 mL of water). Those who had 2-h plasma glucose value of  $\geq 11.1$  mmol/L (200 mg/dL) (based on the World Health Organization Consulting Group criteria) were labeled as “newly detected diabetes subjects” ( $n=222$ ). Subjects who had fasting plasma glucose  $< 5.6$  mmol/L (100 mg/dL) and a 2-h plasma glucose value of  $\leq 7.8$  mmol/L (140 mg/dL) were categorized as normal glucose tolerance<sup>9</sup> and labeled as normal glucose-tolerant (NGT) ( $n=1,736$ ). The total number of diabetes subjects in the CURES study population is 1,604 (1,382 known diabetes subjects + 222 newly detected diabetes subjects). From these 1,604 diabetes subjects, 1,010 subjects were randomly selected for the present study; similarly, from the 1,736 NGT subjects, 1,016 subjects were chosen randomly. Informed consent was obtained from all the subjects who participated in this study, and the study was approved by the institutional ethical committee.

### Biochemical measurements

Anthropometric measurements including weight and height were obtained using standardized techniques. The body mass index (BMI) was calculated as the weight in kilograms divided by the square of the height in meters. Biochemical analyses were carried out on a Hitachi-912 autoanalyzer (Hitachi, Mannheim, Germany) using commercial kits (Roche Diagnostics, Mannheim). Fasting plasma glucose was estimated using the glucose oxidase–peroxidase method. Serum cholesterol was estimated using the cholesterol oxidase–phenol 4-amino-antipyrene peroxidase method. Serum triglyceride was estimated using the glycerol phosphatase oxidase–phenol 4-amino-antipyrene peroxidase method. High-density lipoprotein cholesterol was estimated using the polyethylene glycol–pretreated enzyme method, and low-density lipoprotein cholesterol was calculated using the Friedewald formula. Glycated hemoglobin (HbA1C) was estimated by high-performance liquid chromatography using the Variant™ machine (Bio-Rad, Hercules, CA). Serum insulin concentration was estimated using Dako (Glostrup, Denmark) kits.

### Genotyping

DNA was isolated from whole blood using the phenol-chloroform method. Sequencing of the *INSR* gene was carried out on 25 NGT and 25 T2D subjects on an ABI 310 genetic analyzer (Applied Biosystems, Foster City, CA) using previously described primers.<sup>3</sup> The His1085His single nucleotide polymorphism was genotyped by polymerase chain reaction (PCR)–restriction fragment length polymorphism. A final reaction volume of 15  $\mu$ L for PCR contained 100 ng of genomic DNA, 5 pmol of each primer, PCR buffer with 1 mM MgCl<sub>2</sub>, each deoxynucleotide triphosphate at 100  $\mu$ M, and 0.5 U of *Taq* polymerase (Life Technologies, Carlsbad, CA). PCR was carried out on a Peltier thermal cycler (MJ Research Inc., Waltham, MA) using the following conditions: 95°C for 5 min, followed by 34 cycles of (95°C for 30 s, 60°C for 30 s, 72°C for 30 s) and a final extension of 72°C for 9 min. PCR products were digested with 2 units of HpyCH4IV enzyme and electrophoresed on a 3% agarose gel. To assure that the genotyping was of adequate quality, random duplication in 20% of the samples was performed by a technician who was masked to the phenotype, and there was 99% concordance in the genotyping.

### Statistical analysis

SPSS Windows version 10.0 (SPSS, Inc., Chicago, IL) was used for statistical analysis. One-way analysis of variance was used to compare groups for continuous variables. The  $\chi^2$  test was used to compare the proportions of genotypes or alleles. Regression analysis was done using diabetes as the dependent variable and the genotypes as independent variables. Power was estimated using an online post hoc power computation tool ([www.dssresearch.com/toolkit/spcalc/power\\_p2.asp](http://www.dssresearch.com/toolkit/spcalc/power_p2.asp)).

## Results

### Sequencing of the *INSR* gene

The crucial exons in the *INSR* gene such as exon 2, which codes for the insulin-binding domain, and exons 17–21, which encode the protein tyrosine kinase domain, were sequenced in 25 NGT and 25 T2D subjects. This revealed only one previously reported polymorphism, the His1085His [rs1799817, (CAC→CAT)] polymorphism in exon 17. The His1085His polymorphism was further genotyped in 1,016 NGT and 1,010 T2D subjects.

### Subject characteristics

The diabetes subjects were older compared with the NGT subjects ( $49 \pm 10$  and  $46 \pm 11$  years, respectively) (Table 1). A comparison between diabetes and NGT subjects showed that the age- and sex-adjusted BMI, waist circumference, fasting plasma glucose, HbA1C, fasting serum insulin, total cholesterol, serum triglycerides, and low-density lipoprotein cholesterol were all significantly higher in the T2D subjects ( $P < 0.001$ ).

### Genotype and allele frequency

The genotype and allele frequencies of the His1085His variant are presented in Table 2. The genotypic distribution was in Hardy–Weinberg equilibrium. The frequency of the “T” allele was significantly lower in the T2D subjects (31%)

TABLE 1. CLINICAL AND BIOCHEMICAL CHARACTERISTICS OF THE STUDY SUBJECTS

	NGT subjects	T2D subjects	P value <sup>a</sup>
<i>n</i>	1,016	1,010	—
Male/female ratio	394/622	469/541	—
Age (years)	46 ± 11	49 ± 10	—
BMI (kg/m <sup>2</sup> )	23.7 ± 4.6	25.1 ± 4.3	<0.001
Waist circumference (cm)	84.4 ± 11.9	90.4 ± 10.0	<0.001
Fasting plasma glucose (mmol/L)	4.66 ± 0.5	9.27 ± 4.0	<0.001
2-h post-load plasma glucose (mmol/L)	5.66 ± 1.1	—	—
Fasting serum insulin (μIU/mL)	8.7 ± 6.2	—	—
Glycated hemoglobin (%)	5.6 ± 0.5	8.6 ± 2.1	<0.001
Total cholesterol (mmol/L)	4.75 ± 0.98	5.14 ± 1.04	<0.001
HDL cholesterol (mmol/L)	1.14 ± 0.26	1.09 ± 0.23	<0.001
LDL cholesterol (mmol/L)	2.99 ± 0.78	3.32 ± 1.01	0.001
Serum triglycerides (mmol/L)	1.18 ± 0.01	1.72 ± 0.01	<0.001

Data are mean ± SD values. The 2-h post-load plasma glucose and fasting serum insulin were not considered for analysis in type 2 diabetes (T2D) subjects.

<sup>a</sup>P value adjusted for age and sex.

BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NGT, normal glucose-tolerant.

compared with the NGT subjects (35%) ( $P=0.019$ ). The genotypic distribution of the His1085His polymorphism was significantly different between the NGT and T2D groups ( $P=0.011$ ). In order to confirm this, logistic regression analysis was performed taking T2D status as the dependent variable and the genotypes as independent variable (Table 2). Although the TT genotype showed significant protection against diabetes over both the CC (odds ratio [OR] 0.64, 95% confidence interval [CI] 0.47–0.86,  $P=0.003$ ) and CT (OR 0.66, 95% CI 0.49–0.89,  $P=0.008$ ) genotypes, the CT genotype did not show any protection against diabetes compared with the CC genotype (OR 0.96, 95% CI 0.79–1.15,  $P=0.67$ ). This showed that the genotypic distribution was not compatible under a codominant model.

Analysis coding CC genotype as 0 (and indicating it as reference) and CT+TT genotypes as 1 did not show any as-

TABLE 2. FREQUENCY OF THE His 1085His INSR VARIANT IN THE STUDY SUBJECTS

Genotype	NGT subjects (n = 1,016)	T2D subjects (n = 1,010)
Genotype frequency		
CC	441 (43.4%)	468 (46.3%)
CT	444 (43.7%)	453 (44.9%)
TT	131 (12.9%)	89 (8.8%)
P value ( $3 \times 2 \chi^2$ )	0.011	
Allele frequency		
"C" allele	1,326 (65%)	1,389 (69%)
"T" allele	706 (35%)	631 (31%)
P value	0.019	

NGT, normal glucose-tolerant; T2D, type 2 diabetes.

TABLE 3. ASSOCIATION OF THE His 1085His INSR VARIANT WITH TYPE 2 DIABETES

Genetic model	Unadjusted OR; 95% CI; P value	Adjusted OR; 95% CI; P value <sup>a</sup>
Codominant model		
CC vs. CT	0.96; 0.79–1.15; 0.67	0.97; 0.78–1.21; 0.82
CC vs. TT	0.64; 0.47–0.86; 0.003	0.70; 0.49–1.00; 0.051
CT vs. TT	0.66; 0.49–0.89; 0.008	0.71; 0.50–1.02; 0.067
Dominant model		
CC vs. CT+TT	0.88; 0.74–1.06; 0.18	0.91; 0.74–1.12; 0.40
Recessive model		
CC+CT vs. TT	0.65; 0.49–0.86; 0.003	0.71; 0.50–0.99; 0.048
OR for the "T" allele	0.85; 0.75–0.97; 0.019	

<sup>a</sup>Odds ratio (OR) adjusted for age, sex, and body mass index. CI, confidence interval.

sociation with diabetes (for CT+TT: OR 0.88, 95% CI 0.74–1.06,  $P=0.18$ ), ruling out the possibility of a dominant mode of inheritance (Table 3). However, when the regression analysis was performed according to a recessive model (taking CC+CT as reference), the TT genotype was significantly protective to diabetes with an OR of 0.65 (95% CI 0.49–0.86,  $P=0.003$ ). After adjusting for age, sex, and BMI, the OR for the TT genotype was nominally significant (OR 0.71, 95% CI 0.50–0.99,  $P=0.048$ ) (Table 3). Adjusting this  $P$  value by the number of competing models (three) using Bonferroni's correction, we found that the association finding did not remain significant as the nominal  $P$  value is higher than the Bonferroni threshold of 0.05/3 (=0.016). However, the "T" allele showed significant protection against diabetes with an allelic OR of 0.85 (95% CI 0.75–0.97,  $P=0.019$ ).

#### Association of the His1085His single nucleotide polymorphism with clinical and biochemical parameters

When the clinical and biochemical characteristics of the NGT subjects in relation to the CC, CT, and TT genotypes were compared, none of the parameters showed any significant differences among the genotypes. Because the TT genotype showed significant protection against diabetes under a recessive model, the biochemical parameters were also analyzed accordingly, and no significant differences were found in any of the parameters between the CC+CT genotypes and the TT genotype.

#### Discussion

In the present study, exon 2, which encodes the insulin-binding domain of the INSR protein, and exons 17–21, which encode the protein tyrosine kinase domain, were sequenced, and only one previously reported polymorphism, His1085His [rs1799817, (CAC → CAT)], in exon 17 was detected. No other novel variants were found in any of the exons sequenced in these 50 study subjects. This is similar to the report of Kusari et al.,<sup>10</sup> who sequenced exons 16–22 in six T2D subjects and did not find any new mutations. Other than the His1085His

polymorphism, no other known polymorphisms were detected in the present study. This is in contrast with the previous studies that have reported the presence of Lys1068Glu, Val985Met, and Arg1164Gln variants in Caucasian,<sup>11</sup> Italian,<sup>12</sup> and Chinese<sup>13</sup> subjects. The reason for the absence of these variants in this study could be that they are present at a very low frequency in this population and hence went undetected in our sequencing effort.

The presence of the silent polymorphism His1085His (rs1799817) in exon 17 has been reported in many studies,<sup>11,14–17</sup> but the frequency of this polymorphism in control and diabetes subjects was compared only in Danish Caucasian<sup>18</sup> and Chinese<sup>13</sup> populations, and no association with diabetes was seen. In this study, the frequency of the “T” allele was found to be 0.31 in the T2D subjects and 0.35 in the NGT subjects, and the difference was statistically significant ( $P=0.019$ ). This is in contrast to the study in Danish Caucasians,<sup>18</sup> where the frequency of the “T” allele was found to be similar in the NGT (0.17) and T2D (0.16) groups, showing no association with diabetes. Similarly, the overall prevalence of the “T” allele was found to be 0.50 in the 30 Japanese subjects screened by Kim et al.,<sup>15</sup> and there was no difference in the allele frequency between the normal and T2D subjects. In a Chinese study on the relationship between insulin resistance and His1085His polymorphism,<sup>13</sup> although the frequency of the “T” allele was higher in the control male subjects (0.42) compared with the insulin-resistant subjects (0.36), the difference was not statistically significant (0.30).

#### Significance of His1085His “silent polymorphism”

The present study is the first study to demonstrate a protective role of the His1085His polymorphism against T2D in a study population of 1,016 NGT subjects and 1,010 T2D subjects. No other study has shown either a protective or a susceptible effect of His1085His polymorphism with T2D.<sup>13,15,18</sup> However, studies have shown that the His1058 variant is associated with polycystic ovary syndrome.<sup>19,20</sup>

The His1085His polymorphism might play an important role because the ATP binding site between sites 1003 and 1030 of exon 17 is located in this region, which is a key factor to fix phosphorus during autophosphorylation responses.<sup>3</sup> Although it is unclear how a silent polymorphism can alter the risk of diabetes, there is emerging evidence for a possible role of silent polymorphisms in altering protein function.<sup>21</sup> The role of silent polymorphism in T2D has been already demonstrated in the *PPARGC1A* gene, where the minor allele “A” of the Thr394Thr polymorphism is associated with T2D.<sup>22</sup> In addition, a polymorphism need not be functionally relevant by itself, but can be in complete or near-complete linkage disequilibrium with a yet unidentified variant that has functional relevance.

One of the limitations of this study is its power. Although the sequencing study had >99% power to identify alleles with frequency as low as 0.05, it had only 60% power to detect alleles with frequency as low as 0.01. Thus, it is possible that some variants with frequency in the range 0.01–0.05 went undetected. A more extensive sequencing study might be needed to detect the variants with frequency 0.01–0.05. The association study of the His1085His polymorphism with T2D had a power of 0.79 to detect the difference between the TT genotype and CC+CT genotype at a significance of 5%.

In conclusion, the “T” allele of the His1085His polymorphism in the *INSR* gene shows significant protection against diabetes. This study gains importance because there are no data available to date on the role of the His1085His polymorphism in T2D in this urban South Indian population. However, replication in another study in the same ethnic group would validate our findings.

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#### Author Disclosure Statement

No competing financial interests exist.

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