Study of the *LMNA 1908 C/T* gene polymorphism in type 2 diabetic Egyptians with vascular complications

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other The study was carried out in the Internal Medicine Clinics, Diabetes Clinic and Internal Medicine Hospital of Kasr El Aini Hospital, Faculty of Medicine, Cairo, Egypt

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Aims/introduction
Vascular complications are the main cause of morbidity and mortality in type 2 diabetic patients. Genetic susceptibility is associated with the evolution of diabetic complications. One such gene is the *lamin A* and *C* gene located on chromosome 1q21, a susceptibility locus for type 2 diabetes mellitus, and encodes nuclear *lamins A* and *C*. The *LMNA 1908 C/T* polymorphism has been reported to be associated with dyslipidemia, metabolic syndrome, and obesity, suggesting that this polymorphism increases the risk of atherosclerosis and vascular disease. The present study aims to elucidate the association between the *LMNA 1908 C/T* single nucleotide polymorphism and the prevalence of vascular complications in a sample of type 2 diabetic Egyptian patients.

Materials and methods
Genomic DNA from 47 type 2 diabetic patients with vascular complications and 20 control participants was analyzed for the *LMNA 1908 C/T* polymorphism using PCR-RFLP.

Results
Carriers of the *LMNA 1908* T-allele showed a significantly higher prevalence in patients with diabetic nephropathy than carriers of the C-allele ($P < 0.05$). Multiple regression analysis showed that the *LMNA 1908* T-allele tended to be independent risk factor for diabetic nephropathy ($P = 0.012$, odds ratio = 5.460).

Conclusion
The findings of this study suggest that the *LMNA 1908 C/T* single nucleotide polymorphism is associated with the development of diabetic nephropathy in Egyptian type 2 diabetic patients.

Keywords:
LMNA 1908 C/T, type 2 diabetes, vascular complications

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Introduction

Type 2 diabetes mellitus is a major health problem worldwide. In Egypt, type 2 diabetes is a major health issue. The current prevalence of diabetes in Egypt is among the world's 10 highest countries. The expected number of individuals with diabetes in Egypt (20–79 age groups) will be 8.6 million in 2030 [1–3].

Type 2 diabetes is characterized by peripheral insulin resistance with an insulin-secretory defect that varies in severity. Insulin resistance in the offspring of patients with type 2 diabetes has been known to be the best predictor of the development of the disease [4].

Vascular complications are the main cause of morbidity and mortality in diabetes mellitus. Hyperglycemia, obesity, hypertension, dyslipidemia, and insulin resistance are factors that contribute toward the development of diabetic vascular complications [5]. Diabetes mellitus and subsequent microvascular and macrovascular complications occur as a result of interactions between environmental factors and genetic susceptibility [4,6].

The *LMNA* gene is located on chromosome 1q 21, a susceptibility locus for type 2 diabetes. Mutations in the *LMNA* gene, which encodes the nuclear structural proteins *lamins A* and *C*, cause one form of familial partial lipodystrophy, a monogenic syndrome of extreme insulin resistance, abnormal fat distribution, dyslipidemia, hypertension, diabetes, and vascular complications [7,8]. The *LMNA 1908 C/T* polymorphism is the most common *LMNA* single nucleotide polymorphism (SNP) that encodes a silent mutation that alters the relative amounts of *lamins A* and *C*. It has been shown to be associated with dyslipidemia as it is considered to be a high risk factor for atherosclerosis and vascular disease; thus, *LMNA 1908 C/T* SNP might play a role in susceptibility to diabetic vascular complications [5].

Materials and methods

This was a prospective study carried out over a period of 1 year and included 47 type 2 diabetes patients with diabetic vascular complications who were attending the Internal Medicine Clinics, Diabetes Clinic, and Internal Medicine Hospital of Kasr El Aini Hospital, Faculty of Medicine, Cairo University, and 20 healthy control participants.

All patients and control groups were subjected to the following:

(1) Full assessment of history including age of onset of diabetes, duration of diabetes, and complications.

(2) Thorough clinical examination, general, cardiac, neurological, and fundus examination, and calculation of BMI.

Laboratory investigations: fasting blood glucose (mg/dl), serum lipid profile including total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol (mg/dl), fasting serum insulin level (μU/ml), calculation of insulin resistance by homeostasis model assessment (HOMA-IR) as fasting insulin concentration (μU/ml)×fasting glucose concentration (mg/dl)/405 [9], urinary albumin excretion (mg/24 h), and genomic DNA extraction, and analysis of the *LMNA 1908 C/T* gene polymorphism using PCR, followed by restriction fragment length polymorphism analysis.

Exclusion criteria

Patients with nondiabetic renal disease, microscopic hematuria, clinical pyuria, or evidence of bacterial infection in urine culture were excluded; also in addition, patients with apparent cardioembolic cerebral infarction or an undetermined subtype of infarction were excluded. The protocol of this study was approved by the review board of the department of Internal Medicine according to the Declaration of Helsinki.

Statistical methods

The SPSS 10.0 for windows (SPSS Inc., Chicago, Illinois, USA) was used for data management and analysis. Quantitative data were presented as mean ±SD. For comparison of the means of both groups, Student's *t*-test was used. The association between qualitative data was assessed using the χ^2-test. Risk estimate was carried out by odds ratio (OR). A *P* value was considered significant at 0.05.

Results

A total of 47 patients and 20 healthy controls were enrolled. The patient group included 20 men and 27 women (ranging age between 35 and 67 years), whereas the control group included 11 men and nine women (ranging in age between 32 and 63 years).

The BMI and the laboratory parameters among the studied groups are compared in Table 1, which showed statistical significance for fasting blood sugar (FBS), total cholesterol, LDL-C, triglycerides, HOMA, and microalbumin urea in diabetic patients in comparison with the control group.

The number of patients and percentages of different vascular complications in group I (diabetic patients) were as follows: diabetic nephropathy (DN) (*N* = 17) (36.2%), retinopathy (*N* = 16) (34%), neuropathy (14) (29.8%), coronary heart disease (CHD) (*N* = 10) (21.3%), and cerebrovascular disease (CVD) (*N* = 8) (17%).

Allele frequency and risk ratio of the *LMNA 1908 C/T* allele between cases and controls are shown in Table 2, which showed statistical significance in the diabetic group in the allele mutation in comparison with the control group (*P* = 0.031).

The *LMNA 1908 C/T* genotype frequency and risk ratio in cases and controls are shown in Table 3. Table 4 shows a comparison of the clinical features and laboratory parameters among the diabetic group according to the *LMNA 1908 C/T* genotype.

The *LMNA 1908 C/T* genotype frequency and risk ratio in cases with nephropathy, CVD, CHD, retinopathy, and neuropathy are shown in Tables 5 and 6.

Table 1 Comparison of laboratory parameters among the studied groups

Parameter	Group I diabetic patients (*n* = 47)	Group II control group (*n* = 20)	*P* value
BMI (kg/m^2)	31 ± 4.2	27.9 ± 2.9	0.451
FBG (mg/dl)	251.2 ± 120.2	91.2 ± 10.1	0.000
Total cholesterol (mg/dl)	201.8 ± 53.9	156.4 ± 30.3	0.000
HDL-C (mg/dl)	43.4 ± 7.6	41.7 ± 9.5	0.440
LDL-C (mg/dl)	120.2 ± 46.5	91.2 ± 28.1	0.012
Triglycerides (mg/dl)	195.1 ± 80.1	117.0 ± 25.1	0.000
Fasting insulin (mlU/ml)	18.4 (10.3–43.8)	13.7 (9.5–18.0)	0.700
HOMA	10.2 (4.5–23.7)	3.14 (2.1–3.8)	0.000
Microalbumin (mg/24 h)	20.5 (11.3–80.8)	11.1 (6.5–14.2)	0.001

The data are represented as mean ± SD or median (25th–75th percentile); FBG, fasting blood glucose; HDL-C, HDL-cholesterol; HOMA, homeostasis model assessment; LDL-C, LDL-cholesterol.

Table 2 Allele frequency and risk ratio of the *LMNA 1908 C/T* allele between cases and controls

LMNAallele	*n* (%)		OR	95% CI	*P* value
	Cases (*n* = 47)	Controls (*n* = 20)			
Normal allele C	27/47 (76.6)	17/20 (92.5)	3.76	1.059–1341	0.031
Mutated allele T	20/47 (23.4)	3/20 (7.5)			

CI, confidence interval; OR, odds ratio.

Table 3 Comparison between the *LMNA 1908 C/T* genotype frequency and risk ratio in cases and controls

LMNA 1908 C/T genotype	*n* (%)		OR	95% CI	*P* value
	Group I diabetic group (*N* = 47)	Group II control group (*N* = 20)			
Wild CC	27/47 (57.4)	17/20 (85)			
Heterozygous CT					
Homozygous TT	20/47 (42.6)	3/20 (15)	4.19	1.081–16.302	0.048

CI, confidence interval; OR, odds ratio.

Table 4 Comparison of clinical features and laboratory parameters among the diabetic group according to the *LMNA 1908 C/T* genotype

Parameter	CC wild (*n* = 88)	CT (heterozygous) TT (homozygous) polymorphic (*n* = 46)	*P* value
BMI (kg/m^2)[a]	29.4 ± 3.9	31.3 ± 4.1	0.065
FBG (mg/dl)[b]	109 (88.7–248.5)	237 (134–358)	0.004
Total cholesterol (mg/dl)[b]	175 (139–204)	197 (179–233)	0.061
HDL-C (mg/dl)[a]	42.8 ± 7.6	43.1 ± 9.3	0.899
LDL-C (mg/dl)[b]	103.7 (66.7–131.3)	119.4 (101–147)	0.065
Triglycerides (mg/dl)[b]	143 (108–216)	174 (145–198)	0.146
Fasting insulin (m IU/ml)[b]	12.2 (9.8–18.4)	37.9 (15.4–62.4)	0.000
HOMA[b]	3.7 (2.5–8.0)	14.2 (7.3–40.6)	0.000
Microalbumin (mg/24 h)[b]	12.4 (7.9–26.7)	20.4 (14.1–430.0)	0.010

FBG, fasting blood glucose; HDL-C, HDL-cholesterol; HOMA, homeostasis model assessment; LDL-C, LDL-cholesterol; [a]Mean ± SD; [b]Median (25th–75th percentile).

Table 5 *LMNA 1908 C/T* genotype frequency and risk ratio in cases with nephropathy

Genotype	*n* (%)		OR	95% CI	*P* value
	Nephropathy positive (*N* = 17)	Nephropathy negative (*N* = 30)			
Wild CC	6/17 (35)	21/30 (70)			
Heterozygous CT					
Homozygous TT	11/17 (65)	9/30 (30)	4.278	1.208–15.151	0.021

CI, confidence interval; OR, odds ratio.

Table 6 *LMNA 1908 C/T* genotype frequency in cases with cerebrovascular disease, coronary heart disease, retinopathy, and neuropathy

Genotype	*n* (%)		*P* value
	CVD positive (*N* = 8)	CVD negative (*N* = 39)	
Wild CC	3/8 (37.5)	24/39 (62)	
Heterozygous CT			
Homozygous TT	5/8 (62.5)	15/39 (38)	0.258
	CHD positive (*N* = 10)	CHD negative (*N* = 37)	
Wild CC	7/10 (70)	20/37 (54)	
Heterozygous CT			
Homozygous TT	3/10 (30)	17/37 (46)	0.481
	Retinopathy positive (*N* = 16)	Retinopathy negative (*N* = 31)	
Wild CC	10/16 (62.5)	17/31 (55)	
Heterozygous CT			
Homozygous TT	6/16 (37.5)	14/31 (45)	0.615
	Neuropathy positive (*N* = 14)	Neuropathy negative (*N* = 33)	
Wild CC	8/14 (57.1)	19/33 (58)	
Heterozygous CT			
Homozygous TT	6/14 (42.9)	14/33 (42)	0.978

CHD, coronary heart disease; CVD, cerebrovascular disease.

Carriers of the *LMNA 1908* T-allele showed a significantly higher prevalence of DN than carriers of the C-allele ($P < 0.05$). Multiple regression analysis showed that the *LMNA 1908* T-allele tended to be an independent risk factor for DN ($P = 0.012$, OR = 5.460).

Discussion

Type 2 diabetes is a major global health problem that affects over 170 million individuals worldwide; it will be certainly one of the major diseases of the 21st century and should be recognized as a priority [4].

The genetic component in the pathogenesis of type 2 diabetes and development of vascular complications is very significant, but has been shown to be complex, as single gene disorders are only seldom responsible. Patterns of inheritance suggest that type 2 diabetes is both polygenic and heterogeneous as multiple genes are involved and different combinations of genes play a role in different subsets of individuals [4].

One of these contributor genes is the *LMNA* gene; the most common genetic variant in the *LMNA* gene is the C to T substitution at position 1908 in exon 10. Although it is a silent mutation, it could alter mRNA splicing and thus the relative amount of lamins A and C mRNA and related proteins [5]. Conflicting results have been reported on the association of *LMNA* SNPs and diabetes mellitus, some of which suggest modest positive associations, whereas others did not report any convincing association [10]. The aim of this study, therefore, was to investigate the association between the *LMNA 1908 C/T* polymorphism and the prevalence of vascular complications in a group of Egyptian patients with type 2 diabetes.

This study included two groups: group I included 47 type 2 diabetic patients with vascular complications; there were 20 men (42.6%) and 27 women (57.4%) ranging in age from 35 to 67 years (52.9+8.9). Group II included 20 healthy adult volunteers as a control group: 11 men (55%) and nine women (45%) ranging in age from 32 to 63 years (43.9+10.6).

According to the type of macrovascular or microvascular complications, group I included 17 patients with DN (36.2%), 16 patients with retinopathy (34%), 14 patients with neuropathy (29.8%), 10 patients with CHD (21.3%), and eight patients with CVD (17%).

The mean age of patients recruited in this study with the wild genotype (CC) was 49.5+10.4 years, whereas the mean age of patients with the mutant genotype (CT and TT) was 51.6+10.1 years. This was in agreement with the study by Liang *et al.* [5] who reported a mean age of 56 (46–62) and 54 (44–63) years for patients with wild and mutant genotypes, respectively.

Comparison of the clinical features and laboratory parameters of the study participants according to the *LMNA 1908 C/T* genotype indicated a statistically significant increase in fasting plasma glucose levels in carriers of the T-allele than carriers of the C-allele ($P = 0.004$). This finding was in agreement with that of Wegner *et al.* [11] who reported, in a Danish cohort study, that the T-allele was associated with increased fasting glucose concentration ($P = 0.008$). Also, fasting insulin and HOMA-IR showed a statistically significant increase in carriers of the T-allele than carriers of the C-allele, with *P*-values of 0.00 and 0.00, respectively, which indicates that the *LMNA 1908 C/T* SNP may play a role in hyperinsulinemia and development of insulin resistance in type 2 diabetes patients. These findings were not in agreement with those of Murase *et al.* [12] who reported, in a Japanese study, that *LMNA 1908 C/T* SNP was a factor predisposing to insulin resistance ($P = 0.01$); also, Mukti *et al.* [13] reported that this gene variant is associated with insulin resistance and generalized obesity in Indians.

In the present study, 24 h urinary microalbumin was significantly increased in patients with the T-allele than those with the C-allele ($P = 0.01$), which indicates that *LMNA C/T 1908* SNP may lead to the risk of developing nephropathy in type 2 diabetic patients.

This study found no statistically significant difference in BMI between carriers of the T-allele and the C-allele ($P = 0.065$), and this result was in agreement with Wolford *et al.* [14] in a Pima Indian study and Liang *et al.* [5] in a Japanese study. This result is not in agreement with the study by Hegele *et al.* [15] who reported that carriers of *LMNA 1908 C/T* SNP had higher BMI and indices of obesity. The discrepancy between the previous observations may be because of linkage disequilibrium of the 1908 T SNP with other ethnicity-specific polymorphisms.

In the current study, genomic DNA analysis of the *LMNA 1908* gene showed that the frequency of the wild genotype (CC) was 57.4 and 85%, whereas that of the mutant genotype (CT and TT) was 42.6 and 15% in the patient and control groups, respectively. This indicated a statistically significant increase in the frequency of the mutant genotype in the diabetic patients compared with the control participants [OR = 4.19, 95% confidence interval (CI) = 1.081–16.302, $P = 0.048$]. This result was in agreement with the study by Wegner *et al.* [11] ($P = 0.04$).

In this study, the frequency of the T-allele was 23.4 and 7.5%, whereas that of the C-allele was 76.6 and 92.5% for cases and controls, respectively; there was a statistically significant increase in the mutant allele in cases compared with the control participants, with P = 0.031, OR = 3.76, and 95% CI = 1.059–13.41. When subgroup analysis of type 2 diabetic patients was stratified according to their vascular complications, it was found that carriers of the mutant genotype (CT and TT) were more prevalent in diabetic patients with nephropathy than those with the wild genotype (CC) (OR = 4.27, 95% CI = 1.20–15.15, P = 0.021). Also, the frequency of the T-allele was 38.3 and 15%, whereas the frequency of the C-allele was 61.7 and 85% in diabetic patients with nephropathy and those without nephropathy, respectively (P = 0.011); on estimating the risk ratio of the mutant allele, the OR was 3.508 and 95% CI was 1.303–9.443.

These results indicate that type 2 diabetic patients harboring the *LMNA 1908* T-allele showed a higher risk for development of DN than those harboring the C-allele. These findings are in agreement with those of Liang and colleagues, who carried out their study on Japanese participants and reported that the frequencies of *LMNA 1908 C/T* and TT genotypes were 65.7 and 45%, whereas the frequencies of the of CC genotype were 34.3 and 55% in patients with DN and those without DN, respectively (P = 0.036); they also reported that the frequency of the T-allele was 38.6 and 24%, whereas that of the C-allele was 61.4 and 76% in patients with DN and those without DN, respectively (P = 0.022) [5].

This study did not find an association between *1908 C/T SNP* and development of CVD in type 2 diabetic patients (P = 0.258); this was in contrast to the result reported by Liang *et al.* [5], who found an association between this SNP and development of CVD in type 2 diabetes patients (0.003). This may be attributed to the relatively smaller number of participants included in the present study.

The present study found no association between *1908 C/T SNP* and development of CHD, retinopathy, or neuropathy in type 2 diabetes patients (P = 0.481, 0.615, 0.978, respectively). These results were in agreement with those of Liang *et al.* [5]. By applying a logistic regression analysis for risk factors for DN on the data of the present study, it was found that the *LMNA T* genotype is an independent risk factor for development of DN, with OR = 5.460, 95% CI = 1.464–20.366, and P = 0.012. This is in agreement with the study by Liang *et al.* [5], who found a significant correlation between the *LMNA 1908* T-allele and DN (OR = 2.97, 95% CI = 0.89–9.94, and P = 0.047).

In conclusion, these results suggest that the *LMNA 1908 C/T* SNP is associated with the development of DN in Egyptian type 2 diabetic patients.

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Conflicts of interest
There are no conflicts of interest.

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