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Research Article
Genetic Variations in Diabetic Patients with Proliferative and Non-proliferative Retinopathy

[1,2]Shirel Weiss, [4]Merav Gabbay, [1]Olga Dratviman-Storobinsky, [3]Mohamed Atamney, [2,3]Ruth Axer-Siegel, [5,6]Uri Gabbay, [7]Yoram Cohen and [1,2,8]Nitza Goldenberg-Cohen

[1]Krieger Eye Research Laboratory, Felsenstein Medical Research Center, 4941492 Petach Tikva, Israel
[2]Sackler School of Medicine, Tel Aviv University, 6997801 Tel Aviv, Israel
[3]Department of Ophthalmology, Rabin Medical Center-Beilinson Hospital, 4941492 Petach Tikva, Israel
[4]Kiryat Ono Community Health Center, Clalit Health Services, 4941492 Dan-Petach Tikva District, Israel
[5]Department of Epidemiology and Preventive Medicine, Sackler Faculty of Medicine, School of Public Health, Tel Aviv University, 6997801 Tel Aviv, Israel
[6]Quality Unit, Rabin Medical Center-Beilinson Hospital, 4941492 Petach Tikva, Israel
[7]Gynecology Research Laboratory, Department of Gynecology, Sheba Medical Center, 52621 Tel Hashomer, Israel
[8]Pediatric Ophthalmology Unit, Schneider Children's Medical Center of Israel, 4920235 Petach Tikva, Israel

Abstract

Background and Objective: Gene variants associated with diabetes type-2 in the Scandinavian population are population specific and are not predictive in Israelis except for the FTO variant. Of all variants examined, proliferative diabetic retinopathy was associated with KCNJ11 variant only. This study objective was to determine if genes associated with the risk of diabetes mellitus type-2 in the Scandinavian population predict the development and progression/severity of diabetes mellitus type-2 in the Israeli population. **Materials and Methods:** The study sample included 146 patients with diabetes mellitus type-2 of more than 20 year's duration, 16 with proliferative diabetic retinopathy and 130 without and 51 healthy controls. Demographic and clinical data were derived from the medical files. Genetic analysis was performed with the chip-based matrix-assisted laser desorption-time-of-flight mass spectrometer (Sequenom) on DNA extracted from peripheral blood leukocytes. Sixteen single nucleotide polymorphisms in 16 candidate genes identified in the Scandinavian population were investigated: TCF7L2, PPARG, FTO, KCNJ11, NOTCH2, WFS1, CDKAL1, IGF2BP2, SLC30A8, JAZF1, HHEX, CDKN2, TSPAN8, ADAMTS9, CDC123 and THADA. Main outcome measures were frequencies compared between study patients and controls, study patients with and without proliferative diabetic retinopathy among Israeli, Scandinavian and European populations. **Results:** Unlike the Scandinavian findings, only the FTO variant (rs9939309) was significantly associated with diabetes mellitus type-2 in the Israeli patients ($p = 0.031$). Regarding retinopathy, patients with proliferative diabetic retinopathy had a significantly higher frequency of the risk allele in KCNJ11 than patients without proliferative diabetic retinopathy ($p = 0.03$). Frequencies for 11 single nucleotide polymorphisms were similar in the healthy Israeli and European populations. No similarities were found between the Scandinavian and European populations. **Conclusion:** The gene variants associated with DM2 in the Scandinavian population are apparently population-specific. In Israelis, the FTO variant may play a predictive role in diabetes mellitus type-2 and the KCNJ11 variant in proliferative diabetic retinopathy. More studies are needed in larger samples with a longer duration of proliferative diabetic retinopathy.

Key words: Gene variants, type-2 diabetes, DM2, NPDR

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Corresponding Author: Nitza Goldenberg-Cohen, Pediatric Ophthalmology Unit, Schneider Children's Medical Center of Israel, 4920235 Petach Tikva, Israel

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The main risk factors for the progression of diabetes mellitus type-2 (DM2) are duration of diabetes, poor control of glucose and high arterial blood pressure[1-3]. It is now well recognised that genetic factors may play a contributory or interactive role as well[4-6]. Of the many candidate genes identified in early genome-wide association studies[7], variants in three have been consistently associated with DM2: TCF7L2, KCNJ11 and PPARG[8,9]. More recently, several gene variants were shown in reproducible studies to increase susceptibility to DM2 (CDKAL1, IGF2BP2, locus on chromosome 9 close to CDKN2A/CDKN2B, FTO, HHEX, SLC30A8 and WFS1)[10,11] and a meta-analysis reported six novel gene variants that affect pancreatic beta-cell function: JAZF1, CDC123/CAMK1D, TSPAN8/LGR5, THADA, ADAMTS9 and NOTCH2[12].

In 2008, Lyssenko *et al.*[4] genotyped candidate gene variants for DM2 in 18,831 Scandinavia, Swedish and Finnish, subjects of whom 12% had acquired DM2 during a median follow-up time of 23.5 years. Of the 16 single nucleotide polymorphisms (SNPs) investigated, 11 were found to be significantly associated with the risk of DM2 independent of clinical risk factors; eight were associated specifically with impaired beta-cell function. Prompted by these findings, this study sought to examine the predictive value of the same 16 SNPs for DM2 in Israeli patients.

MATERIALS AND METHODS

Study population: A double case-control study design was used. The study group consisted of 146 adults (age range 70.67±9.9) with DM2 attending the Department of Ophthalmology of a Tertiary Medical Center in 2009-2014. Given the importance of environmental factors such as glucose control on the outcome and complications of DM2, this study selected only individuals who were known to have had the disease for more than 20 years. Sixteen had been diagnosed with PDR within 10 years of diagnosis and 130 did not have PDR. The control group included 51 sex and age-matched nondiabetic subjects attended the Ophthalmology outpatient clinic.

Data collection: Data on medical history and clinical and fundus examinations were derived from the medical charts. Only patients for whom complete data were available were included in the study. Genetic analysis was performed using a chip-based matrix-assisted laser desorption-time-of-flight (MALDI-TOF) mass spectrometer (Sequenom, San Diego, CA, USA) as previously described[13]. Blood was withdrawn (5 mL) from each study participant under approval of the National and Institutional Review Boards and genomic DNA was extracted from peripheral blood leukocytes using the Puregene DNA Purification Blood Kit (Gentra Systems, Minneapolis, MN). Sixteen risk SNPs in 16 genes derived from the study of Lyssenko *et al.*[4] were genotyped: HHEX, PPARG, IGF2BP2, ADAMTS9, FTO, NOTCH2, SLC30A8, CDKAL1, CDKN2, JAZF1, TCF7L2, TSPAN8, KCNJ11, THADA, WFS1 and CDC123. In brief, assay design software (Sequenom) was used to design specific primers flanking mutation sites and extension primers that bind adjacent to the mutation site (primers available upon request). After the region of interest was amplified, the primer extension reaction was performed including sequence-specific hybridization and sequence-dependent termination. This generated different products for the mutated and wild-type alleles, each with a unique mass value. The extension products were spotted onto silicon chips preloaded with proprietary matrix (SpectroChip, Sequenom) which were subsequently read with the MALDI-TOF mass spectrometer.

Study procedure: Clinical and genetic parameters were compared between patients with DM2 and nondiabetic controls within the DM2 group between patients with and without PDR. In addition, allele frequencies in the study patients were compared with the reported data in the large Scandinavian study (16,061 subjects from the Malmo Preventive Project, 2063 with DM2 and 2770 subjects from the Botnia studies, 138 with DM2) and on European SNP database (Hapmap Project using http://asia.ensembl.org/ (379 healthy subjects).

Statistical analysis: Categorical variables were expressed as frequency distributions and compared between groups with Fisher's exact test. Normally distributed continuous data were expressed as Means±Standard Deviations (SD) and compared between groups with Student's t-test. All tests were two-tailed. Statistical significance was defined as a p-value of less than 0.05. All analyses were conducted using SPSS 21.0 (SPSS, Inc.; Chicago, IL, USA).

RESULTS

The demographic and clinical characteristics of the cohort are shown in Table 1. By contrast to the Scandinavian study in which 11 of the same 16 SNPs tested were found to be

Table 1: Demographic and clinical data of Israeli patients with diabetes, with or without PDR

Characteristics	Subgroup	N	Mean±SD	Mean±SE	p-value (t-test)
Age (year)	NDR	93	69.43±9.76	1.01	0.03
	PDR	14	63.50±7.94	2.12	
Disease duration (year)	NDR	85	19.27±8.19	0.89	0.00
	PDR	15	4.87±4.60	1.19	
Age at diagnosis (year)	NDR	84	49.63±10.13	1.11	0.01
	PDR	13	57.46±9.32	2.59	
Last HbA1C (%)	NDR	86	7.45±1.37	0.15	0.15
	PDR	14	8.33±2.10	0.56	
Body mass index	NDR	86	28.77±5.30	0.57	0.92
	PDR	12	28.61±2.61	0.75	
Highest triglycerides (mg dL^{-1})	NDR	86	194.43±106.10	11.44	0.61
	PDR	13	178.77±70.17	19.46	

NDR: No diabetic retinopathy and PDR: Proliferative diabetic retinopathy

Table 2: Frequency of risk alleles in Israeli patients with or without DM2 and European population

Chr	Gene	SNP	RA	IL-DM2 RA (%)	N	IL-control RA (%)	N	IL-DM2 vs IL-control (p-value)	Europe RA (%)	N	IL-control vs Europe (p-value)
10	HHEX	rs1111875	C	50	125	56	48	NS	31	379	0
3	PPARG	rs1801282	C	93	102	88	42	NS	78	379	0.007
3	IGF2BP2	rs4402960	T	21	122	16	49	NS	9	379	0
3	ADAMTS9	rs4607103	C	46	125	33	45	NS	62	379	0
16	FTO	rs9939609	A	35	124	18	51	0.031	21	379	0.003
1	NOTCH2	rs10923931	T	1	128	0	48	NS	2	379	NS
8	SLC30A8	rs13266634	C	65	117	60	48	NS	52	379	NS
6	CDKAL1	rs7754840	C	15	123	12	49	NS	13	379	NS
9	CDKN2	rs10811661	T	72	119	58	48	NS	71	379	NS
7	JAZF1	rs864745	T	26	121	26	47	NS	22	379	NS
10	TCF7L2	rs7903146	T	13	120	13	48	NS	11	379	NS
12	TSPAN8	rs7961581	C	13	121	19	48	NS	8	379	NS
11	KCNJ11	rs5219	T	14	129	19	48	NS	10	379	NS
2	THADA	rs7578597	T	86	127	85	48	NS	84	379	NS
4	WFS1	rs10010131	G	45	130	55	49	NS	38	379	NS
10	CDC123	rs12779790	G	8	107	0	38	NS	5	379	NS

Chr: Chromosomea, SNP: Single nucleotide polymorphism, RA: Risk allele, IL: Israeli and DM2: Diabetes mellitus type-2

significantly more frequently in patients with DM2 than in subjects without DM2[5] in this Israeli cohort, the only significant difference between the study and control groups was a higher frequency of the FTO gene risk allele (rs9939609) in the diabetic patients ($p = 0.031$) (Table 2). There were no significant between-group differences in any of the other variants investigated.

Within the study group, comparison of the clinical parameters between the diabetic Israeli patients with and without retinopathy revealed that the patients with proliferative retinopathy were significantly older at diagnosis ($p = 0.01$) and had had diabetes for a significantly shorter time ($p = 0.001$). There were no between-group differences in mean HbA1C concentration, triglyceride level or body mass index (Table 1). The patients with PDR had a significantly higher frequency of the KCNJII risk allele (rs5219) ($p = 0.03$) and a higher frequency of the WFS1 risk allele (rs10010131) which achieved borderline significance ($p = 0.066$).

Comparison of the Israeli nondiabetic control group and the reported frequency of the European population in the Hapmap project for the same SNPs identified in the Scandinavian study[5] revealed differences in five. The frequencies of the HHEX, PPARG and IGF2BP2 risk alleles were higher in the Israeli cohort than the European population and the frequencies of ADAMTS9 and FTO alleles were higher in the European population (Table 2). The ADAMTS9 was not associated with DM 2 in the Israeli study while FTO was the only gene associated with DM2 in the present study. There was no difference in frequency of any of the other variants tested (NOTCH2, SLC30A8, CDKAL1, CDKN2, JAZF1, TCF7L2, TSPAN8, KCNJ11, THADA, WFS1 and CDC123) between the Israeli cohort with or without PDR and the European population.

On further comparison of the same SNPs between the Scandinavian and European populations, significant differences were found for all 16 (Table 3).

Table 3: Frequency of risk alleles in European (Hapmap) and Scandinavian populations[4]

Chr	Gene	SNP	RA	Europe RA (%)	N	Malmo RA (%)	N	Botnia RA (%)	N	p- value (Europe vs Malmo+Botnia)
10	HHEX	rs1111875	C	31	379	60	12127	61.0	3815	0.000
3	PPARG	rs1801282	C	78	379	87	12155	88.0	3838	0.000
3	IGF2BP2	rs4402960	T	9	379	32	11641	32.0	3516	0.000
3	ADAMTS9	rs4607103	C	62	379	76	11959	77.0	3770	0.000
16	FTO	rs9939609	A	21	379	44	12117	43.0	3814	0.000
1	NOTCH2	rs10923931	T	2	379	10	11878	12.0	3711	0.000
8	SLC30A8	rs13266634	C	52	379	70	12109	70.0	3822	0.000
6	CDKAL1	rs7754840	C	13	379	33	11750	34.0	3737	0.000
9	CDKN2	rs10811661	T	71	379	85	11460	85.0	3672	0.000
7	JAZF1	rs864745	T	22	379	52	12119	54.0	3826	0.000
10	TCF7L2	rs7903146	T	11	379	30	12015	32.0	3800	0.000
12	TSPAN8	rs7961581	C	8	379	26	11883	27.0	3711	0.000
11	KCNJ11	rs5219	T	10	379	40	11779	42.0	3821	0.000
2	THADA	rs7578597	T	84	379	90	11894	90.0	3726	0.000
4	WFS1	rs10010131	G	38	379	60	12121	59.0	3823	0.000
10	CDC123	rs12779790	G	5	379	19	11849	19.0	3698	0.000

Chr: Chromosome, SNP: Single nucleotide polymorphism, RA: Risk allele and EU: European

DISCUSSION

Genetic factors contribute to the onset and progression of chronic complications of DM2 but the genes conferring susceptibility to these disorders remain unclear[14,15].

In the present investigation of 16 gene polymorphisms found to be associated with the risk of DM2 in a Swedish and Finnish population[4], only one was significantly associated with DM2 in this Israeli cohort. Despite the heterogeneity of the Israeli population, a similarity in the frequency of the risk alleles was noted on comparison of Israeli cohort with a European population. Interestingly, when the Scandinavian and European populations were compared, significant differences were found for all the published risk alleles (Table 3). Together, these findings suggest that the Scandinavian population is not representative of the European population and the majority of the SNPs identified as associated with DM2 in the Scandinavian study are population-specific.

The sole SNP of the 16 evaluated that occurred at a significantly different frequency between patients with DM2 and healthy subjects in both the present Israeli study and the Scandinavian study[4] was rs9939309 in the FTO gene. This was one of the five variants that differed between the Israeli and European populations. Hence, this study concluded that this gene is a risk allele for the development of DM2 but it is population-dependent. Interestingly, the FTO gene has been associated with a risk of obesity in white Europeans. However, in Southern Indians, the association of the FTO gene with diabetes is apparently not mediated by body mass index[16,17].

In this DM2 group, there were two different phenotypes of retinal disease with (n = 16) and without (n = 130) PDR. The patients with PDR had a significantly higher than expected frequency of the rs5219 polymorphism in KCNJ11 (p = 0.03) and developed PDR in short duration since diagnosed. They also had a higher frequency of the rs10010131 polymorphism in WFS1 but the difference reached only borderline significance (p = 0.066). Thus, although these variants were not associated with the risk of developing DM2, they were associated with the risk of more severe disease with retinal complications.

Genetic association studies of PDR in other specific and universal populations identified additional candidate genes[18-20]. Significant associations with four SNPs in the VEGF gene were reported in a Chinese population (p<0.001-0.006)[18] and a study of Caucasians in the UK yielded a significantly different distribution of the VEGF-460 genotype between patients with PDR (n = 69) and other grades of retinopathy, independently of blood pressure, glycemic control, duration of diabetes[19] and obesity (p = 0.02). In the Indian population, screening of nine loci (15 polymorphisms) selected from previous studies (RAGE, PEDF, AKR1B1, EPO, HTRA1, ICAM and HFE) or on the basis of their role in biological pathways involved in PDR (CFH and ARMS2) yielded one, rs2070600 (G82S) in the RAGE gene that occurred at a significantly higher frequency in patients with PDR than in diabetic patients without PDR[20,21]. This study did not examine these SNPs because a recent meta-analysis that stratified patients by ethnicity (Asian and Caucasian) failed to support these findings[22,23]. Furthermore, the genes associated with the risk of DM2 and diabetic nephropathy did not appear to be consistently associated with the risk of PDR[24]. Several other genetic variants found to be associated with PDR, particularly three in SELP (especially rs6128) and one in IDUA (rs6856425 tagging α-l-iduronidase) require further investigation[20].

This study is unique in the strict definition of the study group wherein we compare patients with no PDR despite a long duration of DM2 and patients with PDR within a short period of disease onset. This led to small group of PDR patients.

New technologies are being developed to explore genetic risk factors for DM2 and its complications. A recent genome-wide analysis of gene-SNPs (SWAP) performed in a large cohort of Japanese patients with PDR, with or without nephropathy and highlighted the usefulness of SWAP for studying susceptibility to common diseases[15]. Others have described the application of next-generation parallel sequencing using whole-genome or target region resequencing to in examine individual genetic variations in DM[24,25]. These data, combined with clinical studies of complications, disease progression and response to treatment have important implications for improving the outcome of patients with DM2.

CONCLUSION

The gene variants associated with DM2 in the Scandinavian population are apparently population-specific. In Israelis, the FTO variant may play a predictive role in DM2 and the KCNJ11 variant in PDR. More studies are needed in larger samples with a longer duration of PDR.

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