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ORIGINAL ARTICLE

Association of *IGF1* and *VEGFA* polymorphisms with diabetic retinopathy in Pakistani population

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Abstract

Aims The incidence of microvascular complications, including diabetic retinopathy (DR), increases with duration of type 2 diabetes (T2D). Meta-GWAS have reported numerous single-nucleotide polymorphisms (SNPs) associated with T2D; however, no loci, achieving genome-wide significance has been reported for DR. Vascular endothelial growth factor A (VEGFA) and insulin-like growth factor 1 (IGF1) are considered as potential genetic candidates involved in T2D and DR progression. Moreover, the association of serum levels of these proteins with diabetes-related traits is controversial. Therefore, the current study was designed to evaluate the possible genetic predisposition and role of these circulating growth factors in serum in the pathophysiology of T2D and DR.

Methods A cohort of 1126 individuals with T2D was collected including those without retinopathy (DNR = 573), non-progressive diabetic retinopathy (NPDR = 301) and progressive diabetic retinopathy (PDR = 252), and 348 healthy controls. Genomic DNA was isolated, and six SNPs: rs833061, rs13207351, rs1570360, rs2010963, rs5742632 and rs6214, were genotyped and results statistically analyzed. ELISA was performed on a subset of the samples to measure serum levels of IGF1 and VEGFA.

Results The minor allele of rs6214 was associated with T2D [OR = 1.67 (95% CI 1.39–2.01, $p = 4.9E{-}8$)], rs13207351 was associated with NPDR [OR = 1.97 (95% CI 1.28–3.03, $p = 9.0E{-}3$)]when compared with DNR, and rs5742632 showed positive association with PDR [OR = 1.66 (95% CI 1.33–2.05, $p = 1.0E{-}4$)] compared to DNR. Lowered IGF1 serum levels were found to be associated with T2D, NPDR and PDR.

Conclusions *IGF1* was found to increase the T2DM susceptibility as well as advanced DR, i.e., PDR, while *VEGFA* was found to be associated with early DR stage, i.e., NPDR.

Keywords GWAS · Diabetic retinopathy · *VEGFA* · *IGF1* · Logistic regression

Introduction

Type 2 diabetes (T2D) is a metabolic disorder characterized by insulin resistance that leads to hyperglycemia, which subsequently results in disturbances in carbohydrate, fat and protein

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metabolism. Persistent hyperglycemia during diabetes leads to progressive macrovascular and/or microvascular complications including that of heart, kidney, nerve or retina [1].

Diabetic retinopathy (DR) is one of the damaging microvascular complications of T2D and is the major cause of visual impairment worldwide in adults aged 20–74 years. In 2010, an estimated 285 million people worldwide were affected with diabetes, of which over one-third developed DR and were at risk of loss of vision due to proliferative diabetic retinopathy (PDR) [2]. Retinal changes in affected individuals include thickening of the basement membrane, loss of pericytes, microaneurysms, dysfunction of the endothelial cell, microvascular infarcts and neovascularization leading to hemorrhage and retinal detachment, which eventually result in vision loss. Clinically, DR starts with non-proliferative diabetic retinopathy (NPDR) with enhanced vascular permeability, which progresses to PDR with vascular closure and new blood vessel formation (angiogenesis) in the retina [3].

Angiogenesis is the process of new blood vessel formation normally occurring in early developmental processes: In healthy adults, vascular turnover is extremely low and angiogenesis is rare. This process is modulated by VEGFA; a glycoprotein secreted by pericytes (retinal pigment epithelial cells), glial and endothelial cells; therefore, VEGFA is considered among the most specific and powerful angiogenic mediators. Angiogenesis progression to DR is also modulated by VEGFA [4]. Numerous studies have tested multiple *VEGFA* SNPs for association with DR in different populations [5, 6] but none from the Pakistani population.

Insulin-like growth factor 1 (IGF1) has a vital role in growth and cellular proliferation in humans [7]. It has a structural homology with the insulin receptor, and therefore, IGF1 can bind with either IGF1 or insulin/IGF1 hybrid receptors [8]. This can stimulate glucose transport into cells in fat and muscle tissues causing decreased blood glucose levels and insulin secretion. Serum IGF1 concentrations are also governed by a balance of hormones such as growth hormone and insulin [9]. Normal levels of circulating IGF1 in serum are important for maintaining glucose metabolism. However, decreased levels of IGF1 have been reported to be associated with increased insulin insensitivity that might lead to T2D and its complications [10].

In the current study, we aim to investigate the association of *VEGFA* and *IGF1* SNPs and their serum levels with T2D as well as DR in the Pakistani population.

Methodology

Cohort and sample collection and DNA extraction

The cohort selection, sample collection and DNA isolation details are given in supplementary material "Cohort and sample collection and DNA extraction [11–14]." The study conforms to the Declaration of Helsinki and was approved by the Ethics Review Board of the Department of Biosciences at COMSATS University Islamabad, Pakistan. All subjects provided written informed consent.

Genotyping of *VEGFA* and *IGF1* SNPs

In total, six SNPs were selected (for selection criteria of the SNPs, see Supplementary Material: SNPs Selection) including four in *VEGFA* (rs833061, rs13207351, rs1570360 and rs2010963) and two in *IGF1* (rs5742632 and rs6214). rs833061, rs13207351 and rs1570360 were genotyped by Sanger sequencing [11, 12], while rs2010963 [13], rs5742632 and rs6214 were genotyped by PCR–RFLP [14]. A subset of samples was Sanger-sequenced to verify the PCR–RFLP analysis. Details are given in Supplementary Table 1.

Association analysis

All analyses were performed in R v3.2.2 [15]. Association of the SNPs was tested with T2D (T2D vs. control) and retinopathy (retinopathy vs. non-retinopathy in subjects with T2D only) using logistic regression under an additive genetic model; the results were adjusted for age and gender. SNPs were also tested for association with retinopathy (non-retinopathy, NPDR and PDR) in subjects with T2D using multinomial logistic regression implemented in multinom from nnet package version# 7.3–12 under an additive genetic model [16–18]. The results were adjusted for gender, diabetes duration and age at T2D diagnosis, in order to address multiple testing the effective number of independent SNPs; and new threshold was calculated as described previously [19] and was 0.008. Power calculations for genetic analyses were performed by Quanto (Version 1.2.4).

Serum *VEGFA* and *IGF1* concentration

VEGFA and IGF1 serum levels were measured by sandwich enzyme-linked immunosorbent assay (ELISA) (Cat. No. E-EL-H0111; E-EL-H0086 Elabscience, Houston, TX) in a subset of cases and controls. The assay was directed against amino acid 27–91 of VEGFA and full-length protein for IGF1. The cases and controls for VEGFA and IGF1 ELISA were selected based on genotype (Supplementary Table 2). The serum VEGFA and IGF1 levels were not normally distributed (Fig. 1) even after transformation; so the difference between the levels was calculated using the nonparametric Mann–Whitney *U* test. Univariate logistic

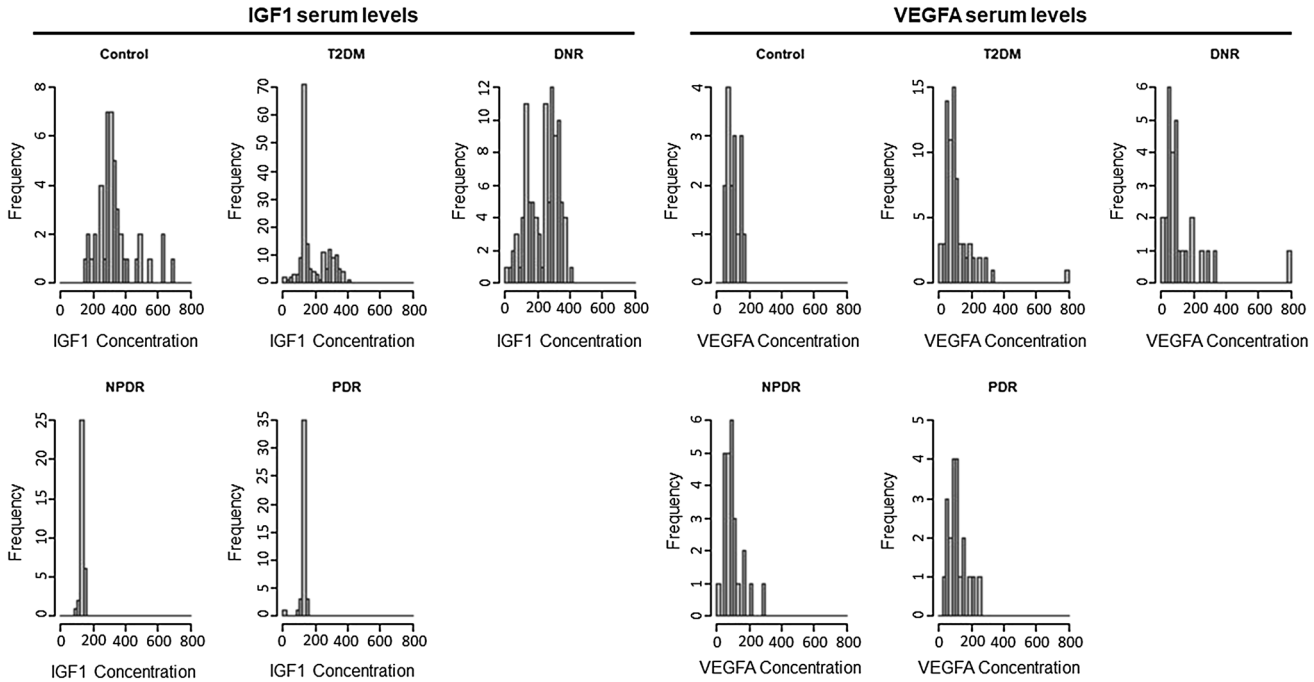

Fig. 1 *IGF1* and *VEGFA* serum levels in 5 sub cohorts. Left panel represents *IGF1* levels while right panel *VEGFA* levels. The distribution of *IGF1* and *VEGF* levels are not normally distributed, hence, the difference between the levels was calculated using the non-parametric Mann-Whitney U test

regression was used to test the association of IGF1 levels, age and gender with T2D and DR separately. In order to evaluate the role of age or gender-related decrease in serum IGF1 levels among cases, the results were also adjusted for both these variables.

Results

Cohort descriptive analysis

Subject characteristics of overall cohort are summarized in Table 1 (For phases 1 and 2 subject characteristics separately, see Supplementary Table 3).

A total of 1126 subjects with T2D were recruited (mean age (SD) = 53.5 (10.6) yrs) including 518(46.0%) males. Out of these T2D patients, 553(49.1%) subjects had developed retinopathy. DR subjects included 301(54.4%) NPDR and 252(45.6%) PDR. Subjects with T2D were significantly older than the control subjects ($p < 2.2E{-}16$).

Subjects with missing glucose measurements were significantly younger, but with longer diabetes duration and were more likely to be males as compared to the subjects with glucose measurements. DNR subjects with FPG or RPG had older age and higher proportion of males at the time of diabetes diagnosis as compared to the subjects with missing FPG and RPG. No such trend was observed in NPDR; however, in PDR, cases with missing FPG and RPG were older, diagnosed at older ages and were mostly males as compared to DNR with missing data. It was also observed that a significantly higher proportion of people with PDR are on insulin therapy as compared to DNR and NPDR (Supplementary Table 4).

SNPs

Of the six SNPs genotyped, rs1570360 and rs2010963 in *VEGFA* were not in HWE ($p = 8.01E{-}5$ and $1.96E{-}4$, respectively, Table 2); moreover, rs2010963 had a low call rate (83%) as compared to the other five SNPs (call rate > 90%). In order to determine the difference in population structure with that reported in the 1000 Genomes Project Punjabi population from Lahore (PJL) [20], the genotype and allele frequencies of all these six SNPs were retrieved and compared with the current data (Supplementary Table 5). We found significant differences between the genotype and allele frequencies for all SNPs. This could be a result of the fact that the two populations are geographically 400 km apart; in addition, the SNPs are all in non-coding sequence so their genotype calls in the 1000 Genomes Project derive from low coverage whole genome sequencing data, which does not produce high-quality genotypes (see Fig. 1b [21]). LD patterns for PJL were retrieved from

Table 1 Descriptive features of overall cohort

Subjects	Controls		T2DM cases		T2DM cases versus controls
Total (*N*)	348		1126		
Sex (male)	177 (50.9%)		518 (46.0%)		0.11*
Age (years)	47.05 (11.29) (*n* = 331)		53.54 (10.59)		< 2.2e−16**
T2DM Sub classes	DNR	NPDR	PDR	DNR Vs NPDR	DNR Vs PDR
Total (*N*)	573	301	252		
Sex (male)	273 (47.6%)	132 (43.9%)	112 (44.4%)	0.77*	0.83*
Age (years)	54.32 (10.04)	53.43 (10.82)	51.9 (11.36)	0.24**	3.67E − 3**
Diagnosis age (years)	41.55 (10.35)	40.65 (10.40)	39.89 (10.79)	0.23**	0.04**
Diabetes duration (years)	12.73 (9.23)	12.78 (8.48)	12.01 (7.69)	0.94***	0.25***
Random glucose (mg/dl)	266.9 (103.71) (*n* = 146)	233.1 (109.08) (*n* = 34)	261.68 (112.21) (*n* = 25)	0.09***	0.68***
Fasting glucose (mg/dl)	250.1 (113.41) (*n* = 114)	256.3 (124.32) (*n* = 37)	253.56 (108.36) (*n* = 34)	0.80***	0.60***
Medication					
Metformin	182 (31.7%)	92 (30.6%)	84 (33.3%)	0.59*	0.34*
Sulfonylureas	181 (31.6%)	81 (26.9%)	62 (24.6%)	0.09*	0.07*
Insulin	73 (12.8%)	81 (26.9%)	97 (38.5%)	2.05E−7*	< 2.2e-16*
No record	137 (23.9%)	47 (15.6%)	9 (3.6%)		

Values are *N* (%) or Mean (SD), *X2 *p* value, ***t* test *p* value, ***Wilcoxon *U* test *p* value

LDLink v 3.3.0 [22] and were calculated for our data (Supplementary Table 6) with variations between them.

Association of SNPs with T2D

Both *IGF1* rs5742632 (T > C, OR (95% CI) = 1.49 (1.18–1.90) $p = 1.00E{-}3$) and rs6214 (G > A, OR (95% CI) = 1.67 (1.39–2.01), $p = 4.92E{-}8$) were significantly associated with T2D in the multivariable analysis adjusted for age and gender (Table 2). These two SNPs are in LD with each other ($D' = 0.24$, $R^2 = 0.06$); therefore, logistic regression analysis with both of these SNPs as predictors in the same model showed association of rs5742632 (OR (95% CI) = 1.06 (1.02–1.10), $p = 1.00E{-}3$) and rs6214 with T2D (OR (95% CI) = 1.09 (1.06–1.12), $p = 9.12E{-}9$); however, the effect size for rs6214 is attenuated. We also performed logistic regression with interaction of rs5742632 and rs6214 as predictor and found the interaction is negatively associated with the disease (OR (95% CI) = 0.92 (0.87–0.97), $p = 1.88E{-}3$) when results were adjusted for age and gender (see Supplementary Table 7). However, none of the *VEGFA* SNPs showed significant association with T2D (Table 2).

Association of SNPs with DR

VEGFA rs13207351 was marginally associated with NPDR (G > A, OR (95% CI) = 1.97 (1.28–3.03), $p = 9.00E{-}3$) in the multivariable analysis (Table 3); and rs5742632 was associated with PDR (T > C, OR (95% CI) = 1.66 (1.33–2.05), $p = 1.00E{-}4$) in the multivariable analysis adjusted for age of onset, diabetes duration and gender (Table 4).

Power analysis of association of serum VEGFA and IGF1 levels with T2D and DR

Descriptive characteristics of selected cohort are summarized in Supplementary Table 8. We calculated the sample size needed to attain 80% power to detect the effect size previously described [23] using power and sample size calculator by using *t* test option [24]. For VEGFA T2D vs controls, the calculation was not performed due to lack of effect size data reported from different populations. However, power was calculated for NPDR and PDR as compared to DNR at $\alpha = 0.05$ from Ozturk et al. [21]. For NPDR, the standardized difference (δ) was reported 39.78 and our sample size (n) was 25 NPDR and power of our study was 0.35. For PDR, $\delta = 32.59$ and our sample size was 20 PDR and power was 0.18. These calculations suggested our study to be underpowered so it cannot detect true difference in means at $\alpha = 0.05$ in the two studied groups. For IGF1, most of the studies reported interquartile differences and we did not analyze our data in this way so we could not calculate power of our study.

Table 2 Association of the SNPs with T2D

Gene	SNP	Location in gene	MAF	Control (aa, Aa, AA)	Cases (aa, Aa, AA)	Genotype call rate %	HWE *p* value	Univariable analysis			Adjusted for age and gender		
								OR	95% CI	*p* value	OR*	95% CI	*p** value
VEGFA	rs833061	Promoter	*T* = 0.48	200 (55/88/57)	364 (106/135/123)	98.8	0.09	1.06	0.85–1.33	0.59	1.06	0.83–1.34	0.64
	rs13207351	Promoter	*G* = 0.49	206 (51/96/59)	360 (112/128/120)	99.1	0.33	0.63	0.45–0.90	1.00E−03	0.66	0.45–0.96	0.03
	rs1570360	Promoter	*A* = 0.38	207 (41/69/97)	357 (85/111/161)	98.7	8.01E−5	0.90	0.62–1.30	0.57	0.95	0.64–1.41	0.80
	rs2010963	Promoter	*G* = 0.47	170(60/60/50)	304 (76/120/108)	83.0	1.96E−4	0.77	0.60–0.97	0.03	0.76	0.58–0.98	0.03
IGF1	rs5742632	Intron	*G* = 0.23	348 (12/124/212)	1127 (47/429/651)	98.3	0.24	1.12	0.91–1.38	0.27	1.49	1.18–1.90	0.001
	rs6214	3′UTR	*T* = 0.44	348 (49/149/150)	1127 (265/533/329)	98.3	0.27	1.61	1.35–1.92	8.98E−8	1.67	1.39–2.01	4.92E−8

p value is unadjusted, while *p** value is adjusted for age and gender, the significance threshold is according to Li and Jee correction, i.e., 0.008 a = minor allele, A = major allele

Association of serum VEGFA and IGF1 levels with T2D and DR

For the determination of the difference in serum VEGFA among T2D cases and controls, 73 T2D cases (DNR = 28, NPDR = 25 and PDR = 20) and 16 controls were selected based on genotype. The Mann–Whitney U test results showed no statistically significant differences in serum VEGFA concentration either between T2D subjects and controls or between sub-categories of retinopathy with DNR ($p > 0.05$). Moreover, logistic regression analyses did not reveal association between serum VEGFA levels with T2D, NPDR or PDR (Supplementary Table 9A–9C).

For IGF1, the subset of subjects consisted of 175 T2D cases (DNR = 98, NPDR = 34 and PDR = 43) and 44 healthy controls chosen based on genotypes. Serum concentrations of IGF1 were significantly lower in T2D cases as compared to healthy controls ($p = 5.12E{-}13$). Lower IGF1 levels were observed in NPDR and PDR as compared to DNR ($p = 1.54E{-}7$ and 9.14E−9, respectively). The univariate analyses showed both IGF1 levels and age to be associated with T2D even after adjustment for age and gender in T2D subjects (OR = 0.98, 95% CI = 0.98–0.99, $p = 8.06E{-}9$) (Supplementary Table 9D). Using multinomial regression analyses, decreased IGF1 levels were found to be significantly associated with NPDR and PDR as compared to DNR with and without adjusting for age and gender (OR = 0.98, 95% CI = 0.97–0.99, $p = 4.07E{-}6$, OR = 0.98, 95% CI = 0.97–0.99, $p = 1.09E{-}6$, respectively) (Supplementary Table 9E–9F). In linear regression model, IGF1 serum levels were not found to be significantly associated with any of the two SNPs rs5742632 ($\beta = -13.75$, SE = 15.39, $p = 0.37$) and rs6214 ($\beta = 11.40$, SE = 11.00, $p = 0.302$).

Discussion

This study assessed the role of VEGFA and IGF1 serum levels for association with T2D and its retinal complications along with the genetic association of four *VEGFA* and two *IGF1* SNPs. SNPs showed association with retinal complications in both the genes, and increased IGF1 levels have also been found in patients with T2D and DR. However, the study highlighted the role of both growth-promoting factors for the first time in our population.

The most recent large-scale meta-GWAS analyses on a large cohort of European ancestry subjects reported a number of SNPs to be associated with T2D, yet the number of loci reaching genome-wide significance is still limited [25, 26]. Interestingly, these meta-analyses did not reveal association of any of our six SNPs with T2D (Supplementary Table 10). On the contrary, we highlighted the association of both *IGF1* SNPs with T2D. However, it should be pointed

Table 3 Association of SNPs with NPDR

Gene	SNP	Control (aa, Aa, AA)	Cases (aa, Aa, AA)	Univariable analysis			Adjusted for onset age, disease duration and gender		
				Odds ratio	95% CI	*p* value	Odds ratio*	95% CI*	*p* value*
VEGFA	rs833061	193 (65/71/57)	92 (18/40/34)	1.41	1.08–1.85	0.03	1.41	1.08–1.85	0.03
	rs13207351	193 (60/60/73)	89 (30/44/19)	1.99	1.30–3.05	7.0E−3	1.97	1.28–3.03	9.00E−3
	rs1570360	192 (36/58/98)	93 (23/33/37)	1.27	0.82–1.97	0.37	1.26	0.81–1.96	0.39
	rs2010963	111 (23/47/41)	89 (31/34/24)	1.51	1.12–2.04	0.02	1.55	1.15–2.09	0.02
IGF1	rs5742632	569 (21/187/361)	301 (14/115/172)	1.24	1.01–1.53	0.07	1.25	1.01–1.54	0.08
	rs6214	569 (136/281/152)	301 (70/145/86)	1.00	0.75–1.17	0.97	0.99	0.84–1.16	0.89

p value is unadjusted while *p** value is adjusted for age and gender, the significance threshold is according to Li and Jee correction, i.e., 0.008

Table 4 Association of SNPs with PDR

Gene	SNP rs ID	Control (aa, Aa, AA)	Cases (aa, Aa, AA)	Univariable analysis			Adjusted for onset age, disease duration and gender		
				Odds ratio	95% CI	*p* value	Odds ratio*	95% CI*	*p* value*
VEGFA	rs833061	193 (65/71/57)	79 (23/24/32)	1.28	0.97–1.70	0.14	1.30	0.98–1.73	0.12
	rs13207351	193 (60/60/73)	74 (30/24/20)	1.06	0.66–1.72	0.83	1.00	0.62–1.63	0.99
	rs1570360	192 (36/58/98)	73 (27/20/26)	0.87	0.53–1.44	0.65	0.87	0.50–1.39	0.56
	rs2010963	111 (24,49,38)	73 (22,28,23)	0.86	0.63–1.74	0.43	0.91	0.66–1.26	0.65
IGF1	rs5742632	569 (21/187/361)	252 (11/124/117)	1.65	1.34–2.05	9.0E−5	1.66	1.33–2.05	1.0E−4
	rs6214	569 (136/281/152)	252 (58/105/89)	0.85	0.71–1.01	0.11	0.83	0.70–0.99	0.08

p value is unadjusted, while *p** value is adjusted for age and gender, the significance threshold is according to Li and Jee correction, i.e., 0.008

out that our study only genotyped a small number of SNPs, so it cannot assess whether population structure has led to false positive and/or negative associations. The meta-GWAS analysis for DR identified a few SNPs associated with DR, yet no locus was separately replicated [27].

GWAS analyses were previously conducted for the association of VEGFA serum levels [28] and circulating IGF1 levels with multiple diseased conditions including T2D [29], but only few met stringent GWAS criteria ($p < 5E10{-}8$). All of these large-scale analyses of SNPs, gene expression and protein levels suggested that an interplay between the genes present within the reported loci may affect the circulating levels of both of these growth-stimulating factors.

The small-scale candidate gene approach also elaborated the role of *VEGFA* variants in the angiogenesis-mediated phenotypes including the progression of retinal complications of T2D; however, its role in DR manifestation still remains elusive because of the contradictory findings of the association of different SNPs with DR [30]. Supplementary Table 11 summarizes the previous findings for the association of *VEGFA* variants with DR in different populations that are contrastingly different than our study for few populations, but few studies conducted on Indian, Polish and Slovak populations are in agreement with our study and reported no such associations. These discrepancies could be explained by small sample sizes, population structure differences and the different frequencies of variants in different populations affecting power.

The transcriptional activity and gene expression of *VEGFA* are also known to be regulated by promoter region SNPs [31]; however, different variants are reported from different populations. The expression of the promoter rs833061 along with 5′UTR rs2010963 was studied. No significant association was observed in the production of VEGFA with the genotypes of rs833061 TC and/or CC. However, it was observed that rs2010963 CC was associated with decreased VEGFA secretion [32]. At the molecular level, the binding site of myeloid zinc finger protein (MZF1), a transcriptional factor, is disrupted by the presence of rs2010963 C leading to possible reduced VEGF production [33]. The risk C allele carriers of rs833061 were found to have increased promoter activity of VEGFA [34]. The C allele of rs2010963 was also found to be associated with altered transcriptional activity of *VEGFA* expression in different tissues [35]. However, the effect of the remaining two SNPs on expression is not well understood. The VEGFA-level alterations are not just limited to DR, rather elevated serum VEGFA has also been observed in other angiogenesis-mediated ocular pathologies. These findings suggest that the role of VEGF-mediated angiogenesis is not only limited to DR but also affects other

ocular conditions, suggesting a mechanism-specific role rather than a phenotype-specific role.

Few studies have been conducted which focused on the association of serum VEGFA levels with DR; however, no such association was observed in the current study. A study conducted on vitreous VEGFA levels in PDR cases vs non-PDR cases did not find any significant differences between VEGFA levels among both the classes [36]. On the other hand, another study reported an increased concentration of VEGFA in PDR as compared to DNR cases [37]. These discrepancies highlight the need of replication studies with a larger cohort to get a better insight into the possible mechanism by which these serum, plasma or vitreous VEGFA levels are altered in people with diabetes.

To the best of our knowledge, none of the meta-GWAS identified the association of *IGF1* variants with T2D and DR. However, recent reports of Chinese Han population highlighted the association of *IGF1* variants with T2D susceptibility [38], where the authors observed that two *IGF1* variants rs6218-AG (OR (95% CI) = 1.92 (1.24–2.97), $p = 0.004$) and rs35767-TT (OR (95% CI) = 2.29 (1.13–4.68), $p = 0.02$) were associated with T2D predisposition. Another study showed that rs5742612 ($\beta = 0.13$, $p = 0.008$) and rs2288377 ($\beta = 0.16$, $p = 0.001$) were associated with increased insulin sensitivity and lowered insulin secretion [37]. Zhang et al. [36] also showed that rs6218-AG (OR (95% CI) = 1.77 (1.03–3.06), $p = 0.04$) and rs35767-TT (OR (95% CI) = 2.32 (1.07–5.03), $p = 0.03$) were associated with increased risk of DR. All of these SNPs are in high LD with each other in PJL and Han Chinese Population of 1000 Genomes (Supplementary Table 12). The present study showed the association of *IGF1* variants rs6214 and rs5742632 with T2D and DR progression; however, detailed studies are required to get better insights into their associations with T2D and/or DR.

There are a number of reports that suggested serum IGF1 levels to be associated with T2D. We found that the lowered serum levels of IGF1 are associated with T2D, NPDR and PDR, which are in agreement with the previous studies conducted in different populations [9, 39, 40]. In contrast, a study conducted on the Romanian population [41] showed higher serum IGF1 levels in T2D subjects (mean = 64.62, SD = 4.48 ng/ml) as compared to healthy controls (mean = 36.34, SD = 3.99 ng/ml) ($p < 0.001$), but there were only 30 T2D and 30 control samples in that study. Another study also reported a negative relationship between serum IGF1 levels and insulin sensitivity in the Danish population with a comparatively large cohort ($n = 303$ twin pairs (125 monozygotic and 178 dizygotic)) with $p < 0.0001$ (OR = 0.88, SE = 0.03) [42].

For *IGF1*, out of the two SNPs, rs6214 was found to be genetically associated with T2D and rs5742632 and showed a positive effect with advanced form of retinopathy in our study, but none of these SNPs showed association with lowered IGF1 serum levels, highlighting that there are some other genetic variants in *IGF1* region that influence IGF1 levels and play a role in disease pathophysiology. These gene-promoter regions are the binding sites for different transcriptional factors and through their binding gene promoters play a pivotal role in initiation and regulation of gene transcription. Thus, sequence variation in gene promoters is known to alter the identification and binding of transcription factor and consequently alter gene expression ultimately affecting the biological function [43, 44]. In contrast, another study reported the association of minor allele homozygotes of rs6214 with elevated HbA1c levels showing possible role of this SNP in poor glycemic control in T2D subjects [45].

Despite all of our reported genetic association of the SNPs with the outcome, it must be admitted that most of the analyses were underpowered (See Supplementary Table 13), which may be one explanation why some SNPs did not replicate the association observed in previous studies.

In summary, we studied the association of four SNPs in *VEGFA* and two SNPs in *IGF1* with T2D and DR, while other SNPS in *IGF1* and *VEGFA* must be targeted in larger cohorts to better understand the possible role of those DNA variants in the disease manifestation and progression. We observed a positive association of IGF1 serum levels with T2D and PDR; however, further high-powered studies are needed to evaluate both the vitreous and serum IGF1 along with VEGFA levels in various stages of DR to provide a better insight into the interaction between systemic and local factors responsible for DR development and progression. However, the evidence reported in this study is preliminary and warrants much more in-depth studies.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical statement The study conforms to the Declaration of Helsinki and was approved by the Ethics Review Board of the Department of Biosciences at COMSATS University Islamabad, Pakistan.

Informed consent All subjects provided written informed consent.

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