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Microaneurysm turnover is a predictor of diabetic retinopathy progression

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ABSTRACT

Aim To analyse retinopathy phenotypes and microaneurysm (MA) turnover in mild non-proliferative diabetic retinopathy (NPDR) as predictors of progression to diabetic central-involved macular oedema (CIMO) in patients with type 2 diabetes mellitus (DM) in two different ethnic populations.

Methods 205 patients with type 2 DM and mild NPDR were followed in a prospective observational study for 2 years or until development of CIMO, in two centres from different regions of the world. Ophthalmological examinations, including best-corrected visual acuity (BCVA), fundus photography with RetmarkerDR analysis, and optical coherence tomography (OCT), were performed at baseline and 6 12 and 24 months.

Results 158 eyes/patients reached either the study endpoint, CIMO (24) or performed the last study visit (24-month visit) without developing CIMO (134). From the eyes/patients in analysis, 27 eyes (17.1%) progressed to more advanced ETDRS (Early Treatment Diabetic Retinopathy Study) levels: 6 progressed to mild NPDR (level 35), 15 progressed to moderate NPDR (level 43), 5 progressed to moderately severe NPDR (level 47) and 1 progressed to high risk PDR (level 71). Worsening in ETDRS level is associated with phenotype C (p=0.005). From the 130 eyes/patients with a low MA turnover, 18 (13.8%) eyes/patients had an increase in ETDRS level, and from the 19 eyes/patients with a high MA turnover, 9 (47.4%) had an increase in ETDRS level (p<0.001).

Conclusion Eyes in the initial stages of diabetic retinopathy show different phenotypes with different risks for progression to CIMO. In phenotype C, MA turnover correlates with ETDRS grading worsening and development of CIMO.

INTRODUCTION

Diabetic retinopathy (DR) is a common and serious condition. It is the leading cause of blindness among working-age adults in the USA.[1] Vision loss related to eye disease among people with diabetes is an important disability that threatens independence and can lead to depression, reduced mobility and reduced quality of life. The Eye Diseases Prevalence Research Group classified DR into two major outcomes: any DR, as any DR consisting of mild, moderate or severe DR; and vision threatening DR (VTDR), as DR likely to result in vision loss in the absence of treatment, consisting of proliferative DR, clinically significant diabetic macular oedema (CSMO), or both.[2] This concept is crucial to address the issue of management of DR in order to prevent vision loss and to identify which patients will progress to VTDR (ie, to CSMO and/or proliferative DR). It is now apparent that systemic markers of diabetes do not identify DR progression to VTDR.[3]

It is therefore fundamental to identify organ-specific biomarkers such as retinal lesions and their dynamics in the earlier stages of DR and look for their correlation with worsening of any stage of DR to VTDR.[3]

Previous studies by our group show that some patients progress rapidly to macular oedema in contrast to others that remain stable, even under similar metabolic control. Our group identified three phenotypes with different risks for the development of macular oedema.[4]

Automated image analysis of microaneurysm (MA) turnover performed on colour fundus photographs contributed to the identification of those eyes that were at risk of developing clinical significant macular oedema.[5] In the current prospective study, performed in two centres from different regions of the world, we examine if MA changes occurring in the posterior pole of the eye and detected by automated image analysis are directly correlated with progression of DR represented by worsening in retinopathy severity (Early Treatment Diabetic Retinopathy Study (ETDRS) levels) or development of central-involved macular oedema (CIMO).

METHODS

Details of this study have been previously reported.[6] In brief, one eye from 205 subjects with types 2 diabetes, aged over 35 years, mild NPDR (levels 20 to 35, according the ETDRS diabetic retinopathy severity scale), best-corrected visual acuity (BCVA) >20/25 on the ETDRS chart and glycated haemoglobin (HbA1c) ≤11% were included in a prospective observational study for 2 years or until development of CIMO, at two clinical sites (AIBILI, Coimbra, Portugal; and LV-Prasad Eye Institute (LVPEI), Hyderabad, India). Other inclusion/exclusion criteria were: no previous treatment with laser or anti-vascular endothelial growth factor (anti-VEGF) or steroid intravitreal injections, no other retinal vascular disease or glaucoma, or inadequate ocular media and/or pupil dilatation that did not permit good quality fundus photography. Informed consent was obtained from each patient after explanation of the nature of the study and before any study procedure. The tenets of the Declaration of Helsinki were followed, and approval was obtained from each of the ethics committee (ClinicalTrials.gov number, NCT01607190).

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Data were collected in an initial period of three visits, performed at 6-month intervals, followed by another examination 1 year later for a total of 2 years follow-up.

Baseline and follow-up examinations included BCVA, colour fundus photography (CFP) analysed by automated MA analysis using RetmarkerDR software, Cirrus HD-OCT (optical coherence tomography) (Carl Zeiss Meditec, Inc, Dublin, CA, USA) for retinal thickness (RT) measurements, blood pressure evaluation, $HbA1c_{A1c}$ and lipid blood levels.

CFP was performed according to the ETDRS protocol. An automated computer-aided diagnostic system, RetmarkerDR (Retmarker SA, Coimbra, Portugal) was used to detect MAs automatically on the field-2 colour fundus images. This software includes a patented co-registration algorithm that allows comparison within the same retinal location between different visits for the same eye. The RetmarkerDR computes for each eye/patient the number of MAs at each visit and the number of MAs that appear and/or disappear from one visit to the other, allowing calculation of the number of MAs appearing and/or disappearing per time interval (ie, MA formation rate and MA disappearance rate, respectively). The formation and disappearance rates were calculated for each visit compared with the baseline visit, and the MA turnover was computed as the sum of the MA formation and disappearance rates. Patients were thereafter classified based on the presence of MA formation rate ≥2 and on the presence of an MA turnover ≥6, according to Nunes *et al*,[4 5 7] given that these cut-off values—to separate different mild NPDR phenotypes—have been proposed as being predictive of progression of diabetic retinopathy.

To identify eyes/patients with increased RT in the central subfield (clinical and subclinical macular oedema) and in the inner and outer rings, the reference values established by DRCR.net were used:[8 9]

For clinical macular oedema:

- RT ≥290 µm in women and ≥305 µm in men for Cirrus HD-OCT (Carl Zeiss Meditec, Inc, Dublin, CA).

For subclinical macular oedema:[10]

- RT >260 µm and <290 µm in women and >275 µm and <305 µm in men for Cirrus HD-OCT (Carl Zeiss Meditec, Inc, Dublin, CA).

Patients were classified into one of the three phenotypes of DR progression[4]—phenotype A: low MA turnover and normal retinal thickness (MA turnover <6 and central subfield (CSF) RT <260 µm (women) or CSF RT <275 µm (men)); phenotype B: low MA turnover and increased retinal thickness (MA turnover <6 and CSF RT ≥260 µm (women) and CSF RT ≥275 µm (men)); and phenotype C: high MA turnover (MA turnover ≥6) with or without increased retinal thickness.

Statistical analysis

Frequency and percentages are reported for all categorical measures.

Associations between MA formation rate and MA turnover, at 6 and 12 months, changes in ETDRS level and development of CIMO were tested using χ^2 test.

A multivariate logistic regression was computed with development of CIMO as the dependent variable, and ETDRS changes, phenotypes, HbA1c, body mass index, blood pressure and cholesterol variables at baseline as independent variables.

Correlations between the different parameters were tested using the non-parametric Spearman correlation coefficient.

Statistical analyses were performed using the Stata software version 12.1 (StataCorp LP, College Station, TX, USA). Values of $p \leq 0.05$ were considered to be statistically significant.

RESULTS

Baseline results for the 205 eyes/patients included in the study have been published previously.[6] From these 205 eyes/patients, only 158 eyes/patients reached either the study endpoint, CIMO (24 eyes/patients) or performed the last study visit (24-month visit) without developing CIMO (134 eyes/patients). There were a total of 47 dropouts from the study (one patient died, 11 withdrew consent, two had health problems and 33 were lost to follow-up). Ethnic origin was significantly different between those patients who completed the study and those who dropped out of the study, with more Asians dropping out of the study. Low-density lipoprotein (LDL) cholesterol and diastolic blood pressure were also significantly different between those patients who completed the study and those who dropped out of the study: LDL cholesterol was higher in the group of patients who completed the study, and diastolic blood pressure was higher in the group of patients who dropped out of the study.[6]

Eyes/patients were classified into one of the three phenotypes of diabetic retinopathy progression. Eighty-eight (56.4%) were identified as phenotype A, 49 (31.4%) as phenotype B, and 19 (12.2%) as phenotype C. Comparing both clinical sites, LVPEI had a higher number of patients with phenotype C: in AIBILI, 44 (46.8%) of the eyes/patients were identified as phenotype A, 44 (46.8%) as phenotype B and only 6 (6.4%) as phenotype C; in LVPEI, 44 (71.0%) of the eyes/patients were identified as phenotype A, 5 (8.0%) as phenotype B and 13 (21.0%) as phenotype C.

From the eyes/patients analysed, 27 eyes (17.1%) progressed to more advanced ETDRS levels: six progressed to mild NPDR (level 35), 15 progressed to moderate NPDR (level 43), five progressed to moderately severe NPDR (level 47) and one progressed to high risk PDR (level 71) (table 1).

The majority of eyes/patients who progressed were from LVPEI. In fact, of the 92 eyes/patients from AIBILI only three eyes (3.3%) progressed to mild NPDR (level 35), while from the 57 eyes/patients from LVPEI 24 eyes (42.1%) progressed to more advanced ETDRS levels: three progressed to mild NPDR (level 35), 15 progressed to moderate NPDR (level 43),

Table 1 Eyes/patients with ETDRS changes from baseline to month-24

ETDRS level at baseline	ETDRS level at month 24								
	10	12	14	20	35	43	47	71	Total
20	10	0	0	14	6	3	0	0	33
35	6	5	1	21	65	12	5	1	116
Total	16	5	1	35	71	15	5	1	149

ETDRS, Early Treatment Diabetic Retinopathy Study.

Table 2 Changes between baseline and month 24 in ETDRS level, by phenotype

	DR worsening				DR improving		
Phenotype	≥3 steps	2 steps	1 step	No change	1 step	2 steps	≥3steps
A, n (%)	0 (0.0)	4 (4.6)	13 (14.9)	40 (46.0)	13 (14.9)	1 (1.2)	16 (18.4)
B, n (%)	1 (2.2)	0 (0.0)	3 (6.7)	30 (66.7)	7 (15.6)	0 (0.0)	4 (8.9)
C, n (%)	2 (11.8)	4 (23.5)	1 (5.9)	8 (47.1)	1 (5.9)	0 (0.0)	1 (5.9)
Total	3 (2.0)	8 (5.4)	17 (11.4)	78 (52.4)	21 (14.1)	1 (0.7)	21 (14.1)

DR, diabetic retinopathy; ETDRS, Early Treatment Diabetic Retinopathy Study.

five progressed to moderately severe NPDR (level 47) and one progressed to high risk PDR (level 71).

Changes in ETDRS level from baseline to month 24, by phenotype, are shown in table 2. Phenotype C is associated with a two-step worsening in ETDRS level. Of the phenotype C patients, 41.2% experienced at least a worsening one-step in ETDRS level from baseline to month 24, whereas an improvement was observed in just 11.8% of these patients. The remaining 47.1% remained stable and experienced no change in ETDRS level from baseline to month 24 (table 2).

Phenotype A, although showing a similar percentage of eyes/patients without change in ETDRS level, presented ETDRS level worsening in only 19.5% of the eyes versus the ETDRS level worsening of 41.2% registered in eyes/patients with phenotype C.

From the 107 eyes/patients with an MA formation rate at month 6 <2, only 13 (12.1%) eyes/patients had an increase in ETDRS level; and from the 42 eyes/patients with an MA formation rate ≥2, 14 (33.3%) eyes/patients had an increase in ETDRS level (p=0.003). From the 130 eyes/patients with an MA turnover at month 6 <6, 18 (13.8%) eyes/patients had an increase in ETDRS level; and from the 19 eyes/patients with an MA turnover ≥6, nine (47.4%) eyes/patients had an increase in ETDRS level (p<0.001).

At month 12, from the 99 eyes/patients with an MA formation rate <2, 12 (12.1%) eyes/patients had an increase in ETDRS level; and from the 50 eyes/patients with an MA formation rate ≥2, 15 (30%) eyes/patients had an increase in ETDRS level (p=0.007). From the 119 eyes/patients with an MA turnover <6, 15 (12.6%) eyes/patients had an increase in ETDRS level; and from the 30 eyes/patients with an MA turnover ≥6, 12 (40.0%) eyes/patients had an increase in ETDRS level (p<0.001) (table 3).

A significant association between MA parameters and ETDRS level change was found in LVPEI eyes/patients for MA turnover ≥6 at month 6. From the 44 eyes/patients with an MA turnover at month 6 <6, 15 (34.1%) eyes/patients had an increase in ETDRS level; and from the 13 eyes/patients with an MA turnover ≥6, 9 (69.2%) eyes/patients had an increase in ETDRS level (p=0.024).

Considering the study endpoint, a significant association was found for MA turnover at month 12 and the eyes/patients developing CIMO. From the 123 eyes/patients with an MA turnover <6, 14 (11.4%) eyes/patients developed CIMO; and from the 35 eyes/patients with an MA turnover ≥6, 10 (28.6%) eyes/patients developed CIMO (p=0.012).

For the LVPEI eyes/patients significant associations were found between the development of CIMO and MA formation rate ≥2 at month 6 and MA turnover ≥6 at month 12. From the 32 LVPEI eyes/patients with an MA formation rate at month 6 <2, only two (6.2%) eyes/patients developed CIMO; whereas from the 31 eyes/patients with an MA formation rate ≥2, nine (29.0%) eyes/patients developed CIMO (p=0.022). From the 37 eyes/patients with an MA turnover at month 12 <6, two (5.4%) eyes/patients developed CIMO; and from the 26 eyes/patients with an MA turnover ≥6, nine (34.6%) eyes/patients developed CIMO (p=0.005).

For the AIBILI population, no significant associations could be found between ETDRS level changes, MA parameters and development of CIMO as only three eyes out of 92 showed changes in ETDRS level.

Similarly to patients with phenotype C, development of CIMO tended to be in general more common in patients with ETDRS level worsening (table 4), with more cases of CIMO in those with a three-step or more ETDRS level worsening versus eyes with ETDRS level improvement.

On a phenotype analysis, only one eye/patient identified as phenotype A (1.1%) developed CIMO during the follow-up period. In this patient, the ETDRS level did not change between baseline and month 24. For eyes/patients identified as phenotype B, 13 eyes (26.5%) developed CIMO, of which eight eyes (61.5%) presented no change in ETDRS level. For eyes/patients identified as phenotype C, five eyes (26.3%) developed CIMO, none of which improved on the ETDRS severity scale (table 4).

In a multivariate logistic regression, considering phenotypes, metabolic control and cardiovascular risk variables as predictors to analyse risk of developing CIMO, eyes/patients from phenotype C showed a higher risk of developing CIMO than eyes/patients from phenotype A (OR 44.8, 95% CI 6.8 to 293.8; p<0.001); and eyes/patients from phenotype B showed a higher chance of developing CIMO than eyes/patients from phenotype A (OR 31.4, 95% CI 5.4 to 183.3; p<0.001).

Table 3 MA formation rate and MA turnover, at months 6 and 12, correlated with changes in ETDRS level

	MA formation rate				MA turnover			
	Month 6		Month 12		Month 6		Month 12	
ETDRS level	<2	≥2	<2	≥2	<6	≥6	<6	≥6
Remain or decrease, n (%)	94 (87.9)	28 (66.7)	87 (87.9)	35 (70.0)	112 (86.2)	10 (52.6)	104 (87.4)	18 (60.0)
Increase, n (%)	13 (12.1)	14 (33.3)	12 (12.1)	15 (30.0)	18 (13.8)	9 (47.4)	15 (12.6)	12 (40.0)
P values*	0.003		0.007		<0.001		<0.001	

*χ^2 test.
ETDRS, Early Treatment Diabetic Retinopathy Study; MA, microaneurysm.

Table 4 Changes between baseline and month 24 in ETDRS level, by study endpoint

			DR worsening				DR improving				
Phenotype	Endpoint	# Patients	All	≥3 steps	Two steps	One step	All	One step	Two steps	≥3 steps	No change
A (n=87)	No CIMO	86 (98.9%)	17 (19.8)	0	4	13	30 (34.9)	13	1	16	39 (45.3)
	CIMO	1 (1.1%)	0 (0.0)	0	0	0	0 (0.0)	0	0	0	1 (100.0)
B (n=45)	No CIMO	32 (71.1%)	2 (6.3)	0	0	2	8 (25.0)	6	0	2	22 (68.8)
	CIMO	13 (28.9%)	2 (15.4)	1	0	1	3 (23.1)	1	0	2	8 (61.5)
C (n=17)	No CIMO	12 (70.6)	4 (33.3)	0	3	1	2 (16.7)	1	0	1	6 (50.0)
	CIMO	5 (29.4%)	3 (60.0)	2	1	0	0 (0.0)	0	0	0	2 (40.0)

CIMO, central-involved macular oedema; DR, diabetic retinopathy; ETDRS, Early Treatment Diabetic Retinopathy Study.

Although there were differences in baseline characteristics between the eyes/patients in each clinical site—for example, patients from LVPEI were younger, had poorer metabolic control (higher HbA1c) and lower body mass index, LDL and high-density lipoprotein (HDL) cholesterol—no differences could be found between clinical sites when eyes/patients were grouped by phenotype or analysed by endpoint (development of CIMO). Furthermore, when using multivariate logistic regression analysis in considering phenotypes, metabolic control and cardiovascular risk variables as predictors of developing CIMO, the only significant association was found for phenotypes.[6]

DISCUSSION

This 2-year prospective, longitudinal study of patients with type 2 diabetes and mild NPDR (ETDRS levels 20 and 35, at baseline) shows that MA turnover in field 2 is a good predictor of retinopathy worsening, as demonstrated by step-changes in ETRDS grading and development of macular oedema.

In previous studies demonstration of MA formation rate and turnover, taking into account the exact location of new MA in successive colour fundus photographs, showed higher sensitivity in predicting worsening of the retinopathy in a 10-year follow-up period than simple counting of MA.[5]

Of particular interest in this and previous studies is the observation that MA turnover values determined over a period of only 6 months predict with a high degree of confidence the eyes that do not progress for a period of at least 2 years. This finding has an impact on clinical trial design. To assess efficacy of the drug being tested it is relevant to exclude eyes/patients that are not expected to develop outcomes during the trial. The development of outcomes in the placebo control eyes is fundamental to be able to detect differences between the two arms, placebo versus drug. Our findings suggest that choosing phenotype C would increase the odds of guaranteeing retinopathy worsening in the placebo group of a clinical trial.

Recent studies have shown the relevance of retinopathy severity improvement based on ETDRS level grading as a clinically important outcome. In eyes treated with anti-VEGF agents[11] or with corticosteroids,[12] greater degrees of improvement in ETDRS grading levels correlate with greater magnitudes of functional and anatomic improvement.

This study shows that automated analysis of MA turnover correlates well with changes in severity of ETDRS grading levels, validating its use as a simple to use biomarker of DR progression. Automated analysis techniques offer advantages of repeatability and consistency.

It is also relevant that MA turnover calculated by the Retmarker DR (Retmarker SA) is much less time consuming than ETDRS grading and MA counting by expert graders.

This study confirmed the previously identified distribution of three different phenotypes of DR progression with different risks for the development of diabetic macular oedema (DMO). Phenotype A (50% of the eyes with mild NPDR) shows a very low risk for the development of DMO in contrast to phenotypes B and C that show a much higher risk for progression to DMO. Within phenotype C there is a good correlation between MA turnover, progression in ETDRS levels and development of DMO. However, this correlation is not present in phenotype B. In phenotype B, DMO may occur without ETDRS level changes. The ETDRS severity scale does not take into account the presence or absence of macular oedema, and macular oedema, that is, central-involved macular oedema, may be present in eyes without any or minimal microvascular changes. In order to evaluate progression of DR to VTDR it is necessary to evaluate not only DR worsening by ETDRS scale standards but also retinal thickening measured by OCT.

The majority of eyes/patients who progressed during the study were from LVPEI, in India, where we found a higher number of patients from phenotype C (68.4%). It is of interest that even in this group, phenotype C from the India centre, metabolic control and cardiovascular risk variables did not reach statistical significance.[6] There is previous evidence from aggregation in families and specific ethnic groups that there is a genetic predisposition to develop some diabetic complications such as retinopathy.[13 14] Heritability has been estimated to be as high as 27% for DR and 52% for proliferative DR.[15 16] It is noteworthy that our research group performed a case–control study and found a statistically significant association between different phenotypes of DR progression as described here and different gene variants.[17]

The major limitation of this study is its relatively short duration (2 years) and the fact that phenotype C, associated with a higher number of MAs and increased MA turnover, was mainly present in the clinical site from India.

Finally, the results of this prospective study confirm, in a relatively large number of eyes/patients, that MA turnover values obtained from automated analysis of non-invasive colour fundus photographs and based solely on field 2 images may help to identify the eyes/patients at risk for worsening of their diabetic retinal disease.

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Competing interests None declared.

Patient consent Obtained.

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REFERENCES

1 Fong DS, Aiello LP, Ferris FL, *et al*. Diabetic retinopathy. *Diabetes Care* 2004;27:2540–53.
2 Kempen JH, O'Colmain BJ, Leske MC, *et al*. The prevalence of diabetic retinopathy among adults in the United States. *Arch Ophthalmol* 2004;122:552–63.
3 Hove MN, Kristensen JK, Lauritzen T, *et al*. The relationships between risk factors and the distribution of retinopathy lesions in type 2 diabetes. *Acta Ophthalmol Scand* 2006;84:619–23.
4 Nunes S, Ribeiro L, Lobo C, *et al*. Three different phenotypes of mild nonproliferative diabetic retinopathy with different risks for development of clinically significant macular edema. *Invest Ophthalmol Vis Sci* 2013;54:4595.
5 Nunes S, Pires I, Rosa A, *et al*. Microaneurysm turnover is a biomarker for diabetic retinopathy progression to clinically significant macular edema: findings for type 2 diabetics with nonproliferative retinopathy. *Ophthalmologica* 2009;223:292–7.
6 Ribeiro L, Pappuru R, Lobo C, *et al*. Different phenotypes of mild nonproliferative diabetic retinopathy with different risks for development of macular edema (C-TRACER Study). *Ophthalmic Res* 2018;59:59–67.
7 Haritoglou C, Kernt M, Neubauer A, *et al*. Microaneurysm formation rate as a predictive marker for progression to clinically significant macular edema in nonproliferative diabetic retinopathy. *Retina* 2014;34:157–64.
8 Friedman SM, Almukhtar TH, Baker CW, *et al*. Topical nepafenec in eyes with noncentral diabetic macular edema. *Retina* 2015;35:944–56.
9 Bandello F, Tejerina AN, Vujosevic S, *et al*. EVICR.net. Retinal layer location of increased retinal thickness in eyes with subclinical and clinical macular edema in diabetes type 2. *Ophthalmic Res* 2015;54:112–7.
10 Pires I, Santos AR, Nunes S, *et al*. Subclinical macular edema as a predictor of progression to clinically significant macular edema in type 2 diabetes. *Ophthalmologica* 2013;230:201–6.
11 Ip MS, Zhang J, Ehrlich JS. The clinical importance of changes in diabetic retinopathy severity score. *Ophthalmology* 2017;124:596–603.
12 Wykoff CC, Chakravarthy U, Campochiaro PA, *et al*. Long-term effects of intravitreal 0.19 mg fluocinolone acetonide implant on progression and regression of diabetic retinopathy. *Ophthalmology* 2017;124:440–9.
13 Leslie RD, Pyke DA. Diabetic retinopathy in identical twins. *Diabetes* 1982;31:19–21.
14 Warpeha KM, Chakravarthy U. Molecular genetics of microvascular disease in diabetic retinopathy. *Eye* 2003;17:305–11.
15 Arar NH, Freedman BI, Adler SG, *et al*. Heritability of the severity of diabetic retinopathy: the FIND-Eye study. *Invest Ophthalmol Vis Sci* 2008;49:3839.
16 Hietala K, Forsblom C, Summanen P, *et al*. Heritability of proliferative diabetic retinopathy. *Diabetes* 2008;57:2176–80.
17 Simões MJ, Lobo C, Egas C, *et al*. Genetic variants in ICAM1, PPARGC1A and MTHFR are potentially associated with different phenotypes of diabetic retinopathy. *Ophthalmologica* 2014;232:156–62.