Phenotypes and Biomarkers of Diabetic Retinopathy. Personalized Medicine for Diabetic Retinopathy
The Weisenfeld Award

José Cunha-Vaz

Association for Innovation and Biomedical Research on Light and Image (AIBILI), Coimbra, Portugal
Department of Ophthalmology, University of Coimbra, Coimbra, Portugal

Correspondence: José Cunha-Vaz, AIBILI, Azinhaga de Santa Comba, Celas 3000-548 Coimbra, Portugal; cunhavaz@aibili.pt.

Submitted: May 27, 2014
Accepted: May 27, 2014


I have not yet found the right words to express my gratitude to the persons involved in my nomination for this Award and to the ones who finally made me this year’s choice. It is great to hear someone telling you that your life work has been worthwhile. The Award is named after Mildred Weisenfeld, a person who suffered from a disease that caused her to lose vision progressively. Mildred Weisenfeld dedicated her life to promote and establish eye research, and her commitment was so relevant that she was highly instrumental in the inception of the National Eye Institute (Bethesda, MD, USA). The National Eye Institute has now a unique place in supporting eye research in the United States and worldwide.

When I returned to Europe in 1968 after my stay in the United States, where I realized the unique value of the National Eye Institute, the development of an European Vision Institute following on the steps of the USA National Eye Institute has been always a permanent goal for me. The Weisenfeld Award is one of the main Awards of the Association for Research in Vision and Ophthalmology (ARVO), and ARVO means, to me, Eye Research. Since working under the mentorship of Norman Ashton, in London, I realized that eye research was my calling. Finding new solutions and adding to present knowledge to help human beings who lost vision or are at risk of losing vision clearly was a useful and rewarding challenge.

At ARVO I feel at home, and it is a special feeling to be recognized by my peers. My links to ARVO were initiated a long time ago and became emotional for a variety of reasons. My first ARVO meeting took place in 1977 in Sarasota, Florida. I was invited to lecture at Johns Hopkins (Baltimore, MD, USA) by Arnold Pritz just before the ARVO meeting, and one of my mentors, David Maurice, insisted that I should take the opportunity to participate in the ARVO meeting. He booked me, at the last minute, a room at the Around the World Motel and shepherded me there.

At this same ARVO meeting, Mark Tso and Morton Goldberg invited me to the Illinois Eye and Ear Infirmary Party, which was followed by an invitation to come to Chicago.

Therefore, ARVO 1977 was a turning point in my life and career. Now, in 2014, a few years later, I am here receiving the Weisenfeld Award for Excellence in Ophthalmology. Why am I here? I am here for many reasons, for which I try to mention just a few. On reviewing my life, two words come to my mind, luck and fun. Fun, because I enjoyed very much doing what I have been paid to do. Luck, because of the persons who opened the way for me and supported my first steps.

First, my father, an ophthalmologist who wanted me to become a better ophthalmologist than himself and advised me to learn eye pathology for my medical practice to be based on the best available scientific knowledge.

I then was extremely lucky to have as mentors two of the best minds in the Institute of Ophthalmology in London, UK, where I stayed between 1965 and 1966: Norman Ashton, an ARVO Proctor Awardee, who welcomed me into his Department on March 8, 1965, and David Maurice, an ARVO Friedenwald Awardee, 1 year later. These very different personalities helped me learn to enjoy working in eye research.

Finally, and probably more important of all, I was lucky enough to find the right girl, who has since supported me all the way.

In 1963, when initiating my stay in London, Norman Ashton challenged me to work in diabetic retinopathy (DR), a major cause of blindness, and to focus my research on the retina and retinal vascular disease, a challenge that I accepted and has now brought me here today.

At that time, the work of Majno et al., appeared in the literature, suggesting that the venous side of the capillary circulation was the most susceptible site for alterations of vascular permeability. Considering that this finding could explain the initial changes occurring in DR, we repeated their experiments in the retina and we found, to our great surprise, that the retinal vessels behaved differently from other vessels in other regions of the body or even from other vessels of the eye. These observations led us to the discovery of tight junctions between the endothelial cells of the retinal vessels, making the retinal endothelium function as an epithelial-like barrier (Fig. 1), an observation that was confirmed also to occur in the brain. A specific blood-retinal barrier then could be demonstrated, in the rabbit, to be mainly located in the endothelial layer of the retinal vessels (Fig. 2).

After establishing the morphologic basis of the blood–retinal barrier, the next challenge was to calculate its restricted permeability, and it was then that I started to work also with David Maurice. We were able to measure the permeability of the retinal vessels and to identify, for the first time, the...
presence of an active transport system not only on the retinal pigment epithelium, but also in the retinal vessels themselves.9

I recall proudly that David Maurice used to say that I was the only guy who made him work in the back of the eye. Our work laid the basis for the physiological understanding of the blood–retinal barrier.10

When I returned to Portugal, I had to make a long interval in my research work, because first I had a 2-year period of military service in Angola and, after that, in 1972, back in my University, Coimbra, I had to concentrate on developing a Department of Ophthalmology committed to translational research, bringing together laboratory and clinical research.

My scientific interest remained centered on the blood–retinal barrier and DR, and when I was finally able to assemble my first team in Coimbra, we developed and tested vitreous fluorophotometry as a clinical method to quantitate the permeability of the blood–retinal barrier in patients with diabetes.11

Our studies were published for the first time in 1975, in the British Journal of Ophthalmology, showing that an alteration of the blood-retinal barrier was an early alteration occurring in the retina in diabetic patients (Fig. 3A). This work raised much interest and was the reason for the invitation by Arnall Patz in 1977 and my arrival at ARVO. At the time, our work led to the formation of the International Society of Ocular Fluorometry. The work with vitreous fluorometry in diabetic patients showed that before the development of fundus changes, there was an alteration of the blood-retinal barrier affecting only approximately 30% of the patients. This was the first indication that DR had different characteristics in different patients (Fig. 3B).

Following my invitation to Chicago, I had the unique opportunity to work with a great team at the University of Illinois Eye and Ear Infirmary and later in the Lions of Illinois Eye Research Institute. During the next 8 years, I moved with my entire family to Chicago (twice, with an interval of 3 years in between) and had the opportunity of learning with my third mentor, Morton Goldberg, also a previous ARVO Weisenfeld Awardee, from whom I learned how translational research should be organized efficiently in an eye department. I then was able to continue my work on DR while being exposed to different and excellent laboratory researchers, clinicians, and clinician-scientists. My interest in instrumentation and biomedical engineering was aptly reinforced by the collaboration established with Zeimer et al.12

Finally, I decided to return to Portugal and Europe, maybe because I felt that I could be more useful there and contribute to the development of European ophthalmology. Since then, I have been very much involved in bringing together European ophthalmology and fighting for the creation of an European Vision Institute along the model of the USA National Eye Institute. I was able to initiate the EuroEye Network, which was followed more recently by the European Vision Institute Clinical Research Network, EVICR.net. Back in Europe, I continued my quest for improved understanding and management of DR. We were able to introduce multimodal imaging of the macula, a methodology developed around a modified confocal scanning laser ophthalmoscope, allowing localized mapping of the alteration of the blood-retinal barrier, of particular relevance to study DR due to its initial focal nature, something that was not possible with the initial methodology used to perform vitreous fluorophotometry (Fig. 4).13,14 The application of multimodal mapping of the macula to diabetic patients with mild nonproliferative retinopathy revealed different phenotypes of retinopathy progression, suggesting that different patients had different types of retinal pathology, some characterized by very slow progression with minimal vascular changes occurring over time, whereas another group of patients showed mainly an alteration of the blood-retinal barrier, and, finally, a third group, showed active retinal vascular disease demonstrated by high rates of microaneurysm (MA) formation and disappearance with development of progressive capillary closure.
The initial identification of three major patterns of progression of nonproliferative DR was made using elaborate multimodal imaging methodology involving new examination technologies, some still in the research domain. Furthermore, the sample size was small.

In a more recent study performed by our research group, we were able to follow a much larger population of eyes/patients with well-characterized, mild nonproliferative retinopathy in patients with type 2 diabetes, in an effort to verify if similar phenotypes of progression of mild nonproliferative DR could be identified using only noninvasive procedures that were easy to repeat, such as digital color fundus photography and optical coherence tomography (OCT).

This study was a 2-year prospective, observational study, designed to follow eyes/patients with mild nonproliferative DR (Early Treatment Diabetic Retinopathy Study [ETDRS] grades 20 and 35) for a period of 2 years or until the time of development of clinically significant macular edema (CSME, ETDRS classification). A total of 410 patients was included with adult-onset type 2 diabetes.

Color fundus photography was performed according to the ETDRS protocol. An automated computer-aided diagnostic system, RetmarkerDR (Critical Health SA, Coimbra, Portugal), was used to detect MA automatically on the field-2 color fundus images. This recently developed software, the RetmarkerDR, allows the identification of the exact location of each red dot in successive fundus photographs performed in each eye (Fig. 5). Identification of the exact location of an individual red dot is considered particularly important, because a new MA is considered to develop only once in a specific location, its disappearance being generally associated with capillary closure, leaving in its place mainly remnants of basement membrane.

The Ward method was used for hierarchical clustering. It confirmed the existence of three clusters. The three phenotypes result from the statistically significant differences for the MA and retinal thickness parameters ($P < 0.001$). The first cluster/phenotype was composed of 181 eyes/patients (48.1%), the second cluster/phenotype was composed of 87 eyes/patients (23.2%), and the third cluster/phenotype was composed of 108 eyes/patients (28.7%). Phenotype B is characterized by a higher central subfield retinal thickness without an increase in the MA parameters ($P < 0.001$). Phenotype C is characterized by higher MA parameters,
number, and turnover with variable values of central thickness ($P < 0.001$).

From the 348 eyes/patients who reached the study end point or who completed the 24-month visit, 26 developed CSME needing treatment, 3 (1.8%) from phenotype A, 7 (8.5%) from phenotype B, and 16 (16.2%) from phenotype C. Estimating the risk for CSME development, phenotype B showed a sensitivity and specificity of 88.9% and 60.5%, respectively (when compared to phenotype A), and phenotype C showed a sensitivity and specificity of 94.4% and 55.9%, respectively (when compared to phenotype A). Phenotype A showed a negative predictive value for developing CSME of 99.2%.

This study showed that, using the mathematical model of hierarchical cluster analysis and only noninvasive procedures (fundus photography and OCT), three different phenotypes of DR can be identified, which show different risks of progression to CSME. These three phenotypes are in agreement with the number of patterns of DR progression proposed previously by Lobo et al. in a different and smaller sample. In this original study, three phenotypes of progression of mild DR were characterized: phenotype A as slow progression, phenotype B as “leaky,” and phenotype C as ischemic. Similar categorization was found in this study as a result of hierarchical analysis, phenotype B with predominance of edema without an increase in MA turnover, and phenotype C with predominance of MA turnover (i.e., with increased rates of MA formation and disappearance) and variable degree of edema (Fig. 6).

Our findings showed that increased activity of microvascular disease in the macular region (field 2), demonstrated by increased rates of MA turnover (phenotype C), is associated with higher risk for development of CSME in the relatively short period of 2 years. This phenotype represents approximately 30% of the patients.

Of relevance also is the finding that, on the other hand, phenotype A, which is characterized by low MA turnover and also no signs of macular edema (representing approximately 50% of the patients), has the lowest risk for development of CSME, with a predictive negative value of 99.2%. This observation has clear implications for management of DR. Furthermore, this observation indicated that a large proportion of the eyes with mild DR will progress very slowly, suggesting that these eyes/patients should not be included in clinical trials of therapy for macular edema because of their slow rates of progression.

The results of our research on the characterization of different phenotypes of DR confirm that there are distinct morphologic manifestations of DR (detected on fundus photography and OCT) in different subjects indicating different rates of the progression and presenting different evolution patterns.

Looking at DR and the identification of the three major phenotypes of progression allows an integrated perspective of DR progression. Chronic hyperglycemia induces generalized cell damage to the retina involving the entire retinal neurovascular unit, but causing different degrees of damage in different cells in different individuals. There is increased apoptosis and the characteristic pericyte loss. These patients develop generalized low-grade damage, which manifests as a slow progressing neuropathy with minimal microvascular damage (phenotype A). Other patients develop an early breakdown of the blood-retinal barrier, which is well identified by increased retinal thickening in the OCT, creating the conditions for inflammation and increased disease progression (phenotype B). Finally, another group of patients (phenotype C) is characterized by active remodeling of the retinal microvasculature identified by increased MA turnover. The increased microvascular disease activity and remodeling of the retinal circulation may be due to increased microthrombotic activity. These features are central in the endothelial dysfunction in diabetes and may be due to specific genetic susceptibilities. The increase in hypoxia due to the vascular

![Figure 6](https://example.com/figure6.png)
In the univariate analysis, 15 single nucleotide polymorphisms (SNPs) from nine candidate genes showed statistically significant associations with the different phenotypes: ICAM1 rs5030400 and PPARGC1A rs16874120 are associated with phenotype A; ACE rs446142, AGER rs5131300, AKRIB1 rs1790998, AKRIB1 rs259458, AKRIB1 rs5053, NOS1 rs1552228, and ICAM1 rs1801714 with phenotype B; and AKRIB1 rs1790998, AKRIB1 rs3896278, NOS1 rs1552228, PPARGC1A rs10213440, MTHFR rs1801133, and VEGFA rs3024994 with phenotype C. Only these SNPs were included in the phenotypes Multivariate Logistic Regression analysis.

The results of the Multivariate Logistic Regression analysis show associations between ICAM1, PPARGC1A, and MTHFR, and the phenotypes of DR progression after adjusting for sex, age, diabetes duration, and HbA1C. The SNP ICAM1 rs1801714 was associated with phenotype B. Gene variants PPARGC1A rs10213440 and MTHFR rs1801133 were associated with phenotype C.

The multivariate analysis results also indicated an association between the gene ICAM1 and the development of CSME after adjusting for sex, age, diabetes duration, and HbA1C, suggesting that an abnormal inflammatory response may be the basis for the development of macular edema.

An individual approach to management of DR based on prognostic and predictive biomarkers can be envisioned in the near future. These will involve generalized cost-effective screening, identification of the eyes that show disease activity, and are at risk for progression to vision-threatening DR, and more timely treatment before development of irreversible vision loss.

**PERSONALIZED MANAGEMENT OF DR**

Uncomplicated nonproliferative DR progresses over time with very little vision loss. Vision loss occurs late in the disease process and in direct association with the development of two major complications, clinically significant macular edema and proliferative retinopathy.

The challenge is to treat and stop disease progression before these complications develop.

The course of retinopathy is not linear, and the progression of DR varies in different individuals. The time necessary for the development of sight-threatening complications is much less in some patients than in others. Activity of disease and its progression vary from patient to patient.

We have shown that it is possible to use MA turnover, computed from noninvasive color fundus photographs, as a biomarker to identify eyes/patients at risk of progression to clinically significant macular edema.

Duration of diabetes and level of metabolic control are major risk factors for development of DR. However, these risk factors do not explain the great variability that characterizes the evolution and rate of progression of the retinopathy in different diabetic individuals. Many diabetic patients, after many years with diabetes, never develop sight-threatening retinal changes, maintaining good visual acuity. Other patients, even after only a few years of diabetes, show a retinopathy that progresses rapidly and may not respond to available treatment.

Diabetes mellitus is a metabolic disorder with strong genetic and environmental influence. Presence or absence of genetic factors may have a fundamental role in determining specific pathways of vascular disease and, as a consequence, different progression patterns of diabetic retinal disease. It could be that certain polymorphisms would make the retinal microvasculature more susceptible to an early breakdown of the blood-retinal barrier (type B) or to microthrombosis,
capillary closure and ischemia (type C). The absence of these specific genetic polymorphisms would allow the slowly progressing pattern of type A to remain largely unchanged over the duration of their diabetes, corresponding to the basic retinal response to chronic hyperglycemia. Our observations, analyzed under the light of available literature, depict DR as a disease of the retinal neurovascular unit due to chronic hyperglycemia, in some patients resulting in microvascular damage conditioned in its progression and prognosis by a variety of different genetic polymorphisms, and modulated in its evolution by HbA1C levels, partly genetically determined and partly dependent on individual lifestyle and environment. The interplay of these multiple factors and the duration of this interplay would eventually characterize different clinical pictures or phenotypes of DR.

The next goal, therefore, should be the characterization of relationships between genetic factors (represented by distinct genotypes) and their clinical expression (distinct DR phenotypes). Another consequence of the characterization of different phenotypes of diabetic retinal disease is its role in the design of future clinical trials. Clinical trials should, indeed, consider only groups of patients characterized by their homogeneity and level of disease activity: patients presenting a specific retinopathy phenotype characterized by rapid progression (wet/leaky or ischemic), with similar duration of diabetes and at similar levels of metabolic control (HbA1C values).

It is accepted that in the initial stages of DR, when the fundus alterations detected by ophthalmoscopy or slit-lamp examination are limited to red-dot and hard or soft exudates (i.e., mild nonproliferative DR), an annual examination is indicated for every patient with 5 or more years of duration of their diabetes. This is the recommendation of the American Academy of Ophthalmology Guidelines for DR.29 Our observations and the identification of different DR phenotypes in the initial stages of DR (i.e., mild or moderate nonproliferative DR, characterized by different rates of progression of the retinopathy) suggest that specific approaches should be used when managing these different retinopathy phenotypes.30 A patient with mild or moderate nonproliferative DR, presenting retinopathy phenotype B (wet/leaky), characterized by breakdown of the blood-retinal barrier and an increased central retinal thickness, registered during a period of 2 years, corresponding to the period of follow-up of our study, indicating retinopathy progression, should be watched more closely. Blood pressure values and metabolic control should be monitored closely, and medication given to keep HbA1C levels and blood pressure at recommended values.31,32 Communication channels should be established rapidly between the patients’ ophthalmologist and diabetologist, internist, or general health care provider. Information should be given indicating that the chances of rapid retinopathy progression to more advanced stages of disease are relatively high, calling for immediate tighter control of both glycemia and blood pressure.

A patient with mild or moderate nonproliferative DR presenting retinopathy phenotype C, with ischemic characteristics identified by high red-dot formation rates, similarly would indicate the need for shorter observation intervals than 1 year, with particular attention for other systemic signs of macro- and microvascular disease. Here, however, control of hyperglycemia and blood pressure must be addressed with some degree of caution. Improved metabolic and blood pressure control must be progressive and less aggressive than with phenotype B. It is realized that the ischemia that characterizes phenotype C may become even more apparent in eyes submitted to rapid changes in metabolic control and rapid lowering of blood pressure may increase the retinal damage associated with ischemia.33-34 Finally, a patient with mild or moderate nonproliferative DR, presenting phenotype A, identified by normal retinal thickness, no signs of capillary closure, low red-dot formation rates, and with a diabetes duration of more than 10 years, all signs indicating a slowly progressive subtype of DR, may be followed at intervals longer than 1 year. If the examination performed after a 2-year interval confirms the initial phenotype characterization, the patient and his diabetologist, internist, or general health care provider should be informed of the good prognosis associated with this retinopathy phenotype.

We have come a long way since our initial work on DR with Norman Ashton. Different key pathways of hyperglycemic damage may have specific roles in the development of specific retinopathy phenotypes. A role for inflammation now is generally accepted,35 and inflammation mediators appear to have an adjuvant role accelerating the retinal disease process and facilitating edema, microthrombosis, and capillary closure.36 It is clear now that only a subset of patients with diabetes who develop some form of retinopathy is expected to lose functional vision during their lifetime. Identification of risk factors for progression to visual loss and precise calculations of risk for progression in individual patients over a given time period appear to be crucial steps in the decision process of whom to treat, when to initiate treatment, and how rigorously.

Identifying individual variations in disease progression by characterizing the DR phenotype that each patient falls in and other modulating risk factors, such as HbA1C levels, is opening new perspectives for the management of diabetic retinal disease. If the patients with the greatest risk of progression and with the greatest potential to benefit from treatment can be identified, then fewer patients will need to be treated to prevent an individual case of blindness. This is of extreme importance at a time when scarce resources must be concentrated on the individual cases that need close follow-up and timely treatment.

**Perspectives for Prospective Care and Personalized Health Planning in DR**

The great medical advances that have been made in the last 50 years must be used to create and validate new models of prospective healthcare that determine the risk for individuals to develop specific diseases, detect the diseases at their earliest onset, and prevent or intervene early enough to provide maximum benefits.37

We must address disease prevention and early personalized management of DR progression. It now is possible to consider for each individual a plan formulated to keep his eye health; that is, deal with the DR and minimize the potential problems associated with progression of the retinopathy to vision loss. Very early in the disease process, it is now possible to establish a regular program of MA assessment, based on noninvasive and simple-to-use fundus photography examinations. Assessment of retinal disease progression and phenotype identification may easily be refined by adding regular examinations with OCT; another noninvasive methodology.

Characterization of the phenotype of progression may be evaluated in the near future in the context of other risk factors, such as genetic, environmental, and lifestyle issues. Further genomic and epidemiologic research likely will contribute to more precise characterization of the different phenotypes of DR progression.
The challenge of developing a personalized healthcare plan for each diabetic patient must be addressed when the first MAs are identified by fundus photography screening. Only if early screening is done may this challenge be met with success. Prevention of DR progression to its sight-threatening complications, such as CSME, is possible and already has been demonstrated to be associated with important cost savings.

Diabetic retinopathy is a chronic disease resulting from diabetes itself, which also is a chronic disease. In DR, we have a window of opportunity, in the presymptomatic period, to delay or prevent the sight-threatening complications. We now have many of the risk assessment tools necessary to assign to our patients a planned personalized management program.

Healthcare systems must now take advantage of specific situations, such as the one occurring in DR, to implement prospective healthcare by testing novel ways of healthcare delivery.

Acknowledgments

The author thanks the mentors mentioned above, and colleagues and collaborators in the many publications that the author has been privileged to share with them.

Disclosure: J. Cunha-Vaz, None

References


