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CIBICI - CONICET - UNC

FCQ Facultad de Ciencias Químicas

ISN-CAEN

Progress report Category 1B. Research Supplies for use in the Applicant's home Laboratory Grant – October 2016

Project Title: Functional and biochemical characterization of a new animal model of Metabolic Syndrome that mimics microvascular and neurodegenerative characteristics of diabetic retinopathy.

Pablo F. Barcelona PhD.

Instituto de Investigación en Bioquímica Clínica e Inmunología
(CIBICI-CONICET-Universidad Nacional de Córdoba), Córdoba, Argentina

Córdoba (Argentina) – November 24th, 2017

Dear Dr. Caroline Rae,

I am glad to report you about my first-year ISN research supplies for use in my laboratory in Argentina where I am setting my own research line. This grant has been very meaningful and had an appreciable impact on this difficult early career stage.

After receiving my PhD at the National University of Cordoba in 2010, under the supervision of Prof. Dr. Maria Cecilia Sanchez, PhD, I did my first postdoctoral training studies at the same laboratory where my research was conducted to demonstrate the α_2M/LRP1 participation on Müller glial cell migration by regulating the matrix metalloproteinase (MMPs) activity (Barcelona et al., FASEB 2013). Then, I moved to Montreal to perform my second postdoctoral studies in the laboratory of Dr. H. Uri Saragovi (Lady Davis Institute, McGill University). Those three years were very productive, dedicated to validate the etiological role of $p75^{NTR}$ as well as therapeutic targets on vascular, proinflammatory, and neurodegenerative process in Diabetic Retinopathy models (Barcelona et al, JN 2016). Thanks to the unconditional support from Dr. H. Uri Saragovi I set up the stage to earn a Researcher position in Argentina. During this training period I gained expertise on structural biology, biochemistry, cell biology, neuroscience, as well as broadened my knowledge on retinal neurobiology.

During my first 6 months in Argentina I started my activities as a postdoctoral fellow, under the supervision of Prof. Dr. Maria Cecilia Sanchez, in her Laboratory. She was kind to allow me to establish my independent lines of research and at the same time providing insights, mentoring and lab facilities and reagents to that end. On July 2016, I was appointed as an Assistant Investigator, which is a full-time research position from the Argentinean National Research Council (CONICET). With this position I am setting my laboratory at the Centre for Research in Clinical Biochemistry and Immunology (CIBICI) which is a CONICET and National University of Cordoba University Institution, located in Cordoba, Argentina. I am also teaching cell biology for the undergraduated Clinical Biochemistry program, School of Chemical Sciences, National University of Cordoba (UNC).

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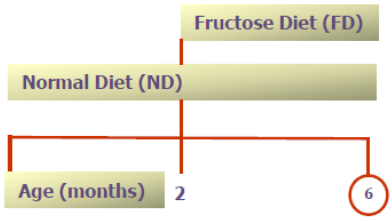
Current research

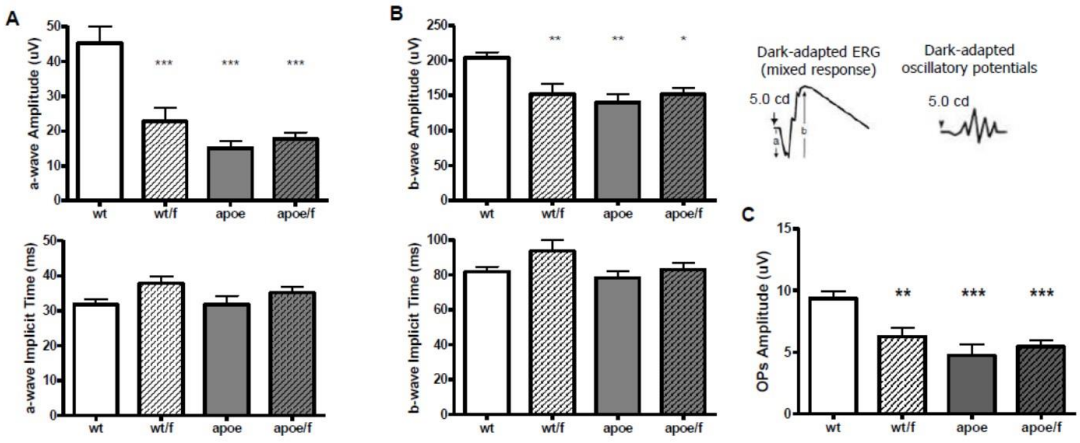
My research goals today merge the expertise I acquired in Montreal with the expertise of the laboratory of Dr. Maria Cecilia Sanchez on retinal vascular disorders. Hence, I am currently part of a group team integrated for Dr. Maria Constanza Paz who is a senior postdoc, she has a great animal handling and she has performed and validated the Electroretinogram (ERG) response. Currently we are writing the manuscript that describe a new model of syndrome metabolic generating in ApoE KO mice fed with fructose 10% on drinking water. In this model we have observed neurodegenerative retinal processes, evidenced by ERG analysis, with loss of neuronal function and vascular alteration manifested by high permeability on retinal vessel. These data are critical to understand why appears the neurogenerative event previous to vascular damage. All the experiments for this publication were running and finished in my current laboratory at CIBICI.

Scientific Progress

Diabetic retinopathy (DR) is the most serious ocular complication associated with Type 2 Diabetes Mellitus (T2DM), which is a metabolic syndrome (MS), and one of the leading causes of blindness. Thus, we proposed to analyze in a MS mouse model, markers of retinal vascular integrity and neuronal functionality, related to early stages of DR.



Animals: C57/BL6 (WT) and apolipoprotein E deficient (ApoE-KO) adult mice (25-30 g). Treatment: normal chow (ND) or a 10% w/v fructose (FD) in drinking water from 2 months of age. Animals were evaluated at 6 months old. Protein expression: western blot of glial fibrilar acidic protein (GFAP), glutamine synthetase (GS), α2macroglobulin (α2M) and albumin respect to tubulin (TUB) or actin, were analysed in retinal homogenates. Electroretinography (ERG): scotopic flash : 5 cd, 0.2 Hz. Vascular morphology: flat mount retinas staining with Alexa Fluor 488-conjugated Griffonia (Bandeiraea) simplicifolia lectin I isolectin B4 together with anti-GFAP were examined by confocal laser-scanning microscopy (Olympus FluoView FV1000 or 1200; Olympus Corp., New York, NY, USA). Vascular permeability: qualitative analysis of flat mount retinas of mice intravenous (i.v) injected with evans blue dye. Statistical Analysis: GraphPad Prism 5. One/Two way-ANOVA followed by Bonferroni or Student Newman-Keuls posthoc tests were used. A value of $p< 0.05$ was considered to be statistically significant (*).



Representative mixed ERG waveforms. Bars represent the average of the amplitude and implicit time of a (A) and b (B) waves, recorded for each experimental group (wt/f, ApoE and ApoE/f) and for the control group (wt). The amplitude of oscillatory potentials (Ops, C) is also represented as the average of the amplitudes summation of OPs2, OPs3, OPs4 in the follow experimental groups (wt/f, ApoE, ApoE/f) and control group (wt). (n= 6-8). One way-ANOVA followed by Bonferroni posthoc test, *p< 0.05, **p< 0.01 and ***p< 0.001 compared to control.

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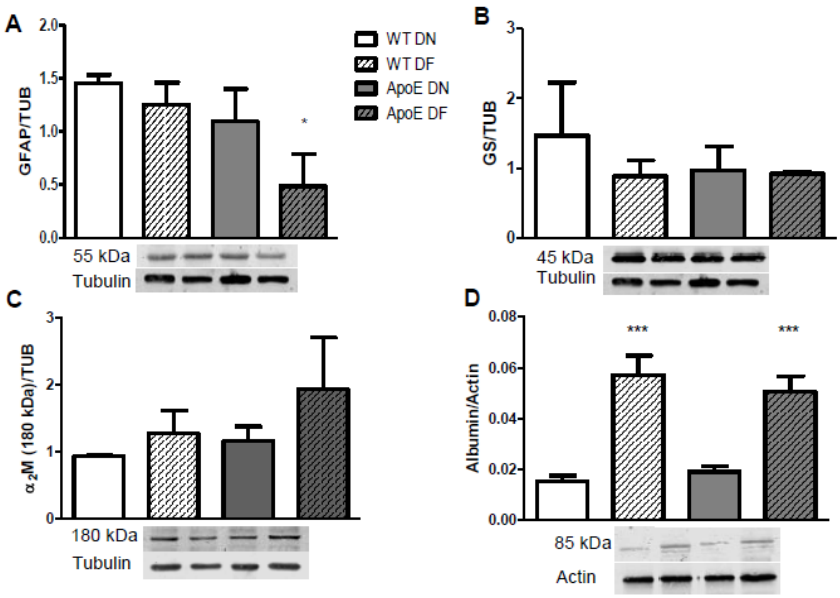
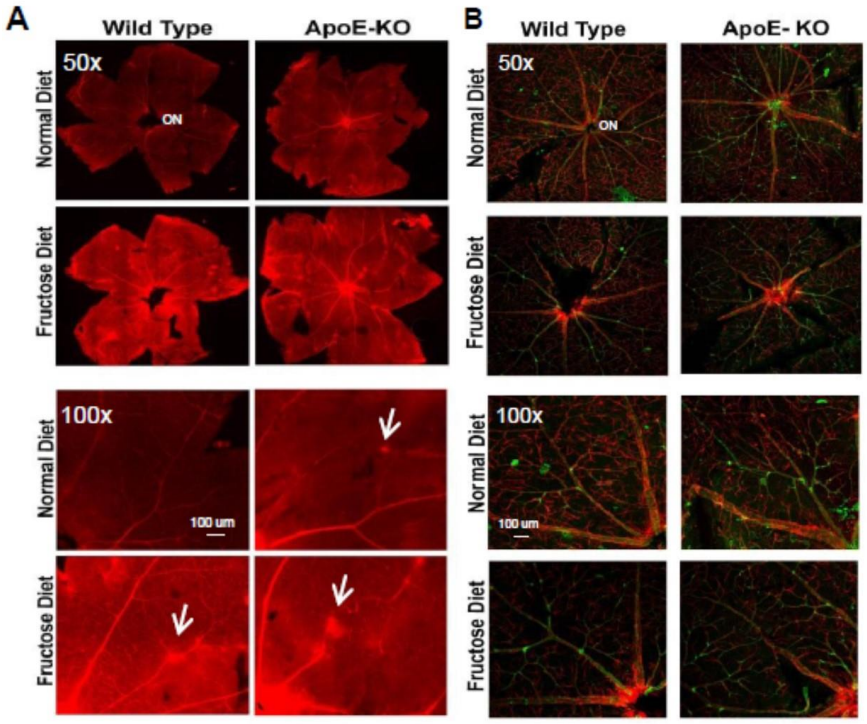
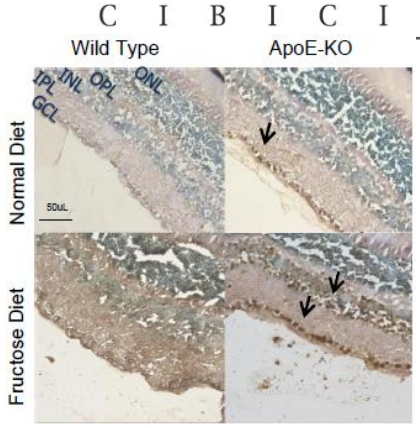


Bar graphics: relative expression of GFPA (A), GS (B), α2M (C) and Albumin (D) in retinal homogenates of mice. Tubulin or Actin is shown as a loading control. Bands were quantified by densitometric analysis, and their relations are represented in the bar graphs. Data are the mean of optic density ± SEM (n= 2-4). One way-ANOVA followed by Bonferroni posthoc test .*p< 0.05, **p< 0.01 and ***p< 0.001, compared to control. Representative blots are showed under each graph.



Permeability assay, 2 days after Blue Evans dye (red) i.v. injected mice whole-mounted retinas showing the leak points with white arrows (A). Analysis of the vascular morphology of the retina using GSA-Lectin (green) and anti-GFAP (red) staining, showing the astrocyte network (B) Representative photomicrographs of the central area (ON) of whole-mounted retina from all the experimental groups. Each image is an stitching result of 10 photos (10 µm) taken at plane z with a magnification of 50x or 100x. ON: optic nerve. Scale bar: 100 µm.



Photomicrographs of retina sections (10µm) of mice, processed by TUNEL/DAB technique with counter staining of methyl green. Cell death was observed in GCL cells (arrow) and in some cells in the INL. Scalebar: 50µm. GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer, ONL: outer nuclear layer.

In summary the ERG a- and b-waves amplitudes and the OPs amplitudes were significantly decreased in retinas of ApoE-KO after 4 month of FD vs WT DN, correlating with an increase in TUNEL positive cells. For another hand higher vascular permeability was observed in ApoE-KO FD, evidenced by the Evans blue leakage and the albumin and α_2M extravasation. However, the GS expression pointing out a normal function of Müller cells, a reduction in GFAP expression and immunoreactivity was observed in ApoE-KO FD whole-mounted retinas, which may be linked to a reduced ability to maintain BRB characteristics in ECs. The ApoE-KO mice after 4 months of FD, which represent features of human MS, presented vascular dysfunction and neurodegeneration. This model offers the opportunity to investigate DR at an early stage, whose prevalence has increased substantially worldwide.

Yours sincerely, Pablo F Barcelona

Financial report

Item	U$D
Laboratory Reagents	
Master Mix Syber Green for Real Time PCR	600
Antibodies for WB and IF	750
Cell culture reagents and media	250
Mice supplies	
Service and maintenance of Animals Facility Rack isolated for ApoE KO	800
Animal supplement (Fructose, regular food, ERGs supplies)	500
Surgical instruments (sterilization expensive, anesthesia, globes, mask, etc.)	750
Attending conferences	
Sociedad de Biología de Córdoba registration	75
Sociedad Argentina de Neurociencia registration and meeting inscription	125
Travel costs for national meetings (SAN Annual Meetings 2017)	150
TOTAL	4000

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Activities and publications during the funding period.

Work experience and positions

Jul 2016 - present Researcher, CONICET. At Instituto de Investigación en Bioquímica Clínica e Inmunología (CIBICI-CONICET-UNC)

Dic 2015 - Jun 2016 Postdoctoral fellow, Instituto de Investigación en Bioquímica Clínica e Inmunología (CIBICI-CONICET-UNC). Works in the laboratory of Dra Maria Cecilia Sanchez. Studies of retinal dysfunction in early stage of experimental animal's models of diabetic retinopathy.

Publications

"In a mouse model of retinitis pigmentosa neuronal death results from TrkC.T1–dependent ERK activation upregulating TNF-α production in retinal glia. Galan A*, Jmaeff S*, Barcelona PF, Sarunic MV and Saragovi HU. 2017 Cell Death and Disease in Press. *co-first authors.

"$p75^{NTR}$ antagonists attenuate photoreceptor cell loss in murine models of retinitis pigmentosa". Platón-Corchado M*, **Barcelona PF***, Marchena M, Hernández-Pinto AM, Jmaeff S, Hernández-Sánchez C, Saragovi HU, de la Rosa EJ. 2017 Cell Death and Disease Jul 13;8(7):e2922. *co-first authors.

"Mechanisms of neuroprotection of subconjuntival delivery of small molecule antagonist of $p75^{NTR}$ in diabetic retinopathy". Galan A, **Barcelona PF**, Nedev H, Sarunic MV, Saragovi HU. 2017 Journal IOVS ;58(7):2852-2862.

"TrkC receptor isoforms transduce NT-3-dependent signals that are either trophic or toxic for motor neurons, and selective targeting is therapeutic in an ALS model". Fouad Brahimi F*, Maira MH *, **Barcelona PF**, Aboulkassim T, Teske K, Rogers ML, Bertram L, Wang J, Yousefi M, Rush R, Fabian M, Cashman N, and Saragovi HU. 2016 PLoS One. 3;11(10):e0162307. *co-first authors.

"$p75^{NTR}$ and its ligand proNGF activate paracrine mechanisms etiological to the vascular, inflammatory, and neurodegenerative pathologies of diabetic retinopathy". **Barcelona PF***, Sitaras N*, Galan A, Esquiva G, Jmaeff S, Jian Y, Sarunic MV, Cuenca N, Sapieha P, Saragovi HU. 2016 J Neurosci. 24;36(34):8826-41. *co-first authors

Manuscripts prepared to be submitted

"Alterations in vascular integrity and neuronal functionality related to early stages of diabetic retinopathy in a metabolic syndrome mouse model". Paz MC*, **Barcelona PF***, Subirada PV, Ridano ME, Chiabrando GA, Castro C, Sánchez MC. *co-first authors

Research Grants

As Responsible Investigator

2016 Research grant. ANPCyT, PICT-Class B. MinCyT-Argentina. 2018 per 2 year grant to conduct research in the field of Autophagy in No proliferative Diabetic Retinopathy.

As collaborator

2014 Research grant. ANPCyT, PICT-Class A. MinCyT-Argentina. 2016 per 3 year grant to conduct research in the field of Proliferative Retinopathies: Low- density lipoprotein-relatein protein-1 LRP1 and its ligand α_2M as neuroprotective strategy.

2015 SECYT de la UNC (366/16) New model of diabetic retinopathy: Functional structural, biochemist and molecular studies.

Abstracts in Scientific Meetings

Selected oral presentations during the first year of funding

"A novel therapeutic target for neurodegeneration and vascular damage in retinopathies". **Barcelona PF**. SAN 2017 (XXXII Annual meeting of Argentine Society of Neuroscience). Young Investigator Symposium. Sep 25-27, 2017, Mar del Plata, Buenos Aires, Argentina.

"Neovascularización y neurodegeneración retinal: modelos animales". **Barcelona PF**. SAO / CAO 2017 (Annual meeting of Ophthalmology Society). AIVO Symposium: "Research of Vision Sciences". May 17, 2017, Buenos Aires, Argentina.

"P75^{NTR}–dependent neurodegeneration and vascular defect in diabetic retinopathy". **Barcelona PF**. AIVO 2015 (XII National Annual Meeting of Research in Vision and Ophthalmology). Dic 11, 2015. Córdoba, Argentina.

Poster presented in the first year of funding

"Isquemic neovascular retinopathies: Autophagy flux deregulation in glial Müller cells under oxygen deprivation conditions". Subirada PV, Paz MC, Ridano ME, **Barcelona PF**, Chiabrando GA, Sanchez MC. ARVO's 2017 (Annual Meeting of The Association for Research in Vision and Ophthalmology). May 7-11, 2017. Baltimore, Maryland, US.

"Retinal dysfunction in early stages of metabolic syndrome established on a new experimental mouse model. Paz MC, **Barcelona PF**, Subirada PV, Ridano ME, Sánchez MC. ARVO's 2017 (Annual Meeting of the Association for Research in Vision and Ophthalmology). May 7-11, 2017. Baltimore, Maryland, US.

"Elevated α_2M in the aqueous humor is associated with glaucoma but not with PXF alone". Eiger-Moscovich M, **Barcelona PF**, Schaap-Fogler M, Saragovi UH, Kramer M. ARVO's 2016 (Annual Meeting of the Association for Research in Vision and Ophthalmology). May 3-7, 2016. Seattle, Washington, US.

"Insulin induces the exocytic traffic of LRP1 from GSV–like structural vesicles". Actis Dato V, Jaldín-Fincati JR, **Barcelona PF**, Sánchez MC and Chiabrando GA. SAIB 2016 (XLVII Annual Meeting of Argentina Society of Biochemistry and Molecular Biology). Nov 7-10, 2016. Córdoba, Argentina.

"Impaired autophagy flux in Müller glial cells exposed to hypoxia: *In vitro* and *In vivo* models". Subirada Caldarone PV; Ridano ME; Paz MC; **Barcelona PF**; Fader Kaiser C; Sanchez MC. SAIB 2016 (XLVII Annual Meeting of Argentina Society of Biochemistry and Molecular Biology). Nov 7-10, 2016. Córdoba, Argentina.

"Expresión y localización de galectina 1 durante el desarrollo de retinopatía inducida por oxígeno (OIR)". Ridano ME, Subirada PV, Paz MC, Lorenc VE, Luna JD, Croci DO, Barcelona PF, Rabinovich GA and Sánchez MC. AIVO 2016 (XI National Annual Meeting of Research in Vision and Ophthalmology, III Joint Meeting AIVO-BRAVO). Oct 20-21, 2016. Capital Federal, Buenos Aires, Argentina.

The New administratioon route of a $p75^{NTR}$ antagonist small molecule on a diabetic retinopathy animals models. **Barcelona PF**, Galan A, Nedev H, Saragovi HU. AIVO 2016 (XI National Annual Meeting of Research in Vision and Ophthalmology, III Joint Meeting AIVO-BRAVO). Oct 20-21. Capital Federal, Buenos Aires, Argentina.

"Autophagy in a mouse modelo f retinopathy induce by oxygen: Same effect for each cell type?". Subirada PV, **Barcelona PF**, Paz MC, Ridano ME, Bonacci GR, Chiabrando GA and Sánchez, MC. AIVO 2016 (XI National Annual Meeting of Research in Vision and Ophthalmology, III Joint Meeting AIVO-BRAVO). Oct 20-21. Buenos Aires, Argentina.

"Is the autophagy process require to neovascularization progress at retinal level". Subirada PV, **Barcelona PF**, Paz MC, Ridano ME, Bonacci GR, Chiabrando GA. and Sánchez MC. First Scientific and professional Meeting of Biochemistry 2016. Oct 5-8, 2016. Córdoba, Argentina.

"The Apolipoprotein E deficit and a fructose diet induce No proliferative diabetic retinopathy in mice". Paz MC, Subirada PV, **Barcelona PF**, Ridano ME, Castro C, Sánchez MC; First Scientific and professional Meeting of Biochemistry 2016. Oct 5-8, 2016. Córdoba, Argentina.

Post-Graduate Courses

2016 "Cellular and molecular neurobiology: Structure-function and neuronal pathology" Córdoba, Argentina, 2016. Pre meeting course SAIB 2016.

Fellowships and Awards

Dic 2015- Aug 2016 Posdoctoral Fellowship from CONICET, Argentina. Works in the laboratory of Dra Maria Cecilia Sanchez. CIBICI–CONICET–UNC.

Teaching

Aug 2015- 2017 Assistant Professor in Clinical Biochemistry, School of Chemical Sciences, National University of Cordoba (UNC).

Sep 2017-Present Associate Professor in in Clinical Biochemistry, School of Chemical Sciences, National University of Cordoba (UNC).

Acknowledgement to the ISN-CAEN

I am very grateful for the support from the ISN-CAEN. The ISN-CAEN research supplies for use in the applicant's home laboratory grant that will allow me to finish a paper on collaboration with Dra. Sanchez MC, helped me to set my research line on the laboratory and office, and to generate results which were/are utilized to apply for national and international funding. The ISN-CAEN will be acknowledge in future publications and meeting presentations.

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