NAME: Giuseppe, Fiermonte

POSITION: Full Professor of Biochemistry

EDUCATION

INSTITUTION AND LOCATION	DEGREE	Date	FIELD OF STUDY
Università degli Studi di Bari-Aldo Moro, Bari, Italy	Pharm.D	04/89	Pharmacy

A. Personal Statement

I have a broad background in the biochemical characterization of the MCF members, in 1993 in John Walker's lab (Cambridge, UK) I developed, an experimental approach[1] used to successfully characterized most of the MCF members known up to date. Since then, my research group in Bari is the worldwide renown group with respect to the mitochondrial substrate carriers with more than 25 of these proteins characterized by reconstitution experiments coupled to cellular studies[2,3]. More recently, I have definitively demonstrated that UCP2 is not a canonical uncoupler but it is a metabolites transporter explaining the key role of UCP2 in the mitochondrial bioenergetic control, glutamine oxidation, insulin secretion and Warburg effect[4]. I successfully administered the projects (e.g. staffing, research protections, budget), collaborated with other researchers, and produced several peer-reviewed publications from each project. In summary, I have a demonstrated record of accomplished and productive research projects in an area of functional characterization of mitochondrial carriers, and my expertise and experience have prepared me to lead the RU of the proposed project.

1. **Fiermonte, G.,** *et al* Abundant bacterial expression and reconstitution of an intrinsic membrane-transport protein from bovine mitochondria. ***Biochem. J.*** **294 (Pt 1),** 293–299 (1993).
2. **Fiermonte G,** *et al.* The sequence, bacterial expression, and functional reconstitution of the rat mitochondrial dicarboxylate transporter cloned via distant homologs in yeast and Caenorhabditis elegans. ***J. Biol. Chem.*** **273,** 24754–24759 (1998).
3. **Fiermonte, G.,** *et al.* A novel member of solute carrier family 25 (SLC25A42) is a transporter of coenzyme A and adenosine 3',5'-diphosphate in human mitochondria. ***J. Biol. Chem.*** **284,** 18152–18159 (2009).
4. Vozza A, Parisi G, De Leonardis F, Lasorsa FM, Castegna A, Amorese D, Marmo R, Calcagnile VM, Palmieri L, Ricquier D, Paradies E, Scarcia P, Palmieri F,Bouillaud F, **Fiermonte G.** UCP2 transports C4 metabolites out of mitochondria, regulating glucose and glutamine oxidation. ***Proc. Natl. Acad. Sci. U.S.A.*** **111,** 960–965 (2014). (**corresponding author**)

B. Positions and Honors

<u>Positions and Employment</u>

1990-1991 Fellow "Biotechnologies and Bioinstrumentations Finalized Project" CNR, Italy
1991-2001 Laureate technician "Department of Pharmaco-Biology, University of Bari, Italy
2001-2005 Researcher, "Department of Pharmaco-Biology, University of Bari, Italy
2005-2016 Associate Professor in Biochemistry, Department of Biosciences, Biotechnologies and Biopharmaceutics, University of Bari, Italy
10-1-2016 Full Professor in Biochemistry, Department of Biosciences, Biotechnologies and Biopharmaceutics, University of Bari, Italy

Other Experience and Professional Memberships

Visiting scientist at MRC, Laboratory of Molecular Biology, Cambridge, U.K. under the supervision of the Nobel Prize, Sir. John E. Walker in the following periods: 1/4/1991-26/7/1991; 3/11/1991-30/4/1992; 18/4/1994-17/5/1994; 2/2/1997-2/3/1997

1990- Member, Italian Society of Biochemistry and Molecular Biology

1998- ad hoc reviewer

2013- Ad hoc reviewer for the Italian *"Ministero dell'Istruzione, dell'Università e della Ricerca"*

C. Contribution to Science

1. Up to the beginning of the nineties, the primary structure the mitochondrial carrier family (MCF) members was determined by direct amino acid analysis or by DNA sequencing, upon their purification. Since the purification of the ADP/ATP carrier (AAC) in 1974 and the huge effort of many research groups, in more than 20 years only 6 members were identified, this was mainly due to the very low expression of these proteins. The first six sequenced members exhibited a tripartite structure consisting of three tandemly repeated domains of about 100 amino acids in length. Each domain contains two hydrophobic stretches separated by hydrophilic regions and a signature sequence motif, suggesting they belonged to a family of proteins. Then, I decided to use these features to find other members of unknown function in all available eukaryotic databases, the gene products were expressed in Escherichia coli, purified and incorporated into liposomes, and the recombinant proteins then functionally characterized by transport assays into liposomes. In 1993, for the first time, in J.E. Walker's lab, I successfully developed this new approach on two already known mitochondrial carriers the AAC and the oxoglutarate carrier[1], since then it has been used in my lab to identify more than 25 new members of the MCF[5-8], and up-to-date it is of the most used experimental approach for the functional characterization of membrane transporters.

5. **Fiermonte G,** *et al.* Identification of the human mitochondrial oxodicarboxylate carrier. Bacterial expression, reconstitution, functional characterization, tissue distribution, and chromosomal location. ***J. Biol. Chem.*** **276,** 8225–8230 (2001).
6. **Fiermonte, G**., *et al.* Identification of the mitochondrial ATP-Mg/Pi transporter. Bacterial expression, reconstitution, functional characterization, and tissue distribution. ***J. Biol. Chem.*** **279,** 30722–30730 (2004).
7. **Fiermonte, G**., *et al.* Identification of the mitochondrial glutamate transporter. Bacterial expression, reconstitution, functional characterization, and tissue distribution of two human isoforms. ***J. Biol. Chem.*** **277,** 19289–19294 (2002).
8. **Fiermonte, G.** *et al.* The mitochondrial ornithine transporter. Bacterial expression, reconstitution, functional characterization, and tissue distribution of two human isoforms. ***J. Biol. Chem.*** **278,** 32778–32783 (2003).

2. In the 2001, by following the experimental approach reported above, with a team of collaborators, I identified the mitochondrial transporter of thiamine pyrophosphate and deoxynucleotides (TPC/DNC)[9,10]. One year later, in collaboration with a research group in US, I demonstrated that the missense point mutation found in patients affected by Amish microcephaly, which produced a G177A amino acid change, drastically reduced the transport activity of the recombinant TPC/DNC[11]. In further studies carried out on a murine model and into liposomes we confirmed that the clinical phenotype shown by Amish microcephaly patients was mainly due to a reduced transport of thiamine pyrophosphate into the mitochondria which lowered the pyruvate and 2-oxoglutarate dehydrogenase activities[12] and produced a severe defect in the mitochondrial energy production.

9. Dolce, V., **Fiermonte,** G., *et al.* The human mitochondrial deoxynucleotide carrier and its role in the toxicity of nucleoside antivirals. ***Proc. Natl. Acad. Sci. U.S.A.*** **98,** 2284–2288 (2001). (**Co-first authorship**)
10. Walker, J., Runswick, M., Palmieri, F., **Fiermonte, G.** & Dolce, V. Transport protein for use in the treatment of mitochondrial, tumor, muscle and viral diseases, ***Patent N. US 20030036111A1***. (2001).
11. Rosenberg MJ1, Agarwala R, Bouffard G, Davis J, **Fiermonte G,** *et al.* Mutant

deoxynucleotide carrier is associated with congenital microcephaly. ***Nat. Genet.* 32,** 175–179 (2002).
12. Lindhurst MJ, **Fiermonte G,** *et al.* Knockout of Slc25a19 causes mitochondrial thiamine pyrophosphate depletion, embryonic lethality, CNS malformations, and anemia. ***Proc. Natl. Acad. Sci. U.S.A.* 103,** 15927–15932 (2006). **(Co-first authorship)**

3. My experience and unique technical skills in the field of the functional reconstitution of recombinant transporters into liposomes support many clinicians and geneticists to shed light on metabolic phenotypes of many human diseases due to the deficiency of a specific mitochondrial carrier. Over the years I developed several national and international collaboration which helped to draw detailed genotype-phenotype correlations in human diseases such as the type II citrullinemia due to the deficiency of the mitochondrial aspartate/glutamate carrier[13,14]; the neonatal myoclonic epilepsy due to the deficiency of the isoform 1 of the mitochondrial glutamate carrier[15]; the HHH syndrome due to the deficiency of the mitochondrial ornithine/citrulline carrier[16].

13. **Fiermonte G,** *et al*. An adult with type 2 citrullinemia presenting in Europe. ***N. Engl. J. Med.*** 358, 1408–1409 (2008).
14. **Fiermonte G,** *et al.* A new Caucasian case of neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD): a clinical, molecular, and functional study. ***Mol Genet Metab* 104,** 501–506 (2011). (corresponding author)
15. Molinari F, Raas-Rothschild A, Rio M, **Fiermonte G**, *et al.* Impaired mitochondrial glutamate transport in autosomal recessive neonatal myoclonic epilepsy. ***Am. J. Hum. Genet.* 76,** 334–339 (2005).
16. Tessa A, **Fiermonte G,** *et al.*. Identification of novel mutations in the SLC25A15 gene in hyperornithinemia-hyperammonemia homocitrullinuria (HHH) syndrome: a clinical, molecular, and functional study**. *Hum. Mutat.* 30,** 741–748 (2009). (Co-first authorship)

Complete List of Published Work in MyBibliography:

https://www.ncbi.nlm.nih.gov/myncbi/browse/collection/48261304/?sort=date&direction=ascending

Research Support

<u>Ongoing Research Support</u>

Fiermonte00459814Airc (AIRC, project 15404) Fiermonte (PI)
01/016/2015 - 31/12/2018
Role of mitochondrial uncoupling protein-2 in pancreatic ductal adenocarcinoma tumorigenesis and maintenance. Role: PI

Fiermonte00459809Prin (University of Bari resources) Fiermonte (PI)
01/10/2013 -
Biochemical and functional characterization of UCP2, UCP3, UCP4, BMCP1 and KMCP1 human mitochondrial uncoupling protein 1 (UCP1) homolog. PI

20158EB2CM_002 PRIN Italian Ministry of Research Urbani (PI)
2017-2020
Project title; Hydrogen sulphide as a new player of Amyotrophic Lateral Sclerosis: focus on mitochondrial homeostasis. RU_PI

2017PAB8EM_002PRIN Italian Ministry of Research Indiveri (PI)
2018-2023
Project title; Membrane transporters, the doors of cellular metabolism. Investigation of biochemical features structure/function relationships, metabolic regulation and physio-pathological aspects

by in vitro and in vivo experimental models.. RU_PI