BIOGRAPHICAL SKETCH

NAME: Piccolo, Pasquale

POSITION TITLE: Assistant Investigator, Telethon Institute of Genetics and Medicine (TIGEM), Italy

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	Completion Date	FIELD OF STUDY
University of Naples Federico II	M.Sc.	2005	Medical Biotechnology
University of Naples Federico II	Ph.D.	2008	Human genetics
Baylor College of Medicine	Post-doctoral	07/09	Adenoviral gene therapy
Telethon Institute of Genetics and Medicine	Post-doctoral	07/16	Liver gene therapy

A. Personal Statement

My career has been devoted to elucidating disease mechanisms and developing of innovative therapeutic approaches. During my training, I gained extensive experience in the fields of liver inherited metabolic diseases and gene therapy. My primary research focus has been liver-directed gene therapy using helper-dependent adenoviral (HDAd) vectors and he worked extensively on vector-host interactions and strategies to improve the efficacy and safety of the vectors for human applications. Later in my training, my work has been focused on understanding the pathogenesis of liver disease due to α1-antitrypsin deficiency. My effort in this disease has also been successful and I published important findings about the disease in the high-impact journals. I recently started my independent research activity that is focused on the development of novel molecular therapies for Wilson disease and Progressive Familial Intrahepatic Cholestasis. I have been able to secure funding from European and US funding agencies and my research activity has also been recognized on both sides of the ocean by prestigious awards.

Ongoing projects that I would like to highlight include:

Alpha-1 Foundation – Funding period: 2022 - 2024
Title: 'Mitochondrial dysfunction in α1-antitrypsin deficiency-associated liver disease'
Role: PI

European Association for the Study of the Liver - Funding period: 2022 – 2024
Title: 'Promoterless liver genome editing for Progressive Familiar Intrahepatic Cholestasis type 3'
Role: PI

European Innovation Council - Funding period: 2022 – 2026
Title: 'AAVolution-Next-generation AAV vectors for liver-directed gene therapy'
Role: co-PI

European Joint Program-Rare Disease – Funding period: 2021 – 2024
Title: 'WilsonMed-Multi-molecular targeting of copper overload in Wilson disease'
Role: co-PI

PSC Partners Seeking a Cure - Funding period: 2020 – 2022
Title: 'Micro-RNA based therapy for Primary Sclerosing Cholangitis'
Role: PI

Citations:

a. **Piccolo P***, Ferriero R, Barbato A, Attanasio S, Monti M, Perna C, Borel F, Annunziata P, Carissimo A, De Cegli R, Quagliata L, Terracciano LM, Housset C, Teckman JH, Mueller C and Brunetti-Pierri N*. Up-regulation of miR-34b/c by JNK and FOXO3 protects from liver fibrosis**.** *Proc Natl Acad Sci USA 2021, March 9; 118(10) e2025242118* *co-corresponding authors

b. **Piccolo P**, Ferriero R, Brunetti-Pierri N. Use of microRNAs in the treatment of fibrosis. *Application number: PCT/EP2022/055005. Filing date: 28/02/2022*

c. Auricchio A, Lyubenova H, **Piccolo P**, Monti M., Padula A., Esposito F. Constructs comprising inteins. *Application number: PCT/EP2021/059841 Filing date: 15/04/2021*

d. **Piccolo P**, Annunziata P, Soria LR, Attanasio S, Barbato A, Castello R, Carissimo A, Quagliata L, Terracciano LM and Brunetti-Pierri N. Downregulation of HNF-4α and defective zonation in livers expressing Z α1-antitrypsin. *Hepatology, 2017 Jul;66(1):124-135*

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2002 – 2005	Intern, Department of Pediatrics, University of Naples Federico II, Italy
2005 – 2008	Graduate student, Department of Pediatrics, University of Naples Federico II, Italy
2008 – 2009	Post-doctoral fellow, Department of Molecular and Human Genetics, Baylor College of Medicine, USA
2009 – 2016	Post-doctoral fellow, Telethon Institute of Genetics and Medicine, Italy
2016 – 2018	Staff scientist, Telethon Institute of Genetics and Medicine, Italy
2018 – 2020	Junior Principal Investigator, STAR program, Department of Translational Medicine University of Naples Federico II, Italy
2019 – to date	Assistant Investigator, Telethon Institute of Genetics and Medicine, Italy

Honors

2013	Travel award (American Society of Gene and Cell Therapy - ASGCT)
2016	Alpha-1 Antitrypsin Laurell's Training Award (Grifols, S.A.)
2017	Gordon L. Snider Scholar Award (Alpha-1 Foundation)
2018	Young Investigator Bursary (European Association for the Study of the Liver - EASL)
2022	Daniel Alagille Award (European Association for the Study of the Liver - EASL)

C. Contributions to Science

During the last years my research has been focused on liver disease related to α1-antitrypsin deficiency. First, I was instrumental in the identification of lysosomal master gene Transcription Factor EB (TFEB) as a new therapeutic target for the disease. Next, I studied the role of transcription factors regulating α1-antitrypsin expression in disease pathogenesis. I identified downregulation of Hepatic Nuclear Factor-4α (HNF-4α) and impairment of liver zonation as new features of liver disease associated to AAT deficiency, that are likely involved in metabolic disturbances and in the increased risks of hepatocellular carcinoma in patients with AAT deficiency. I also identified CHOP and c-JUN to upregulate α1-antitrypsin transcription and play an important role in hepatic disease by increasing the burden of proteotoxic, particularly in the pediatric population. More recently, using multiple approaches I found the involvement of the JNK1/2-FOXO3-miR-34b/c axis in liver disease associated to AAT deficiency and in other liver diseases with fibrosis. This study paved the way to the development of a miR-34b/c-based antifibrotic drug.

e. Pastore N, Blomenkamp K, Annunziata F, **Piccolo P**, Mithbaokar P, Maria Sepe R, Vetrini F, Palmer D, Ng P, Polishchuk E, Iacobacci S, Polishchuk R, Teckman J, Ballabio A, Brunetti-Pierri N. Gene transfer of master autophagy regulator TFEB results in clearance of toxic protein and correction of hepatic disease in α1-antitrypsin deficiency. *EMBO Mol Med. 2013 Mar;5(3):397-412.*

f. **Piccolo P**, Annunziata P, Soria LR, Attanasio S, Barbato A, Castello R, Carissimo A, Quagliata L, Terracciano LM and Brunetti-Pierri N. Downregulation of HNF-4α and defective zonation in livers expressing Z α1-antitrypsin. *Hepatology, , 2017 Jul;66(1):124-135*

g. Attanasio S, Ferriero R, Gernoux G, De Cegli R, Carissimo A, Nusco E, Campione S, Teckman J, Mueller C, **Piccolo P***, Brunetti-Pierri N*. CHOP and c-JUN up-regulate the mutant Z α1-antitrypsin, exacerbating its aggregation and liver proteotoxicity. *J Biol Chem. 2020 Sep 18;295(38):13213-13223* *co-corresponding authors

h. **Piccolo P***, Ferriero R, Barbato A, Attanasio S, Monti M, Perna C, Borel F, Annunziata P, Carissimo A, De Cegli R, Quagliata L, Terracciano LM, Housset C, Teckman JH, Mueller C and Brunetti-Pierri N*. Up-regulation of miR-34b/c by JNK and FOXO3 protects from liver fibrosis**.** *Proc Natl Acad Sci USA 2021, March 9; 118(10) e2025242118* *co-corresponding authors

I have also explored the potential of small molecules for treatment of inherited diseases. In particular, I found losartan, an inhibitor of angiotensin-II type 1 receptor, as a potential treatment for the extracellular matrix deposition defect in rare connective tissue disorders like Myhre syndrome and Geleophysic dysplasia. Based on the results of these studies a clinical trial is currently ongoing to investigate the efficacy of losartan in human patients with Myhre syndrome.

a. **Piccolo P**, Mithbaokar P, Sabatino V, Tolmie J, Melis D, Schiaffino MC, Filocamo M, Andria G, Brunetti-Pierri N. SMAD4 mutations causing Myhre syndrome result in disorganization of extracellular matrix improved by losartan. *Eur J Hum Genet. 2014 Aug;22(8):988-94*

b. **Piccolo P**, Sabatino V, Mithbaokar P, Polishchuk E, Hicks J, Polishchuk R, Bacino CA, Brunetti-Pierri N. Skin fibroblasts of patients with geleophysic dysplasia due to FBN1 mutations have lysosomal inclusions and losartan improves their microfibril deposition defect. *Mol Genet Genomic Med. 2019 Sep;7(9):e844*

c. **Piccolo P**, Sabatino V, Mithbaokar P, Polishchuck E, Law SK, Magraner-Pardo L, Pons T, Polishchuck R, Brunetti-Pierri N. Geleophysic dysplasia: novel missense variants and insights into ADAMTSL2 intracellular trafficking. *Mol Genet Metab Rep. 2019 Sep 5;21:100504.*

At the early stages of my career, my interest was focused on gene therapy with helper-dependent adenoviral vectors (HDAd). Using the HDAd, I first showed the efficacy of these vectors in transducing ependymal cells and neurons (both mature and precursors) for long-term expression. These results hold potential for HDAd application in the gene therapy for several metabolic diseases with brain involvement. As a post-doctoral fellow, my work has continued to be focused on this vector platform: specifically, I investigated HDAd-host interactions and I identified two new receptors for HDAd, Scavenger Receptor-A (SR-A) and Scavenger Receptor expressed on Endothelial Cells-I (SREC-I) that resides on liver macrophages and endothelial cells. Both these cells are considered important players in viral particle uptake by the liver and in the innate immune response triggered by the vector. Next, I exploited this new knowledge and I developed two small peptides which improve the therapeutic index of the HDAd vectors by blocking vector uptake by Kupffer cells and liver sinusoidal endothelial to enhance hepatocyte gene transfer, an application that also have the potential for improving gene therapy for metastatic cancer.

a. Dindot S*, **Piccolo P***, Grove N, Palmer D, Brunetti-Pierri N. Intrathecal injection of helper-dependent adenoviral vectors results in long-term transgene expression in neuroependymal cells and neurons. *Hum Gene Ther. 2011 Jun;22(6):745-51.* *Equal contribution

b. **Piccolo P**, Annunziata P, Mithabokar P, Brunetti-Pierri N. SR-A and SREC-I blocking peptides increase HDAd-mediated liver transduction. Gene Ther. 2014 Nov; 21(11):950-7.

c. **Piccolo P**, Vetrini F, Mithbaokar P, Grove NC, Bertin T, Palmer D, Ng P, Brunetti-Pierri N. SR-A and SREC-I are Kupffer and endothelial cell receptors for helper-dependent adenoviral vectors. *Mol Ther. 2013 Apr;21(4):767-74.*

Given my expertise in HDAd vectors, I had indeed the opportunity to collaborate to several studies on liver directed gene therapy for multiple disease targets, such as haemophilia B, Crigler-Najjar syndrome, Wilson disease, and primary hyperoxaluria type I.

a. Brunetti-Pierri N, Liou A, Patel P, Palmer D, Grove N, Finegold M, **Piccolo P**, Donnachie E, Rice K, Beaudet A, Mullins C, Ng P. Balloon catheter delivery of helper-dependent adenoviral vector results in sustained, therapeutic hFIX expression in rhesus macaques. *Mol Ther. 2012 Oct;20(10):1863-70.*

b. Pastore N, Nusco E, **Piccolo P**, Castaldo S, Vaníkova J, Vetrini F, Palmer DJ, Vitek L, Ng P, Brunetti-Pierri N. Improved efficacy and reduced toxicity by ultrasound-guided intrahepatic injections of helper-dependent adenoviral vector in Gunn rats. *Hum Gene Ther Methods. 2013 Oct;24(5):321-7*.

c. Polishchuk EV, Concilli M, Iacobacci S, Chesi G, Pastore N, **Piccolo P**, Paladino S, Baldantoni D, van IJzendoorn SC, Chan J, Chang CJ, Amoresano A, Pane F, Pucci P, Tarallo A, Parenti G, Brunetti-Pierri N, Settembre C, Ballabio A, Polishchuk RS. Wilson Disease Protein ATP7B Utilizes Lysosomal Exocytosis to Maintain Copper Homeostasis. *Dev Cell. 2014 Jun 23;29(6):686-700.*

d. Castello R, Borzone R, D'Aria S, Annunziata P, **Piccolo P**, Brunetti-Pierri N. Helper dependent adenoviral vectors for liver-directed gene therapy of primary hyperoxaluria type 1. *Gene Ther. 2016 Feb;23(2):129-34.*

I have always been interested in rare genetic conditions as these disorders might help us understanding biological pathways. Using whole exome sequencing, I identified *MIB2* mutations affecting NOTCH pathway as causative for a rare hearth malformation, known as left ventricle hypertrabeculation/non-compaction, associated to a proliferative gastropathy.

a. **Piccolo P**, Attanasio S, Secco I, Sangermano R, Strisciuglio C, Limongelli G, Miele E, Mutarelli M, Banfi S, Nigro V, Pons T, Valencia A, Zentilin L, Campione S, Nardone G, Lynnes TC, Celestino-Soper PBS, Spoonamore KG, D'Armiento FP, Giacca M, Staiano A, Vatta M, Collesi C, Brunetti-Pierri N. MIB2 variants altering NOTCH signalling result in left ventricle hypertrabeculation/non-compaction and are associated with Ménétrier-like gastropathy. *Hum Mol Genet 2017 Jan 1;26(1):33-43*

Complete List of Published Work in MyBibliography:

https://www.ncbi.nlm.nih.gov/myncbi/1hiHik7vcuj/bibliography/public/