BIOGRAPHICAL SKETCH

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NAME: Stephanie Cherqui, Ph.D

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POSITION TITLE: Associate Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Paris Diderot, Paris, France	BS	1993-1997	Molecular Biology and Genetics
University of Paris, Descartes, Paris, France	MS	1997-1998	Molecular Genetics
University of Paris, Descartes, Paris, France	Ph.D	1998-2002	Human Genetics

A. Personal Statement

My laboratory focuses on developing stem cell and gene therapy strategies for degenerative multi-systemic disorders, and to understand the mechanisms by which hematopoietic stem and progenitor cells (HSPCs) can lead to tissue repair in non-hematopoietic genetic diseases. My Ph.D research project focused on the molecular characterization of cystinosis and the generation of the mouse model, $Ctns^{-/-}$ mice (Inserm, Necker Hospital, France). As a postdoctoral fellow at the Scripps Research Institute (TSRI), I focused on stem cells and lentiviral-mediated gene therapy and its application to vascular diseases. I latter transitioned to a tenure-track Assistant Professor position at TSRI and developed the project stem cell and gene therapy for cystinosis. Currently, I am developing a phase I clinical trial for autologous transplantation of HSPCs *ex vivo* gene-corrected using a lentivirus vector for cystinosis. My research has transition from the gene to a gene therapy approach for cystinosis heading towards discovery of a new mechanism of rescue after HSPC transplantation and opening new therapeutic potential for HSPCs. I have now applied this principle to other disorders such as Friedreich's ataxia, a mitochondrial disease for which we now want to develop the HSPC gene therapy approach. I am very involved in the field of gene therapy and I am now the Chair of the American Society of Gene and Cell Therapy for which I review abstracts and organize the conference symposium for the Gene & Cell Therapy of Genetic and Metabolic Diseases section. I am also the Chair of an elective course I organized at UCSD: "Gene Therapy: Principles and Clinical Applications" (MED271). I am also a member of the Center for Genetic Therapies currently in development at UCSD with Dr. Theodore Friedmann.

B. Positions and Honors

Employment

2002 - 2006 Post-doctoral fellow, The Scripps Research Institute, Department of Molecular and Experimental Medicine, La Jolla, California, USA - *Supervisor: Daniel R. Salomon, M.D*

2006 - 2009 Staff Scientist, The Scripps Research Institute, Department of Molecular and Experimental Medicine, La Jolla, California, USA - *Supervisor: Daniel R. Salomon, M.D*

2009 - 2012 Tenure-track Assistant Professor, The Scripps Research Institute, Department of Molecular and Experimental Medicine, La Jolla, California, USA
Department head: Jeffery Kelly, Ph.D.

2012 - 2016 Tenure-track Assistant Professor, University of California, San Diego, Department of Pediatrics, Division of Genetics, La Jolla, California, USA
Department head: Gabriel Haddad, M.D

2016-present Tenure-track Associate Professor, University of California, San Diego, Department of Pediatrics, Division of Genetics, La Jolla, California, USA
Department head: Gabriel Haddad, M.D

Honors

1998 – 2001 Graduate student fellowship from the Minister of National Education, Research and Technology (France)
2001 Prize of the Association for Information and Research on Genetic Renal Diseases (France)
2001 – 2002 Graduate student fellowship from *Vaincre les Maladies Lysosomales* Foundation (France)
2001 – 2002 Post-doctoral fellowship from Inserm (USA)
2003 Prize of the Philippe Foundation (New York, USA)
2003 – 2005 Post-doctoral fellowship from *Juvenile Diabetes Research Foundation* (USA)
2012 Assembly Resolution from the California Legislature Assembly for Dr. Cherqui's Research on Cystinosis

Professional Societies and Public Advisory Committees

2010 - present Scientific Review Board member for the Cystinosis Research Foundation
2012 – present Member of the Board of Trustees for the Cystinosis Research Foundation
2010 - present Cure Cystinosis International Registry (CCIR) Medical and Scientific Council member
2014 – present Chair of the Cystinosis Stem Cell and Gene Therapy Consortium
2011 - 2017 American Society of Gene and Cell Therapy (ASGCT) Gene & Cell Therapy of Genetic and Metabolic Diseases Committee member
2017 - present Chair of the American Society of Gene and Cell Therapy (ASGCT) Gene & Cell Therapy of Genetic and Metabolic Diseases Committee

C. Contributions to Science

1- My contribution to Science starts with Cystinosis, a multi-systemic degenerative disorder. As a graduate student, I participated in the identification of the gene involved in this disease, *CTNS* gene (**a**), and in the characterization of the function of the encoded protein, cystinosin. We showed that cystinosin is a lysosomal cystine transporter with two lysosomal sorting motifs (**b**; **c**). I also identified the murine homologue of *CTNS* and generated the mouse model for cystinosis, the $Ctns^{-/-}$ mice (**d**). This mouse model is now used by over ten investigators internationally.

(a) Town M, Jean G, **Cherqui S**, Attard M, Forestier L, Whitmore SA, Callen DF, Gribouval O, Broyer M, Bates GP, van't Hoff W, Antignac C. (1998) A novel gene encoding an integral membrane protein is mutated in nephropathic cystinosis. *Nature Genet*. 18(4): 319-324. PMID: 9537412
(b) Kalatzis V, **Cherqui S**, Antignac C, Gasnier B. (2001) Cystinosin, the protein defective in cystinosis, is a H^+-driven lysosomal cystine transporter. *EMBO J*. 20(21): 5940-5949. PMID: 11689434
(c) **Cherqui S**, Kalatzis V, Trugnan G, Antignac C. (2001) The targetting of cystinosin to the lysosomal membrane requires a tyrosine-based signal and a novel sorting motif. *J Biol Chem*. 276: 13314-13321. PMID: 11150305
(d) **Cherqui S**, Sevin C, Hamard G, Kalatzis V, Sich M, Pequignot M.O, Gogat K, Abitbol M, Broyer M, Gubler M.C, Antignac C. (2002). Intra-lysosomal cystine accumulation in mice lacking cystinosin, the protein defective in cystinosis. *Mol Cell Biol.* 22(21): 7622-7632. PMID: 12370309

2- The current treatment for cystinosis is the drug cysteamine, which has severe side effects and only delays the progression of the disease. As an Assistant Professor, my goal was to apply a gene therapy strategy for cystinosis. As *CTNS* is ubiquitously expressed, this disease was particularly challenging. With this in mind, I decided to use bone marrow stem cells as a vehicle to bring the healthy *CTNS* gene to tissues. While mesenchymal stem cells had only a short-term limited impact on the disease, the hematopoietic stem and progenitor cells (HSPCs) demonstrated a major therapeutic benefit in the $Ctns^{-/-}$ mice. Cystine content was dramatically reduced in all organs tested and the kidneys were preserved for the life of the mice (**a**; **b**). This work represents the first proof of concept for using HSPC transplantation as a therapy for cystinosis. This work also demonstrates that HSPCs have not only the ability to rescue blood-related disorders but also to treat tissue degenerative disorders. Thus, this study might change the course of treatment for other disorders, for

which bone marrow transplantation has not even been considered as an option. Because of the risks associated with allogeneic stem cell transplantation, our goal is to develop an autologous HSPC transplantation strategy. This therapy may represent a one-time treatment for cystinosis as compared to the daily medication uptake (up to 60 pills per day). We have established an autologous HSPC transplantation and gene therapy protocol using a self- inactivating (SIN)-lentivirus vector expressing a human *CTNS* cDNA. We showed in the *Ctns*$^{-/-}$ mice that transduced cells were capable of decreasing cystine content in all tissues and led to kidney function improvement (**c**). We submitted a pre-Investigational New Drug (IND) in March 2013 and the FDA approved our plan for the pharmacology/toxicology studies to test the safety of our strategy *in vitro* and *in vivo*. These studies will be included in an IND application that we are currently preparing for a phase I clinical trial for autologous, lentiviral vector-modified, CD34^{+} HSPC transplantation for cystinosis. I am the Chair of the Cystinosis Stem Cell and Gene Therapy Consortium who will design and conduct this clinical trial at UCSD.

(a) Syres K, Harrison F, Tadlock M, Jester J, Simpson J, Roy S, Salomon DR, **Cherqui S.** (2009). Successful treatment of the mouse model of cystinosis using bone marrow cell transplantation. *Blood*. 114:2530-2541. **Cover photo**. PMID: 19506297 [Featured in: Terryn S, Devuyst O, Antignac C. (2010). Cell therapy for cystinosis. *Nephrol Dial Transplant*. 25(4):1059-1066.]

(b) Yeagy BA, Harrison F, Gubler M.C, Koziol JA, Salomon DR, **Cherqui S.** (2011). Kidney preservation by bone marrow cell transplantation depends on the level of stem cell engraftment in hereditary nephropathy. *Kidney Intern*. 79(11):1198-1206. **Cover photo**. PMID: 21248718 [Featured in: Pinkernell K. (2011). Cellular therapies: what is still missing? *Kidney International*. 79(11):1161-1163.]

(c) Harrison F, Yeagy BA, Rocca CJ, Kohn DB, Salomon DR, **Cherqui S**. (2013). Hematopoietic stem cell gene therapy in the mouse model of cystinosis. Molecular Therapy. *Mol Ther*. 21(2):433-444. PMID: 23089735

3- The extent of efficacy of HSPCs to rescue cystinosis was surprising especially considering that cystinosin is a transmembrane lysosomal protein. Addressing the mechanism by which Ctns-expressing HSCs led to tissue repair in the Ctns$^{-/-}$ mice, we showed that most of the HSPCs differentiated into macrophages, which generated long tubular extensions known as tunneling nanotubes (TNTs) capable of mediating the transfer of cystinosin- bearing lysosomes into deficient host cells (**a**). We also showed for the first time that TNT could cross the renal tubular basement membrane *in vivo* and transfer cystinosin-bearing lysosomes to the proximal tubular cells, providing a mechanism underlying the long-term kidney preservation after HSPC transplantation in the Ctns$^{-/-}$ mice. While cross-correction has already been demonstrated in several lysosomal storage disorders due to defective soluble lysosomal enzymes by secretion-recapture or enzyme replacement therapy, our study is the first demonstration of cross-correction in the context of a lysosomal transmembrane protein, creating the concept of lysosomal cross-correction. We recently demonstrated the same mechanism in the cornea showing that HSPC transplantation also mediated the rescue of the eye defects in the Ctns$^{-/-}$ mice leading to clearance of corneal cystine crystals, improved ocular structure and intraocular pressure (**b**). This work is the first demonstration that HSPCs could rescue corneal defects and brings new perspective for ocular regenerative medicine. The impact of the HSPCs on the thyroid has been studied in collaboration with Dr. Pierre Courtoy (de Duve Institute, Belgium). He first showed that Ctns$^{-/-}$ mice presented with sustained TSH activation combined with morphological evidence for increased thyroglobulin synthesis. HSPC engraftment in *Ctns*$^{-/-}$ thyroid drastically decreased cystine accumulation, normalized TSH level and corrected the structure of a large fraction of thyrocytes (**c**). Here again, the mechanism involved TNT extensions crossing the follicular basement laminae but also the passage of the HSPCs themselves into follicles, allowing extensive contact with thyrocytes. Because TNTs can transfer not only lysosomes but also other cellular organelles such as mitochondria, we tested HSPC transplantation in the mitochondrial disorder Freidreich's ataxia for which no treatment is currently available. Using the mouse model, we showed for the first time that transplantation of wildtype HSPC led to the complete rescue of the neurologic and muscular complications of FRDA (**d**). We also showed that the underlying mechanism involved the transfer of frataxin from HSPC-derived microglia/macrophages to the diseased host cells. Our findings bring new perspectives to regenerative medicine, as they should be applicable to other multi-compartment disorders involving deficient intracellular organelles. I am co-inventor on a patent entitled "Methods of treating lysosomal disorders" (#20378-101530) and inventor on a patent entitled "Methods of treating mitochondrial disorders" (#20378-201301)

(a) Naphade S, Sharma J, Gaide Chevronnay HP, Shook MA, Yeagy BA, Rocca CJ, Ur SN, Lau AJ, Courtoy PJ, **Cherqui S**. (2015) Lysosomal cross-correction by hematopoietic stem cell-derived macrophages via tunneling nanotubes. *Stem Cells*. 33(1):301-309. PMID: 25186209

(b) Rocca CJ, Kreymerman A, Ur SN, Frizzi KE, Naphade S, Lau AJ, Tran T, Calcutt NA, Goldberg JL, **Cherqui S**. (2015) Treatment of inherited eye defects by systemic hematopoietic stem cell transplantation. *Invest Ophthalmol Vis Sci.* 56(12):7214-7223. PMID: 26540660

(c) Gaide Chevronnay HP, Jansen V, Van Der Smissen P, Rocca CJ, Liao XH, Refetoff S, Pierreux CE, **Cherqui S***, and Courtoy P*. (2016) Hematopoietic stem cell transplantation can normalize thyroid function in a cystinosis mouse model. *Endocrinology.* 157(4):1363-1371. PMID: 26812160
*co-senior author

(d) Rocca CJ, Goodman SM, Dulin JN, Haquang JH, Gertsman I, Blondelle J, Smith JLM, Heyser CJ, **Cherqui S**. (2017) Hematopoietic stem cell transplantation prevents development of Friedreich's Ataxia in a humanized mouse model. *Sci Transl Med*. 9(413). PMID: 29070698

4- We also developed a novel strategy for kidney-targeted gene delivery for the treatment of monogenic hereditary nephropathies. We are using a retrograde renal vein injection of adeno-associated virus (AAV) in mice as a model for a minimally invasive treatment in humans. In its clinical form, we propose that it would follow a similar procedure as implemented in renal venography. Optimal AAV serotype for kidney-delivery has been identified and the procedure optimized (**a**). As AAV is a safe viral vector for gene delivery this strategy could be translated to humans to prevent kidney transplantation. We are now establishing the proof of concept of this strategy in the mouse model of cystinosis, which could become a treatment for the patients with the juvenile form of cystinosis who develop solely ocular anomalies and end-stage renal failure. We are also working in collaboration with Dr. Janice Chou (NIH/NICHD) and applied this strategy in her mouse model of glycogen storage disease type Ia (GSD Ia). Indeed, the curative treatment of this disorder requires the glucose-6- phosphatase enzyme to be expressed both in the liver and kidney. Using tail vein injection of rAAV8-G6Pase, they could only reach partial correction of this disorder because G6Pase was found only expressed in the liver. The results were promosing the mice not only improve their fasting blood glucose levels but also present a normal renal function after 52 weeks post-injection.

(a) Rocca CJ, Ur SN, Harrison F, **Cherqui S**. (2014) rAAV9 combined with renal vein injection is optimal for kidney-targeted gene delivery: conclusion of a comparative study. *Gene Ther*. 21(6):618-628. PMID: 24784447

5- In collaboration with other groups, we participated in the study of the pathogenesis of cystinosis and of the other cellular functions of the protein cystinosin. Our role mostly involved the *in vivo* studies using the $Ctns^{-/-}$ mice. We participated in Dr. Fabrizio De Benedetti's study (Bambino Gesu Children's Hospital, Italy) showing *in vitro* and *in vivo* that cystine crystals activate inflammasome in the kidney that may enhance the renal defects (**a**). With Dr. Sergio Catz (The Scripps Research Institute), we showed that cystinosin is involved in vesicular trafficking (**b**) and in chaperone-mediated autophagy (**c**, **d**). This work may lead to the identification of new drug targets to improve cystinosis phenotype.

(a) PrencipeG, Caiello I, **Cherqui S**, Whisenant T, Petrini S, Emma F and De Benedetti F. (2014). Cystine crystals are an inflammasome activating danger signal: possible implications for the pathogenesis of cystinosis. *J Am Soc Nephrol*. 25(6):1163-1169. PMID: 24525029

(b) Johnson JL, Napolitano G, Monfregola J, Rocca CJ, **Cherqui S** and Catz SD. (2013). Upregulation of the Rab27a-dependent trafficking and secretory mechanisms improves lysosomal transport, alleviates endoplasmic reticulum stress and reduces lysosome overload in cystinosis. *Mol Cell Biol*. 33(15):2950- 2962. PMID: 23716592

(c) Napolitano G, Johnson JL, He J, Rocca CJ, Monfregola J, Pestonjamasp K, **Cherqui S**, Catz SD. (2015) Impairment of chaperone-mediated autophagy leads to selective lysosomal degradation defects in the lysosomal storage disease cystinosis. *EMBO Mol Med*. 7(2): 158-174. PMID: 25586965

(d) Zhang J, Johnson JL, He J, Napolitano G, Ramadass M, Rocca C, Kiosses WB, Bucci C, Xin Q, Gavathiotis E, Cuervo AM, **Cherqui S**, Catz SD. Cystinosin, the small GTPase Rab11, and the Rab7 effector RILP regulate intracellular trafficking of the chaperone-mediated autophagy receptor LAMP2A. J Biol Chem. 2017 May 2. [Epub ahead of print] PMID: 28465352

Complete list of publications: http://www.ncbi.nlm.nih.gov/pubmed/?term=cherqui+s

D. Research Support

Ongoing Research Support

California Institute of Regenerative Medicine (CIRM) CLIN1-09230 (PI: Cherqui) 11/01/2016 - 10/31/2018
Ex vivo transduced autologous human CD34+ hematopoietic stem cells for treatment of cystinosis

To complete the pharmacology/toxicology studies required by the FDA, purchase the GMP lentivirus preparation, perform the manufacturing development and write the IND for autologous transplantation of *ex vivo* gene- modified hematopoietic stem cells using a lentivirus vector containing *CTNS* transgene.

NIH/NIDDK 2R01-DK090058-6 (PI: Cherqui) 01/01/2016 - 01/31/2020
Lentiviral-transduced hematopoietic stem cell transplantation for cystinosis
To investigate strategies to improve the stem cell-gene therapy approach for cystinosis and to investigate which patients' genetic profile will benefit the most from the future clinical trial.

Cystinosis Research Foundation CRFS-2015-002 (PI: Cherqui) 09/01/2015 - 08/31/2019
Mechanism of bone marrow stem cell-mediated therapy in the mouse model of cystinosis
To investigate the mechanisms by which transplantation of hematopoietic stem cells expressing a functional *Ctns* gene lead to cystine decrease and tissue preservation in the mouse model for cystinosis.

Friedreich's Ataxia Research Alliance (FARA) (PI: Cherqui) 02/01/2018 - 01/31/2019
Stem Cell Gene Therapy for Friedreich's Ataxia
The major goal of this project is to develop a new stem cell gene therapy approach for Friedreich's Ataxia.

NIH/NIDDK R01-DK110162-01A1 (PI: Catz, co-PI: Cherqui) 08/09/2017 - 04/30/2020
Molecular and Cellular Mechanisms of the Lysosomal Storage Disease Cystinosis
The major goal of this research project is to develop new understanding of the molecular mechanism regulating Chaperone mediated autophagy in nephropathic cystinosis.

Completed Research Support

Sanford Stem Cell Clinical Center 86G25A (PI: Cherqui) 09/01/2015-10/31/2016
A one arm, open label, single treatment safety and efficacy study of pCCL-CTNS modified CD34+ hematopoietic stem cells after autologous transplantation in patients with nephropathic cystinosis
To complete the pharmacology/toxicology studies required by the FDA for autologous transplantation of *ex vivo* gene-modified hematopoietic stem cells using a lentivirus vector containing *CTNS* transgene.

NIH/NINDS R21-NS090066 (PI: Cherqui) 08/15/2014-07/31/2016
Hematopoietic stem cell-based therapy for Friedrich Ataxia
To evaluate the impact of hematopoietic stem and progenitor cell transplantation on Friedrich Ataxia in the mouse model.

Cystinosis Research Foundation 20134334 (PI: Cherqui) 08/01/2013 - 07/31/2016
Pharmacology/Toxicology studies for gene-modified stem cell transplantation for cystinosis
To evaluate the impact of cysteamine on the stem cell transplantation and to coordinate a medical panel for clinical trial and collect information for natural history report on cystinosis.

NIH/NIDDK R01-DK090058-01 (PI: Cherqui) 01/01/2011-12/31/2015
Lentiviral-transduced hematopoietic stem cell transplantation for cystinosis
To establish the proof-of concept for the transplantation of autologous hematopoietic stem cells genetically modified *ex vivo* to express a functional *CTNS* gene using a lentiviral vector.

NIH/NIDDK R01-DK090058-01 (PI: Cherqui) 07/01/2013-06/30/2015
Toxicology studies for gene-modified stem cell transplantation for cystinosis
To perform the pharmacology/toxicology studies required to obtain an IND for a phase I clinical trial for an autologous transplantation of *ex vivo* gene-modified hematopoietic stem cells using a lentivirus vector containing a functional *CTNS* transgene for cystinosis.

NIH/NIDDK R21-DK090548-01A1 (PI: Cherqui) 09/01/2011-08/31/2013
Kidney-targeted gene delivery for cystinosis
To demonstrate that renal vein injection of adeno-associated virus (AAV) expressing a functional *CTNS* gene will treat or prevent the renal defects in cystinosis when delivered very early in the disease and ameliorate the renal disease if administered to older patients.