

BIOGRAPHICAL SKETCH

NAME: TUPLER, ROSSELLA G

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
University of Pavia	BS (with honors)	1977	Human Physiology
University of Brescia	MD (with honors)	1985	Medicine
University of Pavia	PHD (with honors)	1990	Human Molecular Genetics
Kinderspital, Basel	Other training	1990	Molecular Cytogenetics
University of Toronto	Postdoctoral Fellow	1991	Human Molecular Genetics
University of Pavia	Specialization (with honors)	1993	Medical Genetics

A. Personal Statement

My research expertise is in medical and human molecular genetics of human degenerative diseases. My laboratory is experienced in genotype-phenotypes studies connecting genetic defects with clinical and cellular phenotypes. My experience bridges the clinical knowledge with molecular biology. My laboratory has made several significant contributions in the field of facioscapulohumeral muscular dystrophy (FSHD), taking different approaches to study the pathogenic mechanism of disease. **(1)** We studied the molecular characteristics of the D4Z4 repeat to assess its functional role in biology and disease by using biochemistry and molecular biology (Cell 110:339-348, 2002; Hum Mol Genet 19:3114-23, 2010; Int J Mol Sci 21:2635, 2020, medRxiv 2023.06.13.23291337) and defined the functional features of the FSHD locus at 4q35 (bioRxiv 2024.03.18.585486). **(2)** We generated and characterized mouse models expressing candidate genes for FSHD by using several methodologies (Nature 449:973-977, 2006; J Physiol, 584: 997-1009, 2007; Exp Neurol 220:212-6, 2009; Mol Ther 19:2048-54, 2011; Am J Physiol Regul Integr Comp Physiol 306:R124-37, 2014; PLoS One 10:e0117665, 2015). **(3)** We established a standardized clinical evaluation of myopathic patients and generated clinical categories to classify subjects in FSHD families. (Muscle and Nerve, 42:213-7, 2010; J Neurol. 263:1204-14, 2016); **(4)** I coordinated of the Italian Clinical Network for FSHD and the Italian National Registry for FSHD. with genetic, molecular and clinical information over 4,000 subjects (Orphanet J Rare Dis 16:470, 2021) **(5)** for genotype-phenotype and epidemiology studies (Am J Hum Genet 90:628-35, 2012, Brain 136:3408-17, 2013; BMJ open, 6:e007798, 2016; JAMA Open Network, 2020 3:e204040; European Journal of Pediatric Neurology, 2020; Sci Rep 10:21648, 2020). At present all our studies are benefitting of the complete haplotype level assemblies from the T2T-CHM13 human genome and the human pangenome project and we are pursuing the use of artificial intelligence and deep learning to obtain a complete phenotypic description of myopathic patients integrating clinical, genomic, imaging, and morphological data.

B. Positions and Honors

Employment

1992 - 2005	Assistant Professor , Institute of General Biology and Medical Genetics, University of Pavia, Italy
1996 - 1998	Visiting Scientist , Howard Hughes Medical Institute, University of Massachusetts Medical School, Worcester, MA.
1998 - 2002	Instructor , Program in Molecular Medicine, UMMS, Worcester, MA.
2002 – 2023	Assistant Professor , Department of Molecular Cell and Cancer Biology, UMMS, Worcester, MA.
2005 – present	Associate Professor of Medical Genetics , Department of Biomedical Metabolic and Neural Sciences, University of Modena e Reggio Emilia, Italy

Positions

1998 - 2022	FSHD Italian Clinical Network, Coordinator
2000 - 2005	Laboratory for Neurogenetic Disorders Diagnosis, Pavia, Director
2004 - 2009	FSH Club, Association Française contre les myopathies, Coordinator
2006 - present	Miogen Laboratory for Research and Diagnosis of Neuromuscular Disorders, University of Modena and Reggio Emilia, Director

2006 – 2019	Graduate School in Molecular and Regenerative Medicine University of Modena and Reggio Emilia, Coordinator
2007 - present	Italian National Registry for FSHD, Curator
2007 - 2010	Ethics Committee of the province of Modena, member
2008 - 2012	Center Advisory Board (CAC) of Paul D. Wellstone Muscular Dystrophy Cooperative research Center BBRI, Watertown, USA, member
2010 – 2012	AFM Strategic and Therapeutic Development Committee (COSET), member
2010 – 2012	Association Française contre les myopathies Scientific Council, member
2012	Study Section (Skeletal Muscle and Exercise Physiology) at Center for Scientific Review, National Institute of Health, USA, <i>ad hoc</i> member
2013 - 2015	Massachusetts Wellstone Cooperative Research Center for FSHD Research, University of Massachusetts Medical School, Worcester, Member
2015	2015/10 ZRG1 GGG-Q Innovative Therapies and Tools for Screenable Disorders in Newborns Study Section, Center for Scientific Review, NIH, USA, <i>ad hoc</i> member
2015	2015/10 TAG (Therapeutic Approaches to Genetic Diseases) Study Section, Center for Scientific Review, National Institute of Health, USA, <i>ad hoc</i> member
2016-2019	Strategic Committee Unione delle Associazioni, Italy, member
2016-2020	International Consortium for FSHD, organizer
2018-04/2023	Li Weibo Institute for Rare Diseases Research at the University of Massachusetts Medical School, member
2019-2022	TREAT-NMD: Global database oversight committee-TGDOC, Disease Subgroup Lead for FSHD

Honors

1991	Anna Villa Rusconi Postdoctoral Fellowship
1997	CNR (National Research Centre) Senior Scientist Fellowship
1998	FSH Society - Delta Railroad Construction Postdoctoral Fellowship
2000	Worcester Foundation Award
2000	Legato Ferrari Award.

C. Contribution to Science

1. Definition of the genetic characteristics of FSHD.

At start, in 1992, I analyzed chromosomal structural anomalies involving the 4q35 region to refine the FSHD locus. I demonstrated that haploinsufficiency of the FSHD locus does not cause FSHD. I also established the differential clinical expression of FSHD in presence of identical genetic background. I conducted a pioneer study of differential gene expression in FSHD muscles of subject. All these studies have laid the ground for the continuous development of studies in FSHD genetics.

- Tupler R**, et al. Monosomy of distal 4q does not cause facioscapulohumeral muscular dystrophy. *J Med Genet*, 33:366-370, 1996.
- Tupler R**, et al. Identical “de novo” mutation at D4F104S1 locus in monozygotic male twins affected by facioscapulohumeral muscular dystrophy (FSHD) with different clinical expression. *J Med Genet*, 35:778-783, 1998.
- Tupler R**, et al. Profound misregulation of muscle-specific gene expression in facioscapulohumeral muscular dystrophy. *Proc Natl Acad Sci U S A* 96:12650-12654, 1999.
- Salsi V, Chiara M, Pini S, ... **Tupler R**. A human pan-genomic analysis reconfigures the genetic and epigenetic make up of facioscapulohumeral muscular dystrophy medRxiv 2023.06.13.23291337

2. Definition of the biological function of D4Z4 repetitive elements and their role in disease.

Almost all FSHD patients carry rearrangements occurring in a 3.3 kb tandemly repeated sequence (D4Z4) located at the 4q subtelomere. Each repeated unit sequence contains heterochromatic DNA. To define the role of D4Z4 in regulating gene transcription we characterized the protein binding activity of D4Z4, and the protein binding element within D4Z4 (D4Z4 Binding Element). Through biochemical purification methods and mass spectrometry microsequencing, we isolated three proteins, YY1, HMGB2B, and nucleolin that are part of a multi-protein complex binding D4Z4. Through several experiments we demonstrated that the three proteins were specifically binding D4Z4 in vitro and in vivo. Reduction of YY1, HMGB2, and nucleolin protein level resulted in over-expression of the 4q35 gene FRG2. This seminal work laid the basis for all the subsequent studies on FSHD molecular mechanism and on the molecular organization of the 4q35 region.

- a. Gabellini D., Green M.R., **Tupler R.** Inappropriate gene activation in FSHD: A repressor complex binds a chromosomal repeat deleted in dystrophic muscle. *Cell*, 110:339-348, 2002.
- b. Forlani G, Giarda E, Ala U, Di Cunto F, Salani M, **Tupler R**, Kilstrup-Nielsen C, Landsberger N. The MeCP2/YY1 interaction regulates ANT1 expression at 4q35: novel hints for Rett syndrome pathogenesis. *Hum Mol Genet* 19:3114-23, 2010
- c. Salsi V, Losi F, Salani M, Kaufman PD, **Tupler R** Post-transcriptional RNA stabilization of telomere-proximal RNAs FRG2, DBET, D4Z4 at human 4q35 in response to genotoxic stress and D4Z4 macrosatellite repeat length *Clin Epigenet* 17:73, 2025.

3. Generation of the first animal model for FSHD.

Three genes, *FRG2*, *FRG1*, *ANT1*, were found overexpressed at the 4q35 region in biopsies from FSHD patients. We generated transgenic mice independently overexpressing these genes. Among them, mice overexpressing *FRG1* display an overt dystrophic phenotype with features of disease. *FRG1* is a RNA binding protein and its overexpression causes the aberrant splicing of numerous genes. We demonstrated that the aberrant splicing of Troponin T determines reduced response to Ca^{++} and strength of mouse muscle fibers and it is predominant in muscle from FSHD patients. At present *FRG1* transgenics are the only mouse model mimicking the FSHD muscle pathology. They have been used to test possible therapeutic interventions in FSHD and to study genetic interactions.

- a. Gabellini D, D'Antona G, Moggio M, ... **Tupler R.** Facioscapulohumeral muscular dystrophy in mice overexpressing *FRG1*. *Nature*, 449:973-977, 2006.
- b. Wallace LM, Garwick-Coppens SE, Tupler R, Harper SQ. RNA Interference Improves Myopathic Phenotypes in Mice Over-expressing FSHD Region Gene 1 (*FRG1*). *Mol Ther*. 19:2048-54, 2011
- c. Sancisi V, Germinario E, Esposito A, ... **Tupler R.** Altered *Tnnt3* characterizes selective weakness of fast fibers in mice overexpressing FSHD region gene 1 (*FRG1*). *Am J Physiol Regul Integr Comp Physiol* 306:R124-37, 2014.
- d. Feeney SJ, McGrath MJ, Sriratana A, Gehrig SM, Lynch GS, D'Arcy CE, Price JT, McLean CA, **Tupler R**, Mitchell CA. FHL1 Reduces Dystrophy in Transgenic Mice Overexpressing FSHD Muscular Dystrophy Region Gene 1 (*FRG1*). *PLoS One* 10:e0117665, 2015.

4. Establishment of a standardized methodology to study FSHD patients and families.

FSHD is characterized by great clinical variability and incomplete penetrance sometimes with unexpected mode of inheritance. To create tools for the systematic clinical study of FSHD families we created a evaluation form to collect clinical information and designed a standard protocol that quantifies muscle weakness by combining the functional evaluation of six muscle groups affected in FSHD and discovered that the current diagnostic molecular marker for FSHD diagnosis is a common polymorphism. We also established that gender and degree of kinship contribute to FSHD development in relatives carrying the FSHD molecular signature. Our results suggest that the genetic basis of FSHD, which is remarkably heterogeneous, should be revisited since this has important implications for genetic counseling and prenatal diagnosis of at-risk families. This large cohort of FSHD families provides a massive source of information that will support the validation of the generality of our findings and facilitate the translation of our study to general practice.

- a. Ricci G, Scionti I, Sera F, ... **Tupler R.** Large-scale genotype-phenotype analyses indicate that novel prognostic tools are required for facioscapulohumeral muscular dystrophy families, *Brain* 136:3408-17, 2013
- b. Ricci G, Ruggiero L, Vercelli L, Sera F, ... **Tupler R.** A novel clinical tool to classify facioscapulohumeral muscular dystrophy phenotypes. *J Neurol*. 263:1204-14, 2016
- c. Ruggiero L, Mele F, Manganelli F, ... **Tupler R.** Phenotypic variability among Patients with D4Z4 reduced allele Facioscapulohumeral muscular dystrophy, *JAMA Open Network*, 2020 3:e204040.
- d. Vercelli L, Mele F, Ruggiero L, ... **Tupler R.** A five-year longitudinal study from the Italian National Registry for FSHD *Journal of Neurology*, 268:356-366, 2020

5. Establishment of the Italian National Registry for FSHD and initiative for interpretation of molecular data in FSHD counselling

Guidelines for FSHD diagnosis are based on the molecular features of the D4Z4 locus at 4q35, even though great clinical variability can be associated with these molecular features, including healthy carriers from the general population. We established a novel methodology to approach myopathic patients with suspected FSHD and collected clinical and molecular data systematically in a large database that constitutes the basis of the Italian National Registry for FSHD.

- a. Scionti I, Greco F, Ricci G; ... **Tupler R**. Large scale population analysis challenges the current criteria for the molecular diagnosis of facioscapulohumeral muscular dystrophy (FSHD) *Am J Hum Genet* 90:628-35, 2012
- b. Nikolic A, Jones T, Govi M, ... **Tupler R**. Interpretation of the Epigenetic Signature of Facioscapulohumeral Muscular Dystrophy in Light of Genotype-Phenotype Studies. *Int. J. Mol. Sci.* 21:2635, 2020.
- c. Bettio C, Salsi V, Orsini M, ... **Tupler R**. The Italian National Registry for FSHD: an enhanced data integration and an analytics framework towards Smart Health Care and Precision Medicine for a rare disease. *Orphanet J Rare Dis.* 16:470, 2021
- d. Pini S, Napoli FM, Tagliafico E, ... **Tupler R**. De novo variants and recombination at 4q35: Hints for preimplantation genetic testing in facioscapulohumeral muscular dystrophy. *Clin Genet.* 103:242-246, 2023
- e. Bettio C, Banchelli F, Salsi V, ... **Tupler R**. Physical activity practiced at a young age is associated with a less severe subsequent clinical presentation in facioscapulohumeral muscular dystrophy. *BMC Musculoskelet Disord.* 25:35, 2024

6. Discovery of a novel family of lncRNAs relate facioscapulohumeral muscular dystrophy to nucleolar organization and protein synthesis rate

Aberrant transcription of FRG2A inversely correlating with the size of the D4Z4 array at 4q35, has been reported in FSHD muscle. We demonstrated that FRG2A is one member of a family of long non-coding RNAs (lncRNA) expressed at elevated levels in skeletal muscle cells in an individual-specific manner. We found that FRG2A lncRNA preferentially associates with rDNA sequences and centromeres and promotes the three-dimensional association of centromeres with the nucleolar periphery in FSHD samples. We showed that the elevated FRG2A expression in cells from FSHD patients reduces rDNA transcription and protein synthesis rates. Our results frame a new disease model in which elevated lncRNAs expression mediated by deletions of D4Z4 macrosatellite repeats leads to a diminished protein synthesis capacity, thereby contributing to muscle wasting.

- a. Salsi V, Losi F, Fosso B, Ferrarini M, Pini S, Manfredi M, Vattermi G, Mongini T, Maggi L, Pesole G, Henras AK, Kaufman PD, McStay B, **Tupler R** Nucleolar FRG2 lncRNAs inhibit rRNA transcription and cytoplasmic translation, linking FSHD to dysregulation of muscle-specific protein synthesis *Nucleic Acids Res.* 2025 53gkaf643. doi: 10.1093/nar/gkaf643.

D. Research Support

Ongoing Research Support

Cariplo-Telethon GJC Project-GJC23060-09/01/23 – 08/31/26

Title: Characterization of FRG2 gene family in the framework of FSHD

Aims: This proposal aims at deciphering the function and the role of FSHD Region Gene 2 (FRG2) paralogs in the framework of FSHD.

Role: Coordinator

HORIZON-HLTH-2022-TOOL-12-01 01/05/2023 – 30/04/2027

Title: Computational Models for new Patients Stratification Strategies of Neuromuscular Disorders

Aims: This proposal aims at the creation of computational tools for the integration of clinical, genomic, imaging, and morphological data to support the stratification of patients with Neuromuscular Disorders and provide new classification and diagnostic tools.

Role: Coordinator

2022_PNRR_HEAL_SPK2AFF_E93C22001860006 01/12/23 – 05/31/26

Title: "HEAL ITALIA" Health Extended Alliance For Innovative Therapies, Advanced Lab-research, and Integrated Approaches of Precision Medicine_PE00000019

Aims: This proposal aims at creating tools to support the classification and the advanced diagnosis in human disease towards the implementation of personalized medicine strategies.

Role: Investigator

Ministry of University and Research – PRIN 2022X8PMEA 09/01/23 – 08/31/25

Title: ML-based investigation of exome variants to inspect the phenotypic heterogeneity of Facioscapulohumeral muscular dystrophy

Aims: This proposal aims at developing a ML model integrating clinical, phenotypic and genomic data using clinical and WES data to predict the myopathic profile in a newborn.

Role: Investigator

FSH -Amis METIN-FSHD (RU-PI)

Title: *Unraveling metabolic involvement in facioscapulohumeral dystrophy through metabolomics*

Duration: 09/01/24 – 31/08/25

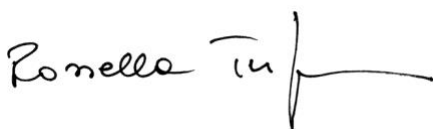
Aims: This proposal aims at identifying biomarkers to monitor FSHD development.

Role: Investigator

E. Relevant Publications

1. Tupler R., Perini G, Pellegrino MA, Green MR. Profound misregulation of muscle-specific gene expression in facioscapulohumeral muscular dystrophy. *Proc Natl Acad Sci U S A* 96:12650-12654, 1999.
2. Tupler R., Perini G, Green MR. Expressing the Human Genome. *Nature*, 409:832-3, 2001.
3. Gabellini D., Green M.R., Tupler R. Inappropriate gene activation in FSHD: A repressor complex binds a chromosomal repeat deleted in dystrophic muscle. *Cell*, 110,339-348, 2002.
4. Gabellini D, D'Antona G, Moggio M, Prelle A, Zecca C, Adami R, Angeletti B, Ciscato P, Pellegrino MA, Bottinelli R, Green MR, Tupler R. Facioscapulohumeral muscular dystrophy in mice overexpressing FRG1. *Nature*, 449:973-977, 2006.
5. Scionti I, Greco F, Ricci G; Govi M, Arashiro P, Vercelli V, Berardinelli A, Angelini A, Antonini G, Cao M, Di Muzio A, Moggio M; Morandi L Ricci E; Rodolico C; Ruggiero L, Santoro L, Siciliano G; Tomelleri G, Trevisan CP; Galluzzi G; Wright W, Zatz M, Tupler R. Large scale population analysis challenges the current criteria for the molecular diagnosis of facioscapulohumeral muscular dystrophy (FSHD) *Am J Hum Genet* 90:628-35, 2012
6. Ricci G, Scionti I, Sera F, Govi M, D'Amico R, Frambolli I, Mele F, Filosto M, Vercelli L, Ruggiero L, Berardinelli A, Angelini C, Antonini G, Bucci E, Cao M, Daolio J, Di Muzio A, Di Leo R, Galluzzi G, Iannaccone E, Maggi L, Maruotti V, Moggio M, Mongini T, Morandi L, Nikolic A, Pastorello E, Ricci E, Rodolico C, Santoro L, Servida M, Siciliano G, Tomelleri G, Tupler R . Large-scale genotype-phenotype analyses indicate that novel prognostic tools are required for facioscapulohumeral muscular dystrophy families, *Brain* 136:3408-17, 20134.
7. Ruggiero L, Mele F, Manganelli F, Bruzzese D, Ricci G, Vercelli L, Govi M, Vallarola A, Tripodi S, Villa L, Di Muzio A, Scarlato M, Bucci E, Antonini G, Maggi L, Rodolico C, Tomelleri G, Filosto M, Previtali S, Angelini C, Berardinelli A, Pegoraro E, Moggio M, Mongini T, Siciliano G, Santoro L, Tupler R. Phenotypic variability among Patients with D4Z4 reduced allele Facioscapulohumeral muscular dystrophy, *JAMA Open Network*, 2020 3:e204040.
8. Nikolic A, Jones T, Govi M, Mele F, Maranda L, Sera F, Ricci G, Ruggiero L, Vercelli L, Portaro S, Villa L, Fiorillo C, Maggi L, Santoro L, Antonini G, Filosto M, Moggio M, Angelini C, Pegoraro E, Berardinelli A, Maioli M, D'Angelo G, Di Muzio A, Siciliano G, Tomelleri G, D'Esposito M, Della Ragione F, Brancaccio A, Piras R, Rodolico C, Mongini T, Magdinier F, Salsi V, Jones P, Tupler R. Interpretation of the Epigenetic Signature of Facioscapulohumeral Muscular Dystrophy in Light of Genotype-Phenotype Studies. *Int. J. Mol. Sci.* 2020, 21, 2635;
9. Vercelli L, Mele F, Ruggiero L, Sera F, Tripodi S, Ricci G, Vallarola A, Villa L, Govi M, Maranda L, Di Muzio A, Scarlato M, Bucci E, Maggi L, Rodolico C, Moggio M, Filosto M, Antonini G, Previtali S, Angelini A, Berardinelli A, Pegoraro E, Siciliano G, Tomelleri G, Santoro L, Mongini T, Tupler R A five-year longitudinal study from the Italian National Registry for FSHD *Journal of Neurology*, 268:356-366, 2021.
10. Salsi V, Losi F, Salani M, Kaufman PD, **Tupler R** Post-transcriptional RNA stabilization of telomere-proximal RNAs FRG2, DBET, D4Z4 at human 4q35 in response to genotoxic stress and D4Z4 macrosatellite repeat length *Clinical epigenetics* 17: 73, 2025

Modena, September 26th, 2025



Rossella Tupler