UNIVERSITÀ DI SIENA
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DIPARTIMENTO DI MEDICINA MOLECOLARE E DELLO SVILUPPO

Al collegio docenti del Dottorato in Medicina Molecolare

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Scientific report (2nd year of PhD Fellowship), academic year 2022/2023

Introduction

Drug resistant focal epilepsy (DR-FE) accounts for a significant proportion of epilepsy burden worldwide. Surgery is often the best option to remove the area of the brain where seizures occur. Germline or brain-only somatic variants are a recognized genetic determinant of FE. Recurrent genes and hot-spot mutations are found in patients carrying circumscribed lesions - e.g. Focal Cortical Dysplasia (FCD) type II and glioneuronal tumors. A genetic origin is suspected in other histological subtypes, but it is still largely unexplored.

Materials and methods

DNA was successfully extracted from matched Fresh Frozen (FF) brain–blood and in some cases from Formalin-Fixed, Paraffin-Embedded (FFPE) samples of 130 surgically treated FE patients. We designed separate panels covering FCD and brain tumor-associated genes (24 total genes) using single molecule Molecular Inversion Probes (smMIPs) and performed the set-up of these gene panels on matched blood-brain samples of FCD and tumor patients. For other epileptogenic lesions we made deep whole-exome sequencing (WES).

Results

smMIPs sequencing obtained an adequate read depth required for the detection of low variant allele frequency (VAF) variants. We obtained 3000X mean coverage for uncollapsed reads, and target regions were fully covered with >200 unique reads. In the 112 tested samples, we identified a pathogenic variant in SLC35A2 with VAF 2.49% in FF lesional tissue of a patient with FCD type I and not present in the blood, two somatic mutation in MTOR with VAFs 1.07% and 1.44%, two germline variants in TSC1 and TSC2 genes in brain-blood matched samples from FCD type II patients. We also detected BRAF somatic mutation with VAFs ranging from 2% to 35% across brain samples and TP53 and IDH1 mutations in glioneuronal tumor patients. For FFPE FCD type II samples, we discovered low VAFs (from 1.10% to 2.68%) mutations in MTOR, two somatic mutation in RHEB with VAFs about 2.0%, a somatic mutation in TSC2 with VAFs about 3.86% and a germline pathogenic mutation in TSC1. To date, we obtain a patient diagnostic rate of 34%. We aim also to characterize somatic genetic variants in the other resected brain specimens using data from deep WES (>350X).

Abstracts and Conferences

• *Title*: Single-molecule Molecular Inversion Probes provide an accurate diagnostic approach for the detection of low VAF somatic variants in focal cortical dysplasias and brain tumors
Authors: **Ester Cifaldi**, Paola Dimartino, Lorenzo Ferri, Raffaella Minardi, Laura Licchetta, Marco Seri, Marco De Curtis, Laura Rossini, Rita Garbelli, Laura Tassi, Michele Rizzi, Matteo Martinoni, Elena Pasini, Roberto Michelucci, Francesca Bisulli, Leonardo Caporali, Tommaso Pippucci
Conference site/date: 55th ESHG-European Society of Human Genetics conference. Glasgow, United Kingdom. June 10-13, 2023.

• *Title*: Single-molecule Molecular Inversion Probes provide an accurate diagnostic approach for the detection of low VAF somatic variants in focal cortical dysplasias and brain tumors.
Authors: **Ester Cifaldi**, Paola Dimartino, Lorenzo Ferri, Raffaella Minardi, Laura Licchetta, Marco Seri, Marco De Curtis, Laura Rossini, Rita Garbelli, Laura Tassi, Michele Rizzi, Matteo Martinoni, Elena Pasini, Roberto Michelucci, Francesca Bisulli, Leonardo Caporali, Tommaso Pippucci
Conference site/date: SIGU – Società Italiana Genetica Umana, XXVI Congresso. Rimini, October 4-6, 2023.

• *Title*: Identificazione di varianti somatiche e germinali in pazienti sottoposti a chirugia dell'epilessia.
Authors: L. Ferri, **E. Cifaldi**, L. Licchetta, R. Minardi, B. Mostacci, P. Dimartino, E Pasini, L. Rossini, M. Rizzi, A. Perciavalle, G. Marucci, M. Martinoni, C. Pastori, M. Seri, R. Michelucci, M. De Curtis, L. Caporali, R. Garbelli, T. Pippucci, L. Tassi, F. Bisulli
Conference site/date: LICE– Lega Italiana Contro l'Epilessia, XXXXVI Congresso Nazionale. Napoli, June 7-9, 2023.

• *Title*: Detection of somatic and germline pathogenic variants in epileptogenic lesions.
Authors: L. Ferri, **E. Cifaldi**, L. Licchetta, R. Minardi, B. Mostacci, P. Dimartino, E Pasini, L. Rossini, M. Rizzi, A. Perciavalle, G. Marucci, M. Martinoni, C. Pastori, M. Seri, R. Michelucci, M. De Curtis, L. Caporali, R. Garbelli, T. Pippucci, L. Tassi, F. Bisulli
Conference site/date: SIN – Società Italiana di Neurologia. Napoli, October 21-24, 2023.

***Courses**, in addition to the ones attended during the 1st year of PhD Fellowship:*

Alberto Mantovani: "Come pubblicare e vivere felici (lo stesso)". 23 novembre 2022. Aula Magna, complesso didattico Le Scotte, Siena.
Stefano Landi: "How to study genetics in complex diseases".19 gennaio e 26 gennaio 2023, Dipartimento di Biologia dell'Università di Pisa.
Laura Governini: "Microvesicles in cell-to-cell communication", Alice Luddi: "Biomarkers discovery in precision medicine". 27 Febbraio 2023, Complesso Istituti Biologici San Miniato, Siena.
Anthony Hessel: "Through thick and thin...and titin: Defining titin's role to muscle function."
Leonardo Sacconi:" Advanced optical methods to monitor and control the cardiac electrical activity".

Pieter de Tombe: "Cellular mechanisms of heart failure". Elisabetta Cerbai and Elisabetta Bigagli: "Drug development, sex-dependent difference, pharmacovigilance", 4 aprile 2023, Aula Magna, Ex Presidenza di Medicina, Careggi, Firenze.
Anna Pelagotti: "Money for nothing and chicks for free: the story of the European Union and its funding". 8 maggio 2023, Auditorium Santa Chiara, Siena.
"I finanziamenti europei nel settore digitale". 11 maggio 2023.
Maria del Mar Mira: "Last update in Sustainability reporting regulation". 7 settembre 2023.
Fausta Cosci e Maria Cristina Costantini: "Ricerche bibliografiche, bibliometria e open access & science". 14 settembre 2023, Auditorium Santa Chiara, Siena.
Carlo Carobbi e Gianluca Murgia: "The role of standardization in innovation development". 20 settembre 2023, Auditorium Santa Chiara, Siena.