Relazione di fine secondo anno di Dottorato in Medicina Molecolare
Ciclo XXXII, aa 2017/2018

Università degli Studi di Siena- Dipartimento di Medicina Molecolare e dello Sviluppo

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La sottoscritta Dott.ssa **Angelica D'Amore**, come previsto dal DD n. 1601/2016, presenta la seguente relazione sulle attività intraprese durante il secondo anno di dottorato, svolto presso il Laboratorio di Medicina Molecolare, IRCCS Fondazione Stella Maris di Calambrone (Pisa).

INTRODUCTION: Hereditary spastic paraplegia (HSP) is an umbrella term used to refer to a group of rare (about 1.8 individuals in 100 000) genetically heterogeneous neurodegenerative motor neuron disorders characterized by progressive age-dependent loss of corticospinal motor tract function, leading to lower limb spasticity, urinary bladder dysfunction and weakness. Next generation sequencing (NGS) methods have recently emerged as the best approach for the genetic study of this pathology. To date, 85 different spastic gait disease loci, and 79 known causative SPastic Gait (SPG) genes, have been identified, of which 10 have been identified with NGS methodologies in the past four years. Next generation sequencing (NGS) technologies can facilitate the identification of new disease genes adopting whole-exome sequencing techniques.

METHODS: In the genetic part of my research, peripheral blood samples were collected from over 200 HSP patients in the study. Total DNA purified from patients was studied using a NGS platform powered by Illumina NextSeq adopting a targeted multigene panel specifically designed for HSP. Gene variants were analyzed with several *in silico* tools to identify every pathogenic changes which could be disease-responsible. We also checked segregation in the families and effects in multiple families using *in-house* and public databases and the gem.app/GENESIS application enlisting WES data from over 550 unsolved HSP cases. One largest family from Sardinia – showing HSP phenotype in six individuals – also underwent whole exome sequencing, after the first NGS panel and their results are still under analyses.
At the present, I am also studying the functional role of SPG52/*AP4S1*, a relatively new HSP-related gene using *Danio rerio* (zebrafish) as *in vivo* model and generating morphants, that is, larvae where gene function has been transiently knocked-down. Mutant and wild-type embryos are evaluated for both coiling frequency and tracking movement at 30 and 120 hours post fertilization (hpf). Further investigation of gene expression and whole mount hybridization are ongoing.

PARTIAL RESULTS: To date, we have collected over 200 HSP samples fulfilling my genetic aim. After NGS studies, we identified 85 pathogenic mutations in 70 patients, with a positive diagnostic yield of 29%. This could allow us to affirm that NGS could be use as first-tier approach in NGS diagnosis.
At the same time, for the functional part, we have shown that 30hpf morphant embryos with the XXX genetic background had increased coiling frequency (five times higher), phenotypic traits resembling some of the features seen in SPG52 patients (i.e. microcephaly), and manifested a

significant delay in time and space during locomotion by motor tracking evaluation. These data mimick at least in part the effects on moror function seen in HSP patients.

PUBLICATIONS:

- Pizzino G, Irrera N, Galfo F, Oteri G, Atteritano M, Pallio G, Mannino F, **D'Amore A**, Pellegrino E, Aliquò F, Anastasi GP, Cutroneo G, Squadrito F, Altavilla D, Bitto A. Adenosine Receptor Stimulation Improves Glucocorticoid-Induced Osteoporosis in a Rat Model. Front Pharmacol. 2017 Sep 5;8:558.
- Pizzino G, Irrera N, Galfo F, Pallio G, Mannino F, D'amore A, Pellegrino E, Ieni A, Russo GT, Calapai M, Altavilla D, Squadrito F, Bitto A. Effects of the antagomiRs 15b and 200b on the altered healing pattern of diabetic mice. Br J Pharmacol. 2018 Feb;175(4):644-655. doi: 10.1111/bph.14113. Epub 2018 Jan 18. PubMed PMID: 29178246; PubMed Central PMCID: PMC5786458.

ABSTRACT:

- D'Amore A, Tessa A, Rubegni A, Barghigiani M, Galatolo D, Bruno I, Cereda C, Dato C, Felicori M, Gallone S, Graziano C, Gurrieri F, Mandich P, Pochiero F, Seri M, Stanzial F, Tinelli F, Valente MR, Gemignani F, Santorelli FMS. Next Generation Sequencing molecular diagnosis in Hereditary Spastic Paraplegia: a cross-sectional study. POSTER ACCEPTED SIGU CONGRESS (Catania - 24-27 October, 2018)

EDUCATIONAL ACTIVITIES:

1) "Drosophila melanogaster as a model organism to study and cure mitochondrial disease". IRCCS Fondazione Stella Maris, Calambrone – Pisa, 04/04/2018
2) "Miglioramento della comunicazione tra servizi nel Dipartimento Clinico". IRCCS Fondazione Stella Maris, Calambrone – Pisa, 19/09/2018
3) "Malattie da accumulo lisosomiale: Fabry – Gaucher – Hunter". IRCCS Fondazione Stella Maris, Calambrone – Pisa, 27/09/2018
4) Corso di Formazione: "Sicurezza nel laboratorio di Medicina Molecolare". IRCCS Fondazione Stella Maris, Calambrone –Pisa, 7 Maggio, 10 Luglio e 9 Ottobre 2018.

SATISFACTION LEVEL OF PhD PROGRAM:

I am fully pleased with how this PhD program is enriching my scientific background and provides the required tools for the advancement of my research project.

Dottoranda
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