Relazione di fine primo anno di Dottorato in Medicina Molecolare
Ciclo XXXII, aa 2016/2017

Università degli Studi di Siena- Dipartimento di Medicina Molecolare e dello Sviluppo
Borsa di Studio Pegaso con finanziamenti POR F.S.E 2014/2020, messi a disposizione dalla Regione Toscana

La sottoscritta Dott. Angelica D'Amore, come previsto dal DD n. 1601/2016, presenta la seguente relazione a proposito delle attività svolte durante il primo anno di dottorato, svolto nel Laboratorio di Medicina Molecolare con sede presso l'IRCCS Fondazione Stella Maris di Calambrone (Pisa).

"Identification of new genes implicated in hereditary spastic paraplegia (HSP) through NGS analysis and in vitro and in vivo functional studies".

BACKGROUND: Hereditary spastic paraplegias (HSPs) are a group of genetically heterogeneous motor neuron disorders characterized by progressive age-dependent loss of corticospinal motor tract function. To date, over 70 different spastic gait disease-loci have been identified, and more than 50 spastic paraplegia genes (SPGs) have been cloned. However, there is room for additional genes since the present classification accounts for about 70% of autosomal dominant and roughly 50% of the autosomal recessive HSP genes. Next generation sequencing (NGS) technologies can facilitate the identification of new disease genes adopting whole-exome sequencing techniques.

AIM: The aim of the PhD research program is 3-fold. First, we plan to study genotype-phenotype correlations in a cohort of patients with mendelianly-inherited HSP and to identify new HSP causing genes, using NGS and whole-exome sequencing analysis. Second, we have the ambition to create an in vitro model, using Human Embryonic Kidney (HEK) 293 cells, to test the identified causing genes, through their reversible silencing, and to evaluate cell growth and effects on cytoplasmic and mitochondrial functions. Third, to employ an *in vivo* zebrafish model to study effects on gene mutations/functions on locomotion and behavior.

METHODS: DNA from the patients will be studied using the NGS platform powered by Illumina MiSeq for an initial analysis and they will be included in several targeted panel, specifically designed for HSP. Then the samples will be analyzed using either whole transcriptome or exome analyses using a NextSeq500 automatic Illumina sequencer. To validate a possible new HSP gene we will check segregation in the family and effects in multiple families using also the gem.app/GENESIS application enlisting WES data from over 550 cases of unsolved HSP patients.
We will hopefully identify new genes, never related to the pathology and an additional cohort of patients with diagnosed HSP could be screened for mutations to confirm their pathological role. Then it will be possible to create an *in vitro* model using HEK 293 cells, which will be transfected with several cDNAs encoding possible causative genes. To evaluate the role of these genes, it might be possible to silence them in a reversible manner, using CRISPR/Cas9 methodology. To understand the potential role of novel HSP disease genes and to understand how they act, we will

perform functional tests in vivo, using zebrafish, performing knockdown experiments and functional assays.

EXPECTED RESULTS: Our goal is to identify at least one new HSP gene and to dissect the way it leads to motor neuron disease. The new potential gene(s) could be used to predict the risk of HSP in a larger cohort of patients and attempt to identify new possible targets to therapies.
To date, I am selecting patients for the exome sequencing, having performed the initial screening of 50 patients with several panels which run on the MiSeq Illumina Platform. At the same time, I am training with the *in vivo* model, zebrafish, and the use of morpholino oligonucleotides to knockdown SPG genes.

PUBLICATION:

- 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.

EDUCATIONAL ACTIVITIES:

1) "Zebraday", 30 Novembre 2016, Calambrone - Pisa
2) La genetica di nuova generazione per la distrofia muscolare di Duchenne e le altre miopatie, percorso diagnostico-terapeutico personalizzato. Napoli, 1 Aprile 2017
3) Dall'esoma per tutti al genoma di tutti. Verona, 19 Aprile 2017
4) Corso di Formazione: "Seminari di Genetica". IRCCS Stella Maris, Calambrone –Pisa, 15 Novembre e 13 Dicembre 2016 + 10 Gennaio e 20 Aprile 2017
5) La spettrometria di massa al letto del malato. CNR Pisa, 18 Settembre 2017
6) Corso di Formazione: "Sicurezza nel laboratorio di Medicina Molecolare". IRCCS Stella Maris, Calambrone –Pisa, 8 Maggio, 10 Luglio e 9 Ottobre 2017

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
Angelica D'Amore

Tutor
Prof. Federica Gemignani

Co-Tutor
Prof. Filippo M. Santorelli