CAUSATIVE NETWORK ANALYSIS OF THE PHENOTYPE-GENOTYPE CORRELATION OF THE TRANSTHYRETIN RELATED-AMYLOIDOSIS

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TTR amyloidosis (ATTR) is the most common condition among the hereditary systemic amyloidosis with an overall prevalence of 0.87–1.1 per 1, 000, 000 persons. *TTR* mutations cause peptide misfolding, resulting in amyloid fibrils formation that affect the peripheral nerves and heart. The disorder is featured by a wide range of clinical signs, including peripheral neuropathy (sensory and motor), autonomic neuropathy, gastrointestinal impairment, cardiomyopathy, nephropathy or ocular deposition. Notwithstanding these clinical signs might be scored in patients with different *TTR* mutations, the correlation between point mutations and phenotypes is not straightforward. The extreme heterogeneity could be related to the age of onset as well as the penetrance and the clinical display that could be ascribed to several genetic and/or epigenetic factors. Thus, the aim of this study will be the evaluation of different molecular machineries in order to identify the ATTR causative relationships among genetics, epigenetics, gene expression, and epistasis. This aim will be pursued through the following roadmap: epigenetic investigation, to determine the methylation pattern of CpG islands around *TTR* gene through EPIC methylation assay; mRNA quantification, to check *TTR* gene expression in ATTR patients blood through Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) method; test of epistatic effect of gene-gene interactions between *TTR* gene and loci selected for putative functional interaction (i.e., co-expression, experimental evidence and database information) by a computational approach.

The methylation analysis has been already carried out on 16 out of a whole sample of ca. 100 people. The DNA was extracted by standard protocol (Budowle et al., 2000). For the DNA methylation analysis, the EZ-96 DNA Methylation kit (Zymo Research, CA, USA) has been used to treat 500 ng of DNA from each sample with sodium bisulfite. DNA methylation has been quantified using the Illumina Infinium MethylationEPIC Chip (with over 850,000 methylation sites; Illumina Inc.).

This study is quite innovative in its combination of multiple molecular analyses based on systems biology and advanced statistics to investigate the genotype-phenotype correlation in ATTR. To the best of our knowledge, no previous studies have applied a similar approach to investigate a rare genetic disorder. Accordingly, this proposal hints pioneering aspects both in the investigation of TTR amyloidosis and in basic genetics of rare diseases. This strategy will move beyond the identification of new candidate mechanisms related to disorder etiopathogenesis in order to discover the causative networks responsible for the course of this disease.