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TESI DI DOTTORATO (Dott.ssa Eleonora Guagliano): "Modulation of cellular stress response in aging and neurodegenerative disorders**".**

Abstract

Emerging evidence indicated that oxidative stress has been implicated in mechanisms leading to neuronal cell injury in various pathological states of the brain, including neurodegenerative disorders such as Alzheimer's disease (AD) and aging **(1, 3, 8, 12, 36, 48, 66)**. Increasing evidence supports the notion that reduction of cellular expression and activity of antioxidant proteins and the resulting increase of oxidative stress are fundamental causes in the aging processes and neurodegenerative diseases **(1, 9, 12, 34, 96, 249)**. As one of the main intracellular redox systems involved in neuroprotection, the *vitagene system* is emerging as a *neurohormetic* potential *target* for novel cytoprotective interventions **(15, 17-18)**. In this study the importance of *vitagenes* in the cellular stress response and the potential use of dietary antioxidants in the prevention and treatment of neurodegenerative disorders, aging and systemic pathologies is discussed **(12, 17, 251)**. The strong evidence that the *vitagene network* operates as a defense system in the brain during oxidative and nitrosative stress open new perspectives in the treatment of neurodegenerative disorders and other age-related diseases **(2, 12, 14, 31, 249, 251)**. Taken together, these results suggest that the expression of Hsps increases with age and occurs as a consequence of redox state perturbation and this may have a role to limit the deleterious consequences associated with protein denaturation **(1, 3, 5, 8, 12)**. Relevant to this, we devoted our recent interest to the development of nutritional interventions, including carnosine, able to *target* redox-sensitive cytoprotective genes, called *vitagenes*, involved in the homeostatic control of so-called longevity-assurance processes **(2, 123,**

251). In this study we tested the hypothesis that neurotoxicity is an important primary mediator of injury in Meniere's disease and may be reflected in measurable increases in *markers* of *cellular stress response* and oxidative stress in the peripheral blood of patients with Meniere's disease. The search for novel and more potent inducers of *vitagenes* will facilitate the development of pharmacological strategies to increase the intrinsic capacity of vulnerable ganglion cells to maximize antidegenerative mechanisms, such as *stress response* and thus cytoprotection **(16, 18, 249, 251)**.

Neurofibromatosis type 1 (NF1) is one of the most common autosomal dominant neurogenetic disorder. The mechanisms underlying NF1 clinical variability remain poorly understood, probably because of the involvement of complex pathophysiology and multiple factors. Neurofibromatosis type 1 (NF1) is a common monogenic tumor-predisposition disorder with an autosomal dominant *pattern* of inheritance that arises secondary to *haploinsufficiency* of the tumor suppressor gene NF1 **(149, 284)**. The allelic heterogeneity of the constitutional NF1 mutation may be one of the factors explaining the disease phenotypic variability **(285)**. Studies provided evidence for a strong genetic component and suggested the involvement of unlinked *modifying genes* and perhaps also of the normal NF1 allele, in the variable expression of the disease **(285)**. The NF1 gene product, neurofibromin, is a cytoplasmic ubiquitously expressed protein of 2818 amino acids long, with structural and functional similarities to the mammalian GTPase-activating protein (GAP)-related protein family, a group of evolutionarily conserved proteins. Analysis of the predicted sequence of neurofibromin revealed that it likely functions as a negative regulator of Ras, a key intracellular *signaling* protein that is important for regulating cell growth and survival. Significant advances in the understanding of the pathophysiology of NF1 have been made in the last decade. Molecular testing is not indicated for the routine clinical care of patients with NF1, but can be helpful in individuals suspected of having NF1 (e.g. a young child with multiple café-au-lait spots and unaffected parents or a patient with a single *criterion*) or when prenatal or preimplantation genetic diagnosis is desired. Recently molecular testing for NF1 has become clinically available **(188, 190)**. Because of the large size of the NF1 gene and the lack of mutation *hotspots*, a *multi-step* detection protocol is preferred. Efforts to identify and characterise all NF1 gene mutations continue to present a considerable research and diagnostic challenge due to a combination of the large gene size, the absence of any localised mutation *clustering*, little evidence of repeat mutation and the wide diversity of mutation types observed. Furthermore, the presence of a number of highly homologous partial NF1 *pseudogene-like* sequences located throughout the human genome has increased the

complexity of PCR-based mutation analysis **(150)**. According to this evidence, data illustrated in the present study clearly reported a wide spectrum of different types of mutations, including pathogenetic *missense*, *nonsense*, small deletions/insertions, which lead to the synthesis of an aberrant protein product. The data presented in this study indicate that *splicing* in NF1 gene is extremely complex **(242-243)**. In fact, we identified heterozygous *missense* mutations in acceptor site or intraexonic causing codons *in frame* microdeletion or activating a *criptic* exon *splicing* site or causing exon *skipping*. All these mutations altered canonic mechanism of normal *splicing* causing aberrant transcripts formation **(242-243)**. To better investigate smaller NF1 *rearrangements*, we performed multiplex ligation-dependent probe amplification (MLPA) to screen of a number patients affected by NF1 for whom the presence of point mutations, small deletions and insertions had been previously excluded by sequencing analysis. Single and multi-exon NF1 copy-number changes, exclusively represented by NF1 gene entire deletion in our genetic test, were found in two different clinic case of patients with NF1. Our study enlarge knowledge and spectrum of NF1 gene mutations and underlie the importance of molecular-genetic tests on the basis also of the Neurofibromatoses clinic field characterization.