

DENATURING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (dHPLC)
SCREENING TO FIND OUT GENETIC POLYMORPHISMS IN THE SIRTUIN GENE
FAMILY RELEVANT FOR ALZHEIMER'S DISEASE SUSCEPTIBILITY

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Sirtuins are a class of enzymes with deacetylase activity conserved from bacteria to mammals. Mammal sirtuins are encoded by seven different genes (SIRT1 to SIRT7) and the resulting proteins are localized in different subcellular compartments: the nucleus (SIRT 1, -2, -6, -7), the cytosol (SIRT2) and the mitochondria (SIRT3, -4, -5). Sirtuins are widely involved in pivotal physiological processes as aging, apoptosis, stress response and several data suggest the involvement of SIRT1 and SIRT2 in aging-associated disorders like Alzheimer's disease (AD) or Parkinson's disease (PD). We have decided to search for frequent single nucleotide polymorphisms (SNPs) in sirtuins' genes that might be associated to sporadic AD risk modulation in the Italian population. We have selected a group of 96 independent subjects (48 AD and 48 matched controls). After PCR amplification of all the sirtuins' exons the presence of heterozygous polymorphisms has been investigated by dHPLC (Denaturing High Performance Liquid Chromatography). Aberrant chromatograms have been sequenced to identify the mutation. In AD patients we have found 17 intronic SNPs distributed in the seven sirtuin genes. Moreover, we have also identified some coding polymorphisms, most of them rare (for instance, Glu_Lys at codon 535 of SIRT1, Val_Ile at codon 207 and Pro_His at codon 262 of SIRT3). We have verified that sirtuins show a genetic variability between AD e matched controls in the Italian population,. We have now selected a panel of 35 most frequent SNPs (including our own) covering most of sirtuins' variability that will be genotyped in an enlarged case-control study (190 AD and 380 controls) to quantitatively assess their influence in sporadic AD risk modulation.