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Poster Abstract - A.08

EXPRESSION OF APOA-I HUMAN APOLIPOPROTEIN MUTANTS IN TOBACCO PLANTS

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Apolipoprotein A-I (apoA-I) is the predominant apolipoprotein of HDL particles. This is a 243-amino acid protein that binds and transports plasma lipid and increases cholesterol efflux from peripheral tissues in a process called "reverse cholesterol transport".

Variant forms of apoA-I are the precursor proteins in some forms of systemic desease known as amyloidosis. In all cases, a N-terminal fragment of apoA-I, ranging in length from 70 to 100 residues, is incorporated into amyloid deposits. To date, nine different mutations in the apoA-I gene have been linked to various forms of hereditary amyloidosis. One of these (named ApoA-I(L174S)) is a missense mutations that occur in position 174. It has been shown that the presence of the mutation causes the formation of amyloid fibrils composed primarily of a N-terminal-derived polypeptide of 93 amino acids (Apo-93).

So far, ApoA-I(L174S) and Apo-93 proteins are not express in any biological system. Biochemical and physiological studies are carried out only on fibrils extracted from the heart of a single dead patient. To facilitate the investigations, we have started a research project to express a suitable amount of these mutants proteins into tobacco cells. We cloned two DNA sequences (one coding for ApoA-I(L174S) obtained by site-specific mutagenesis from ApoA-I wilde-type, and the other coding for the N-terminal 93-amino acid fragment of ApoA-I) into two plant binary expression vectors. Each gene were put under the CaMV 35S promoter.

After co-cultivation with *Agrobacterium tumefaciens*, some transgenic tobacco shoots were obtained from both constructs. Molecular analysis on putative transgenic shoots showed the stable integration of the transgenes. Moreover, transcripts were detected by RT-PCR analyses. Recombinant Apo-93 polyptide fragment wasn't revealed by western blotting. Instead, recombinant ApoA-I(L174S) protein was detected. Morphological analyses of ApoA-I(L174S) and Apo-93 transgenic plants did not show any phenotypic variation in confront with control plants. Further experiments are under way to determine the yield of human recombinant ApoA-I(L174S) protein in transgenic tobacco plants and to develop new strategies to obtain recombinant Apo-93 in plant.