A. Abstract.

Machado Joseph disease (MJD), also called spinocerebellar ataxia 3 (SCA3), is a progressive autosomal dominant inherited neurodegenerative disorder. The gene (*MJD1*) has been mapped to chromosome 14q24.3-q32.1 (Kawaguchi, *et al.* 1994). An unstable expansion of CAG repeats in the *MJD1* gene, translated in a polyglutamine stretch at the protein level, has been discovered to be responsible for the mutation (Ikeda, *et al.* 1996). The function of the normal ataxin-3 protein is still unknown.

In this study, the subcellular localisation of the full-length ataxin-3 protein with a glutamine sequence in the normal range (Q_3KQ_{22}) was analysed in mammalian cell lines. Using two affinity-purified polyclonal antibodies raised against the N-terminal or C-terminal portion of ataxin-3, the protein was detected predominantly, but not exclusively, in the nucleus of COS-7 as well as neuroblastoma cells (SHSY5Y) by immunofluorescence and confocal laser scanning microscopy (CLSM). The distribution of the protein in these cellular compartments was confirmed by biochemical subcellular fractionations. Furthermore, CLSM revealed that the ataxin-3 protein present in the nucleus of neuroblastoma cells is associated with the inner nuclear matrix. In a lymphoblastoid cell line derived from a MJD patient, expressing the protein with a number of glutamines in the pathological range (Q55-84), ataxin-3 protein was detected mostly in the cytoplasm. Taken together the results, I have demonstrated that the localisation of the ataxin-3 protein is dependent upon the particular cell line studied and the length of the polyglutamine stretch is not important for the intracellular localisation of the protein.

In the second part of this work, a biochemical analysis of the ataxin-3 protein, expressed in *E. coli*, containing polyglutamine repeats in the normal (Q12-40) and pathological range (Q55-84) was performed. Electron microscopic analysis of protease-treated GST-ataxin-3 proteins with 42, 64, 71 and more than 150 glutamines showed the formation of insoluble aggregates with a fibrillar morphology reminiscent of β-amyloids in Alzheimer`s disease.

Cellulose acetate filter assay (CAFA) confirmed the presence of these aggregates. Moreover transient expression of ataxin-3 constructs with 131 and 165 CAG repeats in COS-7 cells led to the formation of peri- and intranuclear inclusions as determined by immunofluorescence microscopy.