Clinicopathological analysis of neurodegenerative proteinopathies

PhD thesis

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I. INTRODUCTION

The topic selection of this study would like to emphasize the importance of neurodegenerative diseases clinical neurological experienced in practice. Within neurodegenerative illnesses we deal in detail with clinicopathology of prion disease. One form of prion diseases (*PRNP* gene with E200K mutation genetic CJD) occurs more frequently in Hungary than it is expected on the bases on the literature. The clinician doctors can meet these cases more often and may face differencial diagnosis during the examinations. The neurodegenerative illnesses show progressive run off neurologic disorders, in which neuronal dysfunction and death can be observed during the course of the disease. These diseases characterized by anatomical and physiological sense affect neuronal population in the same system. It is the principle of selective vulnerability. The development of this disease process genetic, enviromental, endogenous and epigenetic factors play role.

Neurodegenerative diseases characteristic of the clinical and pathological sense of diversity. Despite this diversity in the pathogenesis of disease similar processes play role: cell death,

oxidative damage, abnormal conformation protein aggregation. Recently raised the possibility that neurodegenerative diseases pathologically altered proteins can spread cell to cell. The accumulation of abnormal proteins emphasizes the role of the proteins involved in the breakdown of cellular systems. Balance between the production and degradation of proteins, the degradation of abnormal proteins is essential for maintaining a healthy functioning.

Neurodegenerative diseases can be classified as the clinical symptoms based on the process involved in an abnormal conformation of proteins, and aggregating proteins according to cellular and anatomical distribution. Important proteins in neurodegenerative diseases: amyloid-ß, peptid, tau protein, α-synuclein, TDPP-43 protein, FUS and FET proteins, prion protein, polyglutamin.

This thesis focuses on the observations of prion diseases. Human prion diseases are kept the archetype of the conformational neurodegenerative diseases. Infectious nature of this diseases can separate from other neurodegenerative illnesses. Division of the human prion diseases can happen to etiology, clinicopathological phenotype, the prion protein gene constellation and the physico-

chemical features of prion protein. Prion disease can be obtained, sporadic or genetic. The genetic basis of human prion disease, we know that the development of disease substitution and insertion mechanisms are involved and polymorphisms are known. Encoding the prion protein genes at least 30 mutations are known. The best known and most studied mutation is E200K-129M haplotype. The most common form of human prion diseases is the sporadic Creutzfeldt-Jakob disease (CJD), it represents 80-85 % of all prion illnesses. Forms linked to gene mutatons (gCJD) are estimated to 10-15%. In Hungary the incidence rate of genetic forms are higher than that would be expected based on the literature. Among the genetic prion diseases the most common form is associated with E200K mutation. It is typical in prion disease pathomechanism that in the absence of normal conformation protein can not arise prion illness. Process leading to neuronal damage developes in a complex way and more front lines. The tissue damage is the result of more parallel to each other, in interaction or in succession following processes. Neuropathological features of prion disease: 1. progressive neuronal cell loss; 2. reactive gliosis; 3. vacuolation of neuropil (spongiform encephalopathia); 4. abnormal conformation prion protein deposits appearance in the brain. In some forms of prion

diseases the pathological variations of type and localizations are different. In the clinical diagnosis of prion diseases greatly assist the physician the magnetic imaging methods, EEG and liquor exams (markers of neuronal damage; eg: 14-3-3 protein). Each prion disease can be characterized by different clinicopathological phenotype. On the basis of modern molecular allocation the subtypes of the diseases codon 129 genotype (MM, MV, VV) and subtype of prion protein (PrP 1 and 2) can be separated . In mutation cases also applies to the patients' clinicopathological phenotype of the disease is influenced by polymorphism of *PRNP* gene codon 129 and the molecular feature of the abnormal prion protein (PrP^{Sc}).

II. OBJECTIVES

1. Analyses of differentialdiagnistic problems related to prion diseases.

In practice, well known the difficulties of clinical diagnosis of prion diseases. According to the WHO criteria system to set up a definitive diagnosis can be possible with neuropathological examination and/or immunohistochemistry and/or Western blot analyses verified with the protease-resistant PrP. One of the topics

imagery enumeration of the most common aspect of differentialdiagnostic problems.

2. **Determination of E200K mutation cases clinicopathological phenotypes.**

In Hungary the most common *PRNP* mutation is E200K. Our purpose is to analyse the clinicopathological phenotype of E200K mutation cases. Determine the predictive value of early clinical picture and compare them to the most frequent sporadic CJD molecular subtype (sCJDMM-1).

3. **Characterization of proteinopathies occuring in E200K mutation cases.**

Characterization of E200K mutation cases as complex proteinopathies. We analysed that beside the abnormal prion protein which form occurs tau-pathology, moreover A-ß and α-synuclein subsidence.

4. **Analysing of pyroglutamate A-ß oligomers in E200K mutation cases.**

In E200K mutation cases we analyzed whether Alzheimer disease compare to AßpE3 oligomers can also been seen in E200K gCJD cases, furthermore the ratio of these deposit of Aß are different comparing to Alzheimer disease.

5. Clarification and characterization of heredodegenerative etiology of disease.

Analyzing the hereditary neurodegenerative disorders we noticed a rare heredodegenerative medical image. We studied two brothers cases in the same family who have very similar symptoms and serious progress. Systematic neuropathological examination and genetic analyses was done to determine the clinical picture.

III. METHODS

Retrospective clinical data collection

In our work we used the retrospective clinikal data collection method when we studied such cases from materials of Semmelweis University Neuropathological and Prion diseases Reference Center which were proved as E200K mutations. Processing and assessing the avaible clinical data and instrumental test results were made by retrospective methods.

Immunohistochemical examination

Immunohistochemistry was used for testing proteinopathies to identify the pathological conformation proteins (PrP^{Sc}, tau protein, α-synuclein, TDP-43, AßpE3 oligomer, β-amyloid).

Prion genetical examination

Prion genetical examination to identify the PRNP gene mutation. The DNA was extracted from white blood cells.

The PRNP coding region was amplified with PCR technique, then using sequencing kit four overlapping segments were analyzed with fluorescenty labeled primers.

Statistical methods

The different age-groups of prion disease and the clinical course duration was compared with non-parametric test (Mann-Whitney U-Test). The groups did not have to assume a normal distribution. The individual symptoms and the frequency of test results were compared with Chi^2 test.

IV. RESULTS

Ad.1.

Analyses of differentialdiagnostic problems associated with prion disease. Pellagra encephalopathy as a possible differental diagnosis of CJB.

In our study we analyzed such cases which were sent to Prion Disease Reference Center based on clinical signs and tests they referred to Creutzfeldt-Jakob disease (CJD). We studied 59 cases, of which CJD has not been proved, pellagra encephalopathy (PE) was verified in 5 cases. The 59 cases were divided into five diagnostic groups.

Detailed clinicopathological characterization was done in PE cases. Based on the results of the tests and clinical symptoms could be classified as a probable case of sCJD group according to WHO criteria. We described the most common clinical differentialdiagnostic aspect of CJD and drew attention to the fact that PE can imitate the symptoms of CJD.

Ad.2.

Determination of E200K mutation cases clinicopathological phenotype.

In this work the most common *PRNP* mutation prion disease bound to E200K mutation cases was done to determine the clinicopathological phenotype. We made an attempt to determine the predictive value of early clinical symptoms. We studied 75 E200K mutation cases clinical phenotypes and the relationship between the syptoms and codon 129 genotype. According to our results based on the clinical signs –in the absence of family history – is not possible to distinguish the E200K genetic CJD cases from the most common sporadic CJD molecular subtype.

Ad.3.

Neuropathological characterization of the E200K mutation cases

In this work 39 E200K mutation cases clinical, neuropathological and biochemical process were performed. These CJD suspected cases were sent to the management system after the rating on the basis of clinical criteria. The postmortem neurological examination confirmed the CJD. We retrospectively collected and

summarized the details of the clinical and neuroradiological studies. We describe the clinical signs, the results of the instrumental examinations and the nature of the neuropathological lesions. We give a detailed description of prion protein, tau, α-synuclein pathology and amyloid-ß deposits. We conclude that in mutation cases of E200K often appear other proteinopathies. It draws the attention to the fact that *PRNP* can play the role in pathomechanism of other neurodegenerative illnesses.

Ad.4.
Examination of pyroglutamate Aß deposits in E200K mutation cases

As we often observed in addition to histological signs of prion disease characteristic of other neurodegenerative disease in E200K genetic CJD of younger patients (60), therefore, in the following study, we examined that the deposition of Aß in E200K cases whether the so called pyroglutamate- oligomers contains similar amount of Alzheimer disease. According to our results in Aß plaques there are pyroglutamate-Aß oligomers in E200K PRNP mutation cases, furthermore according to our preliminary observation the rate of this can be greater within Aß plaque than in Alzheimer disease.

Ad.5.

Clarification and characterization of etiology of heredodegenerative disease

(cerebrotendinosus xanthomatosis)

In our study we analyzed the case of two brothers where progressive neurodegenerative disorder causing metabolic disturbance was confirmed by genetic and biochemical test. We describe in detail the brothers' symptoms, the results of the studies and the found neuropathological lesions. We drew attention that in this case the lipid metabolism disorder is associated with the appropriate tau pathology in early aging of the brain.

V. CONCLUSIONS

Ad.1.

Pellagra encephalopathia (PE) as differential diagnosis of sporadic CJD.

In cases of prion diseases surveillance system five patients were found where in the background of rapid progression of dementia PE was confirmed by neuropathological examination. According

to our studies PE may arise as a differential diagnosis with patients who consume alcohol regularly in cases of rapid progression of dementia. In our tested material the following disorders were justified: neurodegenerative illnesses, tumor, cerebrovascular disease, inflammatory, metabolic background processes, mixed pathologies.

PE is probably more common disease than the diagnosis is established (this is due to the ratio of the autopsy).

Nevertheless, one case of many others was classified as possible sCJD according to WHO prion disease surveillance criteria before the neuropathological examination. It points to the importance of brain autopsy in case of rapid progression of dementia in prion disease surveillance system.

Ad.2.

E200K mutation associated with CJD clinical phenotype

Our results are from material of 75 patients (gCJB E200K mutation case) tests examination. Our results regarding to symptoms and their incidence do not differ significantly from those reported in the literature. We could not demonstrate a significant relationship between the symptoms and the tested data

as well as the codon 129 genotype. Namely clinical symptoms alone do not have predictive value for anyone to carry the mutation. In our study the clinical phenotype do not differ substantially in cases of E200K-129MM homozygous and E200K-129MV heterozygous. The same result was obtained when the duration of disease were divided into early and late periods and accordingly early and late symptoms were distinguished. The symptoms were not proved predictive value in E200K mutation carriers for codon 129 genotype. Comparison of the result obtained in E200K cases and sporadic CJBMM1 group, gCJD can not be isolated from sporadic cases based on age, duration of illness and clinic. Details of the said criteria and our study underline the importance of in vivo genetic testing. The results of genetic testing may assist, help to interpret the avaible clinical data.

Ad.3.

E200K mutations associated with gCJB: complex proteinopathies

Our study has proved that the abnormal PrP deposition or other neuropathological lesions and proteionopathies appear in various

forms in PRNP mutation cases. The most important result is that besides the prion protein other abnormal protein deposits were verfied.

Our results indicate that the mutant PrP metabolism and intracellular turnover interfere with other proteins production, processing and it leads to formation of abnormal deposits. Based on these E200K mutation can be an important model to understand what kinds of molecular interactions can be between proteins related to neurodegeneration.

Ad.4.

Examination of pyroglutamate Aß deposits in E200K mutation cases

Our studies have demonstrated that AßpE3 oligomers are present in E200K PRNP mutation cases as well as in AK. However it seems AK compared to E200K mutation cases oligomer molecules are present higher proportion in a given Aß plaque than in AK.

Ad.5.

Cerebrotendinosus xanthomatoses: neurodegenerative disorder showing pathological signs of premature aging

We presented two cases of CTX (brothers) clinical phenotype spectrum show compliance with cases reported in literature. During our work the older brother's cerebrum neuropathological process was performed. During tau-immunoreactivity detailed processing revealed that tau-pathology mainly affects the limbic system, and shows the advanced marks of argyrophil grain disease (AGD). AGD is known as age-associated disease.

Own publications related to the topic of the thesis

1. **Kapás I**, Majtenyi K, Törö K, Keller E, Voigtländer T, Kovacs GG. (2012) Pellagra encephalopathy as a differential diagnosis for Creutzfeldt-Jakob disease. Metab Brain Dis. 2: 231-5

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3. **Kapás I,** Katkó M, Harangi M, Paragh G, Balogh I, Kóczi Z, Regelsberger G, Molnár MJ, Kovacs GG. (2014) Cerebrotendinous xanthomatosis with the c.379C>T (p.R127W) mutation in the CYP27A1 gene associated with premature age-associated limbic tauopathy. Neuropathol Appl Neurobiol. 3: 345-50

Publication and presentation related not close to the thesis:

1. Vincze A, **Kapás I**, Molnar MJ, Kovács GG. (2010) Clinicopathological variability in neurodegeneration with brain iron accumulation. Ideggyogy Sz. 3-4: 129-35.