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The First Case of Genetic Creutzfeldt-Jakob Disease with the Rare Mutation R208H, Methionine/ Valine Heterozygous at Codon 129 of the Prion Protein Gene

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Abstract

The first case of Creutzfeldt-Jakob disease, methionine / valine heterozygous at codon 129 with a rare mutation at codon 208 (mutation R208H) of the prion protein gene is presented. Comparison to previously described patients with the same mutation R208H, but methionine or valine homozygous at codon 129 was performed. It revealed similarity in the duration of clinical manifestation, in histopatological lesions and in negative family history, Heterozygosity in codon 129 was characterized by similar clinical symptoms as described in 129MM cases, with dominant psychiatric symptomatology also in prodromal phase, by slightly higher age at death and markedly prolonged prodromal stage of the disease.

Keywords

Creutzfeldt-Jakob Disease, Duration, Mutation R208H, Polymorphism M129V

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1. Introduction

Molecular genetic analysis of the prion protein gene (PRNP) revealed that all patients affected with familial Creutzfeldt-Jakob disease (CJD) have point mutation (insertion/deletion) i.e. they belong to a group of genetic CJD (gCJD). Nevertheless, genealogical studies of gCJD cases demonstrate that not all gCJD patients have a positive family history (1, 2) the range in individual mutations is 12-88% (3) and also their clinical course reminds sporadic CJch (sCJD).

A typical representatives of such „sporadic-like“ gCJD are patients with the most frequent mutation E200K, but also patients with rather rare mutation R208H. In mutation R208H, known since 1996 (4) the adenine substitution for guanine at the second position of codon 208, which results in substitution of histidine for arginine was described. All previously reported patients with PRNP mutation R208H ($gCJD^{R208H}$) were homozygous at codon 129. Four cases were methionine (Met) homozygots (4-8) and two were valine (Val) homozygous (9,10). The first heterozygote Met/Val case of $gCJD^{R208H}$ is now reported and compared with heterozygous patients carrying the same mutation.

2. Patient and Methods

The patient was a 73- years-old woman, graduated in high school, lifelong working as book-keeper, retired. She had a personal history of arterial hypertension, thyreoidectomy (1964), hysterectomy, ovarectomy and cholecystectomy (1998). She was treated in psychiatry for relapsing depressive syndrom since 1998 (cca 9 years).

In September 2006 she developed behavioural abnormalities (nocturnal confusions and walking), apathy, withdrawal. In January 2007 she was hospitalized for obvious intellectual decline, rapid memmory loss, insomnia, lasting cca 2 months. In admission she was depressed, anxious, disoriented in time.

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Gradually she developed dysarthria, mild gait disturbances, spastic quadruparesis and myoclonus.

CT revealed status lacunaris bilateralis in the capsula interna. EEG showed slow activity. No P 14-3-3 or genetic testing were done during the hospitalisation. She died of pneumonia in March 2007, 7 months after the onset of clinical signs.

Her father died 44- years old (renal failure), her mother died at the age of 83 years (heart attack). One brother died as 2 years old. She has 3 healthy (53, 48, 41 years old) married children. No neuropsychiatric disease was reported in the family. All relatives refused genetic testing (Fig.2).The working diagnosis was sporadic CJD. Postmortal differential diagnosis was performed on formaline fixed and frozen brain excisions.

Histopathological and immunohistochemical analyses were done as described previously (2). Detection of protease resistant prion protein (PrP^{res}) was performed by monoclonal antibodies (MoAb) 3F4 (DAKO) and 6H4 (Prionics).

Molecular genetic testing: DNA was extracted from frozen brain by standard method and amplified (PCR reaction performed with thermal cycler PTC-200 from MJ research). The complet open reading frame of PRNP was analysed by sequencing the PCR product using the Big dye terminator 3.1 sequencing kit in ABI prism 3130- XL sequencing machine (Applied Bio-system). For PCR reaction was used primer pairs with M13 plasmid gene:

PRNP for 5´ -
TGTAAAACGACGGCCAGTTCAACATAAATATGGGAC TCTGACG

PRNP rev 5´-
AGCGGATAACAATTTCACACAGGAGCCTATCCGGGA CAAAGAG

And for sequencing were used M13 plasmid geneprimers :

For 5´- TGTAAAACGACGGCCAGTTCA

Rev 5´- AGCGGATAACAATTTCACACA

Immunobloting: Brain homogenates (10%) were prepared in PBS from the frozen brain with or without proteinase K digestion, denaturated with sodium dodecyl sulphate and separated by polyacrylamide gel electrophoresis; separated proteins than transferred on to nitrocellulose membranes. PrPres was detected using MoAbs 3F4.

3. Results

Histopathological examination showed: Cortical atrophy, neuronal loss, diffuse, moderate spongiform changes in all layers (Fig. 1A.), most developed in the occipital region, hypertrophy, proliferation and clasmatodendrosis of astrocytes (Fig. 1B.) more severe in the gray matter.

In the cerebellum was observed mild loss of granular as well as Purkinje cells and incipient spongiosis in the molecular layer. Immunostaining (3F4, 6H4) shows synaptic and fine granular positivity in the molecular layer and granular positivity mainly in glomeruli cerebellares in the granular layer (Fig.1C.).

Western blot analysis has shown type 1 PrP^{res} with dominance of monoglycosylated band (Fig. 3).

Molecular genetic analysis revealed the R208H mutation coupled with heterozygosity Met/Val at codon 129 (Fig.4).

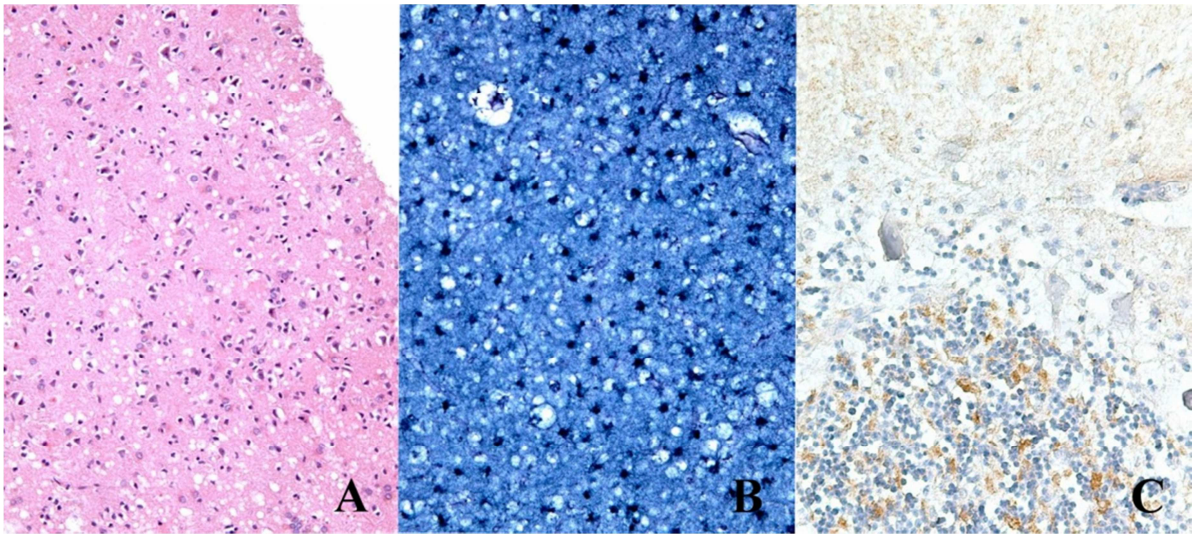

Fig. 1. A) Neuronal loss and diffuse, moderate cortical spongiform changes. (H& E); B) Hypertrophy, proliferation and clasmatodendrosis of cortical astrocytes (Cajal, modification for paraffin slides); C) Immunostaining (3F4) shows synaptic and fine granular positivity in the molecular layer and granular positivity in the granular layer.

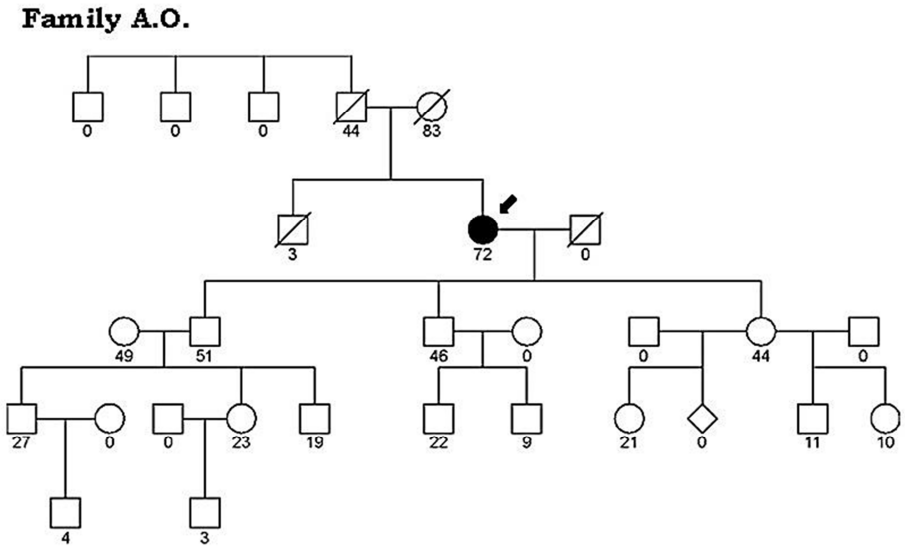

Fig. 2. The pedigree of the patient with gCJDR208H heterozygous at codon 129 of PRNP. All relatives refused up to now a genetic testing.

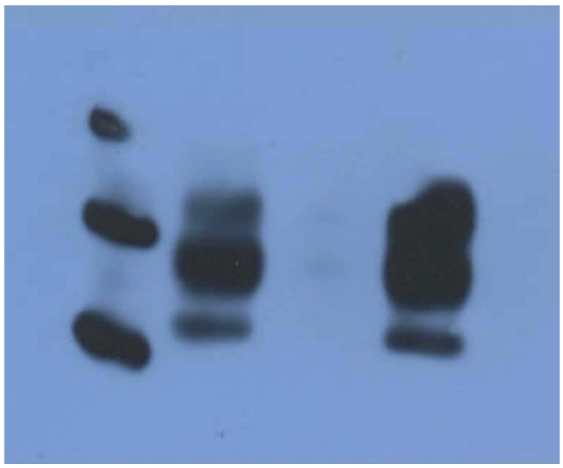

Fig. 3. Western blot of the PK treated brain homogenates: gCJDR208H (lane 1), control, not CJD patient (lane 2), sCJD subtype MM1 (lane 3).

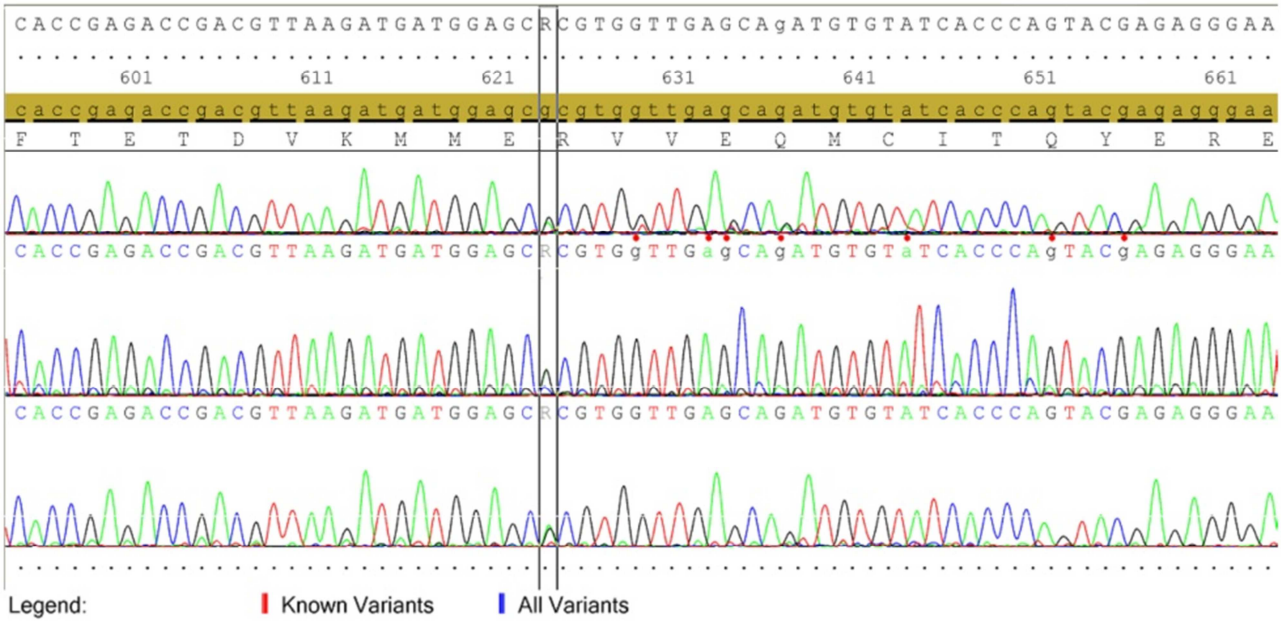

Fig. 4. Sequence of the PRNP gene with mutation at codone 208 in patient with confirmed diagnosis of CJD.

4. Discussion

More than 30 disease-specific point mutations of the PRNP have been described in human genetic prion diseases (PD). In genetic PD, single nucleotide mutations in the gene encoding prion protein (PRNP) can increase the likelihood of aggregation and neurodegenration (11), but the mechanism by which mutations cause the disease is not yet fully understood. Salt bridges between residues in different parts of a protein structure are involved in stabilization of its tertiary structure and six known PD-specific mutations can affect such salt-bridge interactions. According to Zuegg and Grady (12), also in the mutation R208H the loss of salt bridge between E146 and R208 may facilitate the conversion to PrP^{res}, but studies of Bamdad and Naderi-Manesh (13) indicate that the early stages of conformational changes are not associated with the breaking of the salt bridge.

Cardone et al. (14) analysing the quantitative ratio of mutant/wild type of PrP^{CJD} in R208H and another rare V210I gCJD found that the mutant allotypes moderately exceeds the amount of wild type counterpart, suggesting the pathogenic effect of both mutations. For V210I they noted that observed ratios were not affected by the M129V polymorphism.

The mutation R208H is rare, but the number of $gCJD^{R208H}$ patients is slowly increasing. Four reported cases (4-8) are Met/Met homozygous at codon 129, two are homozygous for Val/Val (9, 10). This "disproportion" reminds the prevailing methionine homozygots observed in all forms of CJD (2, 15, 16, 17), as well as in vCJD (18), indicating increased susceptibility to PDs and described also in the general population of certain countries (17, 19).

Due to small numbers, comparison of hetero- and homozygous $gCJD^{R20H}$ patients provides information of limited value. The mean age at death shows slightly increasing values from methionine homozygous to heterozygous patients (Met/Met 58 yrs., Val/Val 61,5 yrs., the single Met/Val case 73 yrs.). The duration of clinical stage showed no difference between homozygous subgroups (7,5 Met/Met, 7 months Val/Val), or in comparison to the heterozygous patient (7 months). Striking difference was found only in the mean prodromal stage (9,3 months Met/Met, 22,5 months Val/Val and 108 months Met/Val). Western blot analysis disclosed type 1 protease resistant prion protein in Met/Met subgroup, type 2 in Val/Val cases and type 1 in Met/Val patient (according to Parchi et al. (20)).

All compared patients characterized: progressive memory loss and cognitive decline, ataxia and gait disturbances. Besides that in Met/Met subgroup were frequently seen anorexia, insomnia, agitation, agressivity, hallucinations, involuntary movements, tremor, dysarthria, while in Val/Val cases behavioural changes, apathy, depression and walking disturbances.

In presented, Met/Val heterozygous patient, psychiatric symptoms appears to be more dominant in prodromal as well as in the clinical stage and instead of agitation, the clinical course was characterized by apathy, withdrawal, passive motionless pose together with pyramidal and extrapyramidal symptomatology.

In summary: The first heterozygous (methionine/valine) gCJD patient with PRNP mutation R208H ($gCJD^{R208H}$) is presented. The clinical course supports the predominantly psychiatric phenotype with prodromal phase, preceding 9 years the clinical onset of CJD. Heterozygosity Met/Val in $gCJD^{R208H}$ patient was characterized by neurological symptoms similar as described in 129MM cases, psychiatric symptomatology dominant also in prodromal stage, by slightly higher age at CJD onset and markedly prolonged prodromal stage of the disease, indicating the influence of the M129V polymorphism also in gCJD with the mutation R208H.

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