Mutations in the neurofilament light chain gene (NEFL) — a study of a possible pathogenous effect

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Neurofilaments (NFs) have been shown to be involved in the molecular pathology of numerous neurodegenerative human disorders. Recently a set of mutations in the neurofilament light gene (NEFL) was reported in patients suffering from axonal and demyelinating forms of Charcot-Marie-Tooth disease (CMT1 and CMT2). Although a few of the NEFL gene sequence variants have been shown to be rather pathogenous mutations than harmless polymorphisms, the status of some of these variants remains unclear. The aim of this study was to analyse a potential pathogenous effect of the mutations in the NEFL gene identified in CMT affected patients.

key words: pathogenous effect of a mutation, neurofilaments, CMT2 disease

INTRODUCTION
Neurofilaments (NFs) are widely expressed neuronal intermediate filament proteins. According to their molecular weight, NFs have been divided into three groups: neurofilament light (NF-L) (68 kDa), neurofilament medium (NF-M) (145 kDa) and neurofilament heavy (NF-H) (200 kDa) [6].

Neurofilaments are assembled by the co-polymerisation of NF-L, NF-M and AF-H. Their ability to co-polymerise results from formation of coiled-coil dimers involving a common central domain of approximately 310-amino acids which share three subunits [2].

Although the function of NFs in neurons remains unclear, it is believed that neurofilaments are major constituents of neuronal cytoskeleton, which controls the axon calibre. Thus, the hypotrophy of axons has been shown both in the neurofilament-deficient mice and in the Japanese quail mutant lacking NF-L due to the nonsense mutation in the NEFL gene [12, 18].

The abnormal neurofilament deposits have been described in amyotrophic lateral sclerosis, Parkinson’s disease, Alzheimer’s disease, Lewy body dementia and Guam-parkinsonism [9].

Although the neurofilament deposits have been detected in many neurodegenerative disorders, the “primary lesion” might not be associated with mutations within the neurofilament coding genes.

Accumulation of neurofilaments in the axonal form of Charcot-Marie-Tooth disease (CMT2) was reported for the first time in 1985 [15].

The first Gln333Pro mutation in the NEFL gene has been detected in a large six-generation CMT2 family originating from Moldavian Republic (Russia) [10].

By 2004, eleven mutations in the NEFL gene have been reported [http://molgen-www.uia.ac.be/CMTMutations].
The aim of this study was to analyze a possible pathogenetic effect of the mutations in the NEFL gene.

**MUTATIONS: P8R AND Q333P**

The Q333P mutation was the first mutation reported in the NEFL gene in humans [10]. The Q333P substitution is located in highly conserved coil 2b of the rod domain of the NEFL gene. Glutamine at codon 333 is present even in Xenopus laevis. A high degree of conservation indicates an important biological function of the codon 333. In fact, the rod-domain of the protein, where Q333P mutation is located, is believed to be responsible for neurofilament assembly [1].

The P8R substitution was reported for the first time in a three-generation CMT family of a Belgian origin [3]. The second family (one affected parent and three affected children) harbouring the heterozygous P8R substitution was of an American origin [8].

Although twelve mutations in the NEFL gene have been reported up to date, only two substitutions, i.e. Q333P and P8R, have been analysed for their potential pathogenetic effect in transiently transfected cells. Both mutations have been shown to abolish the ability of NEFL proteins to self-assemble into a filamentous network. Instead of filamentous network in the Q333P and P8R transfected cells, small (Q333P) and huge (P8R) aggregates were observed. Interestingly, the mutant NEFL proteins cotransfected with wild-type NEFL gene completely disrupted the formation of the not mutated proteins.

In addition, the Q333P and P8R mutated NEFL proteins have been shown to abolish the formation of the NEFL/NEFH filamentous network. The formation of the filamentous network with vimentin in cells expressing Vimentin was also impaired in both Q333P and P8R mutants.

In contrast to vimentin, the Q333P and P8R mutated NEFL did not affect the microtubule network [13]. Although the Pro8Gln and Pro8Leu (reported in two CMT families) mutations are located at the same codon as Pro8Arg mutation, their possible pathogenetic effect is not comparable with that of P8R substitution.

**MUTATIONS: Pro22Thr, Pro22Ser, Asn97Ser, Ala148Val, Glu89Lys AND Glu397Lys**

The Pro22Thr substitution results in a change of a hydrophobic amino acid to the hydrophilic Proline.

Although the Pro22Thr substitution represents a rather powerful chemical change, this mutation was detected so far in two CMT patients from one family. Electrophysiological examination confirming CMT diagnosis was performed only in one patient and the type of CMT was not specified. The Pro22Thr was found in two CMT affected subjects of a Japanese origin. In the control group consisting of 248 healthy individuals of a Japanese origin, the Pro22Thr substitution was not found.

Unfortunately, there are no data describing segregation of the Pro22Thr substitution with the CMT phenotype.

Similarly, the cellular and tissue effects of the Pro22Thr substitution are not known since neither sural nerve biopsy nor functional analysis was performed [17].

The status of the Ala148Val and Asn97Ser substitutions detected in two Japanese CMT affected patients also remains unclear.

Their pathogenetic nature may be only supported by the absence of Ala148Val and Asn97Ser substitutions in the 248 healthy individuals of a Japanese origin [17].

The Glu89 substitution found in a patient of a Mexican-American origin was not detected in 130 chromosomes from the 65 healthy Caucasians [10].

In contrast to the poorly documented Pro22Thr mutation, the Pro22Ser substitution was found in a large five-generation family originating from Slovenia. The Pro22Ser was detected in nine CMT affected family members showing complete segregation with the CMT phenotype. Electrophysiological examination performed in nine CMT affected family members confirmed the diagnosis of CMT2 [5].

The Glu397Lys substitution was identified in a four-generation CMT family. The Glu397Lys substitution was shown inherited as autosomal dominant trait in 4 CMT affected members of this family. Electrophysiological examination confirmed the CMT2 diagnosis in four affected members of the family [19].

In conclusion, only two mutations out of six, i.e. Pro22Ser and Glu397Lys, have been shown to segregate with CMT phenotype. Similarly, CMT2 diagnosis was confirmed by histopathologic studies of sural nerve biopsy only in two CMT patients harbouring the Glu89Lys and Glu397Lys mutations.

**Glu528Del SUBSTITUTION**

A PATHOGENOUS MUTATION OR HARMLESS POLYMORPHISM?

The Glu528del was detected for the first time in a Bulgarian patient with an unspecified CMT phenotype. Although the Glu528del substitution was found in one patient, it was considered inherited in an autosomal dominant mode since other unexamined members of the family were reported to have CMT.
The Glu528del substitution was not found in the control group consisting of 65 healthy Caucasians [10]. In 2004, the Glu528del was reported in four CMT affected patients from three Japanese families. In one family the Glu528del mutation was detected in two brothers.

Unfortunately, analysis of the control group consisting of 248 healthy Japanese disclosed a heterozygous Glu528del mutation with a frequency (f) of 0.018.

In addition, the Glu528del substitution was detected in patients suffering from familial amyloid neuropathy (f = 0.025), spinal muscular atrophy (f = 0.016), amyotrophic lateral sclerosis (f = 0.031) and spinocerebellar ataxia types 1 (SCA) (f = 0.01) [16].

Thus, the Glu528del mutation was detected in healthy individuals, CMT affected patients and subjects with other neurodegenerative disorders.

Since the neurofilament deposits were observed in neurones in patients suffering from ALS and other neurodegenerative disorders, the role of the Glu528del in their pathogenesis remains unclear.

Although the functional analysis of the effects of Glu528del mutation may shed some light on its potential pathogenous nature, its character will still be unclear.

Mutations of the NEFL gene located in the tail of the NEFL protein seem to be rather harmless.

Yoshihara has reported on a Glu530del in five CMT affected patients and nine healthy individuals [17]. The other mutation, i.e. D469N, located in the tail of the NEFL protein, although found in the ALS affected patients, did not segregate with the ALS phenotype [14]. The Thr118Met mutation located in the Peripheral Myelin Protein 22 gene (PMP22), however, was shown to induce the apoptotic-like phenotype in the HeLa transfected cells and was also found in healthy individuals originating from northern Sweden with a frequency of 1.9% [4, 11].

CONCLUSIONS

In this paper, the possible pathogenic nature of mutations in the NEFL gene has been discussed. Knowledge of the pathogenic character of the sequence variants of the NEFL gene is important not only for the understanding of their role in the pathogenesis of CMT disease and other neurodegenerative disorders, but also for the practice of medical genetics.

In fact, knowledge of the nature of NEFL substitutions determines the diagnosis, genetic counselling and possible rationale for the prenatal diagnostics in CMT diseases.

ACKNOWLEDGEMENTS

I wish to thank Prof. Irena Hausmanowa-Petrusewicz for critical reading of the manuscript.

The study was supported by the Polish State Committee for Scientific Research (grant N. P05B, mapping of a locus of Charcot-Marie-Tooth type 2J disease).

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