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MINIREVIEW

Advances in Charcot-Marie-Tooth Disease Research: Cellular Function of CMT-Related Proteins, Transgenic Animal Models, and Pathomechanisms

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The First Workshop of the European Consortium on Charcot-Marie-Tooth (CMT) disease brought together neuroscientists, molecular and cell biologists, neuropathologists, neurologists, and geneticists with a common interest in the understanding of the fundamental mechanisms that underlie the pathogenesis of CMT. The interdisciplinary group of 25 expert scientists discussed recent advances in (i) molecular genetics and histopathology of CMT, (ii) development of suitable animal models, (iii) understanding of the cellular function of CMT-related proteins, and (iv) studies using nerve biopsies from CMT patients. In this minireview, we summarize the key findings presented and discuss their impact on CMT research.

GENETICS AND HISTOPATHOLOGY

Molecular genetics

CMT1 is genetically heterogeneous and has been shown to be associated with at least four distinct chromosomal loci, providing the genetic rationale for a classification of CMT1 disease forms (for review see De Jonghe et al., 1997). CMT1 genes have been identified on chromosomes 17 (CMT1A, hereditary neuropathy with liability to pressure palsies (HNPP)), 1 (CMT1B), and X (CMTX). The most common form, CMT1A, is predominantly associated with a 1.5-Mb duplication that appears to arise from unequal recombination at repeat sequences of insect origin and includes the gene of the peripheral myelin protein PMP22 (Suter & Snipes, 1993). Deletion of the same chromosomal region that is duplicated in CMT1A is the most frequent genetic defect causing HNPP. The duplication and deletion frequencies in CMT1 or HNPP patients were estimated by the European CMT Consortium to be approximately 71 and 84%, respectively (Nelis et al., 1996).

Thus far, 12 point mutations (9 missense, 1 frame shift, and 2 splice site mutations) have been identified...
within regions of the PMP22 gene that encode potential transmembrane domains giving rise to three distinct disease phenotypes (CMT1A, Dejerine-Sottas Syndrome (DSS) and HNPP). Thirty-six different mutations (25 missense, 3 nonsense, 5 frame shift, and 3 deletion mutations) within the major myelin protein Po (MPZ) gene on chromosome 1 have been linked to CMT1B (28 mutations), DSS (7 mutations), and congenital hypomyelination (1 mutation). The extracellular domain of the Po protein is predominantly, but not exclusively, affected (Warner et al., 1996).

Genetically, DSS appears not to be a distinct disease entity, but is rather caused by mutations of either the PMP22 or the Po gene (V. Timmerman). In CMTX, 58 different mutations were found within the connexin32 (Cx32; GJB1) gene, comprising 43 missense, 7 nonsense, 4 frame shift, 2 deletion, and 2 combined deletion-frame shift mutations (E. Nelis). Other investigators have collected more than 80 mutations within the coding region of Cx32 (Scherer et al., 1997). N. Haites reported on the interesting finding that 10 of 36 analyzed X-linked CMT families carried noncoding region mutations. Thus far, mutations were detected (a) in the 5′-untranslated region of exon 1B, creating a potential donor splice site or a translational start site, and (b) in the P2-promoter region of Cx32, possibly altering the rate of transcription (Söhl et al., 1996). By analogy, it is anticipated that noncoding region mutations in the PMP22 and/or Po genes might also occur in CMT1A and CMT1B families.

In addition to CMT1, rare CMT forms including recessive CMT4 (recessive HMSN), CMT2A-D (HMSN II), axonal motor-sensory neuropathy with deafness and mental retardation (CMT2X), the spinal form of CMT (HMSN II, V), hereditary neuralgic amyotrophy (HNA), and hereditary sensory neuropathy (HSN I, II) have been linked to at least 15 different chromosomal loci (V. Timmerman).

**Histopathology**

In CMT1A sural nerve biopsies, the profile of myelinated fibers generally indicates that a significant proportion of large and small myelinated fibers are lost. However, there are remarkable differences between patients with a PMP22 duplication and those carrying point mutations with respect to g-ratio, total transverse fascicular area (TTFA), and onset and degree of onion bulb formation in young patients (point mutations \( \gg \) duplication; A. Gabreëls-Festen, M. Schröder; Gabreëls-Festen et al., 1995). The morphological criteria for HNPP include tomacula, some de- and remyelination leading to a few onion bulbs, variable axon loss, and normal g-ratio of myelinated fibers (Windebank, 1993). However, similar to CMT1A, the phenotype of HNPP frame-shifting mutations in PMP22 differs from the deletion phenotype with respect to the amount of onion bulbs and increase in TTFA (point mutations \( \gg \) deletion). On the other hand, CMT1B could clearly be divided into two morphologically distinct groups, one group featuring mainly uncompacted myelin and the other group containing many focally folded myelin sheaths (A. Gabreëls-Festen). In some CMT1B sural nerve biopsies, the frequency of tomacula was even higher than in HNPP. It will be fascinating to determine whether morphologically distinct phenotypes can be correlated with specific positions of mutations in the Po gene. Even in CMT1A, rare tomacula can be found. Thus, it became evident that morphological criteria seem to be less reliable in CMT1A, CMT1B, and HNPP than previously anticipated and the suggestion was made to also include motor nerve autopsies of genetically diagnosed patients into morphological analyses. Furthermore, it was pointed out that part of the variability observed in the morphological spectrum of CMT1A (e.g., in myelinated axon profiles) may be due to age-dependent alterations (M. Schröder; Thomas et al., 1997).

**Transgenic Animal Models**

Several groups reported on recently established transgenic and gene knock-out rodent models concerning PMP22, Po, Cx32, MAG, and Krox-20.

**PMP22**

U. Suter introduced several mouse models that cover a range of PMP22 gene dosages including animals entirely devoid of PMP22, others carrying only one copy, and others with an overdose of 16 or 30 copies of the PMP22 gene (Adlkofer et al., 1995, 1997; Magyar et al., 1996; Huxley et al., 1996). The findings indicate that impairment of myelination depends on PMP22 gene copy numbers. In homozygous PMP22\(^{-/-}\) mice, the onset of myelination is retarded and abundant tomacula develop during early postnatal development followed by severe demyelination, axonal loss and functional impairment. PMP22\(^{+/+}\) mice are less affected but develop morphological features that make these animals an excellent model for HNPP. Conversely, the marked increase in PMP22 gene dosage
caused an almost complete loss of myelin and the formation of numerous basal lamina onion bulbs, whereas moderately enhanced PMP22 gene dosage (approx. three-fold) in a transgenic rat model, as reported by K. Nave (Sereda et al., 1996), led to hypomyelination and onion bulb formation that closely mimic that in the human CMT1A phenotype. The rat model further demonstrated that myelination of sensory and motor nerves is differently affected. While motor fibers of the ventral horn are severely hypomyelinated, dorsal root sensory fibers are much less affected.

Po

R. Martini reported on recent studies with homo- and heterozygous Po-deficient mouse mutants that develop dysmyelinating and demyelinating phenotypes characteristic of either severely affected DSS or the mild CMT1B neuropathy (Giese et al., 1992; Martini et al., 1995). While Po−/− mice develop myelination abnormalities including altered myelin compaction, hypomyelination, and degeneration of myelin and axons early in life, heterozygous Po-deficient mice initially develop quite normal myelin but show signs of demyelination and remyelination from postnatal week 16 onward. Similar to the PMP22-transgenic rats, motor nerves of heterozygous Po-deficient mice were more severely affected than sensory nerves.

Connexin 32

A Cx32 knock-out mouse was introduced by K. Willecke as the first animal model for CMTX (Nelles et al., 1996; Anzini et al., 1997). Since the mutants develop liver tumors, it was suggested that Cx32 may serve a yet unknown tumor suppressor function. Comments of neurogeneticists and neuropathologists, however, pointed out that CMTX patients do not suffer from an increased incidence of tumors. As in CMTX patients, neurological symptoms develop in these animals rather late in life. Electron microscopic examinations revealed that the periaxonal cytoplasm is unusually enlarged in both motor and sensory nerves, possibly due to lack of efficient connections between adaxonal and abaxonal cytoplasm through Schmidt–Lanterman incisures and "paranodal loops." In addition, complex interdigitations of axon and Schwann cell membranes have been observed. Schwann cell onion bulb formation was much more prominent in motor nerves compared to sensory nerves.

Myelin-Associated Glycoprotein (MAG)

M. Schachner presented a MAG gene knock-out mouse model that is mainly characterized by oligoden-droglia and myelin abnormalities in the CNS, but also shows degenerating myelin profiles and Schwann cell onion bulbs in the PNS of adult mice (Montag et al., 1994; Fruttiger et al., 1995).

In summary, pathological alterations typical for particular genetic defects could be characterized, while dysmyelination- and demyelination-induced onion bulb formation may rather represent less specific histopathological changes possibly reflecting common secondary phenomena.

Krox-20

G. Levi reported on a Krox-20 gene knock-out mutant mouse suffering from slight trembling, lack of PNS myelin sheath, and deficiencies in myelin gene expression (Topilko et al., 1994). Since Schwann cells in Krox-20-deficient mice still contact and enwrap the axons by approximately 1.5 turns, it was suggested that this Zn-finger transcription factor may regulate subsequent steps in myelination. Comparison of the expression of Krox-20 and Krox-24 in rodents demonstrated an inverse regulation of these related transcription factors during normal PNS development and myelination (upregulation of Krox-20, downregulation of Krox-24 in myelinating Schwann cells) as well as after nerve lesion (reversed patterns of Krox-20 and Krox-24 regulation). Additional studies on human CMT1A pathology revealed that Krox-24 is abnormally upregulated and often coexpressed with Krox-20 in onion bulb Schwann cells.

CELLULAR FUNCTION OF CMT-RELATED MYELIN PROTEINS

The presenters of this session focused on recent biochemical, molecular, and cell biological studies to characterize the biological function of known CMT-related myelin proteins as well as potential new myelin disease proteins.

PMP22

H. W. Mueller reported on the experimental proof for a growth modulatory function of PMP22 using retrovirally transduced rat Schwann cell and fibroblast cultures expressing altered levels of PMP22 (Zoidl et
al., 1995, 1997). While moderate overexpression of PMP22 retards progression of both cell types into and through the cell cycle, a small but significant proportion of fibroblasts, but not Schwann cells, show typical signs of apoptotic cell death. These results indicate that PMP22 participates in fundamental mechanisms of cell growth and further demonstrate that there are cell type-specific differences in the ability to tolerate enhanced levels of PMP22. In agreement with these findings, C. Schneider provided further evidence for a link between PMP22 overexpression and programmed cell death using an expression plasmid microinjection paradigm (Fabbretti et al., 1995). In contrast to PMP22, the overexpression of Po protein causes no apoptosis, indicating marked differences in growth-related cellular functions of myelin proteins. These data indicate an important role for PMP22 in regulating cell proliferation and death on one hand and cell differentiation and myelination on the other.

**Po**

P. Bolhuis presented models on the putative effects of various Po mutations on protein structure and discussed the possible impact on protein transport and function (Shapiro et al., 1996). A functional test system for Po mutants in *Drosophila* Schneider cells was introduced by B. Rautenstrauss.

**Connexin 32**

R. Dermietzel summarized work of his group on the localization and expression of Cx32 and Cx45 in the PNS (Spray and Dermietzel, 1995). He showed that Cx32 may participate in reflexive gap junctions at nodes of Ranvier and Schmidt–Lanterman incisures, thus establishing intracellular short cuts between paranodal loops and myelin layers. These gap junctions might shorten the time and distance required for the perpendicular diffusion of small molecules through the myelin sheath roughly by a factor of 1000 (Scherer et al., 1995). Due to its localization, Cx45 is suspected to be involved in intercellular coupling between two adjacent Schwann cells at the nodal region.

L. Barrio presented a physiological study on the effect of CMTX-type and other Cx32 mutations on the coupling of paired oocytes expressing the protein variants (Bruzzone et al., 1994). The degree of functional coupling strongly correlated with the type of mutation and the length of the C-terminal portion of the molecule, but specific gene mutations and physiological data have yet to be correlated with the severity of CMTX disease phenotypes.

**New Potential CMT-Related Myelin Proteins**

Following the known CMT-related proteins, C. Gillen and M. Frank reported on the full-length cloning, expression, and localization of plasmolinip and rMAL/MVP-17, respectively (Gillen et al., 1996; Schaeren-Wiemers et al., 1995). Encoding tetraspan myelin proteins, these two genes are potential candidate genes for CMT or related neuropathies (Magyar et al., 1997). Both plasmolinip and rMAL are predominantly expressed in Schwann cells of the PNS and in the kidney, but to a lower degree also in oligodendrocytes of CNS white matter. The specific functions of these proteins are unknown.

**PMP22 EXPRESSION IN CMT1A AND HNPP**

In leaving animal and cell culture models, the last session was devoted to observations on CMT1A and HNPP nerve biopsies and human Schwann cell cultures. O. Hanemann described the developmental changes in gene expression (e.g., for PMP22, p75-NGF receptor, NCAM), myelination (from myelinated to demyelinated state), cell proliferation, and onion bulb formation in sural nerve biopsies of young and adult CMT1A patients with confirmed PMP22 gene duplication (Hanemann et al., 1996, 1997). He emphasized that prior to apparent histological changes in myelin profiles, Schwann cells acquire an abnormal pattern of gene expression, indicating the early manifestation of a pathological phenotype. The abnormal molecular differentiation is considered as a consequence of the specific influence of PMP22 on fundamental mechanisms of cell growth as already demonstrated *in vitro* (see above). Based on his results, O. Hanemann presented a hypothesis of sequential stages in pathogenesis of CMT1A neuropathy leading from the initial PMP22 gene duplication via altered levels of PMP22 expression, modulation of growth behavior, and abnormal molecular differentiation of the Schwann cell to secondary events such as disturbance of myelin maintenance, stability, and/or turnover causing demyelination and onion bulb formation. Since the secondary histopathological events are frequently observed in human CMT1 and in transgenic animal models as demonstrated during the workshop, it has been suggested that the delayed events may represent the common terminal pathway of CMT1 neuropathies. Thus, future work will have to focus on the primary molecular reactions and potential altered protein inter-
actions following abnormal expression or mutation of the CMT-related myelin proteins.

In the final talk, A. Schenone presented data on the reduced PMP22 expression levels in sural nerve biopsies of patients lacking one copy of the PMP22 gene (Schenone et al., 1997). He demonstrated an inverse correlation of the clinical phenotype as defined by a “neuropathy disability score” with the relative amount of PMP22 expressed in HNPP nerves. Conversely, the g-ratio correlated linearly with the relative levels of PMP22. This work is the first human study demonstrating a dose-dependent effect of PMP22 gene dosage on clinical symptoms in HNPP.

CONCLUSIONS

The final discussion between the participants of the workshop revealed that the most important future CMT research directions must include a detailed comparison of the pathology in human and in transgenic animal models, the determination of structure-function-disease correlations on the basis of the mutated proteins, and the search for potential partner molecules interacting with the above-mentioned myelin proteins. The participants agreed that a substantial number of useful animal and cell culture models for CMT and HNPP are now available. These systems are likely to foster further rapid progress in our understanding of the pathomechanisms in CMT and provide the basis for developing successful strategies for therapeutic or preventive treatment in future.

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