Newsletter

der

Deutschen Gesellschaft für Neurogenetik

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Society News

The third workshop Neurogenetics in Germany, and the second annual meeting of the DGNG will be held in Ulm from October 10-12, 1996. Society members have already received the program from Professor F. Lehmann-Horn/Ulm. Please note that the ***deadline for abstracts is August 20.***

Munich Reference Center for Neuro-degenerative Disorders

In the fall of 1994, the Reference Center for Neurodegenerative Disorders was founded at the University of Munich with generous support from the German Government (Bundesministerium für Bildung und Forschung) in order to set up the first Brain Bank in Germany. In the same year, the German Society of Neuropathology and Neuroanatomy became the official sponsor of the Center which also serves as a national diagnostic referral center.

The main task of the Munich Reference Center for Neurodegernative Disorders is the collection and banking of human brain tissue from patients with neurodegenerative diseases and from appropriate controls (brain donor program) in order to provide the neuroscience community with high quality research material. The fulfillment of this task requires detailed diagnostic evaluation of all tissue specimens prior to banking. In addition, brain tissue is banked in a disease-oriented manner with a strong emphasis on common neurodegenerative disorders such as Alzheimer and Parkinson disease. Furthermore, it is a major goal of the center to enhance communication between clinical research groups and the Brain Bank, thus optimizing the collection of data and the collaboration with the Center.

You can contact the Reference Center at **http://www.med.uni-muenchen.de/neuropath/nerz.**

Research Highlights

Molecular mechanisms in CMT1A and HNPP. Charcot-Marie-Tooth syndrome type 1A (CMT1A) is most frequently caused by a duplication of 1.5 Mb of DNA within 17p11.2-p12. Approximately 90% of sporadic and 70% of familial cases carry this mutation. Conversely, hereditary neuropathy with liability to pressure palsies (HNPP) commonly results from a 1.5 Mb deletion of the same region. The duplicated/deleted segment contains the gene coding for peripheral myelin protein 22 (PMP22) and its altered expression is thought to result in the different phenotypes of these two demyelinating peripheral neuropathies.

Low copy number repetitive elements (referred to as the CMT1A-REP repeats) of ~30 kb flank the 1.5 Mb fragment.The findings suggest that recombination events occur at the same locations in both CMT1A and HNPP and that recombinational hotspots border the 1.5 Mb fragment. This hypothesis was examined by Reiter et al. (1996) and by Kiyosawa and Chance (1996). The CMT1A-REP repeats are highly homologous and therefore potentially

allow for homologous recombination throughout this stretch of DNA. Surprisingly, Reiter et al. detected a recombination hotspot of only 1.7 kb within this repeated sequence. In 94 out of a total of 123 (~75% of 104 CMT1A and ~84% of 19 HNPP) patients tested, the DNA rearrangement involved the 1.7 kb hotspot region. A mariner transposon-like element was detected in close proximity to the 1.7 kb segment within the CMT1-REP repeats. This element is being referred to as MITE (Mariner Insect Transposon-like Element). A testis-specific transcript containing portions of the mariner-like element was identified. Testis-specific expression might be functionally significant since de novo recombinations in CMT1A and HNPP occur almost exclusively during male meiosis. The existence of several stop codons within the putative transcript suggests that it is not translated. A role of MITE in the recombination events that underly CMT1A and HNPP was considered. Reiter et al. speculate that MITE "mediates strand exchange events via cleavage by a transposase at or near the 3´end of the element". The apparent lack of translation of the MITE transcript, however, does not support this hypothesis. Further studies are necessary to clarify the role if any of MITE in homologous recombination in CMT1A and HNPP.

Reiter LT, Murakami T, Koeuth T, Pentao L, Muzny DM, Gibbs RA, Lupski J (1996) A recombination hotspot responsible for two inherited peripheral neuropathies is located near a mariner transposon-like element. *Nature Genet* 12, 288-297.

Kiyosawa H, Chance PF (1996) Primate origin of the CMT1A-REP repeat and analysis of a putative transposon-associated recombinational hotspot. *Hum Mol Genet* 5, 745-753.

Identification of the gene defect in Friedreich ataxia. Friedreich ataxia (FRDA) is an autosomal recessive ataxia that occurs at a frequency of 1/50,000. Neurological findings include ataxia (100%), areflexia in the legs (99%), pyramidal weakness (88%), and impaired sense of vibration (84%). Less frequently, nystagmus (20%), optic atrophy (30%), and deafness (8%) occur. Hypertrophic cardiomyopathy is commonly associated with the disorder. Furthermore, diabetes occurs in 10% of the cases. The disease gene was assigned to the proximal long arm of chromosome 9 (9q13-q21.1) more than 8 years ago. Employing a positional cloning approach, Campuzano et al. (1996) have now been able to isolate the gene. It is called *X25* and is composed of 6 exons (5´-3´: 1,2,3, 4, 5a, and 5b). The transcript can be alternatively spliced. The most common splicing variant is composed of exons 1-5a, results in a 1.3 kb transcript and is translated into a polypeptide of 210 amino acids, termed frataxin. Corresponding to the tissues primarily affected in Friedreich ataxia, the highest levels of the 1.3 kb transcript are found in the spinal cord and in the heart. Lower amounts are present in the pancreas and skeletal muscle. A polymorphic trinucleotide repeat, GAA is located in intron 1 of the gene. This trinucleotide is greatly expanded in the majority of patients and appears to interfere with normal splicing of the transcript. As a result, no mature message is generated. The mutation thus results in a loss-of-gene-function.

FRDA is the first trinucleotide repeat disorder that results from the expansion of an intronic repeat. In trinucleotide repeat disorders caused by CAG expansions (Huntington disease, spinocerebellar ataxias type 1 and 3, spinobulbar muscular atrophy, dentatorubral-pallidoluysian atrophy) the repeat is located in an exon. The disease gene is transcribed and translated into a polypeptide with an expanded glutamine stretch. In these disorders, the expansion is thought to give rise to altered gene function (gain-of-function) mutation. The CGG/GCC expansions found in fragile X syndromes A and E are located in the 5´untranslated region of exon 1 of the respective disease gene and the CTG repeat expanded in myotonic dystrophy is located in the 3´ untranslated exon of the myotonic dystrophy protein kinase gene. In these disorders the expansion is thought to result in a loss-of-gene function.

The discovery of the FRDA gene is a first step towards a better understanding of the disorder at the molecular level. Questions as to the role of the FRDA gene product, frataxin, in neuronal and in selected non-neuronal (especially heart and pancreas) tissues can now be addressed. The possibility of a correlation between the type of mutation in the FRDA gene (e.g. GAA expansions vs point mutations) and the highly variable phenotype observed in FRDA will be studied. Furthermore, it is intriguing to learn whether FRDA gene mutations also occur in isolated forms of hypertrophic cardiomyopathy or diabetes without neuronal involvement.

Campuzano et al. (1996) Friedreich´s ataxia: Autosomal recessive disease caused by an intronic GAA triplet repeat expansion. *Science* 271: 1423-1427.

Possible interaction between the gene products of CAG repeat disorders and GAPDH. Expansions of the trinucleotide CAG within a disease gene are the cause of several trinucleotide repeat disorders including Huntington disease (see above). The mechanism by which CAG expansions cause disease is widely unknown. As summarized in *Newsletter 3*, abnormal protein-protein interactions appear to play an important role in the pathogenesis of these diseases. Burke et al. (1996) have now shown that glyceraldehyde-3-phosphate dehydrogenase (GAPDH) preferentially binds to the expanded polyglutamine stretch of both huntingtin and the product of the dentatorubral pallidoluysian atrophy (DRPLA) gene. Since GAPDH is a key enzyme in glycolysis and glucose is the main source of energy in the brain, Burke et al. speculate that the abnormal proteins inactivate GAPDH. So far it has not been shown, however, that the GAPDH - poly-glutamine-containing polypeptide interaction does in fact inactivate the enzyme. In any case, additional tissue-specific proteins probably also play an important role in disease pathology by forming supramolecular complexes with the glutamine-containing proteins. One such protein may be HAP-1 that was found to be associated with huntingtin previously.

Burke J, Enghild JJ, Martin ME, Jou Y-S, Myers RM, Roses AD, Vance JM, Strittmatter WJ (1996) Huntingtin and DRPLA proteins selectively interact with the enzyme GAPDH. *Nature Medicine* 2: 347-350.

Technical advances

Differential display. A large number of genes is expected to be expressed only in the nervous system to a significant extent. Identification and functional analysis of these genes remains a formidable task of the „functional genomics“ which is likely to dominate genetic research in the beginning of the next century. A method, *differential display (DD),* has been developed that will be immensely useful for the identification and isolation of differentially expressed genes, e.g. during development and disease processes. The method relies on the use of PCR to amplify those messages after reverse transcription that are expressed in higher or lower amounts in the tissue under investigation as compared to those of an appropriate control tissue. Following mRNA extraction, reverse transcription with special „anchored“ oligo-dT-N (N=C, G or A) primers generates different pools of cDNAs which are further amplified with complementary anchored oligo-dT primers and an arbitrary 13-mer oligonucleotide. The resulting PCR products of varying lengths are then „displayed“ (visualized) by autoradiography. *Differential display* is performed by comparing the auto-radiographs of the PCR reactions obtained from the experimental and the control tissue. The candidate gene is isolated by recovering the cDNA corresponding to the respective band(s) from the gel. This cDNA may be further subcloned for the production of cRNA probes or used directly for filter hybridizations. Although the technique is relatively easy to perform (kits are available), problems still exist with the identification of unique bands, especially when total tissue is used for the preparation of mRNA.

Recommended reading

1) Liang P, Pardee AB (1992) Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction. Science 257, 967-971

2) Livesey FJ, Hunt SP (1996) Identifying changes in gene expression in the nervous system: mRNA differential display. Trends Neurosci 19, 84-88.

Companies to contact

GenHunter Corp., 50 Boylston Street,
Brookline, MA 02146, Tel. (617) 739-6771,
Fax (617) 734-5482

or

Display Systems Biotech at
http://www.displaysystems.com

We encourage all members of the DGNG to contribute to future issues of the Newsletter.

Looking forward to an exciting meeting in Ulm,

Sincerely yours,

Ulrich Müller

Peter Propping

Manuel B. Graeber