Newsletter
der
Deutschen Gesellschaft für Neurogenetik

July, 1998
DGNG News No. 8

Society News

5th Workshop Neurogenetics in Germany, 4th Annual meeting of the DGNG

The 5th workshop Neurogenetics in Germany and 4th Annual Meeting of the DGNG will be held in Freiburg from October 22-24, 1998. The organizers of the meeting are G.B. Landwehrmeyer, R. Korinthenberg, C.H. Lücking, D. Morris-Rosendahl, and U. Wolf.

Preliminary program & tentative titles

Thursday, October 22, 1998

17.00 Registration

18.30 Membership assembly of the German Society for Neurogenetics (DGNG)

19.30 Refreshments

20.00 Key note lecture: J.-L. Mandel (Strasbourg): Dynamic mutations and neurological diseases: an overview

21.30 Welcome reception

Friday, October 23, 1998

8.15 Introduction

8.30 S.W. Davies (London): Intranuclear inclusions in neurodegenerative disorders

9.15 E.E. Wanker/A. Sittler (Berlin): Protein interactions in Huntington's disease

10.00 Coffee break

10.15 S.E. Antonarakis (Geneva): Introduction into the genetic dissection of pychiatric disorders

11.00 X.O. Breakfield (Boston): Dystonia - what is new?

11.45 Coffee break

Oral presentations I

12.00 NN

12.15 NN

12.30 NN

12.45 NN

13.00 Lunch

14.00 Poster session & coffee

15.30 B.A. Oostra (Rotterdam): Fragile X-Syndrome

16.15 A. Wynshaw-Boris (Bethesda): Gene defects in Lissencephaly

16.45 Coffee break

17.00 L. Peltonen (Helsinki): Neuronal ceroid lipofuscinoses

18.30 Guided tour

20.00 Dinner

Saturday, October 24, 1998

8.30 O. Steinlein (Bonn): Ion channel mutations in human idiopathic epilepsies

9.15 P. Szepetowski (Oxford): Genetics of human polygenic idiopathic epilepsies - an approach to epilepsies

10.00 Coffee break

Oral presentations II

10.15 NN

10.30 NN

10.45 NN

11.00 NN

11.15 H.Bujard (Heidelberg): Generating conditional mouse mutants

12.00 Coffee break

12.15 H.Orr (Minneapolis): Mouse models for CAG-repeat disorders

13.00 Light lunch

14.00 End of the conference

Workshop language: English

Submission: Registered participants

Abstracts: Abstracts must be submitted in English. Abstracts may not exceed 2000 characters including title, authors, affiliation, and body of the abstract (see enclosed form). Abstracts should be submitted on disk (PC 3.5'') in MS-Word or ASCII format or can be e-mailed as attached files (Word 6 for Macintosh or for Windows; Word 7 for Windows; WordPerfect 5.0 for Windows; ASCII) to landwehr@sun1.ukl.uni-freiburg.de. Abstracts

will be published in the 3rd issue of 'Medizinische Genetik' 1998 (scheduled to appear at the end of September 1998) and will be posted on the web-site. Free oral communications will be selected from submitted abstracts. Abstract forms and instructions may be downloaded from the web-site **(www.ukl.uni-freiburg.de/neurozen/nlo/neurogen/index.htm)**.

Deadline for the submission of abstracts: August 15, 1998.

Oral presentations: Free oral communications will be selected from submitted abstracts. We are planning to select 8-12 abstracts for oral presentation. The time allotted for each presentation will be 15 min.

Poster session: There will be a poster session during the meeting; posters will be exhibited from Friday morning to the end of the workshop on Saturday. Poster size: 90 cm wide x 120 cm high. The posters will be numbered according to the code listed in the abstract booklet.

Registration fees:

DGNG members: DM 80.-
Non-members: DM 120.-
Students (with student I.D.): free

Accommodation: We suggest that participants of the workshop stay in the Hotel and Boarding-House 'An den Kliniken' which is close to the conference site. Single and double rooms are available at a reduced rate with advance registration. For members, the costs of a workshop-package including 2 nights at the Hotel and Boarding-House 'An den Kliniken', refreshments during the workshop, a reception (Thursday evening) and dinner (Friday evening) and the registration fee would be a total of DM 250.- (single room) and DM 290.- for non-members (single room), respectively. Cost may be reduced by sharing rooms (total cost: DM 190.- per person for members, DM 230.- per person for non-members, shared double room).

Deadline for advance registration: September 15, 1998.

Workshop account:
G.B. Landwehrmeyer/Sonder-konto DGNG-Kongress. Sparkasse Freiburg,
Account number 2015770, BLZ 680 501 01

Bank transfers to the workshop account are preferred. Fees may be paid while registering on-site either with cash or Eurocheques. Please note that we are not able to handle credit cards.

Research News

GCG expansion in oculopharyngeal muscular dystrophy (OPMD). OPMD is an adult onset muscular disorder characterized by dysphagia, ptosis, and proximal weakness of the limbs. The major pathological findings are nuclear filament inclusions in skeletal muscle. OPMD is usually inherited as an autosomal dominant trait but autosomal recessive inheritance has also been reported. The disease locus has been assigned to 14q11.2-q13 in French-Canadian families, and further delineated to a 350 kb cosmid contig flanked by loci D14S990 and D14S1457. Transcripts were assigned to this contig and the gene coding for poly(A) binding protein 2 (PABP2) was analyzed as a candidate. *PABP2* is highly expressed in skeletal muscle, and the protein is exclusively localized to the nucleus. It plays an important role in mRNA polyadenylation. *PABP2* is composed of 7 exons with only parts of exons 1 and 7 being translated. Brains et al. discovered a GCG repeat encoding a polyalanine tract in the N-terminal region of the PABP2 polypeptide. The GCG repeat has a copy number of 6 in 98% of normal persons and of 7 in 2% of controls. In patients the repeat is slightly expanded to a copy number of 8-13. More severe phenotypes were observed in compound heterozygotes for the $(GCG)_9$ and $(GCG)_7$ alleles as compared to heterozygotes carrying a $(GCG)_9$ in addition to a wildtype $(GCG)_6$ allele. Furthermore, homozygosity for the $(GCG)_7$ allele was documented in autosomal recessive OPMD. Thus the $(GCG)_7$ allele can function as a modifier of the autosomal dominant phenotype or as a recessive mutation. The authors argue that the short expansion of the (GCG) trinucleotide results in a gain of *PABP2*

function that culminates in the accumulation of nuclear filaments. Accordingly, the expanded polyalanine tract may cause abnormal polymerization of the PABP2 protein that is usually found in oligomeric or dimeric form only.

1) Brais B, Bouchard J-P, Xie Y-G, Rochefort D.L, Chrétien N, Tomé FMS, Lafrenière RG, Rommens J.M., Uyama E, Nohira O, Blumen S, Korcyn AD, Heutink P, Mathieu J, Duranceau A, Codèdere F, Fardeau, Rouleau GA (1998) Short GCG expansions in the PABP2 gene cause oculopharyngeal muscular dystrophy. Nat. Genet. 18:164-167.

Gene defect in X-linked lissencephaly and double cortex syndrome. Lissencephaly (LIS) describes agyria (absence of gyri) or pachygyria (reduced number of gyri) and is caused by abnormal neuronal migration during development. Histologically, lamination of the six-layered cortex is disturbed and only four layers are demarcated. Subcortical laminar heterotopia (SCLH) is another form of cortical dysgenesis caused by abnormal neuronal migration. It is characterized by bilateral bands of gray matter located beneath and well separated from the cortex and ventricle. Therefore it is also referred to as double cortex syndrome (DC). LIS and DC can occur in a sporadic or hereditary pattern and both syndromes have been observed in single pedigrees. In these pedigrees males are affected by LIS and females by DC. This suggested X-chromosomal inheritance of both disorders with hemizygous males being more severely affected than heterozygous females in whom gene function is only disturbed in those cells where the wild-type X-chromosome is inactivated. Recent mapping efforts have localized LIS/DC to Xq22.3-q23 both by linkage analyses and the identification of an X;autosome translocation in a girl with lissencephaly (Ross et al., 1997). Two groups have now identified the gene causing LIS/DC. Des Portes et al.(1998) constructed a YAC contig of the LIS/DC critical region as defined by analysis of linkage and of the Xq22.3-q23 breakpoint. They assigned 8 ESTs to the contig spanning Xq22.3-q23 from DXS287 to DXS1072. One of these ESTs detected a 9.5 kb transcript that is present in high quantity in fetal brain and becomes undetectable in adult brains. This gene was further characterized and found to consist of 9 exons. An ATG start codon with the first in-frame stop codon (TAA) yields an ORF of 1080 bp. This ORF spanning exons 2 to 6 encodes a polypeptide of 360 amino acids that may be involved in the regulation of a neuronal protein kinase.The authors performed mutation analyses in unrelated patients with DC/LIS syndrome and found point mutations in three families that result in amino acid changes of doublecortin. Furthermore, they observed splicing and nonsense mutations in additional cases. The data suggest that DC/LIS results from a loss of function of the doublecortin gene. Essentially identical data were obtained by Gleeson et al. who identified doublecortin by cloning of the X;2 translocation breakpoint in a patient that disrupts the gene.

1) Gleeson JG, Allen KM, Fox JW, Lamperti ED, Berkovic S, Scheffer I, Cooper EC, Dobyns WB, Minnerath SR, Ross ME, Walsh CA (1998) *doublecortin*, a brain-specific gene mutated in human X-linked lissencephaly and double cortex syndrome, encodes a putative signaling protein. Cell 92:63-72.

2) des Portes V, Pinard JM, Billuart P, Vinet MC, Koulakoff A, Carrié A, Gelot A, Dupuis E, Motte J, Berwald-Netter Y, Catala M, Kahn A, Beldjord C, Chelly J (1998) A novel CNS gene required for neuronal migration and involved

in X-linked subcortical laminar heterotopia and lissencephaly syndrome. Cell 9:51-61.

3) Ross E, Allen K, Srivastava A, Featherstone T, Gleeson J, Hirsch B, Harding B, Andermann E, Abdullah R, Berg M et al. (1997) Linkage and physical mapping of X-linked lissencephaly/SBH (XLIS): a gene causing neuronal migration defects in human brain. Hum Mol Genet 6:555-562.

Further progress in the molecular genetics of Parkinson disease. Parkinson disease (PD) is one of the most common neurological disorders in older people with an overall prevalence of 1- 2 per 1000. The incidence increases to 1.4% at age 55 and 3.4% at 75. The clinical manifestations of this neurodegenerative disorder include resting tremor, muscular rigidity, bradykinesia, and postural instability. The disease is caused by striatal deficiency of dopamine as a result of neuronal death in the substantia nigra. Neuropathological hallmarks of PD are loss of pigmented neurons in the pars compacta of the substantia nigra and neuronal intracytoplasmic inclusions, known as Lewy bodies (LB).

In Newsletter 7 we reported on the identification of a mutation in the gene *α-synuclein* in 4 families with a rare autosomal dominant form of the disease (1). This mutation causes an evolutionarily nonconserved amino acid change (Ala53Thr) due to a G209A transition . While an alanine is present at position 53 of α-synuclein in humans and some other mammals, threonine is found at this position in the rat, mouse, and the canary. Numerous studies to identify the G209A mutation in other families with autosomal dominant PD (ADPD) and in patients with sporadic PD (2,3), including sequence analysis of the entire gene in 30 ADPD families of European and American origin (4), have failed to detect additional PD patients with a mutation in *α-synuclein*. These negative findings raised the possiblility that the Ala53Thr change observed in the original study (1) represents a linked rare polymorphism and not the causative gene. Recent findings have now confirmed that *α-synuclein* mutations can cause PD and that the original amino acid changes observed are probably pathogenic. Studying a German family, Krüger et al (5) have identified a mutation that causes a conservative amino acid change (Ala30Pro) in α-synuclein. This substitution occurs in an evolutionarily highly conserved repeat motif of the synuclein gene and was not observed in more than 1000 chromosomes of age-matched healthy German controls and in 75 DNA samples from human brains without LB histopathology. α-synuclein has now been detected as a major component of LB in PD, LB dementia, and the LB variant of Alzheimer disease. (6). In addition, α-synuclein antibodies detected the protein in abnormal neurites and dystrophic neurites mostly in the synaptic terminals. These findings suggest that both abnormal transport of synaptic proteins and abnormal compartmentalization of α-synuclein might play a role during the progression of Lewy body diseases.

Recently, a second susceptibility locus for PD was mapped to chromosome 2p13 (PD2) (7). The authors tested 6 families with autosomal dominant PD and found statistically significant lod scores for markers from 2p13 in three of them. Statistically insignificant lod scores were found in another family for markers from 4q, the location of *α-synuclein*. However, no mutations were detected in patients in this family (4). The penetrance of the PD2 mutation has

been estimated at less than 40%. This raises the possibility that mutations in the (yet unidentified) PD2 gene are also responsible for some of the much more common sporadic forms of PD.

Another breakthrough in the molecular genetics of PD comes from the investigation of an autosomal recessive form of juvenile parkinsonism (see Newsletter 6). Although neuro-pathological changes in the basal ganglia resemble those found in typical PD, there are no LB. Kitada et al. (8) studied the region of chromosome 6q25.2 to which the disease locus had been mapped and found deletions in a novel gene, *parkin*. Parkin has moderate similarity to ubiquitin at the amino terminus and a RING-finger motif at the carboxy terminus. The authors reported mutations in 5 patients with autosomal recessive juvenile PD (8). The observed deletions in parkin are likely to result in a loss of gene function. This contrasts to the missense mutations found in α-synuclein which are thought to result in a "gain-of-function". It will be interesting to find common mechanisms in the pathogenesis of these two kinds of mutations.

1) Polymeropoulos MH, Lavedan C, Leroy E, et al. (1997) Mutation in the *α-synuclein* gene identified in families with Parkinson's disease. Science 276:2045-2047.

2) Chan P, Tanner CM, Jiang X, Langston JW (1998) Failure to find the *α-synuclein* gene missense mutation (G209A) in 100 patients with younger onset Parkinson's disease. Neurology 50:513-514.

3) Farrer M, Wavrant De Vrieze F, Crook R, et al. (1998) Low frequency of *α-synuclein* mutations in familial Parkinson's disease. Ann Neurol 43:394-397.

4) Vaughan JR, Farrer MJ, Wszolek ZK, et al. (1998) Sequencing of the *α-synuclein* gene in a larger series of cases of familial Parkinson's disease fails to reveal any further mutations. Hum Mol Genet 7:751-753.

5) Krüger R, Kuhn W, Müller T, Woitalla D, Graeber M, Kösel S, Przuntek H, Epplen JT, Schöls L, Riess O (1998) Ala30Pro mutation in the gene encoding *α-synuclein* in Parkinson's disease. Nature Genet 18:106-108.

6) Takeda A, Mallory M, Sundsmo M, Honer W, Hansen L, Masliah E (1998) Abnormal accumulation of NACP/ α-synuclein in neurodegenerative disorders. Am J Pathol 152:367-372.

7) Gasser T, Müller-Myhsok B, Wszolek ZK, Oehlmann R, Calne DB, Bonifati V, Bereznai B, Fabrizio E, Vieregge P, Horstmann RD (1998) A susceptibility locus for Parkinson's disease maps to chromosome 2p13. Nature Genet. 18: 262-265.

8) Kitada T, Asakawa S, Hattori N, et al. (1998) Mutations in the *parkin* gene cause autosomal recessive juvenile parkinsonism. Nature (London) 392:605-608.

Update on Alzheimer disease. Research on Alzheimer disease certainly is one of the most active fields of neurogenetic inquiry. Consequently, there are too many new developments to be highlighted in a single short article making the selection of topics somewhat arbitrary. One of the most interesting recent findings comes from a US-Finnish research team. Crook et al. (1) reported on the discovery of a novel variant of Alzheimer disease in a Finnish pedigree with 17 affected individuals of both sexes in three generations. Affected individuals showed not only progressive dementia but in many cases spastic paraparesis as well. Classical amyloid plaques with core and abnormal neurites ("neuritic plaques") which are found in both sporadic and familial cases of typical Alzheimer disease were not found. Instead, the predominant plaques in the Finnish pedigree resembled cotton wool balls and were immunopositive for Abeta but lacked a congophilic dense core or plaque-related neuritic

pathology. The disease-causing mutation is a deletion of exon 9 of the presenilin 1 gene from the mRNA in the absence of a splice acceptor site mutation. The results of this study essentially represent a blow to the "amyloid cascade hypothesis" (2). According to this hypothesis the pathogenesis of Alzheimer's dementia can be explained by parenchymal deposition of beta amyloid with the formation of neurofibrillary tangles, cell loss and dementia occurring secondarily, i.e., representing consequences of this deposition. The findings of Crook et al. now suggest that the major determinant of dementia in the Finnish patients is not the dense congophilic amyloid deposits so typical of most Alzheimer cases but rather is located upstream of this deposition in the pathogenic cascade.

Mitochondria form the focus of another growing area of Alzheimer disease research. There is increasing evidence that mitochondrial mutations may play a role in the etiology of Alzheimer disease but probably through facilitating rather than directly causing neurodegeneration (3, 4). Recently, two independent groups (5, 6) refuted the widely publicized finding that a large proportion of sporadic Alzheimer cases are associated with heteroplasmic mutations in two mitochondrial genes (CO1, CO2) which encode the catalytic core of cytochrome-c-oxidase (7). As now seems clear, the findings represent artifacts which are due to PCR amplification of nucleus-embedded mtDNA pseudogenes. These pseudogenes are considered to provide a molecular evolutionary "snapshot" of human ancestral mtDNA but are unlikely to play a role in the etiology of Alzheimer disease (8). Such pseudogene sequences clearly pose pitfalls for studies on disease-causing hetero-plasmic mtDNA mutations.

Finally, some good news. The relevance of the apolipoprotein E epsilon 4 allele as a risk factor for Alzheimer's as well as its usefulness in the differential diagnosis of the disease has been consolidated (9). The study confirmed that while apoE genotyping alone does not provide sufficient sensitivity or specificity to be used as a diagnostic test for Alzheimer disease, it improves the specificity of the diagnosis when used in combination with clinical criteria.

The search for brain sections from Alzheimer's original case, Auguste D., has now come to an end, and the histopathological diagnosis of this patient could be confirmed (10, 11). However, no apolipoprotein E epsilon 4 allele was found.

1) Crook R, Verkkoniemi A, Pereztur J, Mehta N, Baker M, Houlden H, Farrer M, Hutton M, Lincoln S, Hardy J, Gwinn K, Somer M, Paetau A, Kalimo H, Ylikoski R, Poeyhoenen M, Kucera S, Haltia M (1998) A variant of Alzheimer's disease with spastic paraparesis and unusual plaques due to deletion of exon 9 of presenilin-1. Nature Med 4:452-455.

2) Hardy J (1997) Amyloid, the presenilins and Alzheimer's disease. Trends Neurosci 20:154-159.

3) Hutchin TP, Heath PR, Pearson RCA, Sinclair AJ (1997) Mitochondrial DNA mutations in Alzheimer's disease. Biochem Biophys Res Comm 241:221-225.

4) Tanno Y, Okuizumi K, Tsuji S (1998) mtDNA polymorphisms in Japanese sporadic Alzheimer's disease. Neurobiol Aging 19:S 47-S 51.Davis JN, Parker WD (1998) Evidence that two reports of mtDNA cytochrome c oxidase mutations in Alzheimer's disease are based on nDNA pseudogenes of recent evolutionary origin. Biochem Biophys Res Comm 244:877-883.

5) Hirano M, Shtilbans A, Mayeux R, Davidson MM, DiMauro S, Knowles JA, Schon EA (1997) Apparent mtDNA heteroplasmy in Alzheimer's

disease patients and in normals due to PCR amplification of nucleus-embedded mtDNA pseudogenes. Proc Natl Acad Sci USA 94:14894-14899.

6) Wallace DC, Stugard C, Murdock D, Schurr T, Brown MD (1997) Ancient mtDNA sequences in the human nuclear genome: a potential source of errors in identifying pathogenic mutations. Proc Natl Acad Sci USA 94:14900-14905.

7) Davis RE, Miller S, Herrnstadt C, Ghosh SS, Fahy E, Shinobu LA, Galasko D, Thal LJ, Beal MF, Howell N, Parker WD (1997) Mutations in mitochondrial cytochrome c oxidase genes segregate with late-onset Alzheimer disease. Proc Natl Acad Sci USA 94:4526-4531.

8) Davis JN, Parker WD (1998) Evidence that two reports of mtDNA cytochrome c oxidase mutations in Alzheimer's disease are based on nDNA pseudogenes of recent evolutionary origin. Biochem Biophys Res Comm 244:877-883.

9) Mayeux R, Saunders AM, Shea S, Mirra S, Evans D, Roses AD, Hyman BT, Crain B, Tang MX, Phelps CH (1998) Utility of the apolipoprotein E genotype in the diagnosis of Alzheimer' s disease. N Engl J Med 338:506-511.

10) Enserink M (1998) First Alzheimer's diagnosis confirmed. Science 279:2037.

11) Graeber MB, Kösel S, Grasbon-Frodl E, Möller HJ, Mehraein P (1998) Histopathology and APOE genotype of the first Alzheimer disease patient, Auguste D. Neurogenetics 1:223-228.

We are looking forward to seeing you in Freiburg.

Sincerely yours,

Ulrich Müller
Olaf Riess
Manuel B. Graeber

Announcements

Gaucher/Niemann-Pick C Disease Reseach Group, Rostock

The Gaucher/Niemann-Pick C Disease Research Group at the University of Rostock (Speaker: Arndt Rolfs) is an interdisciplinary and international consortium of clinical and basic science research groups (e.g. K. Harzer/ Tübingen; M. Horowitz/Tel Aviv). The main aim of our group is the detailed analysis of the pathogenesis of neuronopathic type III Gaucher disease (GD). GD is the most prevalent hereditary metabolic storage disease, and the most common genetic disorder in individuals of Ashkenazi Jewish ancestry. GD transmitted in an autosomal recessive manner is mostly caused by deficient activity of the enzyme glucocerebrosidase (GBA). The clinical phenotypes of GD patients are classified into three groups: non-neuronopathic (type I), which is the most common variant with an incidence of 1 in 30,000, acute neuronopathic (type II), and chronic (subacute) neuronopathic (type III). Although many different point mutations, some insertions, deletions, and more complex disease alleles due to recombination events with the CBA pseudogene (GBAP) have been identified, in about 25-35% of all Caucasian GD patients the mutation is still unknown.

To obtain more detailed information on the distribution and frequency of the mutation pattern in European non-Jewish, Caucasian GD patients our group has analyzed the entire GBA coding region in 75 such patients of whom 11 suffered from the chronic neuronopathic GD variant. The enzyme replacement therapy which is highly effective in patients with type I is only moderately beneficial in patients with type III.

The scientific program includes detailed documentation of the clinical course of these patients with type III, performance of functional, structural and cellbiological analyses, expression studies and in-vitro

mutagenesis trial of the gene, and beginning treatment trials. As part of the genetic research project we are looking for further type III GD patients. The disease is mainly characterized by

- multifocal myoclonus and generalized seizures,
- horizontal supranuclear gaze palsy,
- ataxia,
- spasticity but
- only moderate hepatosplenomegaly and thrombocytopenia

We have established a sequencing procedure of the complete coding glucocererbrosidase gene as well as for the NPC1 gene since Niemann-Pick type C is the major differential diagnosis of GD.

If you have patients with seizures of unclear etiology in combination with hepatosplemomegaly, ataxia, or supranuclear gaze palsy we are offering genetic analysis for both the glucocerebrosidase and NPC1 gene.

For further information please contact Prof. Dr. Arndt Rolfs, Klinik für Neurologie und Poliklinik, Universität Rostock, Gelsheimerstraße 20, 18055 Rostock, Tel.: +49-381-494-9540; FAX: +49-381-494-9542; email: arndt.rolfs@medizin.uni-rostock.de