Newsletter
der
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Society News

8th Workshop Neurogenetics in Germany, 7th Annual Meeting of the DGNG

The 7th annual meeting of the society of Neurogenetics (8th workshop Neurogenetics in Germany) will be held in Magdeburg from October 25 to 27, 2001. The conference will be organized by Professors P. Wieaker, E. Gundelfinger, G. Reiser, and C.-W. Wallesch and by Drs. D. Montag, S. Vielhaber, S. Jakubiczka and M. Stumm. Major topics include „Mental retardation", „ion channel diseases", „mouse models for neurodegenerative diseases", „paraplegias", „muscular dystrophies" and „embryonic stem cells in neurological diseases". Details and registration forms are on the web (http://www.med.uni-magdeburg.de/fme/institute/ihg/tagung.html) Deadline for abstracts is 06/30/2001.

Research News

Mapping of modifier gene in mouse model of ALS. In monogenic disorders identical mutations in a disease gene can result in highly variable phenotypes. This phenomenon has long been recognized and been referred to as reduced penetrance (a phenotypic effect of a mutated gene is not observed in every carrier of a mutation) and variable expressivity (the phenotype of mutation carriers varies greatly between individuals). These phenomena are thought to be primarily caused by genes modifying the effect of the main mutation (in addition to putative environmental influences).

Kunst et al. have mapped a gene locus in a mouse model of amyotrophic lateral sclerosis (ALS) that greatly delays disease onset. The mouse investigated carries a mutation (G86R, corresponding to human G85R mutation) in the superoxide dismutase 1 (SOD1) gene, presently the only disease gene identified in autosomal dominant ALS in humans. The authors found that disease onset is dependent on the mouse strain carrying the mutation. While disease develops at about 100 days in G86R SOD1 mice with the FVB/background, the disease onset is greatly delayed (143 days to >2 years) in mice with the mixed background of C57Bl6/129Sv. Performing linkage analyses in a large pedigree originating from the cross of a C57Bl6/129Sv and a FVB/NJ mouse carrying the G86R mutation, the authors assigned a modifier locus to murine chromosome 13. Since the interval contains the spinal muscular atrophy (SMA)-associated genes Smn (survival motor neuron) and several copies of Naip (neuronal apoptosis inhibitory protein) the authors speculate their finding might indicate a link between ALS and SMA.

Kunst, CB, Messer L, Gordon J, Haines J, Patterson D: Genetic mapping of a mouse modifier gene that can prevent ALS onset. Genomics 70: 181-189 (2000).

Growing number of inducible transgenic mouse models. Although the number of transgenic and knock-out models for human diseases is continuously growing, inducible models overexpressing a specific transgene are rather rare. The major advantage of the latter is the opportunity to turn off and on the

transgene in the mice repeatedly and at different time points. This system will show whether it will be possible to reverse a phenotype by turning off the transgene after the disease has already started. It might therefore also be useful to estimate the efficiency of therapeutic concepts in humans. The most widely used system to turn on and off the transgene is the tetracycline-response transactivator system (tTA) developed by Prof. Bujard (Gossen and Bujard 1992). As we reported in Newsletter 13, the tTA system has been applied to generate an inducible model for Huntington's disease (Yamamoto et al. 2000). Since then two new models for neurodegenerative diseases have been published, one to overexpress dystrophin in *mdx* mice (Ahmad et al. 2000) the other to generate a model for Charcot-Marie-Tooth disease type 1A (CMT1A, Perea et al. 2001). Ahmad and colleagues used the *mdx* mouse, a widely accepted model for Duchenne muscular dystrophy, to assess the potential of gene therapeutic approaches by overexpressing dystrophin in an inducible manner. Based on their data, several important conclusions can be drawn: first, the expressed dystrophin is localized at the exact site in the sarcolemal membrane as normal dystrophin; second, induction of expression is far more effective when initiated in utero than postnatally (expression at later stages did not lead to any benefit in muscle cell morphology); and third, even after turning off transgene expression, dystrophin was still detectable for at least 6 months. These results suggest that future gene transfer experiments might also be beneficial in DMD patients when initiated at early stages even if downregulation of transcription of existing vectors occurs over time. Perea and colleagues did not generate therapeutic models but rather a disease model of a hereditary demyelinating neuropathy. They also used the tetracycline system to overexpress peripheral myelin protein 22 (PMP22). PMP22 causes CMT1A when it is duplicated and therefore present in three copies in the genome of the patient. In their mice, Perea and colleagues were able to generate an inducible model for CMT1A by overexpression of PMP22. Turning off PMP22 transgene expression by administration of tetracycline resulted in the correction of demyelination that started at 1 week and was almost complete at 3 months. After turning on the expression repeatedly, the phenotype developed again in the mice clearly indicating that pmp22 overexpression is the cause of demyelination. This indicates that therapeutic interventions downregulating PMP22 expression might correct demyelination within a few months. Now, the long road to develop and identify drugs or antisense approaches to downregulate PMP22 in CMT1A patients seems more encouraging.

Ahmad A, Brinson M, Hodges BL, Chamberlain JS, and Amalfitano A (2000) *Mdx* mice inducibly expressing dystrophin provide insights into the potential of gene therapy for Duchenne muscular dystrophy. Hum Mol Genet 9: 2507-2515

Gossen M and Bujard H (1992) Tight control of gene expression in mammalian cells by tetracycline-responsive promoters. *Proc Natl Acad Sci USA* 89:5547-5551

Perea J, Robertson A, Tolmachova T, Muddle J, King RHM, Ponsford S, Thomas PK, and Huxley C (2001) Induced myelination and demyelination in a conditional mouse model of Charcot-Marie-Tooth disease type 1A. Hum Mol Genet 10: 1007-1018

Yamamoto A, Lucas JJ, and Hen R (2000) Reversal of neuropathology and motor dysfunction in a conditional model of Huntington's disease. Cell 101: 57-66

HIF regulation of mutant VHL product is preserved in type 2C (pheochromocytoma only) von Hippel-Lindau disease. Von Hippel-Lindau (VHL) disease is a rare hereditary tumor syndrome (incidence about 1/40,000) characterized by cerebellar, spinal, and medullary hemangioblastomas, retinal angiomas, clear-cell renal

carcinomas, and pheochromocytomas. Depending on the combination of tumors present, four types of VHL are distinguished clinically. Type 1 denotes simultaneous occurrence of hemangioblastomas and renal cell carcinoma in the absence of pheochromocytomas. 2A, B, and C describe types in which pheochromocytomas occur. In type 2A pheochromocytomas are associated with hemangioblastomas, in type 2B they occur in the presence of both renal cell carcinomas and hemangioblastomas, and in type 2C only pheochromocytomas are found. All four types of VHL are caused by mutations in the same gene on chromosome 3p25.

The VHL gene is a tumor suppressor gene. Its product (pVHL) associates with elongin B and C, Cul2, and Rbx1 to form a ubiquitin-ligase complex. This complex regulates expression of the hypoxia-inducible factor (HIF) 1 by polyubiquitination (as a first step towards proteasomal degradation) of subunit HIFα in the presence of oxygen. In the absence of oxygen HIF1 is expressed, binds to DNA, and stimulates expression of various target genes that encode polypeptides such as VEGF (vascular endothelial growth factor), PDF B (platelet-derived growth factor B chain), and TGFα (transforming growth factor α). As a result, VHL tumors are characterized by high levels of HIF1 and its target genes. Other functions of pVHL include control of extracellular matrix formation by interacting with fibronectin, and a role in the control of the cell cycle.

Two groups (Clifford et al., 2001; Hoffman et al., 2001) report that mutations in the VHL gene causing only pheochromocytomas (type 2C) differ from those mutations causing other types of VHL. Hoffman et al. studied four type 2C germ line mutations (V84L, L188V, R64P, F119S) and Clifford et al. investigated three (V84L, L188V, S80G). Two of the mutations studied were identical in both investigations. Both groups found that the type C mutations are still capable of downregulating HIF1 to various degrees but are defective for fibronectin binding. Their findings suggest that downregulation of HIF1 is a prerequisite for development of angioblastomas and renal cell carcinomas but not of pheochromocytomas. It appears that disturbed extracellular matrix formation plays an important role in pheochromocytoma development. At this stage, however, it remains unexplained why pheochromocytomas do not occur in type 1 VHL. In type 1 VHL, mutations result in both defective HIF1 regulation and in loss of fibronectin binding, yet pheochromocytomas only occur extremely rarely.

Hoffman MA, Ohh M, Yang H, Klco JM, Ivan M, Kaelin WG (2001) von Hippel-Lindau protein mutants linked to type 2C VHL disease preserve the ability to downregulate HIF. Hum Mol Genet 10: 1019-1027

Clifford SC, Cockman ME, Smallwood AC, Mole DR, Woodward ER, Maxwell PH, Ratcliffe PJ, Maher ER (2001) Contrasting effects on HIF-1α regulation by disease-causing pVHL mutations correlate with patterns of tumourigenesis in von Hippel-Lindau disease. Hum Mol Genet 10: 1029-1038

Genotype-phenotype correlations in tuberous sclerosis. Tuberous sclerosis is an autosomal dominant neurological disorder characterized by hamartomas, epileptic seizures, and mental retardation. Two gene loci have been identified, i.e. *TSC1* on chromosome 9q34, and *TSC2* on 16p13. In both forms the disease genes have been isolated and loss of heterozygosity (LOH) analyses suggest that both *TSC1* and *TSC2* can function as tumor suppressor genes. The polypeptide encoded by *TSC1* is referred to as hamartin and that encoded by *TSC2* as tuberin. In a search for clinical differences between patients with mutations at *TSC1* and *TSC2*,

Dabora et al. (2001) performed genotype-phenotype analyses in a large sample of patients with tuberous sclerosis. They detected mutations in 186 (83%) of 224 cases, in 28 patients at *TSC1* and in 158 at *TSC2*. Although there was clinical overlap of symptoms, *TSC1* patients had fewer seizures and some had milder mental retardation than patients with mutations at *TSC2*. Some features such as kidney and liver angiolipomas, forehead plaques, and retinal hamartomas either did not occur in *TSC1* at all or were much less common than in *TSC2* patients. Given the rare occurrence of tumors such as hamartomas and angiolipomas in *TSC1*, the authors suggest that the simultaneous occurrence of germ line and somatic mutations (the hallmark of tumor suppressor genes) is less common in *TSC1* than in *TSC2*.

Dabora SL, Jozwiak S, Franz DN, Roberts PS, Nieto A, Chung J, Choy Y-S, Reeve MP, Thiele E, Egelhoff JC, Kasprzyk-Obara J, Domanska-Pakiela D, Kwiatkowski DJ (2001) Mutational analysis in a cohort of 224 tuberous sclerosis pateints indicates increased seerity of TSC2, compared with TSC1, Disease in multiple organs. Am J Hum Genet 68: 64-80 (2001)

We are looking forward to seeing you in Magdeburg.

Sincerely yours,

Ulrich Müller
Olaf Riess
Bernhard Landwehrmeyer