

Newsletter der Deutschen Gesellschaft für Neurogenetik

July, 2004
DGNG News No. 20

Society News

The 10th Annual Meeting (11th Workshop "Neurogenetics in Germany") will be held in Hamburg from September 9 to 11, 2004. The conference will be organized by Drs. Ulrich Finckh, Alexander Münchau, and Alexander Spauschus. Main topics of the meeting will be neurodegeneration, cognition, dementia, and dystonias (<http://ihg.uke.uni-hamburg.de/dgn2004>). At this meeting the present heads of the society will step down and a new board will be elected. A second meeting also focussing on neurogenetics will be held in Weimar from September 29 to October 2, 2004. The announcement of this meeting including a flyer have been sent out to DGNG members in March (Newsletter 19a). Additional information can be obtained via the DGNG website.

Research News

Fragile X-syndrome (FXS) and fragile X-associated tremor/ataxia syndrome (FXTAS): Molecular pathology of CGG expansions in FMR1. CGG expansions within the 5' untranslated region (UTR) of the gene *FMR1* can cause at least two clinically distinct neurological disorders, the fragile X-syndrome (FXS) and the fragile X-associated tremor/ataxia syndrome (FXTAS).

Massive trinucleotide repeat expansions (>200 CGG) cause FXS. FXS can affect both sexes and is characterized by

mental retardation, typical facies (in virtually all affected men and in 20%-40% of affected women), and - in men - by macroorchidism. The large CGG expansion results in hypermethylation of the promoter and CGG tract, silencing of *FMR1* transcription, and absence of the *FMR1* protein (FMRP). Absence of FMRP leads to neuronal dysfunction with no evidence of neuronal loss or formation of inclusion bodies.

Moderate repeats (55-200 copies) were formerly considered "premutations" of *FMR1* with no clinical phenotype. Several years ago, however, it was recognized that moderate expansions can be associated with FXTAS in elderly men (Hagerman et al., 2001). [In women premutations are associated with premature ovarian failure in about 20 % of cases (Schwartz et al., 1994; Allingham-Hawkins et al., 1999.)] FXTAS is clinically defined by cognitive decline, memory loss, Parkinsonism, and lower limb proximal muscle weakness. A recent survey of 192 male carriers of premutations demonstrated age-related penetrance of intention tremor and gait ataxia. Symptoms were found in 17% of the 50-59 year-old, in 38% of the 60-69, in 47% of the 70-79 year old participants, and in 75% of those older than 80. In this study no female carrier was diagnosed with probable or definite FXTAS (Jacquemont et al., 2004).

Progressive degeneration of the middle cerebellar peduncles and adjacent cerebellar white matter is found by MRI and considered an important diagnostic feature of FXTAS. Neuropathologically,

there are intranuclear inclusions throughout the cerebrum and brainstem. mRNA is elevated (2-4 fold) but levels of FMRP are either normal or mildly decreased. Therefore, unlike FXS, FXTAS is thought to be caused by an excess of CGG repeat (rCGG) mRNA and not by loss of FMRP.

Jin et al. (2003) have now proven that the fraX-premutation does indeed cause RNA-mediated neurodegeneration. They expressed 90 rCGG in drosophila after cloning 90 CGG repeats and approximately 200 flanking base pairs from a human premutation carrier in Drosophila transformation vector pUAST-EGFP. This vector includes the enhanced green fluorescent protein (EGFP) reporter gene. The CGG repeat was inserted upstream of the translation start site (ATG) of EGFP but downstream of the transcription start site of the gene. This way the repeat was transcribed but not translated. Jin et al. obtained several clones containing 90 CGG repeats and two clones where the repeat was contracted to 60 CGG copies. A construct with no CGG repeat was used as a control. Using the drosophila *gmr*-GAL4 promoter they directed expression of rCGG-EGFP to the drosophila eye. Using different promoters they directed expression to either all neurons of the peripheral and central nervous system (*elav*-GAL4), to all cells of the drosophila embryo (*Act5C*-GAL4), or primarily to epithelial cells (*dpp*-GAL4).

They found dosage - and repeat length - dependent toxicity of the fragile X premutation rCGG. When expressed in the eye they observed progressive neurodegeneration. Expression in the nervous system was lethal as was ubiquitous expression of both 90rCGG and 60rCGG. However, no effect was observed using the *dpp*-GAL4 promoter

indicating that rCGG is more toxic in neuronal than in epithelial cells.

Jin et al. also demonstrated that the observed progressive cell death is indeed neurodegenerative rather than developmental. They conditionally modulated promoter activity and transgene expression levels by keeping the fly cultures at different temperatures. When the construct/transgene was turned on by shifting adult transgenic flies from 18°C to 29°C, similar neuropathological changes were observed as in flies that expressed the transgene throughout life. Comparable to findings in brains of FXTAS patients, transgene-expressing flies also formed ubiquitin-, proteasome-, and heat shock protein (Hsp) 70 chaperone- positive intranuclear inclusions. Unlike the exclusive intranuclear location of these inclusions in postmortem brains of FXTAS patients, the inclusions in the eyes of transgenic flies were present in both nuclei and cytoplasm.

Since hsp70 that was part of the inclusion bodies had previously been shown to suppress neurodegeneration due to misfolded proteins, Jin et al. investigated a possible role of this chaperone in their model. Surprisingly they found amelioration or reversal of symptoms when hsp70 was overexpressed. Given that the FXTAS pathology is caused by RNA and not by protein, the hsp70 effect observed suggests that the CGG RNA might result in neurodegeneration via misfolding of other proteins.

The investigations of Jin et al. are the first direct proof that expanded rCGG RNA is neurotoxic.

Jin P, Zarnescu DC, Zhang F, Pearson CE, Lucchesi JC, Moses K, Warren ST (2003) RNA-mediated neurodegeneration caused by the fragile X premutation rCGG repeats in Drosophila. *Neuron* 39: 739-747.

Jacquemont S, Hagerman RJ, Leehey MA, Hall DA, Levine RA, Brunberg JA, Zhang L, Jardini T, Gane

LW, Harris SW, Herman K, Grigsby J, Greco CM, Berry-Kravis E, Tassone F, Hagerman PJ (2004) Penetrance of the fragile X-associated tremor/ataxia syndrome in a premutation carrier population. *JAMA* 291: 460-469.

Hagerman RJ, Leehey M, Heinrichs W, Tassone F, Wilson R, Hills J, Grigsby J, Gage B, Hagerman PJ (2001) Intention tremor, parkinsonism, and generalized brain atrophy in male carriers of fragile X. *Neurology* 57: 127-130.

Schwartz CE, Dean J, Howard-Peebles PN, Bugge M, Mikkelsen M, Tommerup N, Hull C, Hagerman R, Holden JJ, Stevenson RE (1994) Obstetrical and gynecological complications in fragile X carriers: a multicenter study. *Am J Med Genet* 51: 400-402.

Allingham-Hawkins DJ, Babul-Hirji R, Chitayat D, Holden JJ, Yang KT, Lee C, Hudson R, Gorwill H, Nolin SL, Glicksman A, Jenkins EC, Brown WT, Howard-Peebles PN, Becchi C, Cummings E, Fallon L, Seitz S, Black SH, Vianna-Morgante AM, Costa SS, Otto PA, Mingroni-Netto RC, Murray A, Webb J, Vieri F, et al. (1999) Fragile X premutation is a significant risk factor for premature ovarian failure: the International Collaborative POF in fragile X study-preliminary data. *Am J Med Genet* 83: 322-325.

FGF20, a risk factor for Parkinson Disease (PD). PD is a late-onset neurodegenerative disorder. Although monogenic variants exist (currently 11 loci including 5 genes have been identified in familial forms), PD most commonly has a multifactorial etiology. A whole-genome scan in multifactorial PD identified strongest linkage to chromosome 8p (8p21.3-p22) (Scott et al., 2001). This region harbours the gene coding for fibroblast growth factor 20 (FGF20) that exerts a strong neurotrophic effect. Its preferential expression in dopaminergic neurons of the substantia nigra in both rat and human make FGF20 a good candidate susceptibility gene for PD. Van der Walt et al. (2004) analyzed 5 single nucleotide polymorphisms (SNPs) within the *FGF20* gene in 644 families using

the genotype pedigree disequilibrium test (PDT), the multilocus-genotype PDT, and the family – based association test. They found highly significant association of PD with three SNPs, one intronic and two in the 3' UTR of the gene. Furthermore, they found a haplotype of all five SNPs that is positively associated with risk of PD ($P = 0.0003$) and another haplotype that is negatively associated ($P=0.0009$). The authors discuss that the disease-associated polymorphisms within the 3' UTR region of *FGF20* could disrupt a regulatory binding sequence thus compromising transcript stability, translation or transport to subcellular locations. They also point out the possibility that several SNPs in different regions of the gene act in concert and interfere with transcription. Clearly, functional tests need to be done to figure out the effect of the PD associated SNPs on *FGF20* expression.

Scott WK, Nance MA, Watts RL, Hubble JP, Koller WC, Lyons K, Pahwa R, Stern MB, Colcher A, Hiner BC, Jankovic J, Ondo WG, Allen FH Jr, Goetz CG, Small GW, Masterman D, Mastaglia F, Laing NG, Stajich JM, Slotterbeck B, Booze MW, Ribble RC, Rampersaud E, West SG, Gibson RA, Middleton LT, Roses AD, Haines JL, Scott BL, Vance JM, Pericak-Vance MA (2001) Complete genomic screen in Parkinson disease: evidence for multiple genes. *JAMA* 286:2239-2244

van der Walt JM, Nouredine MA, Kittapa R, Hauser MA, Scott WK, McKay R, Zhang F, Stajich HM, Fujiwara K, Scott BL, Pericak-Vance MA, Vance JM, Martin ER (2004) Fibroblast growth factor 20 polymorphisms and haplotypes strongly influence risk of Parkinson disease. *Am J Hum Genet* 74: 1121-1127.

Looking forward to seeing you in Hamburg and in Weimar.

Sincerely yours,

Ulrich Müller
Olaf Riess
G. B. Landwehrmeyer