New results on the fields of genetics and therapy of neurodegenerative disorders

PhD thesis

Dr. Tamas Benjamin Bereznai

Semmelweis University

PhD School of Neuroscience "Szentágothai János"

Tutor: Dr. med. habil. Mária Judit Molnár

Opponents: Dr. Tibor Kovács, Ph.D.
Dr. Katalin Jakab, Ph.D.

Chairman of the final exam committee: Prof. Dr. Falus András
Members of the final exam committee:: Dr. Ercsey Csaba, Ph.D.
Dr. Valikovics Attila, Ph.D.

Budapest

2008

1. INTRODUCTION

The scientific work of the candidate focused to the genetic background and the not pharmacological therapy of neurodegenerative diseases. His PhD dissertation tries to band together the results of multifaceted methods like clinical neurological, genetic and imaging tests. The scientific work focus on three neurodegenerative diseases: Morbus Parkinson, dystonia and motoneuron disease. Regarding to Parkinson and motoneuron disease genetic and clinical research was performed, for dystonia beside the genetic research new therapeutic approach was investigated.

Going back some more then a decade all researcher agreed about Parkinsonism is not inherited. Research in this field brought a long list of loci, starting with PARK1 up to PARK11. Information about this genes increased our knowledge about pathomechanism of Parkinson disease. Actually 6 genes are known responsible for Parkinson disease. The known mutations in the α-synuclein gene are a rare cause for familial PD but this protein plays an important role in sporadic PD as it is a major component of Lewy bodies. Most of the mutations are directly or indirectly involved in the protein degradation by the ubiquitin-proteasome system, which is responsible for the pathologically formed protein degradation in eucariot cells.

Genetic research of dystonia syndromes made possible to move from the topographic or time-of-onset related classification to an aetiology based classification of dystonia (DYT1-DYT15). This is not only from scientific relevance as different types of dystonia have different response to modern therapeutic approaches like deep brain stimulation (DBS).

Amyotrophic lateral sclerosis (ALS) is a degenerative disease of motoneurons. Similar to PD one can not distinguish between sporadic and familial cases by clinical criteria. Responsible for familial ALS mutations of the SOD1 gene were detected, in the meantime 8 loci and 4 genes are known.

2. OBJECTIVES

Summery of the objectives of the research for this thesis:

1. Investigation of genetic background for monogenic PD and testing of already described mutations and loci for appearance and frequency in European Caucasian population with familial PD.
2. Investigation of genetics of dystonia and genotype-phenotype correlation.
3. Investigation of the genetic background of familial ALS.
4. Investigation of deep brain stimulation in different types of dystonia.

3. METHODS

Patients with Parkinson disease

The PD and non PD members of our families were seen by neurologist, medical history, pedigree of the family and clinical status (in part with video documentation) were taken. In some cases functional imaging with 18F-dopa-PET or 123J-IPT SPECT and autopsy were available, too. Only families corresponding to strong criteria for PD were taken for genetic studies: 3 of the 4 main criteria for PD (akinesia, rest tremor, rigor and postural instability) and a significant levodopa respond were required. The functional imaging was in all cases positive for PD, the autopsy showed PD like degeneration in substancia nigra with typical Lewy bodies. Linkage analysis was performed in 6 families (see II. publ.) and in 13 families (see I. publ.) with 26 respectively 53 affected individuals. The studies were performed in cooperation with "The European Consortium on Genetic Susceptibility in Parkinson Disease" (for coworkers see I., III.,IV. publ.). For mutations analysis of α-synuclein gene (Ala53Th, UCH-L1 gene (Ile93Met) and the NR4A2 gene 77, 11 respectively 44 PD families were tested (see III., IV., and V. publ.).

Patient with dystonia

We tested a family with alcohol-respondive myoclonic dystonia with 9 affected and 25 unaffected members. Video-documentation were taken of the affected members for

second opinion. Also the family members (3 affected, 4 at-risk) with writer´s cramp were tested by neurologists (see VII. publ.). In addition to the clinical investigation the DYT1 sib-pair obtained lab testing, electrophysiological studies, MRI imaging, muscle biopsy and genetic testing (see VIII. publ.). For deep brain stimulation 6 partly different types of dystonia cases were chosen. One DYT1 positive dystonia, patients, three segmental dystonia and 2 cervical dystonia patients were studied. Pre- and postoperative status of all patients were documented by video (see IX. publ.).

Patients with ALS

We studied a large Austrian family with ALS. Beside the index patient and his healthy brother, they reported from two affected sibs, two affected cousins and one affected child of a sib. All other affected members except the index patient have already died. The index patient was scored due to the El Escorial criteria to have definite ALS (see X, publ.).

Clinical methods

All probands involved in genetic studies got a complete neurological investigation, for evaluation and documentation of pre and postoperative status of dystonia we used the BMF dystonia scale, the Tsui scale for cervical dystonia and the SF-36 Quality of Life Survey, each at base line and 3 respectively 12 month. Magnetic resonance imaging studies were performed to show the anatomical localisation of the electrode leads.

Molecular methods

Linkage analysis

Genetic linkage occurs when particular genetic loci or alleles for genes are inherited jointly. Genetic loci on the same chromosome are physically connected and tend to segregate together during meiosis, and are thus genetically linked. For linkage analysis families with at least 2 affected members are necessary. For the primer sequences, for details regarding the two-point and multi-point analysis see the attached publication. PCR amplification of polymorph DNA fragments we used standard technique.

Genehunter and Vitesse software and algorithms were used for multipoint and two-point analysis.

Genotyping

In the two larges PD pedigrees we performed genotyping using 358 polymorph marker. (Human genom screening set, Vers.6) 86% of the marker set were tri- or tetranucleotid repeat marker, average heterozygoty was 76%, the average genetic distance 10 cM. For Genotyping GenScanTM and GenoTyperTM software and LinkRun or LinkScan screening program was applied.

Sequencing

Initially dye terminator PCR sequencing was performed, later on direct sequencing from genomic DNA was performed due to standard technique.

Restriction digestion

For detection of previously described mutations PCR amplification and restriction digestion were performed. For visualization we used radioactive marked amplification or direct visualization by staining with ethidium bromid.

Single-strand conformational polymorphism analysis (SSCP)

For screening for changes of conformation due to sequence changes of the exons 1.2.4 of the SOD1 gene we used non denaturating 6% polyacrilamid gel electrophoresis.

Deep brain stimulation

Under MRI control the electrodes for deep brain stimulation were inserted under local anesthesia. As previously described target point was the same as for Parkinson surgery: 3 mm anterior to the AC-PC midpoint and 18-22 mm lateral to the midline to a depth of 3-6 mm below the intercommissural plane. Intraoperative macrostimulation was performed using a monopolar electrode to evaluate side effects. Once the optimal target site for no side effects had been difined, the testing electrode was replaced by the permanent quadripolar stimulating electrode. After a testing period of 3-6 days we

implanted two programmable pulse generators. The amplitude of stimulation was increased over a period of several days to determine optimal level. MRI studies were carried out to show the anatomical localization of the electrode leads. Outline sketches of the basal ganglia were projected onto the MRI scans in order to visualize electrode lead position.

4. Results

Genetic complexity and Parkinson´s Disease

We have examined polymorphic markers closely linked to the Parkinson locus published by Polymeropoulos on chromosome 4q21-4q23. Multipoint analysis with eight markers spanning the entire 17 cM region likely to contain PD1 in five families excluded linkage. In addition in six families only the polymorphic markers most closely linked to PD1 have been analysed, again without evidence for linkage. We conclude that mutations at the PD1 locus are probably a rare case of autosomal-dominant parkinsonism.

Susceptibility locus 2p13

Due to the results of the simulation study we selected the two most informative family C and D for genom-wide scan. The scan performed using 344 autosomal and 14 X-chromosomal markers showed consistently positive lod scores for several adjacent markers on chromosome 2p. This area of 40 cM was subsequently investigated more closely by typing additional 28 markers in all six families left in our study. Significant evidence for linkage was found with a maximum multipoint lod score of 3,96, considering affected members only, estimating marker allele frequencies from founders in the pedigrees, and allowing for genetic heterogeneity.

Mutation of the α-synuclein gene (Ala53Thr)

We report the results of a screen of 230 European familial index cases of Parkinson´s disease for the recently described Ala53ZThr mutation in the α-synuclein gene in an autosomal dominant Parkinson´s disease kindred. No mutations were found from this

broad white population, and we therefore conclude that this mutation is a very rare cause of familial Parkinson´s disease.

Ubiquitin carboxy-terminal-hydrolase L1 gene (UCH-L1)

Leroy described in a German family with Parkinson´s disease a mutation in the UCH-L1 gene. We performed mutation analysis on the complete coding region of the UCH-L1 gene in one randomly selected sibling from those families in which two affected siblings shared a haplotype for 5 markers of this region. We did not observed the described mutation and any other mutation of the coding exons 1-9 of the UCH-L1 gene. We conclude that the UCH-L1 gene is not a major gene responsible for familial PD.

NR4A4 gene - Exon 1

We screened 30 autosomal-dominant PD families from Caucasian ethnicity for the described NR4A4 gene - Exon 1 mutation. No mutation were found in exon 1 in this families with known parent-child transmission. Our findings show that sequence alterations in exon 1 of hte NR4A2 gene are not a major cause of familial PD in Europa.

Linkage studies in alcohol-responsive myoclonic dystonia

A large German family with myoclonic dystonia with lightning jerks responsive to alcohol was identified. Linkage analysis was performed using simple sequence repeat polymorphisms closely associated with region containing 15 candidate genes: DYT1, 15 subunits of GABA A receptor, 2 subunits of glycine receptor. We excluded that any of these gene-bearing chromosomal regions contain the disease gene in this family.

DYT1

We describe a family with 5 clinically affected individuals positive for DYT1 presented as a dystonic writer´s cramp during late childhood or adolescence, which affected sequentially both sides but did not progress to a generalized form of dystonia. We conclude that familial writer´s cramp may be a manifestation of the DYT1 mutation.

In contrast an other sibling with DYT1 mutation is described with first signs of focal dystonia at age 12 and generalisation over a period of 6-10 years.

Deep brain stimulation in different types of dystonia

The results of deep brain stimulation of the globus pallidus in six patients with generalized, focal and segmental dystonia resistant to other therapy are presented.

One day to 2 weeks after surgery all except one patient experienced a major improvement in motor symptoms. This improvement in motor symptoms was paralleled by reduction of functional disability. In the BFM dystonia rating scale there was a mean reduction of 72,5%, the mean reduction in the Tsui score for cervical dystonia was 63%. The SF-36 self-administrated short form health survey showed a remarkable increment in the scores of a set of eight scales, which gave a profile of health status categories.

MRI imaging studies were performed to show the anatomical localization of the electrode leads, Images were scanned to make exact measurements of electrode lead positions and to project detailed anatomical drawings from Schaltenbrand and Wahren Atlas onto the scans. The mean stereotactic coordinates of the electrode leads were 2,8 mm anterior to MC point and 20,5 mm lateral to midline. The mean ventral distance 5,3 mm. Because of local infection electrodes of patient 2 had to be removed. The difference in electrode position and clinical effect allowed the conclusion that a more lateral position of the lead reduces the clinical benefit of DBS.

Superoxide dismutase (SOD1) mutation in ALS

We report on an multigenerational pedigree with autosomal dominant ALS with a novel SOD1 mutation in exon 1 at Gln8Leu.

5. Conclusions

We report about our results in the most expanding discipline of neuroscience, from the field of neurogenetics and from interventional neurosurgery, exacting from deep brain stimulation used for the treatment of dystonia. The purpose of our scientific work was to determine the genetic background of neurodegenerative diseases with heredity transmission, Parkinson, dystonia and ALS, furthermore to investigate the correlation between phenotype and genotype of these entities and to investigate the therapeutic effect of deep brain stimulation in different types of dystonia.

1. Mutations at the PARK1 locus in the European Caucasoid population are probably a rare cause of autosomal-dominant Parkinsonism, therefore it is not recommended to look for this gene in the first-line routine diagnostics.

2. We describe a new genetic locus that appears to be involved in the development of parkinsonism. This locus, PARK3, was detected in a group of families of European origin.

3. We performed mutation analysis on Caucasoid families with at least two affected sibs. As we did not detect any mutation in the UCH-L1 (PARK5) and NR4A2 gene we conclude that this genes are not responsible for familial PD.

4. A large family with "myoclonic dystonia with lightning jerks responsive to alcohol" was identified. By linkage analysis the candidate genes DYT1, GABA A receptor subunits and glycine receptor subunits could be excluded as the disease gene in this family.

5. We describe a family with 5 clinically affected individuals carrying the DYT1 mutation presented as a dystonic writers cramp without progression to a generalised form of dystonia. In contrast to this phenotype we also describe two brothers with the same genotype (3 bp deletion in exon 5 of DYT1 gene) and a progressive course starting with focal dystonia and generalisation over a period of 6 to 10 years.

6. The results of deep brain stimulation (DBS) of the Gpi in six patients with generalised, focal and segmental dystonia are presented. Clinical symptoms were evaluated before and after surgery using rating scales and SF-36 Health Survey for health status. We conclude that chronic high-frequency Gpi stimulation in different types of dystonia is an effective and safe treatment.

7. We report on a pedigree with autosomal dominant ALS and a novel T to A missense mutation in exon 1 of the SOD1 gene. Preclinical genetic testing for known mutations can contribute to prevention of an untreatable malignant disease. This novel mutation can contribute for understanding the disease mechanism in the future.

6. PUBLICATIONS RELEVANT TO THE DISSERTATION

I. Gasser T, Muller-Myhsok B, Wszolek ZK, Durr A, Vaughan JR, Bonifati V, Meco G, Bereznai B, Oehlmann R, Agid Y, Brice A, Wood N. Genetic complexity and Parkinson's disease. Science. 1997 Jul 18;277(5324):388-9;

II. Gasser T, Muller-Myhsok B, Wszolek ZK, Oehlmann R, Calne DB, Bonifati V, Bereznai B, Fabrizio E, Vieregge P, Horstmann RD. A susceptibility locus for Parkinson's disease maps to chromosome 2p13. Nat Genet. 1998 Mar;18(3):262-5.

III. Vaughan J, Durr A, Tassin J, Bereznai B, Gasser T, Bonifati V, De Michele G, Fabrizio E, Volpe G, Bandmann O, Johnson WG, Golbe LI, Breteler M, Meco G, Agid Y, Brice A, Marsden CD, Wood NW. The alpha-synuclein Ala53Thr mutation is not a common cause of familial Parkinson's disease: a study of 230 European cases. European Consortium on Genetic Susceptibility in Parkinson's Disease. Ann Neurol. 1998 Aug;44(2):270-3.

IV. Harhangi BS, Farrer MJ, Lincoln S, Bonifati V, Meco G, De Michele G, Brice A, Durr A, Martinez M, Gasser T, Bereznai B, Vaughan JR, Wood NW, Hardy J, Oostra BA, Breteler MM. The Ile93Met mutation in the ubiquitin carboxy-terminal-hydrolase-L1 gene is not observed in European cases with familial Parkinson's disease. Neurosci Lett. 1999 Jul 23;270(1):1-4.

V. Zimprich A, Asmus F, Leitner P, Castro M, Bereznai B, Homann N, Ott E, Rutgers AW, Wieditz G, Trenkwalder C, Gasser T. Point mutations in exon 1 of the NR4A2 gene are not a major cause of familial Parkinson's disease. Neurogenetics. 2003 Aug;4(4):219-20.

VI. Gasser T, Bereznai B, Muller B, Pruszak-Seel R, Damrich R, Deuschl G, Oertel WH. Linkage studies in alcohol-responsive myoclonic dystonia. Mov Disord. 1996 Jul;11(4):363-70.

VII. Gasser T, Windgassen K, Bereznai B, Kabus C, Ludolph AC. Phenotypic expression of the DYT mutation: a family with writer's cramp of juvenile onset. Ann Neurol. 1998 Jul;44(1):126-8

VIII. Bereznai B, Baraczka K, Nagy Z, Molnár MJ. DYT1 pozitív dystonia: egy testvérpár esettanulmánya. Clinical Neuroscience (Ideggy Szemle). 2007 Jul: 7-8: 342-7

IX. Bereznai B, Steude U, Seelos K, Botzel K. Chronic high-frequency globus pallidus internus stimulation in different types of dystonia: a clinical, video, and MRI report of six patients presenting with segmental, cervical, and generalized dystonia. Mov Disord. 2002 Jan;17(1):138-44.

X. Bereznai B, Winkler A, Borasio GD, Gasser T. A novel SOD1 mutation in a family with amyotrophic lateral sclerosis. Neuromuscul Disord. 1997 Mar;7(2):113-6.

Posters:

XI. Bereznai B., Müller B., Pruszak-Seel R., Damrich R., Deuschl G., Oertl W., Gasser T., Genetic linkage studies in a family with Myoclonic dystonia, in "Abstracts presented at the 1994 meeting of the european federation of neurological societies", Poznan, Poland, 1994

XII. Bereznai B., Steude U., Gasser T., Boetzel K.: Chronic hight frequency GPI stimmulation in different types of dystonia. A clinical, MRI and video report of 5 patients presenting with generalized and segmental dystonia. ENS Jerusalem 2000. J Neurol. Vol 247, Suppl. 3

7. OTHER PUBLICATIONS

1. Bereznai B, Goebels N, Dang T, Voltz R, Walther E, Zimmermann C, Hohlfeld R. Therapy of multiple sclerosis. Dtsch Med Wochenschr. 1999 May 14;124(19):595-9.

2. Bereznai B and Hohlfeld R. Immunmodulierende Therapie der Multiplen Sklerose mit Betaferon. Münch med Wschr. 1999 Nr. 141, 27-9

3. Deckert J, Hennemann A, Bereznai B, Fritze J, Vock R, Marangos PJ, Riederer P. (3H)dipyridamole and (3H)nitrobenzylthioinosine binding sites at the human parietal cortex and erythrocyte adenosine transporter: a comparison.Life Sci. 1994;55(21):1675-82.

4. Deckert J, Bereznai B, Hennemann A, Gsell W, Gotz M, Fritze J, Riederer P. Nimodipine inhibits [3H]nitrobenzylthioinosine binding to the adenosine transporter in human brain. Eur J Pharmacol. 1993 Jul 6;238(1):131-3.

Posters:

5. Bereznai B., Hennemann A., Gsell W., Götz M., Fritze J., Beckmann H., Riederer P., Deckert J., Inhibition of(3H)NBI binding by adenosine uptake inhibitors and DHP calcium channel antagonists in human brain, Int.J.Purine and Pyrimidine Res., 3(1), 64, 1992

6. Bereznai B., Deckert J., Hennemann A., Gsell W., Götz M., Fritze J., Beckmann H., Riederer P., Hemmung der Bindung von (3H)NBI durch Adenosin Transport Hemmer und DHP Kalzium Kanal Blocker in menschlichem Kortex, Fortschr. Neurol. Psychiat. 60 (Sonderheft 1), 55, 1992

7. Bereznai, B.; Brückmann, H.; Missler, U.; Hamann, G.: Entstehungsmechanismen der Hirnstammischämie bei Megadolichobasilaris. Posterpräsentation DGN München.1998

Book chapter

8. K. Bürger, B. Bereznai.: Diagnose der Vaskulären Demenz. In: H. Hampel und H.J. Möller (Hrsg.) Alzheimer Demenz. Wissenschaftliche Verlagsgesellschaft. Stuttgart, 2003

Book translation

9. Neurológiai kórképek kézikönyve. Parkinson-szindrómák. Thomas Brandt, Johannes Dichgans, Hans Chritoph Diener. Fordította: Dr. Bereznai Benjamin. Budapest 2007

ACKNOWLEDGEMENT

I would like to express my appreciation and gratitude to my teachers and colleges Prof. Dr. Thomas Gasser and PD Dr. Kai Bötzel, for their help during my research.

First of all my gratitude is expressed to my mentor Dr. Mária Judit Molnár for her excellent supervision and guidance.

I am also indebted to Prof. Dr. Zoltán Nagy for all his encouragement and advice.

I would like to express my special thanks to my family, to my parents and to my wife Anja and the children for their patience, encouragement and the maximum possible guidance.

I would like to express my appreciation to the patients and their families for the dedicated cooperation.