Genetic and clinical characteristics of Wilson disease in patients from Hungary

Ph.D. thesis

Kuczyné dr. Folhoffer Anikó

Semmelweis University
Doctoral School for Clinical Science in Medicine

Tutor: Prof. Ferenc Szalay, MD, PhD, DSc

Official reviewers:

András Arató, MD, PhD, DSc
László Szalay, MD, PhD, CsC

Chairmen of the examination board:

Prof. János Banai, head of dept, MD, PhD, CsC

Members of the examination board:

László Herszényi, MD, PhD, CsC
György Székely, head of dept, MD, PhD, CsC

2008 Budapest

INTRODUCTION

Wilson disease (WD) is an autosomal recessive genetic disorder of copper metabolism caused by the mutation of the ATP7B gene, resulting in a defect of biliary copper excretion. Excessive copper in the liver, brain and in other organs leads to hepatologic and neuropsychiatric symptoms.

WD is a relatively rare disease with huge importance because of its serious symptoms and, without treatment, potentially fatal outcome.

The worldwide prevalence of the disease is estimated to be 30 per 1 million, with a gene frequency of 0.56% and a carrier frequency of 1 in 90. The number of WD patients in Hungary is estimated to approximately three hunred.

The WD gene (ATP7B), identified on chromosome 13q in 14-q21 positions, encodes a copper transporting P-type ATPase, which is predominantly expressed in the liver with reduced expression in the kidney, brain and placenta. This gene contains 22 exons spanning a DNA region of ~100 kb encoding a protein of 1465 amino acids.

Since the gene was identified in 1993, the number of known mutations within this region exceeds 350 variations, according to recent data on WD mutation. The most common one is the H1069Q point mutation (50-80%) in Hungary, similarly to other Central, Eastern or Northern European countries.

Genetic analysis has a huge clinical importance. Detection of mutations on both allels makes the diagnosis and treatment possible before the appearance of the first clinical symptoms.

OBJECTIVES

The aim of the present study was to analyze the demographic characteristics and the distribution of the patients and to assess the impact of genetic testing for diagnosis of WD in patients (n=142) from Hungary. In order to discover the rare, new, not yet described mutations, we performed the sequencing on most WD gene exons in collaboration with Prof. Peter Ferenci from the Vienna University.

In my research the following questions were studied:

1. **Genetic mapping of Wilson disease in Hungary:**
    - What is the prevalence of H1069Q point mutation in patients with Wilson disease from Hungary?
    - **Is there any difference between** data of WD patients from **Hungary** compared to those **from neighbouring or distant countries**?
2. **Which mutations of ATP7B gene causing disease can be detected beyond the H1069Q in Hungarian Wilson disease patients?**
3. **Genotype-phenotype correlation analysis:**
    - Is there any **correlation between genotypes and several phenotypic manifestation** of the illness?
    - What is the practical **clinical impact of genetic testing** for the diagnosis of WD?
    - What are the **demographic characteristics of WD** patients in Hungary?
4. **Assessment of the occurrence of acute liver failure in Hungarian Wilson patients:**
    - **What is the incidence of acute liver failure** among the Hungarian Wilson patients? How many **liver transplantation** was done **due to Wilson disease**? What was the prognosis of these patients?
    - What were the genotypes of the affected patients? Is there any possible correlation between the mutations of *ATP7B* and the clinical manifestation?

PATIENTS AND METHODS

We examined 142 patients from 128 families. As a hepatological centre,we are indebted to the colleagues for sending samples from throughout the country, which made it possible to reach this, even on an international scale, large number. There were 81 male and 61 female patients. Their mean age at the time of the onset of symptoms was 19 years, while at the time of diagnosis it was 22 years. So, on average, three years passed until the diagnosis; however, in some exceptional cases, regrettably, this period exceeds more than ten years. We analyzed the data of eight patients post mortem, who had died from fulminant liver failure.

Our diagnosis was established by the international scoring system based on the typical clinical symptoms and laboratory findings. Only patients with a score of ≥4 were included.

Seventy patients had neuropsychiatric symptoms, 66 had hepatic manifestation of the disease, and 6 patients had no clinical symptoms at all. Among the siblings of index patients, 47 were healthy and 14 had Wilson disease.

We also examined 60 healthy blood donors as control subjects to exclude that the new mutations reflect polymorphisms present in the Hungarian population.

Genomic DNA was isolated according to standard protocols. The most common mutation was assessed by seminested PCR-based RFLP assay. Heterozygotes and negative samples were then screened for mutations by denaturating HPLC and then sequenced.

RESULTS

We found 35 mutations altogether, 10 of which have not been described and listed even in the international database yet. Fourty-eight patients were homozygous for one of them (including fourty patients homozygous for H1069Q), and 44 patients were compound heterozygous. In 6 asymptomatic siblings we proved WD by detection of two disease-causing mutations. In 123 patients we found at least one disease-causing mutation.

The most common point mutation was present in 70% of patients (n=100), indicating that the detection of this mutation is very important in the diagnosis of WD in Hungary. Fourty patients were homozygous (28%), 60 patients were heterozygous (42%), and 42 patients (30%) were negative for this mutation. This distribution within the WD population according to the H1069Q genotype is close to the equilibrium calculated from the Hardy-Weinberg estimation using the measured 49.3% allele frequency.

We analyzed the distribution of patients according to the age and manifestation of the disease at the detection of the first symptom. Patients with hepatic manifestation were younger than patients with neurological symptoms. The youngest patient was 3 years old and presented with liver disease; the oldest one was 49 years old presented with neurological symptoms.

After classifying patients according to clinical manifestation and presence of H1069Q mutation, we found that the Kayser-Fleischer ring was more frequent in H1069Q homozygous patients, and their mean age at the time of diagnosis was higher than in patients either heterozygous or negative for H1069Q.

In altogether 60 patients, mutations other than H1069Q were found, among which there were 26 missense, 1 nonsense, 1 splice substitution and 6 deletion (5/6 frameshift and the V1217-1218L with deletion of 6 bases). All of these mutations except Q1351X and K844K-fs appeared only in a few patients or in the index patients' family members.

By further analysis, we identified mutations on both alleles in 92 patients (65%) and one mutation in 31 patients (22%). In 19 patients (13%) both mutations are as of yet unknown , although they are clinically proved WD patients.

Most of the further mutations had already been observed in European patients—except R778G, which was described in Turkish patients, whereas the R778L is frequent in Far-Eastern (28-44%) but not in Central-European countries.

Since this is a hereditary disease, we performed the family screening tests in each index patients' case. As a consequence of recessive inheritance, WD in two consequtive generations is expected to be particularly rare. However, among Hungarian patients with Wilson disease, we found three such families. Therefore, this occurence is not so rare among Hungarian Wilson patients.

We studied the occurrence of acute liver failure in Wilson disease.

Among the 142 WD patients tested genetically and the 8 young patients who had died in fulminant liver failure – most likely due to WD – , 28 had severe liver failure, among whom 19 patients (15 female and 4 male, mean age:16±4) had acute or subacute liver failure. The juvenile deceased patients' data were analyzed post mortem, and in their several siblings Wilson disease was confirmed.

Fourteen ortotopic liver transplantations were done due to Wilson disease at the Department of Transplantation and Surgery of the Semmelweis University. More than 10 years have passed since the first transplantation. This patient is well, is asymptomatic, his ceruloplasmin level is in the normal range, and the Kayser-Fleischer ring has disappeared.

There was no difference in the survival rate calculated by the Kaplan-Meier method between the 23 patients who had received transplants due to metabolic liver disease (among them 14 WD patients) and those who had undergone liver transplantation because of other etiology (p=0,6).

Survival data of OLT patients from Hungary

Survival	Metabolic (n=23)	Non-metabolic (n=298)
1 yr	78%	77%
3 yrs	73%	71%
5 yrs	48%	66%

Twelve out of nineteen patients died in hepatic coma, several of whom had been on the waiting list for transplantation, and some of whom had not even been on the list, because the diagnosis had been established so late.
Fifteen out of nineteen patients with ALF had Coombs negative hemolyis, while in all of the Hungarian Wilson population it was detectable only in 25/142 patients.

Serum ceruloplasmin level was almost normal in fulminant form of WD, since it is an acute phase protein; AST was higher, ALT lower, serum bilirubin was much higher, and alkaline phosphatase was much lower than in the other subgroups. (AST/ALT= 2.25 vs 0.5 and 1).

The fulminant hepatic manifestation of Wilson disease is more frequent in women. It can be assumed that female sex hormones may aggravate hepatic copper accumulation.

CONCLUSIONS

Our previous observations were extend with the data of 142 Hungarian Wilson disease patients screened for most of the exons for mutations. The 35 different mutations found, eight of which had been described only in Turkish, Italian and Albanian patients before, reflect the genetic heterogenity of WD in Hungary, , which could be the genetic consequence of the 150-yearTurkish occupation of Hungary in the 16th and 17th centuries.

The detection of H1069Q point mutation is very important in the diagnosis of WD in Hungary. By standard means (on clinical and chemical findings alone), 44 patients presented with liver disease (66/142) and 7 patients with neurological manifestation (70/142) would have failed to be diagnosed, while,

by adding genetic testing to the diagnostic algorithm, diagnosis could be established, which made further diagnostic tests unnecessary. On the other hand, by using molecular testing, the timeframe from symptoms to diagnosis was shortened in some cases.

Mutation analysis may be helpful in patients in whom typical clinical symptoms or findings of WD are absent: in patients presenting hepatic disease and in asymptomatic siblings. However, mutation analysis is not useable for first line screening, because of the huge number of mutations of the WD gene.

Examination of the index patients' siblings is very important. The presence of disease-causing mutations on both alleles is sufficient to diagnose Wilson disease, even in the absence of any actual clinical symptoms. Treatment started in preclinical stage could prevent the development of clinical symptoms.

OUR NEW RESULTS AND THEIR PRACTICAL SIGNIFICANCE

- In a nationwide collaboration, we extended our previous observations with the analysis of the data **from an internationally significant number of Hungarian Wilson disease patients (n=142)**. We assessed that, on average, a long time (several **years) had passed,** regrettably, **from the first symptom until the diagnosis**. The activity of our workgroup has also contributed to abridging this timeframe.
- We confirmed that **in Hungary**, similary to other Central-Northern-Eastern European countries, **H1069Q was the most frequent** mutation, detected in 100 patients (**70%**), which means a **49% allele frequency.**
- **Thirty-four further mutations** were found by sequencing exon 6-20, including **ten new ones not described before** and not even listed in the international WD mutation database: **L517-fs (c1549-1559del11), N676I, S693Y, Y715H, M769L, W939C, V1001G, V1039F, P1273S and G1281D**. Eight of them had been described, apart from Hungary, only in Turkish, Italian and Albanian patients before, which could be the genetic consequence of the 150-year Turkish occupation of Hungary in the 16th and 17th centuries.
- Our results confirmed the general view of the impact of genetic testing in patients presented with hepatic disease or the asymptomatic siblings of an index case, as we documented in several case reports. Examination of index patients' siblings is especially important, since, in case of two disease-causing mutations, the beginning of the therapy

can be prophylactic in the development of clinical symptoms in preclinical stage, as we confirmed with several case reports.

- Wilson patients' distribution according to gender: In the whole Hungarian WD population, male patients were dominant, while in the subgroup of patients suffering from acute liver failure there were more women. Female sex hormones may aggravate hepatic copper accumulation.
- The distribution of patients according to the age and manifestation of the disease at the detection of the first symptom showed that patients with hepatic manifestation were younger than patients with neurological symptoms.
- The occurrence of acute hepatic failure was relatively frequent among the Hungarian Wilson patients and seemed to be associated with H1069Q heterozygous genotype.
- Wilson disease is a relatively rare cause of acute liver failure. However, it must be kept in mind in case of every liver disease with unclarified origin/etiology, considering that in almost 15% of the fulminant WD serum ceruloplasmin is in the normal range, without the presence of the Kayser-Fleishcer ring and neurologic symptoms.
- We described three Hungarian families, where Wilson disease appeared in two consequtive generation.

THE BIBLIOGRAPHY OF PUBLICATIONS

Publications related to the theme of PhD thesis:

Full papers:

1. **Folhoffer A**, Horváth A, Hegedűs D, Firneisz G, Dunkel K, Willheim C, Ferenci P, Lakatos PL és Szalay F. **ATP7B génmutációk magyarországi Wilson kóros betegekben; esetismertetések a betegség változatos klinikai megjelenésére**

   Orv Hetil. 2003 Dec 21;144(51):2509-15.

2. Nagy J, Vincze Z , **Folhoffer A** , Horváth A, Csák T, Zelkó R. **A Wilson-kór patomechanismusa és gyógyszeres kezelése.**

   Acta Pharmaceutica Hungarica 2003; 73(4): 237-241.

3. Firneisz G, Szonyi L, Ferenci P, Willheim C, Horvath A, **Folhoffer A**, Tulassay Z, Szalay F. **The other mutation is found: follow-up of an exceptional family with wilson disease**.

   *Am J Gastroenterol. 2004 Dec;99(12):2504-5.-**Sci Letter*** (IF: 4,716)

4. Nagy J, **Folhoffer A**, Horváth A, Csák T, Taba G, Szentmihályi K, Szalay F, Zelkó R. **Kinetic study of zinc sulphate release from lipophilic matrices prepared for the therapy of Wilson's disease.**

   *Pharmazie 2005;**60**:(7)524-6.* IF: 0,587

5. **Folhoffer A,** Ferenci P, Csak T, Horvath A, Hegedus D, Firneisz G, Osztovits J, Kosa JP, Willheim-Polli C, Szonyi L, Abonyi M, Lakatos PL and Szalay F. Novel mutations of the *ATP7B* gene among 109 Hungarian patients with Wilson's disease

   Eur J Gastroenterol Hepatol 2007 Feb;19(2):105-11. IF: 1,843

Abstracts quotable in vetted journals:

1. Horvath A, Keresztes K, **Folhoffer A**, Hegedus D, Lakatos PL, Dunkel K, Kempler P, Szalay F. AUTONOMIC AND SENSORY NERVE DYSFUNCTION IN PATIENTS WITH WILSON DISEASE
   1st Department of Internal Medicine, Semmelweis University, Budapest, Hungary
   J Hepatol 2003; 38(Suppl No 2):211.

2. Dunkel K., Horvath A., Keresztes K., **Folhoffer A.,** Hegedűs D., Lakatos P.L., Kempler P., Szalay F. ALTERED HEART RATE VARIABILITY IN PATIENTS WITH WILSON DISEASE

1st Department of Medicine of Semmelweis University, Budapest, Hungary
Z Gastroenterol 2003;41:434.

3. **Folhoffer A**, Horvath A, Lakatos PL, Dunkel K, Beko G, Willheim C, Ferenci P, Szalay F. H1069Q AND FURTHER MUTATIONS OF ATP7B GENE IN WILSON DISEASE PATIENTS FROM HUNGARY
1 st Department of Semmelweis University, Budapest, Hungary, Department of Internal Medicine IV.,Gastroenterology and Hepatology, University of Vienna, AKH, Wien, Austria
Z Gastroenterol 2003;41:436.

4. **Folhoffer A[1]**, Horvath A[1], Hegedus D[1], Firneisz G[1], Dunkel K[1], Willheim-Polli c[2], Ferenci P[2], Lakatos PL[1], Szalay F[1]. H1069Q AND OTHER MUTATIONS OF ATB7B GENE IN HUNGARIAN PATIENTS WITH WILSON DISEASE
[1]1st Department of Medicine, Semmelweis University, Budapest, Hungary, [2] Department of Internal Medicine 4, University of Vienna, Austria
Gut 2003; 52 (Suppl VI) A3

5. **Folhoffer[1],** A. Horvath[1], T. Csak[1], P. L. Lakatos[1], M. Abonyi[1], L. Szonyi[2], C. Willheim-Polli[3], P. Ferenci[3], F. Szalay[1]. LIVER FAILURE AS PRESENTING SYMPTOM IN WILSON DISEASE
[1]1st Dept of Internal Medicine, [2]1st Dept of Pediatrics, Semmelweis University, Budapest, Hungary, [3]4th Dept of Medicine, University Vienna, Vienna, Austria
Gut 2004;53(Suppl VI) A93

6. **Folhoffer A**, Lakatos PL, Horvath A, Csak T, Osztovits J, Bajnok E, Speer G, Nagy Z, Habior A, Tornai I, Lakatos P, Szalay F. CALCIUM-SENSING RECEPTOR (CASR) A986S POLYMORPHISM AND BONE DISEASE IN PRIMARY BILIARY CIRRHOSIS AND WILSON DISEASE
Zeitschrift für Gastroenterologie 2005; 43: 485 (A27)

7. **A Folhoffer**, P Ferenci, T Csak, A Horvath, D Hegedus, G Firneisz, J Osztovits, JP Kosa, C Willheim-Polli, L Szonyi, M Abonyi, PL Lakatos, F Szalay. NOVEL MUTATIONS OF ATP7B GENE AMONG 109 HUNGARIAN PATIENTS WITH WILSON DISEASE. BENEFITS OF GENETIC ANALYSIS
GUT SVII (54), A190

8. **Folhoffer A**., Csák T., Horváth A., Osztovits J., Fuszek P., Zacher G., Szalay F.
LATE DIAGNOSIS OF WILSON DISEASE AFTER ANTI-PARKINSON TREATMENT AND ATTEMPT AT SUICIDE. A CASE OF WILSON DISEASE ASSOCIATED WITH ULCERATIVE COLITIS
Zeitschrift für Gastroenterologie 2006; 43: 1-48; A27

9. Ferenci P, Merle U, **Folhoffer A**, Evstatiev R, Yurdaydin C, Bruha R, Caca K, Willheim C, Demir K, Houwen R, Szalay F, Stremmel W PHENOTYPE-GENOTYPE CORRELATIONS WILSON DISEASE (WD) – RESULTS IN A MULTINATIONAL STUDY IN 1008 PATIENTS *Hepatology*

<u>Publications not related to the theme of Phd thesis</u>
Full papers:

1. Szalay F, Hantos MB, Horvath A, Lakatos PL, **Folhoffer A**, Dunkel K, Hegedus D, Tekes K. **Increased nociceptin/orphanin FQ plasma levels in hepatocellular carcinoma.**
World J Gastroenterol. 2004 Jan;10(1):42-5. IF: …….*.

2. Horvath A, **Folhoffer A**, Lakatos PL, Halosz J, Illyes G, Schaff Z, Hantos MB, Tekes K, Szalay F. **Rising plasma nociceptin level during development of HCC: a case report.**
World J Gastroenterol. 2004 Jan;10(1):152-4. IF: …….*

3. Lakatos PL, Bajnok E, Tornai I, **Folhoffer A**, Horvath A, Lakatos P, Szalay F. **Csökkent csontásványianyag-tartalom és génpolimorfizmus primer biliaris cirrhosisban**
Orv Hetil. 2004 Feb 15;145(7):331-6.

4. Lakatos PL, Bajnok E, Tornai I, **Folhoffer A**, Horvath A, Lakatos P, Habior A, Szalay F. **Insulin-like growth factor I gene microsatellite repeat, collagen type Ialpha1 gene Sp1 polymorphism, and bone disease in primary biliary cirrhosis.**
Eur J Gastroenterol Hepatol. 2004;16(8):753-759. IF: 1,578

5. Keresztes K, Istenes I, **Folhoffer A**, Lakatos PL, Horváth A, Csak T, Vargha P, Kempler P, Szalay F: **Autonomic and sensory nerve dysfunction in primary biliaris cirrhosis**
World J Gastroenterol. 2004; 10 (20):3039-3043 IF: …….*

6. Szalay F, Telegdy L, Szeli D, Csák T, **Folhoffer A**, Horváth A, Abonyi M, Szabó O, Rédei Cs, Nemesánszky E. **Rifaximin a hepaticus encephalopathia kezelésében.**
Lege Artis Medicinae 2004; 14(5): 321-325.

7. **Folhoffer A**, Horváth A, Csák T, Nébenführer L, T, Hazslinszky P, Iványi A, Szalay F. **Neurofibromatosis, melanoma malignum, hyperthyreosis és HCV pozitivitás esete.**
Lege Artis Medicinae 2004; 14(5): 349-352.

8. Csák T, **Folhoffer A**, Horváth A, Lengyel G, Kóbori L, Szalay F: **Májtranszplantáció utáni krónikus C-hepatitis recidívájának kombinált antivirális kezelése.**
Orv Hetil. 2004 145(39):2003-2006.

9. Horvath A**, Folhoffer A**, Csak T, Komoly S, Szalay F. **Cryoglobulinaemiával és súlyos fokú polyneuropathiával járó krónikus C hepatitis gyógyult esete**
Magyar Belorvosi Archívum, 2004

10. Szalay F, **Folhoffer A**, Horvath A, Csak T, Speer G, Nagy Z, Lakatos P, Horvath C, Habior A, Tornai I, Lakatos PL. **Serum leptin, soluble leptin receptor, free leptin index and bone mineral density in patients with primary biliary cirrhosis.**
EurJ Gastroenterol Hepatol. 2005 Sep ;17(9):923-8. IF:1,843

11. Keresztes K, **Folhoffer A**, Lakatos PL, Istenes I, Horváth A, Csák T, Vargha P, Kempler P, Szalay F:
Az autonom és szenzoros neuropathia gyakorisága és rizikófaktorai primer biliáris cirrhosisban
Magyar Belorv Arch 2005; 58; 103-112.

12. Ferencz V, Horvath C, Kari B, Gaal J, Meszaros S, Wolf Z, Hegedus D, Horvath A, **Folhoffer A**, Szalay F. **Bone disorders in experimentally induced liver disease in growing rats.**
World J Gastroenterol. 2005 Dec 7;11(45):7169-73. IF:……*

13. Csak T, **Folhoffer A**, Horvath A, Halasz J, Diczhazi C, Schaff Z, Szalay F. **Holmes-Adie syndrome, autoimmune hepatitis and celiac disease: a case report.**
World J Gastroenterol. 2006 Mar 7;12(9):1485-7. IF:…..*

14. Csák T, **Folhoffer A,** Horváth A, Osztovits J, Halász J, Diczházi Cs, Schaff Zs, Szalay F. **Autoimmun hepatitis, coeliakia és Holmes-Adie szindróma együttes előfordulása**
Magy Belorv Arch 2006; 59: 55-58.

15. Csák T, **Folhoffer A**, Horváth A, Osztovits J, Papp J, Görög D, Kóbori L, Szalay F. **Extrém hypercholesterinaemia és xanthomatosis laparoszkópos cholecystectomia után. Teljes reverzbilitás a iatrogén szűkület műtéti megoldását követően.**
Orv Hetil 2006; 147(15): 705-710.

16. Horváth A, **Folhoffer A**, Csák T, Osztovits J, Szalay F.**Májrák primer biliáris cirrhosisban. Az irodalom áttekintése egy eset kapcsán.**
Magyar Belorv Arch 2006; 61: 207-212.

17. Speer G, Szenthe P, Kósa JP, Tabák AG, **Folhoffer A**, Fuszek P, Cseh K, Lakatos P. **Myocardial infarction is associated with Spl binding site polymorphism of collagen type 1A1 gene**.
Acta Cardiol 2006 Jun;61(3):321-5

18. Ferencz V, Horváth Cs, Folhoffer A, Kári B, Gaal J, Mészáros Sz, Wolf Zs, Mester Á, Hegedűs D, Horváth A, Szalay F. **A csontfejlődés zavara kísérletes májcirrhosisban, növekedésben lévő patkányokban.**
Ca és Csont 2005;8(2):52-58

19. Speer G, Szenthe P, Kósa JP, Tabák GÁ, Nagy Zs, **Folhoffer A**, Cseh K, Lakatos P. **Az 1-es típusú kollagén A1génjének -1245G/T polimorfizmusa összefügg a miokardiális infarktussal: az új patogenetikai faktor?**
Cardiologia Hungarica 2005; 35:64-69

** IF of WJG in 2003: 3,318.*
„Notice from Thomson/ISI 2006-07-14: World Journal of Gastroenterology has been re-accepted for coverage in Current Contents/ Clinical Medicine and SCIE."

Abstracts quotable in vetted journals:

1. Lakatos P László, Éva Bajnok, **Anikó Folhoffer**, Andrea Horváth, István Tornai#, Lakatos Péter, Szalay Ferenc. COLLAGEN TYPE I□1 GENE SP1 POLYMORPHISM AND BONE DISEASE IN PRIMARY BILIARY CIRRHOSIS
   1st Department of Medicine, Semmelweis University, Budapest, #2nd Department of Internal Medicine, Debrecen University, Debrecen, Hungary

   J Hepatol 2003; 38(Suppl No 2):212.

2. Horváth A., Hantos M., Tekes K., Halász J., Illyés Gy., Schaff Zs., **Folhoffer A**., Lakatos PL., Szalay F. HEPATOCELLULAR CARCINOMA IN A PATIENT WITH PRIMARY BILIARY CIRRHOSIS IS NOCICEPTIN A MARKER FOR HCC?
   1st Department of Medicine, Department of Pharmacodynamics and 2nd Department of Pathology of Semmelweis University, Budapest, Hungary
   Z Gastroenterol 2003;41:439.

3. Lakatos PL, Bajnok E, **Folhoffer A**, Horvath A, Tornai I, Lakatos P, Szalay F. INSULIN-LIKE GROWTH FACTOR (IGF-1) GENE MICROSATELLITE REPEAT AND COLLAGENE TYPE I ALPHA 1 Sp1 POLYMORPHISMS AND BONE DISEASE IN HUNGARIAN PATIENTS WITH PRIMARY BILIARY CIRRHOSIS (PBC)
   1st Department of Semmelweis University, Budapest, Hungary, 2nd Department of Internal Medicine, Debrecen University, Debrecen, Hungary
   Z Gastroenterol 2003;41:444.

4. Nemesánszky E[1], Szeli D[1], Telegdy L[2], **Folhoffer A[3]**, Horváth A. [3], Szalay F.[3]
   RIFAXIMIN TREATMENT IN PORTOSYSTEMIC ENCEPHALOPATHY
   [1]Dept. of Gastroenterology, Polyclinic of the Hospitaler Brothers, [2]Dept. of Hepatology of St. Laszlo Hospital, [3]1st Dept. of Medicine, Semmelweis University, Budapest, Hungary
   Z Gastroenterol 2003;41:449.

5. Szalay F.[1], Horvath A.[1], Hantos M.[2], **Folhoffer A.[1]**, Lakatos P.L.[1], Bekő G.[1], Hegedus D[1], Tekes K. [2] EXTREMELY HIGH PLASMA NOCICEPTIN MIGHT BE A MARKER FOR HEPATOCELLULAR CARCINOMA
   1st Dept of Medicine[1] and Dept of Pharmacodynamics[2] of Semmelweis Univ., Budapest

Z Gastroenterol 2003;41:459

6. Szalay F, Hantos MB, Horvath A, Lakatos PL, **Folhoffer A**, Dunkel K, Hegedus D, Tekes K. STRIKING ELEVATION IN PLASMA NOCICEPTIN LEVEL IN PATIENTS WITH HEPATOCELLULAR CARCINOMA
   Gastroenterology 2003;124;4(Suppl):A770-771.

7. Lakatos PL[1], Bajnok E[1], Tornai I[2], **Folhoffer A[1]**, Horvath A[1], Lakatos P[1], Szalay F[1]. INSULIN LIKE GROWTH FACTOR-I (IGF-I) GENE MICROSATELLITE REPEAT, COLLAGEN TYPE IA1 GENE (COLIA1) SP1 POLYMORPHISM AND BONE DISEASE IN PRIMARY BILIARY CIRRHOSIS
   [1]1st Department of Semmelweis University, Budapest, Hungary, [2]2nd Department of Internal Medicine, Debrecen University, Debrecen, Hungary
   Gut 2003; 52 (Suppl VI) A164

8. Horvath A[1], Hantos MB[2], Tekes K[2], Zalatnai A[3], **Folhoffer A**[1], Lakatos PL[1], Szalay F[1]. HIGH PLASMA AND LIQUOR NOCICEPTIN LEVELS IN RAT WITH EXPERIMENTAL HEPATOCELLULAR CARCINOMA
   1st Department of Medicine[1], Department of Pharmacodynamics[2] and 1st Department of Pathology[3] of Semmelweis University, Budapest, Hungary
   Gut 2003; 52 (Suppl VI) A232

9. Szalay F, Horvath A**, Folhoffer A**, Lakatos PL, Zalatnai A, Hantos MB, Tekes K. INCREASED LIQUOR, PLASMA AND TUMOR TISSUE NOCICEPTIN IN RATS WITH EXPERIMENTALLY INDUCED HEPATOCELLULAR CARCINOMA.
   Hepatology 2003;38.No4.Suppl1,412A

10. Horvath A[1], **Folhoffer A**[1], Csak T[1], Lakatos PL[1], Zalatnai A[2], Hantos MB[3], Tekes K[3], Szalay F[1]. INCREASED NOCICEPTIN AND NOCISTATIN IN PLASMA, CEREBROSPINAL FLUID AND TUMOR TISSUE INDICATES ALTERED OPIOID SYSTEM IN RATS WITH EXPERIMENTAL HEPATOCELLULAR CARCINOMA
    1st Dept of Medicine[1], 1st Dept of Pathology[2], Dept of Pharmacodynamics of Semmelweis University
    J Hepatol 2004, 40 (Suppl 1) A250

11. A Horvath[1], **A Folhoffer** [1], T Csak [1], PL Lakatos [1], MB Hantos [2], K Tekes [3], A Szíjártó [4], P Kupcsulik [4], A Kiss [5], J Halasz [5], Z Schaff [5], F Szalay [1]. HIGH NOCICEPTIN CONTENT IN HUMAN HEPATOCELLULAR CARCINOMA TISSUE

[1]1st Department of Medicine, [2] Neurochemical Research Unit of Hungarian Academy of Sciences, [3]Department of Pharmacodynamics, [4]1st Department of Surgery, [5] 2nd Department of Pathology, Semmelweis University, Budapest, Hungary
Gastroenterology 2004, 126 (Suppl 2) A736

12. Csák T, **Folhoffer A,** Horvath A, Szeli D, Lakatos PL, Szalay F. ADIE'S SYNDROME AND MESENTERIAL LYMPHANGIOMA IN A PATIENT WITH CELIAC DISEASE AND AUTOIMMUNE HEPATITIS
Z Gastroenterol 2004; 42:P408

13. **Folhoffer A,** Horvath A, Csák T, Lakatos Pl, Szalay F. NEUROFIBROMATOSIS, CUTAN MELANOMA AND HYPER-THYROIDISM IN A HCV POSITIVE PATIENT TREATED WITH INTERFERON
Z Gastroenterol 2004; 42:P411

14. Horváth A, **Folhoffer A**, Csák T, Zalatnai A, Hantos M, Tekes K, Lakatos PL, Szalay F. NOCICEPTIN IN EXPERIMENTALLY INDUCED HEPATOCELLULAR CARCINOMA
Z Gastroenterol 2004; 42: P415

15. Lakatos PL, Willheim-Polli C, **Folhoffer A**, Horvath A, Csak T, Österreicher C, Habior A, Tornai I, Ferenci P, Szalay F. NOD2/CARD15 SNP8, 12 AND 13 AND OTHER EXON4 MUTATIONS AND PRIMARY BILIARY CIRRHOSIS (PBC) IN HUNGARIAN AND POLISH PATIENTS
Z Gastroenterol 2004; 42:P423

16. Szalay F, **Folhoffer A**, Horvath A, Csak T, Tornai I, Habior A, Speer G, Nagy Z, Lakatos P, Lakatos PL. SERUM LEPTIN LEVELS IN PATIENTS WITH PRIMARY BILIARY CIRRHOSIS
Z Gastroenterol 2004; 42:P440

17. F. Szalay, **A. Folhoffer**, A. Horvath, T. Csak, A. Habior, I. Tornai, G. Speer, Z. Nagy, P. Lakatos, P.L. Lakatos. LOW SERUM LEPTIN LEVEL IN PATIENTS WITH PRIMARY BILIARY CIRRHOSIS
Gut 2004;53(Suppl VI) A64

18. Csák T, **Folhoffer A**, Horvath A, Bíró A, Lakatos PL, Osztovits J, Toth T, Habior A, Bekő G, Tornai I, Karádi I, Füst G, Szalay F. CHOLESTEROL BINDING ANTIBODIES IN PRIMARY BILIARY CIRRHOSIS
Zeitschrift für Gastroenterologie 2005; 43: 481 (A13)

19. Horvath A, Szegedi A, **Folhoffer A**, Csak T, Osztovits J, Bekő G, Vargha P, Szalay F. SERUM ALPHA-FETOPROTEIN (AFP) LEVEL IN NON-TUMOROUS CHRONIC LIVER DISEASE
Zeitschrift für Gastroenterologie 2005; 43: A44

20. Szalay F, Csak T, **Folhoffer A**, Horvath A, Speer G, Nagy Z, Lakatos P, Habior A, Tornai I, Lakatos PL. SERUM LEPTIN, SOLUBLE LEPTIN RECPETOR LEVELS AND FREE LEPTIN INDEX IN PATIENTS WITH PRIMARY BILIARY CIRRHOSIS
Zeitschrift für Gastroenterologie 2005; 43:513 (A126)

21. Keresztes K, Istenes I, **Folhoffer A**, Lakatos P, Horvath A, Csak T, Vargha P, Kempler P, Szalay F. AUTONOMIC AND SENSORY NEUROPATHY IN PRIMARY BILIARY CIRRHOSIS
Zeitschrift für Gastroenterologie 2005; 43:495 (A61)

22. T Csak, **A Folhoffer**, A Horvath, J Osztovits, A Biro, PL Lakatos, T Toth, A Habior, G Bekő, I Karadi, G Füst, I Tornai, F Szalay. ANTI-CHOLESTEROL ANTIBODIES IN PRIMARY BILIARY CIRRHOSIS AND WILSON DISEASE
GUT SVII (54), A12

23. T Csak, D Székács, **A Folhoffer**, A Horvath, J Osztovits, A Zalatnai, MIK Fekete, GM Nagy, I Bodnár, F Szalay. ALTERED NORADRENALINE-DOPAMINE SYSTEM IN EXPERIMENTAL LIVER CIRRHOSIS
GUT SVII (54), A190

24. A Horvath, A Szegedi, **A Folhoffer**, T Csak, J Osztovits, G Bekő, P Vargha, F Szalay. SERUM ALPHA-FETOPROTEIN (AFP) LEVEL IN NON-TUMOROUS CHRONIC LIVER DISEASES
GUT SVII (54), A196

25. F Szalay, T Csak, **A Folhoffer**, A Horvath, A Biro, J Osztovits, PL Lakatos, A Habior, I Tornai, I Karadi, G Fust. ANTIBODIES TO FREE CHOLESTEROL IN PRIMARY BILIARY CIRRHOSIS
HEPATOLOGY; 42 (4): 509A, Suppl. 1

26. Szalay F., Csak T., Biro A., Scherzer T., **Folhoffer A**., Horvath A., Osztovits J., Lakatos P., Karádi I., Füst G., Ferenci P. ANTI-CHOLESTEROL ANTIBODY LEVELS IN CHRONIC HEPATITIS C. HIGH TITERS IN PATIENTS WITH GENOTYPE 3 AND 4, BUT NOT IN GENOTYPE 1B
Zeitschrift für Gastroenterologie 2006;43: 1-48; A122

27. Csák T., **Folhoffer A**., Horvath A., Osztovits J., Papp J., Görög D., Kóbori L., Szalay F.
XANTHOMATOSIS AND EXTREME HYPERCHOLESTEROLEMIA AFTER LAPAROSCOPIC CHOLECYSTECTOMY. RECOVERY FOLLOWING SURGERY OF IATROGENOUS STENOSIS OF COMMON BILE DUCT.
Zeitschrift für Gastroenterologie 2006;43: 1-48; A13

28. Osztovits J., Csák T., **Folhoffer A**., Horváth A., Ónodi L., Schaff Z., Szalay F.
ACUTE CHOLESTATIC HEPATITIS SYNDROME CAUSED BY ECSTASY
Zeitschrift für Gastroenterologie 2006; 43:1-48; A86

29. T Csak, D Szekacs, **A Folhoffer**, A Horvath, J Osztovits, I Bodnar, A Zalatnay, M Fekete, Gy Nagy, F Szalay. ALTERATION OF NORADRENALIN-DOPAMIN SYSTEM IN EXPERIMENTAL LIVER CIRRHOSIS
GASTROENTEROLOGY; 130 (4); Suppl 2; A815 (M-1070)

ACKNOWLEDGEMENTS

I render thanks to **Prof. Rudolf DeChâtel and Prof. Csaba Farsang,** the former Director and **Miklós Szatmári** the present Director of the 1st Department of Medicine of Semmelweis University for supporting me considerably in my work, and for giving me a hand in my research and in my study.
I express my thanks to **Prof Zsolt Tulassay** for giving opportunity to participate in his doctoral program and following my work with care and attention.
I include my respectful gratitude to my Supervisor **Prof. Ferenc Szalay**, who provided support for this research all the way along, sharing his experiences and knowledge in the research and in the medical attandance.

I owe **Dr. Margit Abonyi** thanks for her help to start my calling in the internal medicine and for giving a hand for me any time.

This work could not be completed without the wide **national and international collaboration**. I am indebted to the colleagues for sending samples from throughout the country, making possible to examine a large number of patients. I specially thank **Prof. Peter Ferenci** and **Claudia Willheim-Polli** for supporting my work.

I thank you ever so much to **Prof. János Fehér, Dr. László Szőnyi, Dr. János Schuller, Dr. Enikő Újhelyi, Dr. Judit Csomor, Prof. Dr. László Király, Dr. András Lászik, Prof. Csaba Horváth, Prof. Romána Zelkó, Dr. Judit Nagy, Gabriella Taba, Prof. Ilona Kovalszky, Dr. Anita Kamondi, Dr. Gertrúd Tamás** and the **Transplantation Team** (**Prof. Ferenc Perner, Dr. Jenő Járay, Dr. Dénes Görög, Dr. László Kóbori, Dr. Imre Fehérvári, Dr. Balázs Nemes**) for allowing me to co-operate with them.

I express my gratitude to **Prof. Peter Lakatos** who has accepted me to take part in the life of his high-level genetic research laboratory and I would like to thank him for his valuable practical advices.

I am deeply indebted to **all of my colleagues in the 1st Department of Internal Medicine**, in the **Clinical Research and Isotope Diagnostic Laboratory** and in the **Central Laboratory**, I specially thank **Dr. Béla Büki** and **Dr. Gabriella Bekő, Dr. János Pál Kósa, Szabóné Sinkovits Tünde** and **Györgyi Keresztényi** for their help.

I render thanks to **Dr. Dalma Hegedűs, Dr. Gábor Firneisz** and particularly **Dr. Péter László Lakatos** for supporting me any time.

I express my gratitude to my close colleagues, **Dr. Andrea Horváth, Dr. Tímea Csák, Dr. Viktória Ferenc, Dr. Kinga Dunkel, Dr. János Osztovits and Dr. Zsolt Visnyei**, who have provided me with friendly environment and stimulated my work with a lots of help.

Finally the most important: it is hard for me to find words to express my sincere gratitude **to my Family**: **my Parents**, my sister, **Gyöngyi**, my husband, **Csaba** and our daughter, **Dorottya** for their support in all its form.

I thank to the **Wilson disease patients and their family-members**, for those whom I could help and for those whom it is to be regretted that I could not help. This work has been done also for them.