

## Concomitancy of mutation in FRDA gene and FMR1 premutation in 58 year-old woman

Alena Zumrová, Radim Mazanec, Martin Vyhánek, Anna Křepelová, Zuzana Mušová, Stefanie Krilová, Ludmila Appltová & Markéta Havlovicová

Neurogenetic Centre of 2nd Faculty of Medicine, Charles University and Medical Hospital Motol, Prague, CZECH REPUBLIC

Correspondence to: Alena Zumrová, M.D.  
Department of Child Neurology  
2nd Faculty of Medicine,  
Charles University and Medical Hospital Motol  
V úvalu 84  
150 00 Prague, CZECH REPUBLIC  
TEL: + 420 737857068  
FAX: + 420 224433322  
EMAIL: [alena.zumrova@lfmotol.cuni.cz](mailto:alena.zumrova@lfmotol.cuni.cz)

Submitted: January 27, 2005

Accepted: February 3, 2005

Key words: **very late-onset Friedreich ataxia; FMR1 premutation; Fragile X premutation tremor/ataxia syndrome; repeats disorders; coexistence**

Neuroendocrinol Lett 2005;26(1):71-74 PMID: 15726025 NEL260105C03 © Neuroendocrinology Letters [www.nel.edu](http://www.nel.edu)

### Abstract

DNA testing broadens diagnostic tools available for hereditary ataxias. However, together with current knowledge of genes and their mutations crop up new phenotype figures of diseases already well known. Diagnostic problems in practice can consist in part due to the very similar symptoms of hereditary ataxias and acquaintance in or availability of new techniques such as DNA testing and result in misdiagnosis. We present a case study of a 57 year-old woman with both expansion of the triplet repetitive sequence of FRDA gene and a premutation in FMR1 gene. At present we diagnose her with Very Late Onset Friedreich's ataxia, but we advise of possible combinations or aggravations of her symptoms due to manifestation of Fragile X premutation tremor/ataxia syndrome. In non-typical phenotypes of DNA verifying hereditary ataxias we recommend searching of comorbidity, specifically from a range of hereditary ataxias with very similar spectra of symptoms.

### Abbreviation and units:

DNA	- Deoxyribonucleid acid	MEP	- Motor evoked potentials
FMR1	- Fragile site mental retardation 1 gene	CMCT	- Central motor conduction times
FRDA	- Friedreich's ataxia	SSEP	- Somatosensory evoked potentials
LOFA	- Late Onset Friedreich's Ataxia	PCR	- Polymerase Chain Reaction
VLOFA	- Very Late Onset Friedreich's Ataxia	m	- metre
CAG	- Cytosine Adenine Guanine	m.	- musculus
GAA	- Cytosine Adenine Adenine	s	- second
FXTAS	- Fragile X Premutation Tremor/Ataxia Syndrome	ms	- millisecond
ECG	- Electrocardiograph	uV	- microvolt

## Introduction

Differential diagnosis of hereditary ataxias is very complex and includes a many diseases with identified genetic and/or biochemical defects [1,9,15,19,21]. Friedreich's ataxia (FRDA) is an autosomal recessive ataxia resulting from a mutation of FRDA gene on chromosome 9 [4]. FRDA is the most common inherited ataxia with a prevalence of between 1 and 2 per 100,000 and deduced carrier frequency of 1 in 120 in European populations [5]. FRDA is characterized by a progressive gait and limb ataxia, a lack of tendon reflexes in the legs, distal loss of proprioception and vibration, pyramidal weakness, extensor plantar responses, and dysarthria. Variable features include cardiomyopathy, scoliosis, pes cavus, optic atrophy, diabetes, and sensorineural deafness, all of which may be present [2,10,11]. Identification of the mutation responsible for Friedreich's ataxia has allowed the study of genotype-phenotype correlation. The initial classification, which discounted patients with onset after 25 years, has since been broadened. Patients with late onset Friedreich's ataxia (LOFA) [14], and very late onset Friedreich's ataxia (VLOFA) [17,18] have been identified. Both LOFA and VLOFA account for up to 25% of patients with FRDA. Since the identification of the first CAG repeat-expansion underlying spinocerebellar ataxia type 1 (SCA1) in 1993 [16], more than 25 additional gene loci have been found to be responsible for autosomal dominant inherited forms of SCA. At present, this recent knowledge enlarges differential diagnosis of a group of ataxia, or tremor with unexplained etiology. Fragile X premutation tremor/ataxia syndrome (FXTAS) is a new neurodegenerative disease, for the main, characterized by intention tremor and balance problems (gait ataxia) with/or peripheral neuropathy, mild parkinsonism, lower limb proximal muscle weakness, short-term memory loss, executive function deficits, cognitive decline, and autonomic dysfunction [6,7,13]. It has very similar clinical symptoms like other spinocerebellar ataxias. The frequency of the premutation is about 1 in 259 females and 1 in 810 males in the general population [20], identification of FXTAS frequency (in males) is not finished, and only case reports in female are mentioned in the literature [3,8]. We present a case study of a 57 year-old woman with both expansion of the triplet repetitive sequence of X25 gene and a premutation in the FMR1 gene.

## Case Report

A 57 year-old woman, a younger daughter of non consanguineous parents.

**Family history:** Her father died at the age of 75 (a neoplasm of the brain), her mother at the age of 87 years, cachexy, profound visual impairment. Her sister, a 66 year-old, started to have problems with her gait from 48 years of age, has refused diagnostic investigations. Our patient has a son and a daughter, they are healthy.

**Personal history:** Delivery and post partum development were normal. No increased morbidity. At the age of 30 years cholecystectomy, in 1978 Caesarean section, 1982 appendectomy. From 2002 therapy for high blood pressure and thyreotoxicosis. Menses from 11 years of age, climacterical from 48 years of age. She was employed in a bank, now is an old-age pensioner.

**Current illness:** Patient noticed mild instability at age 49, however she had no limitations in daily life. She didn't seek medical advice. Her problems markedly increased in connection with the death of her husband in 2002 (55 years of age). Her gait was wide-based, and she fell sometimes. Her vision, hearing, and swallowing were intact, bladder and bowel functions were normal. Neurological examinations started in January 2003. At the time she was alert and oriented. Her speech was intelligible, only minimal dysarthria. Cranial nerves were intact, no nystagmus. Muscle strength and tone were normal, deep tendon reflexes C5–8 were quick, L2–4 high, L5–S2 present. She had an inconstant right Babinski sign. Ataxia with mild intention tremor of her right upper limb and lower limbs. No impairment of vibration sense and joint position sense, positive Romberg sign. Her gait was slightly wide based, aggravated by closing her eyes. She was able to walk on her toes, slightly worse on heels, without assistance.

Complete blood cell counts and routine chemistry test results were normal. The level of vitamin B12, and vitamin E were in the low normal range. Thyroid functions were stabilized by therapy.

Brain magnetic resonance imaging scans revealed two nonspecific supratentorial focuses of gliosis. Spinal cord magnetic resonance imaging showed bulging of disks L5–S1, spondylosis of cervical and lumbar vertebral column, degeneration of disks Th8–9, and L4–S1 with normal spinal cord size and signal.

No optic atrophy has been seen, visual evoked potentials were normal.

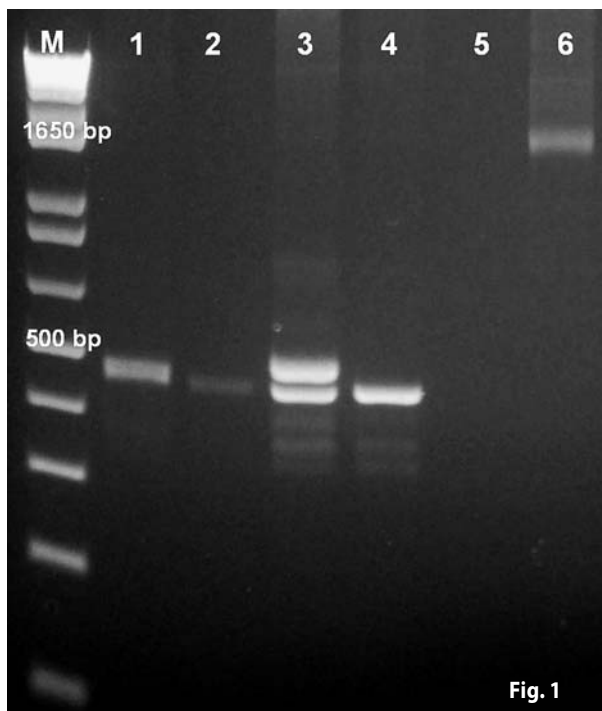
ECG findings were normal.

Psychological examinations revealed no cognitive impairment.

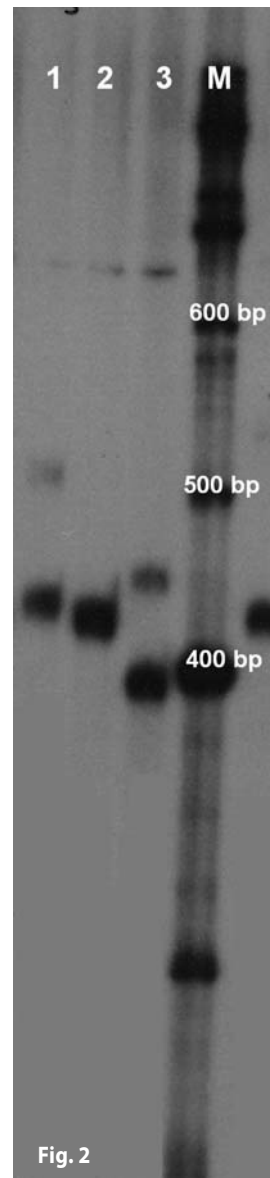
Conduction studies on motor and sensory nerves (2003) were normal on lower limbs (peroneal motor conduction velocity 50m/s, compound motor action potential amplitude 8,7 mV; sural motor conduction velocity 52 m/s, sensory nerve action potential amplitude 22uV). By contrast, motor evoked potentials (MEP) confirmed abnormality of central motor pathway for upper and more for lower limbs. Central motor conduction times (CMCT) for m. abductor digiti quinti were (dx/sin) 4,0/5,0 ms, for m. biceps brachii 5,4/5,6 ms, m. tibialis anterior 10,6/20,0 ms, and m. vastus medialis 8,6/8,4 ms.

Somatosensory evoked potentials (SSEP) revealed no pathology in 2003.

Examinations of cerebrospinal fluid (protein, glucose, cytology, isofocusing, virology, and antibody tests for CNS Lyme disease) were normal.



**Figure 1: PCR analysis of GAA repeat in *FRDA* gene**  
M – marker, 1, 2, 3, 4 – normal, 5 – blank, 6 – our patient (homozygous GAA triplet expansion – approximately length of 400 repeats)



**Figure 2: PCR analysis of CGG repeat in *FMR1* gene**  
M – marker, 1 – our patient (62/35 CGG repeat), 2, 3 – normal

**DNA testing for *FRDA*** (July of 2003): DNA was isolated from blood leukocytes.  $(GAA)_n$  repeat in intron 1 of the *FRDA* gene was amplified with GAAF and GAAR primers according to Campuzano et al, 1996 [4]. PCR products were separated in 2% agarose gel. *Homozygous GAA triplet repeat expansion was detected with approximately length of 400 repeats* (Fig. 1).

In 2004 slowly deteriorate ataxia and dysarthria of our patient. Deep tendon reflexes are retained, but vibration sense markedly decreased in lower limbs. She is uncooperative and disinterested in her treatment, including physiotherapy and further study.

**DNA testing for fragile X:** (December 2004; within the frame of screening of FXTAS in the set of patients with spinocerebellar ataxia): DNA was isolated from blood leukocytes. The size of CGG repeat was detected by polymerase chain reaction (PCR). PCR products were separated in 6% PAGE with urea and lengths of products were sized by Multianalyst software. *In our patient we detected heterozygous premutation in *FMR1* gene in length of 62 CGG repeat.*

## Discussion

We have examined a series of 490 patients from unrelated families receiving genetic testing because of idiopathic, progressive ataxia and inheritance compatible with autosomal recessive or sporadic disease. All patients were of Czech origin except 2 (of Slovak origin). 28 patients from the examined group (5,7%) were homozygous for a GAA triplet-repeat expansion

in intron 1 of the *FRDA* gene. The age of onset has been observed to be between 4–20 years in 18 patients (64%), between 20–25 in 6 patients (21%), between 25–39 years in 3 patients (11,5%), and after 40 years in 1 patient. The last patient is the above mentioned woman with a rare Very Late Onset Friedreich's ataxia and, at present, with unique concomitancy with premutation in the *FMR1* gene. Her clinical progress as well as results of neuroimaging and electrophysiological methods accord with a diagnosis of VLOE, but in the future, we cannot exclude FXTAS signs due to her DNA test results. A combination of clinical symptoms especially radiological criterion for FXTAS will be important, that means we will look for symmetrical regions of increased T2 signal intensity in the middle cerebellar peduncles and adjacent cerebellar white matter [12]. Inherited ataxias represent one of the most heterogeneous groups of diseases in neurology. Advances in molecular genetics have led to identification of an increasing number of genes responsible for them. Their correlation of phenotype/genotype is in progress, so some of patients are denoted by atypical form. Following our observations we recommend considering and searching for comorbidity in nontypical phenotypes of DNA verifying hereditary ataxias, namely from a range of hereditary ataxias with very similar spectra of symptoms, because coexistence of them exists.

## Acknowledgements

This paper was supported by the research grants No NM/7405-3 and NR/8124-3 from Ministry of Medicine, the Czech Republic.

## REFERENCES

- 1 Albin RL. Dominant ataxias and Friedreich ataxia: an update. Review. *Curr Opin Neurol*. 2003; **16**(4):507–14.
- 2 Alper G, Narayanan V. Friedreich's ataxia. Review. *Pediatr Neurol*. 2003; **28**(5):335–41.
- 3 Berry-Kravis E, Potanos K, Weinberg D, Zhou L, Goetz CG. Fragile X-associated tremor/ataxia syndrome in sisters related to X-inactivation. *Ann Neurol*. 2005; **57**(1):144–7.
- 4 Campuzano V, Montermini L, Molto MD, Pianese L, Cossee M, Cavalcanti F, Monros E, Rodius F, Duclos F, Monticelli A, et al. Friedreich's ataxia: autosomal recessive disease caused by an intronic GAA triplet repeat expansion. *Science*. 1996; **271**(5254):1423–7.
- 5 Chakravarty A. Friedreich's ataxia-yesterday, today and tomorrow. Review. *Neurol India*. 2003; **51**(2):176–82.
- 6 Hagerman RJ, Leehey M, Heinrichs W, Tassone F, Wilson R, Hills J, Grigsby J, Gage B, Hagerman PJ. Intention tremor, parkinsonism, and generalized brain atrophy in male carriers of fragile X. *Neurology*. 2001; **10**; **57**(1):127–30.
- 7 Hagerman PJ, Greco CM, Hagerman RJ. A cerebellar tremor/ataxia syndrome among fragile X premutation carriers. Review. *Cytogenet Genome Res*. 2003; **100**(1–4):206–12.
- 8 Hagerman RJ, Leavitt BR, Farzin F, Jacquemont S, Greco CM, Brunberg JA, Tassone F, Hessl D, Harris SW, Zhang L, Jardini T, Gane LW, Ferranti J, Ruiz L, Leehey MA, Grigsby J, Hagerman PJ. Fragile-X-associated tremor/ataxia syndrome (FXTAS) in females with the FMR1 premutation. *Am J Hum Genet*. 2004; **74**(5):1051–6.
- 9 Harding AE. Classification of the hereditary ataxias and paraplegias. *Lancet*. 1983; **21**; **1**(8334):1151–5.
- 10 Harding AE. The inherited ataxias. *Adv Neurol*. 1988; **48**:37–46.
- 11 Harding AE. Friedreich's ataxia: a clinical and genetic study of 90 families with an analysis of early diagnostic criteria and intrafamilial clustering of clinical features. *Brain*. 1981; **104**(3):589–620.
- 12 Jacquemont S, Hagerman RJ, Leehey M, Grigsby J, Zhang L, Brunberg JA, Greco C, Des Portes V, Jardini T, Levine R, Berry-Kravis E, Brown WT, Schaeffer S, Kissel J, Tassone F, Hagerman PJ. Fragile X premutation tremor/ataxia syndrome: molecular, clinical, and neuroimaging correlates. *Am J Hum Genet*. 2003; **72**(4):869–78.
- 13 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. Penetrance of the fragile X-associated tremor/ataxia syndrome in a premutation carrier population. *JAMA* 2004; **28**; **291**(4):460–9.
- 14 Klockgether T, Chamberlain S, Wullner U, Fetter M, Dittmann H, Petersen D, Dichgans J. Late-onset Friedreich's ataxia. Molecular genetics, clinical neurophysiology, and magnetic resonance imaging. *Arch Neurol* 1993; **50**(8):803–6.
- 15 Koenig M. Rare forms of autosomal recessive neurodegenerative ataxia. Review. *Semin Pediatr Neurol*. 2003; **10**(3):183–92.
- 16 Kwiatkowski TJ, Jr, Orr, H T, Banfi, S, McCall, AE, Jodice, C, Persichetti, F, Novelletto, A, LeBorgne-DeMarquoy, F, Duvick, LA, Frontali, M, Subramony, SH, Beaudet, AL, Terrenato, L, Zoghbi, HY, Ranum, LPW. The gene for autosomal dominant spinocerebellar ataxia (SCA1) maps centromeric to D6S89 and shows no recombination, in nine large kindreds, with a dinucleotide repeat at the AM10 locus. *Am. J. Hum. Genet*. 1993; **53**:391–400.
- 17 Lhatoo SD, Rao DG, Kane NM, Ormerod IE. Very late onset Friedreich's presenting as spastic tetraparesis without ataxia or neuropathy. *Neurology* 2001; **26**; **56**(12):1776–7.
- 18 Labauge P. Very late onset Friedreich's presenting as spastic tetraparesis without ataxia or neuropathy. *Neurology*. 2002; **9**; **58**(7): 1136.
- 19 Margolis RL. Dominant spinocerebellar ataxias: a molecular approach to classification, diagnosis, pathogenesis and the future. Review. *Expert Rev Mol Diagn*. 2003; **3**(6):715–32.
- 20 Rousseau F, Rouillard P, Morel ML, Khandjian EW, Morgan K. Prevalence of carriers of premutation-size alleles of the FMR1 gene and implications for the population genetics of the fragile X syndrome. *Am J Hum Genet*. 1995; **57**(5):1006–18.
- 21 Schols L, Bauer P, Schmidt T, Schulte T, Riess O. Autosomal dominant cerebellar ataxias: clinical features, genetics, and pathogenesis. *Lancet Neurol*. 2004; **3**(5):291–304.

# Instructions for authors

Neuroendocrinology Letters ISSN 0172-780X

Editor-in-Chief: Peter G. Fedor-Freybergh

**Neuroendocrinology Letters** is an international, peer-reviewed transdisciplinary journal covering the fields of Neuroendocrinology, Psychoneuroimmunology, Neuropsychopharmacology, Reproductive Medicine, Chronobiology, Human Ethology and related areas for **RAPID** publication of Original Papers, Review Articles, Clinical Reports, and other contributions from all the fields covered by Neuroendocrinology Letters.

## FAST TRACK PUBLICATION

The Neuroendocrinology Letters can publish high-priority papers 2-4 weeks after acceptance. A fast-tracked manuscript will be posted on our website ([www.nel.edu](http://www.nel.edu)) earlier than it appears in the printed journal, as well as sent to Medline ahead of publication.

## AIM & SCOPE

Papers from both basic research (methodology, molecular and cellular biology, anatomy, histology, biology, embryology, teratology, normal and pathological physiology, biophysics, pharmacology, pathology and experimental pathology, biochemistry, neurochemistry, neuropsychopharmacology, enzymology, human ethology, chronobiology, receptor studies, endocrinology, immunology and neuroimmunology, animal physiology, animal breeding and ethology, psychology and others) and from clinical research (reproductive medicine, obstetrics and gynecology, endocrinology, immunology, neuropsychopharmacology, cardiovascular studies, internal medicine, pediatrics, neurology, psychiatry and child psychiatry, oncology and others) will be considered.

The Journal publishes original papers and review articles. Brief reports, special communications, provided that they are based on adequate experimental evidence, clinical studies, case reports, commentaries, discussions, letters to the editor (correspondence column), book reviews, congress reports and other categories of articles (philosophy, art, social issues, medical and health policies, biomedical history, etc.) will be taken under consideration.

The requirements for publishing in NEUROENDOCRINOLOGY LETTERS are in accordance with the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals," 5th edition. JAMA 1997; 277:927-934. For manuscripts submitted after August 23, 2004, the References are adjusted according to the Reference section below.

## SUBMISSION OF PAPERS

It is understood that material submitted to the Journal has not been published previously in print or electronic format and is not under consideration by another publication or electronic medium. **All manuscripts submitted for publication should be written in clear,**

**plain English** (American or British English spelling), and **approved by a native English speaker in case that the correspondent author is not.** Neuroendocrinology Letters undertakes no language correction.

**The correctness of the English language throughout the whole manuscript is entirely the responsibility of the authors. The papers with insufficient English language will be rejected by the Editor-in-Chief immediately after submission, and will be returned to the author without further processing.**

**All authors must give a signed consent for publication in a letter submitted with the manuscript.**

## PROCESSING FEE and PUBLICATION FEE

(page charge):

**Processing fee** – EUR 100 / USD 120

**and Publication fee** – printed page charge – EUR 75 / USD 90 (1 printed page is approximately 2 manuscript pages) **are required for all papers accepted for publication** in the Neuroendocrinology Letters.

**Upon submission, the authors declare the acceptance of the processing fee and page charge, which must be paid to the publisher within one week after the author is notified about the acceptance of the paper for publication in the Neuroendocrinology Letters, and before the galley proofs will be sent and the final date for publication will be settled.**

The author will be notified about the **total cost** (processing fee and publication fee) directly after the acceptance of their manuscript.

**No submission fees** will be required for submitted papers **that will not be accepted for publication.**

While the processing fee is obligatory and unified for all papers accepted for publication, the publication fee is page dependent, and thus the authors may influence the final cost for publication of their papers depending on the length of the paper. **No limitations** to the total length of their paper, independently of the category (Original papers, Review papers, Clinical Reports, Case Reports, etc) will be applied.

ALL MANUSCRIPTS must be sent to the Editor-in-Chief by email only ([editor@nel.edu](mailto:editor@nel.edu)).

The final acceptance of all papers for publication in the Neuroendocrinology Letters is subject for Chief Editor's decision.

The submitted and already accepted papers for publication in the Neuroendocrinology Letters are to be continuously found on [www.nel.edu](http://www.nel.edu) in the section the Latest from the Editor and may be cited as "in press, Neuroendocrinology Letters". The authors will be notified about the publication date in due time.

## LENGTH OF PAPERS / MANUSCRIPT PAGES

**No limitations** to the total length of their paper, independently of the category (Original papers, Review papers, Clinical Reports, Case Reports, etc) will be applied.

Manuscripts should be typed double-spaced on numbered pages and conform to the “Uniform Requirements for Manuscripts Submitted to Biomedical Journals.” **Two manuscript pages is approximately one printed page.**

### REVIEWING

All submitted manuscripts are reviewed initially by the Editor-in-Chief. Those manuscripts with insufficient priority for publication are rejected promptly. Other manuscripts are sent to relevant senior scientists for RAPID peer review. The identities of both peer-reviewers and authors are kept confidential. The comments by the reviewers may be conveyed to the authors by the Editor, at his discretion.

Manuscripts are reviewed with due respect for the author’s confidentiality. At the same time, reviewers also have rights to confidentiality, which are respected by the editor. The editor ensures both the authors and the reviewers that the manuscripts sent for review are privileged communications and private intellectual property of the author.

When submitting a manuscript for consideration for publication, authors may suggest the names of potential reviewers of their choice.

If an author for any reason wishes to withdraw his/her submitted manuscript from publication, the editor will always respect this wish unless the submitted and accepted manuscript has already passed publishing procedures.

The original material of rejected articles will be returned to the authors.

### AUTHORITY & RESPONSIBILITY

The intellectual content of the paper is the responsibility of the authors. **The Editors and the Publisher accept no responsibility for the opinions and statements of the authors.** While every effort will be made by the Editors and the Publisher to avoid inaccurate and misleading data, they accept no liability whatsoever for the consequence of inaccurate information. The authors undertake to keep the Editor and the Publisher fully and effectually indemnified against any liability of claims that may arise from the publication of inaccurate and/or misleading data.

### COPYRIGHT

It is a condition of publication that the authors transfer the world copyright of their manuscripts to the *Neuroendocrinology Letters*. All manuscripts should therefore be accompanied by a signed statement that the article is original, is not under consideration or has not been previously published in another journal. Nevertheless, authors will be entitled to publish any part of their paper elsewhere without permission, provided the usual acknowledgments and reference to the ORIGINAL source are given. Authors will be notified

if a request to publish a part or whole of their paper is received. Illustrations, figures, tables or quotations from other publications are already copyrighted and can be reproduced only with written permission from the copyright owner. Written permission to use these should accompany the manuscript.

### ETHICS

The Editors and the Publisher support the principles of the Declaration of Helsinki of 1975, as revised in 1983, and expect that the authors of papers submitted to the Journal will have obtained ethical consent and followed those legal and regulatory requirements for human experimentation with drugs, including informed consent, according to procedures which apply in their institution and country.

**When reporting experiments on animals, indicate whether the institution’s or a national research council’s guide for, or any national law on, the care and use of laboratory animals was followed.**

### PRESENTATION

Manuscripts should be typed on numbered pages and conform to the “Uniform Requirements for Manuscripts Submitted to Biomedical Journals” except the Reference section (see below).

The pages should be numbered consecutively, beginning with the Title page. The **sections of the manuscript** should be in following sequence: Title page, Abstract (structured for Original papers and non-structured for Review Articles), Key words, Abbreviations, Main text (Introduction, Material and methods, Results and Discussion), Acknowledgments, References, Tables and Figures. **Particular attention should be taken to ensure that the manuscript adheres to the Instructions for Authors of the Neuroendocrinology Letters in all respects.** The use of footnotes is not permitted (numbered comments/footnotes can be added at the end of the main text, before the Reference section).

It is very important that all the text is typed without extra spaces between words and that all text within a paragraph is typed without extra carriage returns between the lines. To make a new paragraph, only a carriage return is allowed (tabs allowed for numbered or bullet lists). For tables, see TABLES.

The Editors reserve the right to alter manuscripts whenever necessary to make them conform to the stylistic and bibliographic conventions of the *Neuroendocrinology Letters*.

### TITLE PAGE

Title page of the manuscript should contain:

1. The title itself.
2. The name(s) of the author(s): **first name(s)** spelled out, **family name** and highest academic degree.
3. Author’s Affiliations: The name(s) of the department(s) or institution(s) from which the study originated.
4. Corresponding Author: The name and full address, including telephone and fax numbers, e-mail

address(es) and other useful information of the corresponding author. The authors are obliged to inform the publisher immediately about any change of their fax, telephone, e-mail and ordinary mail address by e-mail: [info@nel.edu](mailto:info@nel.edu)

5. A “running headline,” a maximum of 40 characters, including word spaces.

#### ABSTRACT and KEY WORDS

Abstract and Key words follow directly after the Title page (no extra page) A **structured abstract (Original Articles) and non-structured abstract (Review papers)** not exceeding 250 words should state what was done, including **objectives, design, setting, results, the main findings, conclusions**, and how the work was interpreted. **Additional headings may be used.**

**At least 5 to 10 key words should be used, which correspond to MESH headings by Medline.** The key words should be separated by semicolons. The key words will be included in the Subject Index of the volume. The next section can start directly after this on the same page.

#### ABBREVIATIONS & UNITS

List of abbreviations and symbols used and spell them out in full. Abbreviations and symbols must be standard, and SI units (The International System of Units) should be used throughout. Drugs should be described by their official names, but trade names should be indicated in brackets the first time a drug is quoted in the main text.

#### MAIN TEXT

The text is conventionally divided into sections headed: Introduction, Material and methods, Results and Discussion. Lengthy papers may require subheadings for clarification.

#### INTRODUCTION

State clearly the purpose of the paper. Do not review the subject extensively and give only pertinent references.

#### MATERIAL & METHODS

Describe your selection of the observational subjects (patients or laboratory animals, including controls) clearly. Describe the study population in detail. Identify the methods and procedures in sufficient detail to allow other workers to reproduce the results. If the methods used are new or substantially modified, describe them and state their limitations.

When reporting research on human beings, the authors must include an assurance that the work was approved by a medical ethics committee and that the subjects gave their informed consent to participate. (See Ethics.)

When reporting experiments on animals, indicate whether the institution's or a national research council's guide for, or any national law on, the care and use of laboratory animals was followed. (See Ethics.)

#### RESULTS

Do not repeat in the text all the data displayed in the tables or illustrations; only important observations should be emphasized or summarized.

#### DISCUSSION

Emphasize only the new and important aspects and conclusions of the study, including the implications and the limitations of the findings and their relation to other relevant studies. The conclusions should be clearly linked with the objectives of the study. Avoid unqualified statements and conclusions that are not supported by the data. Do not claim priority, and do not allude to work in progress. State new hypotheses when warranted, but clearly label them as such. Recommendations, when appropriate, may be included.

#### STATISTICS

Describe statistical methods with enough detail to enable a knowledgeable reader with access to the original data to verify the reported details. The design of the study and the data sources should be clearly identified. The statistical methods used should be described so that it is clear which method was used and where. Give relevant references and additional details if non-standard methods or analyses have been applied. The basic principle is to supply sufficient information about design and analysis to allow the research to be repeated by someone else. The presentation of the analysis should include relevant summaries of the data, not just the results of significance testing. The use of confidence interval is encouraged.

#### REFERENCES

Identify references in the text, tables, figures and legends by Arabic numerals in brackets e.g. [5,1] and number them in alphabetical order in the Reference list. The Reference list is in alphabetical order, and the numbers assigned in the Reference list are inserted in the text in brackets, by example:

Plasma vitamin B12 levels have been previously implicated with cognitive abilities [6,4,5,1]. In this contribution we investigated the correlation between plasma vitamin B12 levels and cognitive impairment in Alzheimer's disease (AD), the most common cause of dementia. Plasma vitamin B12 levels were measured in 241 AD patients diagnosed according to the NINCDS-ADRDA (National Institute of Neurological and Communicative Disorders and Stroke- Alzheimer's Disease and Related Disorders Association) criteria [3,2]. Clinical evaluation included detailed medical history, psychiatric, somatic and neurological status, neuropsychological testing, routine blood tests, an electroencephalogram, a computed tomography scan or magnetic resonance imaging.

#### REFERENCES

- 1 Duthie SJ, Whalley LJ, Collins AR, Leaper S, Berger K, Deary IJ. Homocysteine, B vitamin status, and cognitive function in the elderly. *Am J Clin Nutr* 2002; 75(5):908-913.

- 2 Eussen SJ, Ferry M, Hininger I, Haller J, Matthys C, Dirren H. Five year changes in mental health and associations with vitamin B12/folate status of elderly Europeans. *J Nutr Health Aging* 2002; **6**(1):43-50.
- 3 Ganzer S, Arlt S, Schoder V, Buhmann C, Mandelkow EM, Finckh U, Beisiegel U, Naber D, Muller-Thomsen T. CSF-tau, CSF-Abeta1-42, ApoE-genotype and clinical parameters in the diagnosis of Alzheimer's disease: combination of CSF-tau and MMSE yields highest sensitivity and specificity. *J Neural Transm* 2003; **110**(10):1149-1160.
- 4 Levitt AJ, Karlinsky H. Folate, vitamin B12 and cognitive impairment in patients with Alzheimer's disease. *Acta Psychiatr Scand* 1992; **86**(4):301-305.
- 5 Malouf R, Areosa Sastre A. Vitamin B12 for cognition. *Cochrane Database Syst Rev* 2003; (3):CD004326.
- 6 Nilsson K, Warkentin S, Hultberg B, Faldt R, Gustafson L. Treatment of cobalamin deficiency in dementia, evaluated clinically and with cerebral blood flow measurements. *Aging (Milano)* 2000; **12**(3):199-207.

References within the tables, figures or legends should be numbered as in the main text. The titles of journals should be abbreviated according to the style used in the Index Medicus.

Other examples of the correct form of references:

**1. Standard journal article** List all authors when six or less. When seven or more, list only the first six and add et al. **Example:** Parkin DM, Clayton D, Black RJ, Masuyer E, Friedl HP, Ivanov E, et al. Childhood leukaemia in Europe after Chernobyl: 5 year follow-up. *Br J Cancer* 1996; **73**:1006-1012. If the language is not English, add the translated title in brackets, e.g. Swedin G. Transkutan elektrisk nervstimulering som smärtlindring vid förlossning. [(Transcutaneous electrical nerve stimulation for analgesia in childbirth.) (In Swedish with English abstract.)] *Läkartidningen* 1979; **776**:1946-1948.

## 2. Books and other monographs

**a/** Personal author(s):

Ringsven MK, Bond D. *Gerontology and leadership skills for nurses*. 2nd ed. Albany (NY): Delmar Publishers; 1996.

**b/** Editor(s) as author(s):

Norman IJ, Redfern SJ, editors. *Mental health care for elderly people*. New York: Churchill Livingstone; 1996.

**c/** Chapter in a book:

Phillips SJ, Whisnant JP. Hypertension and stroke. In: Laragh J, Brenner BM, editors. *Hypertension: pathophysiology, diagnosis, management*. 2nd ed. New York: Raven Press; 1995. p. 465-478.

**d/** Conference proceedings:

Kimura J, Shibasaki H, editors. *Recent advances in clinical neurophysiology*. Proceedings of the 10th International Congress of EM and Clinical Neurophysiology; Oct 15-19, 1995; Kyoto, Japan. Amsterdam: Elsevier; 1996.

**e/** Conference paper:

Bengtsson S, Solheim BG. Enforcement of data protection, privacy and security in medical informatics. In: Lun KC, Degoulet P, Piemme TE, Rienhoff O, editors. *MEDINFO 92. Proceedings of the 7th World Congress on Medical Informatics*; Sept 6-10, 1992; Geneva, Switzerland. Amsterdam: North-Holland; 1992. p. 1561-155.

**f/** Dissertation:

Kaplan SJ. *Post-hospital home health care: the elderly's access and utilization (dissertation)*. St. Louis (MO): Washington Univ.; 1995.

**TABLES AND FIGURES (Figures, illustrations, graphs, etc.)** Tables and Figures should be numbered consecutively and provided with a concise title and legend. Be sure that each table and figure is cited in the text. All tables and figures should be specifically referred to in the text, e.g. Table 3, Figure 3. Figures/illustrations, etc must send as a high quality scan (.tiff, .eps or .jpg in high resolution, original Illustrator file .ai or PowerPoint file), **electronically by email**. All figures (figures, illustrations, photographs, representational drawings, graphs, etc.) must be professionally executed; freehand or typewritten lettering is unacceptable.

Figures and Tables including the legends should be placed at the end of the document or in a separate file (not inserted in the main text).

## COLOR

If authors wish to have their figures, tables, or other illustrations to be published **in color**, for every color page (not more than two figures/tables/illustrations etc on one page), **EUR 550.00 (within Europe) or USD 650.00 (outside of Europe) will be charged**.

## Figures must be sent electronically as separate attachments:

**a/** PC compatible Adobe Illustrator 9.0 and 6.0x .AI files or .EPS files (for Mac users .eps) with fonts included in the file (not converted to vector/outline illustration); at the same time the original program files used for illustrations (figures) should also be attached (even original Mac files); as well as .PDF files if possible. Bitmap raster images as .TIF, BMP, GIF or .EPS are accepted if the resolution 300 dpi for published figure size is provided. Files can be compressed with WinZip. JPG files are accepted if saved in high quality compression only.

**b/** Excel and PowerPoint files are accepted (PC compatible) as separate files by email.

**All exceptions to the above should be agreed upon** after contact with our Art Director (art.director@nel.edu) or Publisher (publisher@nel.edu).

## ACKNOWLEDGMENTS

The scientific and material contributions of others to the work should be acknowledged. Any grant supports should be listed and permission for reproduction of published material acknowledged. Authors are responsible for obtaining written permission from anyone acknowledged by name. The acknowledgments will be published as an appendix to the text.

**PROOFS**

Before publication the correspondent author will receive a copy of the final version of the paper, which should be read carefully for errors and returned promptly. Proofs will be sent for the correction of **typographic errors only**. No other changes will be accepted. Proofs not returned within 7 days will be considered as approved by the author(s).

**DOCTORAL THESES & DISSERTATIONS in brief**

This Journal encourages the submission of important works by young investigators, researchers and practitioners. In this section the Editor wishes to recognize innovative research conducted during graduate and postgraduate studies. Each doctoral thesis or dissertation should contain an abstract and provide a concise synopsis (10 manuscript pages maximum) of the major findings presented in the final version.

**LETTERS TO THE EDITOR**

The Neuroendocrinology Letters has a section carrying comments, questions, or criticism about articles that have been published and where the original authors can respond. This section takes the form of Letters to the Editor, where also other topics and views from readers may be published and discussed.

**SUPPLEMENTS**

Monographs or series of articles that have undergone regular scientific review, university approved theses, conference proceedings, symposia on related issues or topics, etc. may be printed as supplements to the Neuroendocrinology Letters. Supplements are published as a separate issues of the Journal and are negotiated in advance with the Editor-in-Chief, and must be **prepaid prior to publication**. The Supplements are not automatically included in the subscription price, but can be purchased separately at publisher@nel.edu

**REPRINTS – PRINT QUALITY PDF**

Instead of Reprints, a **print-quality PDF for unlimited use** can be ordered by the author(s) for the price of EUR (Europe) 165.00 / USD 200.00.

**ADDITIONAL CONTACT INFORMATION**

If you have any additional questions, please do not hesitate to contact us.

E-mail: Correspondence and information:  
info@nel.edu

Correspondence to the Editor-in-Chief:  
editor@nel.edu

Correspondence to the Art & Advertising Director:  
art.director@nel.edu

Publisher contact: Information to our Publisher:  
publisher@nel.edu

Information about subscriptions: publisher@nel.edu

Advertisement proposal to: art.director@nel.edu

**Example of a Structured Abstract in NEL**

**OBJECTIVES:** The distribution of serotonin (5-HT) and its effect on insulin and glucagon secretion were

investigated to examine whether there are changes in the pattern of distribution and effect of 5-HT after the onset of experimental diabetes.

**METHODS:** The pattern of 5-HT and its effect of insulin and glucagon secretion was examined using immunohistochemical and radioimmunoassay techniques, respectively.

**RESULTS:** 5-HT was demonstrated mainly in the neural elements of the pancreas. 5-HT-containing fine varicose nerve fibers were discerned in the wall of blood vessels and pancreatic ducts. 5-HT-containing nerves were also observed in the periacinar and periinsular regions of normal pancreas. The pattern or intensity of the distribution of serotonergic nerves did not change after the onset of diabetes. The perivascular, periductal, periacinar and periinsular regions of diabetic pancreas all contained 5-HT positive nerves. 5-HT elicited marked increases in insulin secretion from normal pancreas but had an inhibitory effect on insulin secretion from diabetic pancreatic tissues. In contrast, 5-HT inhibited glucagon secretion from normal pancreatic tissue fragments but stimulated glucagon release from diabetic pancreatic tissue fragments.

**CONCLUSION:** 5-HT is well distributed in normal and diabetic pancreatic tissues and has stimulatory effects on insulin secretion from normal pancreas and glucagon secretion from diabetic pancreas. This result indicates that although 5-HT may help in the maintenance of the blood sugar level in normal pancreas by increasing insulin secretion and decreasing glucagon secretion, it may also aggravate the hyperglycemia observed in diabetes mellitus and hence exacerbate the symptoms of hyperglycemia in poorly controlled diabetes mellitus.

**Example of References in NEL**

- 1 Adegate E, Donáth T. Intramural serotonin immunoreactive cells in normal and transplanted pancreas. *Biogenic Amines* 1990; 7:385–390.
- 2 Cardinali DP, Larin F, Wurtman RJ. Control of the rat pineal gland by light spectra. *Proc Natl Acad Sci USA* 1972; 69:2003–2005.
- 3 Hellerstrom C, Swenne I, Andersson A. Islet cell replication and diabetes. In: Lefebvre PJ, Pipeleers DG, editors. *The pathology of the endocrine pancreas in diabetes*. Berlin: Springer-Verlag; 1988.
- 4 Karasek M. Zależność ultrastruktury szyszynki szczura od wieku. [(The dependence of white rat pineal gland ultrastructure on age.) (In Polish with English abstract)] *Endokrynol Pol* 1974; 25:275–287.
- 5 Nishino T, Kodaira T, Shina S, Imagawa K, Shima K, Kumahara Y, et al. Glucagon radioimmunoassay with use of antiserum to glucagon C-terminal fragments. *Clin Chem* 1981; 27:1690–1697.
- 6 Pathak MA, Nghiem P, Fitzpatrick TB. Acute and chronic effects of the sun. In: Freedberg IM, Eisen AZ, Wolff K, Austen LA, Goldsmith K, Katz SI, Fitzpatrick TB, editors. *Fitzpatrick's Dermatology in General Medicine*, 5th edition. New York: McGraw-Hill; 1999. p. 1598–1607.