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Panayiotis Vlamos *Editor*

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Contents

Preclinical Validation of the Located Hyperthermia Using Gold Macro-Rods and Ultrasound as an Effective Treatment for Solid Tumors

Andre L.S. Barros, Carlos Austerlitz, Ioannis Gkigkitzis, Diana Campos, Jeyce K.F. de Andrade, Teresinha G. Silva, Silene C. Nascimento, and Ioannis Haranas

Abstract Hyperthermia, the procedure of raising the temperature of a part of or the whole body above normal for a defined period of time, is applied alone or as an adjunctive with various established cancer treatment modalities such as radiotherapy and chemotherapy. In this study used a method for inducing hyperthermia in solid tumors with a combination of gold macro rod (GR) and ultrasound, the feasibility of this technique was described only with computational models and in vitro. The Ehrlich tumor, derived from a *mouse* adenocarcinoma, has been used to investigate the bio-heat transfer and the effect of gold rods irradiated with ultrasound. The in vivo measurements demonstrated that the technique inhibited more 80% of the tumor growth in both experimental models tested. These results not only confirm the bio heat transfer to tissue as predicted by analytical calculation and in vitro measurements, but are also proved to be a potential alternative to kill cancer cells.

Keywords Ultrasound • Gold Macro-Rods • Hyperthermia • Cancer

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1 Introduction

Hyperthermia (also called thermal therapy or thermotherapy) is a noninvasive anticancer approach in which biological tissues are exposed to temperatures higher than normal (up to 41 °C) to promote selective destruction of abnormal cells. In the temperature range of 41–47 °C tumors are selectively destroyed because of their reduced heat tolerance [1]. The elevation from the normal temperature for a certain time interval causes irreversible cell damage by denaturing proteins, which affects the structure of the cell membrane. Thus, hyperthermia for anticancer treatment could inhibit tumor cell proliferation by destroying cancer cells or making them more sensitive to the effects of conventional antitumor therapies, such as radiation or chemotherapy [2]. The use of heating sources conventionally employed for hyperthermia is limited because of the damage they cause to surrounding healthy tissues. Various heating sources ranging from radiofrequency to microwaves as well as ultrasound waves have been used to produce moderate heating in a specific target region [3–5]. The use of gold nanospheres, nanorods, nanoshells and nanocages in vitro assays, ex vivo and in vivo imaging, cancer therapy, and drug delivery, has been discussed by [6], despite of the great effort that has been put in the application of gold nanoparticles (GNPs) for cancer therapy related to thermal therapy, GNPs face some major inconveniences: improvement of delivery methods to induce accumulation of a larger number of particles to the tumor site [7]; vasculature is highly abnormal in tumor [8]. Therefore, it may not be expected that GNPs are homogeneous distributed inside the tumor; and the bio heat transfer from GNPs to the tissue is in order of a few nm, associated with bio distribution, the relatively short range of the bio-heat transferred from the GNPs to the surrounding tissue may result in heterogeneous high-thermal and cold regions in the tumor leading to failed treatments.

A method for inducing hyperthermia in solid tumors with a combination of gold macro rod (GR) and ultrasound has been described elsewhere [9–12]. The feasibility of this method has been demonstrated by means of analytical considerations about bio-heat transfer in tissue [9, 10], infrared pictures of GR exposed to ultrasound [12]. However, there are no data performed with such method based on in vivo tumor cells. Based on the analytical considerations about bio-heat transfer in tissue, the aim of this study was establish a Preclinical Model to confirm the viability of Hyperthermia Using Gold Macro-Rods and Ultrasound on Treatment of Cancer.

2 Materials and Methods

2.1 Animals

In this study, 96 BALB/c mice (male, 25–30 g), were obtained from the Laboratory of Immunopathology Keizo-Asami (LIKA) of the Federal University of Pernambuco (UFPE), Brazil. The animals were kept in cages with free access to food and

water, under 12 h light-dark cycles. The animals were treated in accordance with the International Council for Laboratory Animal Science (ICLAS), and following the ethical principles of the Brazilian Society of Science in Laboratory Animals (SBCAL). All experiments were approved by the Ethics Committee for Animal Experimentation of the Biological Sciences Center of the Federal University of Pernambuco, Brazil, number 23076.013243/2012-04.l.

2.2 Solid Ehrlich Carcinoma (SEC) Tumor Model

Ehrlich ascites carcinoma (EAC) cells were derived from a spontaneous murine mammary adenocarcinoma. EAC cells were maintained in the undifferentiated form by passaging in syngeneic mice by transplanting 2.5×10^6 cells/mL (i.p.) each week. The ascitic fluid was removed by opening the belly and collecting all of the fluid with a sterile syringe. Ascitic tumor cell counts were carried out using the trypan blue dye exclusion method with a neubauer hemocytometer. Animals received 200 μL of a suspension containing 5×10^6 cells/mL (i.p.). Tumor volume was measured using a electronic caliper, assuming a fairly constant relationship of mass to volume, tumor development can be expressed in terms of volume increment. In order to determine tumor volume by external caliper, the greatest longitudinal diameter (length) and the greatest transverse diameter (width) were determined. Tumor volume based on caliper measurements were calculated by the modified ellipsoidal formula [13, 14]:

$$Tumor\ volume\ \left(mm^3\right) = 1/2\left(length \times width^2\right)$$

The inhibition rate (%) was calculated as follows [15]:

$$Inhibition\ rate\ (\%) = [(A–B)\ /A] \times 100$$

where A is the average weight of the non treated group and B is the average tumor weight of the treated group.

2.3 Insertion of Gold Macro-Rods and Ultrasound Irradiation Setup

After 5 days of the transplanted tumor (Tumor volume approximately equal to 350 mm^3), the mice were anesthetized with 0.1 to 0.1 mL/100 g of ketamine and xylazine (1:1), the hair around the tumor area was removed then the gold rod (0.155 cm diameter and 0.54 cm height) was inserted in the center of tumor by means of a trocar needle (Fig. 1). A layer of ultrasound transmission gel was spread

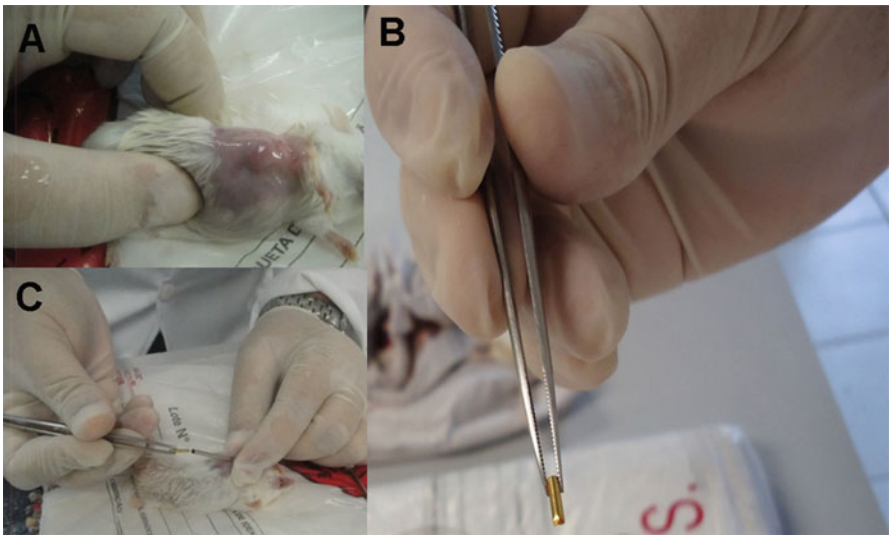

Fig. 1 Insertion of gold macro-rods in the tumor

on the shaved mice's skin as contact media gel and exposed for 15 or 30 min with a 1 cm transducer connected to a GS8 2E ultrasound under a nominal frequency of 1 MHz and about 75 W. The temperature increase was measured every minute in the central and peripheral region of the tumor with needle type thermistor connected to a thermometer FLUKE. Changes in body temperature were valued by rectal temperature.

2.4 Experimental Design

After 5 days of tumor implantation (Tumor volume $= 300 \pm 18$ mm^3), mice were randomly assigned into four groups (6 mice/group) as follows:

Group 1 (Control): untreated control.
Group 2 (GR): gold macro-rods inserted on tumor not irradiated with ultrasound.
Group 3 (U): irradiated with ultrasound only.
Group 4 (GR + U): gold macro-rods inserted on tumor and irradiated with ultrasound.

2.4.1 Treatment Model 1 (Single Dose)

Mice with gold macro-rods inserted on tumor were irradiated with ultrasound for 30 min. Three hour after the irradiation, the mices were sacrificed and the tumors were dissected, weighed and two fragments (central and peripheral region) were fixed in 10% formaldehyde for Histopathological analyzes.

2.4.2 Treatment Model 2 (Three Doses)

Mice with gold macro-rods inserted on tumor were irradiated with ultrasound for 15 min every 5 days, totaling 3 irradiations. Tumor volume was measured from the 5th to the 16th day after implantation of SEC. 24 h after the last irradiation, the mice were sacrificed and the tumors were dissected and weighed, additionally blood samples were collected from each group for evaluation of adverse effects.

2.5 Histopathological Analyzes

After being fixed in formaldehyde, the tumors were put into paraffin. The blocks were cut using a microtome to a thickness of 4 μm, and the slides were stained with hematoxylin and eosin for morphological analysis. Histological analysis was performed by light microscopy and histological sections were photographed with an MC 80 DX camera coupled to a Zeiss Axiophot light microscope, and tumor/necrotic areas were quantified using Image ProPlus 5.1 software.

2.6 Hematological Analysis

Hematological analysis was carried out using an automatic cell counter (ABX-MICROS-60 cell counter Horiba, Inc). The samples were evaluated for the following hematological parameters: number of erythrocytes, concentration of hemoglobin, number of platelets and total count and differential of leukocytes.

2.7 Statistical Analysis

The results are presented as the mean ± standard deviation (SD). One-way ANOVA followed by the Newman-Keuls test was used to evaluate the differences among the treatments. P values <0.05 were considered to be statistically significant.

3 Results

3.1 Treatment Model 1 (Single Dose)

In this study was observed the enhancement in the temperature only in group 4. The temperature in the tumor was measured with the FLUKE thermistor in two regions defined in accordance with the proximity to the GR inserted. In central region, the heat rate produced increased about 0.81 °C/min from 36.6 to 60.9 °C and in peripheral region 0.38 °C/min from 36.3 to 48.8 °C (Fig. 2). Significatives changes in rectal temperature not were observed.

The effect of gold macro-rods irradiated with ultrasound, against solid Ehrlich carcinoma is showed in Fig. 3. The mass of the tumors not presented statistically significant differences between groups, however in the histopathology analysis, the GR + U group showed extensive areas of coagulating necrosis accounting for 81.9 ± 7.2% of the total area (Fig. 4a).

Histological sections from control, GR and U groups showed a typical pattern consisting of viable tumor with pleomorphic polygonal cells, hyperchromatic nucleus, some binucleated cells and cytoplasm limits (Fig. 5a–c). The degeneration characteristics of early stages of coagulative necrosis can be observed in Fig. 5d.

3.2 Treatment Model 2 (Three Doses)

In this treatment protocol, the GR + U group was irradiated three times for 15 min (5, 10 and 15 days after implantation of Erhlich carcinoma). The enhancement in the temperature in central region was from 37.2 to 53.4 °C the heat rate increased about 1.08 °C/min and in peripheral region 0.54 °C/min from 36.7 to 44.8 °C. As

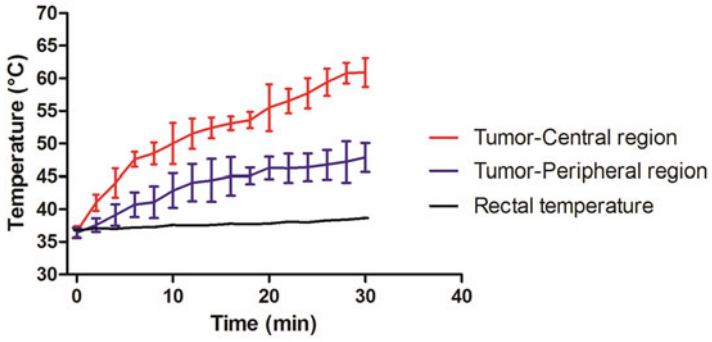

Fig. 2 The heat enhancement on tumor during the irradiation of the gold rod with ultrasound

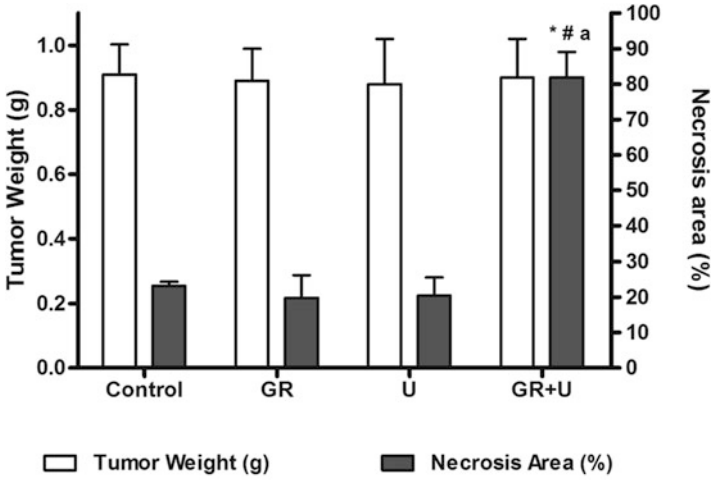

Fig. 3 The effect of gold rods irradiated with single dose of ultrasound for 30 min, in mice transplanted with Ehrlich carcinoma. Data are presented as mean ± standard deviation. * $p < 0.05$ compared to control by ANOVA followed by Newman-Keuls test. # $p < 0.05$ compared to GR by ANOVA followed by Newman-Keuls test. [a] $p < 0.05$ compared to U by ANOVA followed by Newman-Keuls test

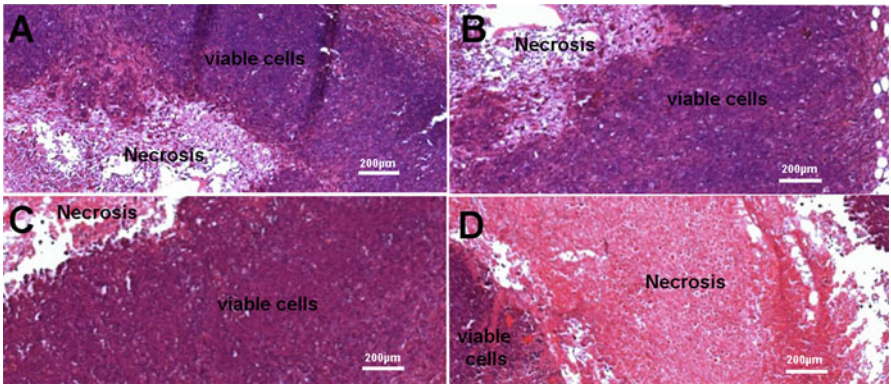

Fig. 4 Ehrlich tumor histopathology after treatment using gold rods irradiated with single dose of ultrasound for 30 min. Sections refer to: (**a**) control, (**b**) GR, (**c**) U and (**d**) GR + U

it is shown in Fig. 6, Ehrlich tumors in GR + U group were significantly inhibited as compared with tumors of untreated mice ($P < 0.05$). Furthermore, there were no differences between control, GR and U groups.

The tumor reduction is described in Fig. 7. The sequential irradiation, blocked tumor development over the trial period. In GR + U group, the inhibition rate was 84.7%. The control group, GR and U not were significantly different. In the Hemogram, the only significant differences induced for GR + U with respect to the other groups were concerning the increased Leukocytes (Table 1).

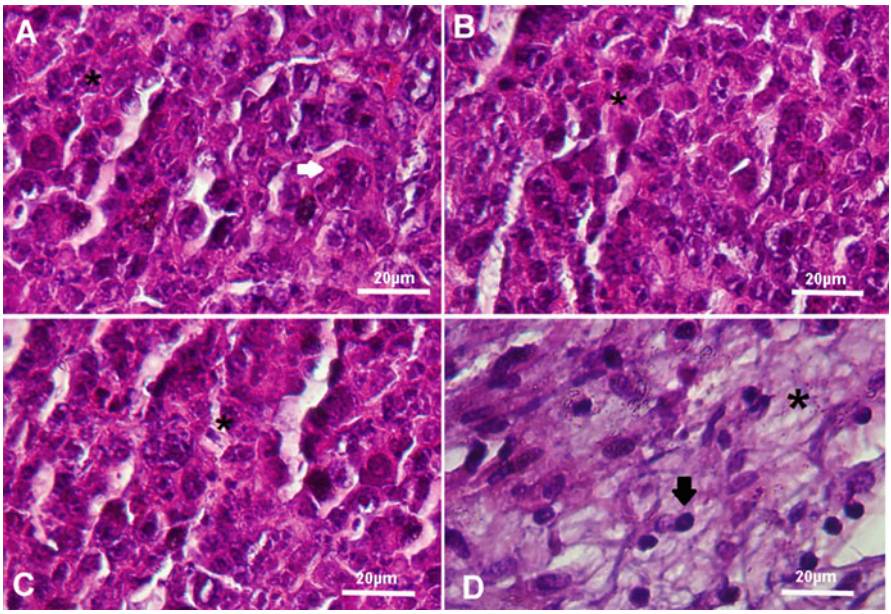

Fig. 5 Photomicrograph showing the histopathology evaluation of the Ehrlich carcinoma from (**a**) animals control: normal cells morphology showing intact membrane and nucleus (*black asterisk*) and cell division (*white arrow*); (**b**) GR: intact cells (*black asterisk*); (**c**) U: intact cells (*black asterisk*) and (**d**) GR+ U: plasma membrane degeneration (*black asterisk*) and intense chromatin condensation (*black arrow*). Analyzed In light microscope

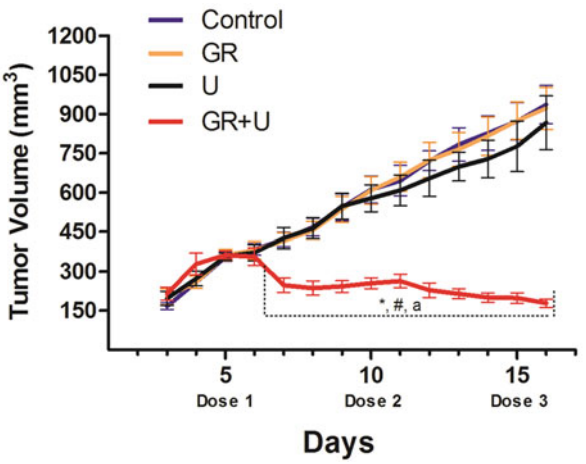

Fig. 6 The effect of gold rods irradiated with three doses of ultrasound for 15 min on the growth curve of Ehrlich tumor. Data are presented as mean ± standard deviation. $^{*}p < 0.05$ compared to control by ANOVA followed by Newman-Keuls test. $^{\#}p < 0.05$ compared to GR by ANOVA followed by Newman-Keuls test. $^{a}p < 0.05$ compared to U by ANOVA followed by Newman-Keuls test

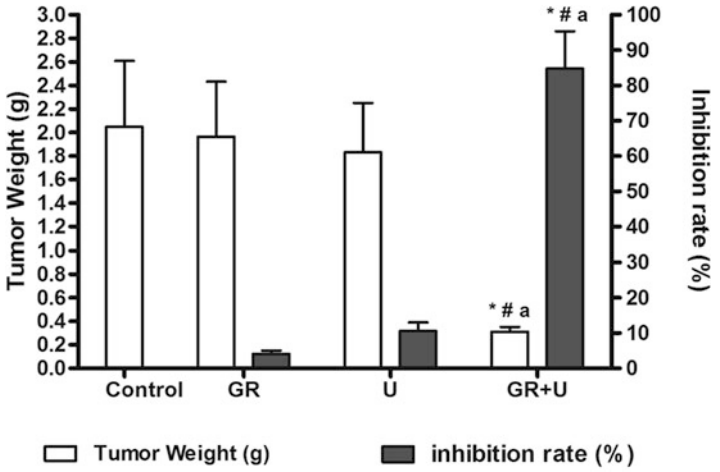

Fig. 7 The effect of gold rods irradiated with three doses of ultrasound for 15 min in mice transplanted with SEC. Data are presented as mean ± standard deviation. * $p < 0.05$ compared to control by ANOVA followed by Newman-Keuls test. # $p < 0.05$ compared to GR by ANOVA followed by Newman-Keuls test. [a] $p < 0.05$ compared to U by ANOVA followed by Newman-Keuls test

Table 1 Effect of gold rods irradiated with three doses of ultrasound for 15 min on hematological parameters of mice 16 days after implantation of Erhlich carcinoma

Parameter	Control	GR	U	GR + U
Red blood cells (10^6/mm^3)	8.9 ± 0.7	8.4 ± 0.82	9.1 ± 0.95	8.7 ± 0.79
Leukocytes (10^3/mm^3)	7.98 ± 0.55	8.06 ± 0.72	8.12 ± 0.68	14.4 ± 1.03*
Platelets (10^3/mm^3)	436 ± 39.3	457 ± 61.3	463 ± 69.07	461 ± 49.6
Hemoglobin (g/dL)	14.67 ± 0.93	14.99 ± 1.02	14.08 ± 0.91	14.7 ± 0.95
Hematocrit (%)	41.09 ± 1.6	42.1 ± 1.8	41.79 ± 1.3	40.89 ± 1.7
VCM (μμg)	48.3 ± 1.62	46.3 ± 1.3	46.2 ± 0.98	45.77 ± 1.76
HCM (pg)	16.44 ± 0.81	16.88 ± 0.67	17.04 ± 0.93	19.97 ± 0.91
CHCM (%)	31.14 ± 1.41	32.04 ± 1.7	31.84 ± 1.52	30.95 ± 1.37

The values are presented as the average ± the standard deviation. * ($p < 0.05$) compared to the control and other groups by ANOVA followed by the Student Newman-Keuls post-test

4 Discussion

The experimental design of this study was based on analytical considerations and measurements in vitro as described by [9–12] and our results confirm what was predicted mathematically by these authors where, the about 1-cm radial bio heat transferred to the tumor from the gold rod causing cell death.

Ehrlich carcinoma is an undifferentiated carcinoma that has high transplantable capability, no regression, rapid proliferation, 100% malignancy and also does not have tumor-specific transplantation antigen [16]. Following the inoculation the

number of cells increases rapidly and after a given time, the host animal died due to the pressure exerted by the tumor volume and/or the damage that resulted from the tumor [17]. In this study the holders SEC animals were treated from the initial proliferative stage of tumor development.

In the tumors with gold rods irradiated for 30 min with ultrasound the temperature in the central region was greater than 42 °C for 28 min while that in the peripheral region for about 20 min. The heat transfer in irradiated tumors for 15 min, temperatures above 42 °C showed by 13 and 5 min in the central and peripheral regions, respectively. Temperatures in the range of moderate hyperthermia can be non-lethal (39–42 °C) or lethal (>42 °C). Temperatures above 42 °C were shown to kill cancer cells in a time and temperature-dependent manner that was measured by the clonogenic cell survival assay [18].

The rate of proliferation of cancer cells makes them more sensitive to the effects of hyperthermia where the intensity of cell death is dependent on the cell cycle phase. In general, high heat sensitivity can be observed during the S and M phases. Microscopic examinations of M-phase cells subjected to hyperthermia show damage of their mitotic apparatus leading to inefficient mitosis and consecutive polyploidy. S-phase cells are also sensitive to hyperthermia, where chromosomal damage is observed. Both S- and M-phase cells undergo a "slow mode of cell death" after hyperthermia, whereas those exposed to heat during G1-phase are relatively heat resistant and do not show any microscopic damage. Cells during G1-phase may follow a "rapid mode of death" immediately after hyperthermia. These variations existing between the different cell cycle phases indicate the possible diversity of molecular mechanisms of cell death following hyperthermia [19–21].

Gold rods irradiated with single dose of ultrasound for 30 min or three doses for 15 min demonstrated ability of tissue destruction in SEC. The histopathological analysis showed extensive areas of coagulative necrosis and progressive inhibition of tumor directly related to heat transfer in tissue. Classical hyperthermia relies on a temperature of 42–45 °C for periods of 30–60 min to cause irreversible cellular damage [21–23]. The inactivation of vital enzymes is a key feature of tissue injury at these temperatures [24]. As the tissue temperature rises to 60 °C, the time required to achieve irreversible cellular damage decreases exponentially. Protein denaturation occurs between 60 and 140 °C and leads to immediate cell death followed by coagulative necrosis [24]. Other cellular changes as membrane rupture, cell shrinkage, pyknosis, hyperchromasia are also observed at temperatures above 60 °C.

The increase in leukocyte numbers shown in the GR + U group can be explained by the indirect effects of hyperthermia. In the area of coagulative necrosis, studies have reported inflammatory infiltrates that include neutrophils, macrophages, dendritic cells (DCs), natural killer (NK) cells, as well as B cells and T cells that are specific to the ablated tissue [25, 26]. Pro-inflammatory cytokines that are released from the injuried tissue or tumour cells, as well as from the disruption of local extracellular matrix and tissue components such as fibrinogen, hyaluronic acid and endothelial cells trigger the release of additional cytokines, chemokines and vascular

adhesion molecules. Levels of serum interleukin-1β (IL-1β), IL-6, IL-8 and tumour necrosis factor-α (TNFα) have all been shown to increase after thermal ablation or hyperthermia (on the timescale of hours to days) [27–31].

In summary, the experimental models tested in this study showed that gold macro rods irradiated with high-frequency ultrasound were effective in cancer cell destruction and furthermore confirmed the bio heat transferred to tissue as predicted by calculation and in vitro measurements.

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Conflicts of Interest The authors declare no conflict of interest.

References

1. van der Zee, J. 2008. Heating the Patient: A Promising Approach? *Annals of Oncology* 13: 1173–1184.
2. Svaasand, L.O., C.J. Gomer, and E. Morinelli. 1990. On the Physical Rationale of Laser Induced Hyperthermia. *Lasers in Medical Science* 5: 121–128.
3. Mirza, A.N., B.D. Fornage, N. Sneige, et al. 2001. Radiofrequency Ablation of Solid Tumors. *Cancer Journal* 7: 95–102.
4. Seki, T., M. Wakabayashi, N. Nakagawa, et al. 1999. Percutaneous Microwave Coagulation Therapy for Patients with Small Hepatocellular Carcinoma, Comparison with Percutaneous Ethanol Injection Therapy. *Cancer* 85: 1694–1702.
5. Ahmed, M., and S.N. Goldberg. 2011. Basic Science Research in Thermal Ablation. *Surgical Oncology Clinics of North America* 20 (2): 237–258.
6. Cai, W., T. Gao, H. Hong, et al. 2008. Applications of Gold Nanoparticles in Cancer Nanotechnology. *Nanotechnology Science and Applications* 1: 17–32.
7. Kennedy, L.C., L.R. Bickford, N.A. Lewinski, et al. 2011. A New Era for Cancer Treatment: Gold-Nanoparticle-Mediated Thermal Therapies. *Small* 7: 169–183.
8. Kennedy, A.S., C. Nutting, D. Coldwell, J. Gaiser, and C. Drachenberg. 2004. Pathologic Response and Microdosimetry of Y-90 Microspheres in Man: Review of Four Explanted Whole Livers. *International Journal of Radiation Oncology Biology Physics* 60: 1552–1563.
9. Gkigkitzis, I., C. Austerlitz, and D. Campos. 2012. The Effect of the Shape and Size of Gold Seeds Irradiated with Ultrasound on the Bio-heat Transfer in Tissue. *American Journal of Clinical Oncology-Cancer Clinical Trials* 35 (2): 195–196.
10. Gkigkitzis, I., C. Austerlitz. East Carolina University, University Committee on Intellectual Property/Patents Review of Case # TT1212; Use of Gold Macro-Rods and Ultrasound as a Hyperthermia Cancer Treatment. Office of Technology Transfer, Division of Research and Graduate Studies June 19, 2012.
11. Austerlitz, C., I. Gkigkitzis, and D. Campos. Macro Gold Rod and Ultrasound: A Potential Alternative to Kill Cancer Cells, East Carolina University, University Committee on Intellectual Property/Patents Review of Case # TT1212; Use of Gold Macro-Rods and Ultrasound as a Hyperthermia Cancer Treatment. Office of Technology Transfer, Division of Research and Graduate Studies June 19, 2012.
12. ———. An Approach to Assess Bio-heat Transfer in Tissue with Infra-Red Camera, East Carolina University, University Committee on Intellectual Property/Patents Review of Case # TT1212; Use of Gold Macro-Rods and Ultrasound as a Hyperthermia Cancer Treatment. Office of Technology Transfer, Division of Research and Graduate Studies June 19, 2012.

13. Euhus, D.M., C. Hudd, M.C. LaRegina, and F.E. Johnson. 1986. Tumor Measurement in the Nude Mouse. *Journal of Surgical Oncology* 31: 229–234.
14. Tomayko, M.M., and C.P. Reynolds. 1989. Determination of Subcutaneous Tumor Size in Athymic (Nude) Mice. *Cancer Chemotherapy and Pharmacology* 24: 148–154.
15. Bezerra, D.P., F.O. Castro, A.P.N.N. Alves, et al. 2008. In vitro and In vivo Antitumor Effect of 5- FU Combined with Piplartine and Piperine. *Journal of Applied Toxicology* 28: 156–163.
16. Kabel AM, Abdel-Rahman MN, El-Sisi Ael-D, Haleem MS, Ezzat NM, El Rashidy MA. Effect of Atorvastatin and Methotrexate on Solid Ehrlich Tumor. European Journal of Pharmacology 2013; 713(1–3):47–53.
17. Ozaslan, M., I.D. Karagoz, I.H. Kilic, and M.E. Guldur. 2011. Ehrlich Ascites Carcinoma. *African Journal of Biotechnology* 10 (13): 2375–2378.
18. Dewey, W.C., et al. 1977. Cellular Responses to Combinations of Hyperthermia and Radiation. *Radiology* 123 (2): 463–474.
19. Laszlo, A. 1992. The Effects of Hyperthermia on Mammalian Cell Structure and Function. *Cell Proliferation* 25: 59–87.
20. Kregel, K.C. 2002. Invited Review: Heat Shock Proteins: Modifying Factors in Physiological Stress Responses and Acquired Thermotolerance. *Journal of Applied Physiology* 92 (5): 2177–2186.
21. Coss, A.R., W.C. Dewey, and J.R. Bamburg. 1982. Effects of Hyperthermia on Dividing Chinese Hamster Ovary Cells and on Microtubules In vitro. *Cancer Research* 42 (3): 1059–1071.
22. Thomsen, S. 1991. Pathologic Analysis of Photothermal and Photomechanical Effects of Laser-Tissue Interactions. *Photochemistry and Photobiology* 53: 825.
23. Hillegersberg, R., M.T. de Witte, W.J. Kort, and O.T. Terpstra. 1993. Water-Jet-Cooled Nd:YAG Laser Coagulation of Experimental Liver Metastases: Correlation Between Ultrasonography and Histology. *Lasers in Surgery and Medicine* 13: 332.
24. Haen, S.P., P.L. Pereira, H.R. Salih, et al. 2011. More than Just Tumor Destruction: Immunomodulation by Thermal Ablation of Cancer. *Clinical & Developmental Immunology* 2011: 1–19.
25. Dromi, S.A., et al. 2009. Radiofrequency Ablation Induces Antigen-Presenting Cell Infiltration and Amplification of Weak Tumor-Induced Immunity. *Radiology* 251: 58–66.
26. Wissniowski, T.T., et al. 2003. Activation of Tumor-Specific T Lymphocytes by Radio-Frequency Ablation of the VX2 Hepatoma in Rabbits. *Cancer Research* 63: 6496–6500.
27. Ali, M.Y., et al. 2005. Activation of Dendritic Cells by Local Ablation of Hepatocellular Carcinoma. *Journal of Hepatology* 43: 817–822.
28. Fietta, A.M., et al. 2009. Systemic Inflammatory Response and Downmodulation of Peripheral CD25+Foxp3+ T-Regulatory Cells in Patients Undergoing Radiofrequency Thermal Ablation for Lung Cancer. *Human Immunology* 70: 477–486.
29. den Brok, M.H.M.G.M., et al. 2004. In situ Tumor Ablation Creates an Antigen Source for the Generation of Antitumor Immunity. *Cancer Research* 64: 4024–4029.
30. Sabel, M.S. 2009. Cryo-Immunology: A Review of the Literature and Proposed Mechanisms for Stimulatory Versus Suppressive Immune Responses. *Cryobiology* 58: 1–11.
31. Erinjeri, J.P., et al. 2013. Image-Guided Thermal Ablation of Tumors Increases the Plasma Level of Interleukin-6 and Interleukin-10. *Journal of Vascular and Interventional Radiology* 24: 1105–1112.

Cerebral Thrombosis: A Neurogenetic Approach

Christos Yapijakis

Abstract Cerebral venous thrombosis (CVT) is a severe multifactorial condition with various clinical manifestations that may include headache, papilledema, seizures, focal deficits, coma and death. The mortality rate of untreated CVT is up to 50%, but it drops to 10% when CVT is properly treated. Prevention of CVT is feasible through healthy lifestyle, genetic counseling, molecular genetic analysis for common thrombophilia-related mutations, and prophylactic anticoagulative medication.

Keywords Cerebral venous thrombosis • Multifactorial condition • Prevention • Thrombophilia • Genetic testing • Genetic counseling • Prophylactic medication

1 Introduction

Cerebral venous thrombosis (CVT) is a rare and severe disease with various clinical manifestations that may include headache, papilledema, seizures, focal deficits, coma and death [1–3]. It is often characterized pathologically by hemorrhagic infarction that contraindicates anticoagulation treatment. CVT symptoms include focal signs (50%), isolated intracranial hypertension (40%), cavernous sinus thrombosis or even unusual presentations such as psychiatric symptoms, migraine and subarachnoid hemorrhage. Percentage of observed symptoms are shown in Table 1.

Cerebral thrombosis was first described in 1825 by Ribes, who reported a 45 years old man that presented severe headache for 6 months, epilepsy and delirium. A postmortem investigation showed superior sagittal sinus, left lateral and left parietal cortical vein thrombosis [4]. A few years later, in 1828 Abercrombie reported a younger woman with postpartum CVT [5].

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Table 1 Observed symptoms in CVT cases [1–3]

Headache	75%
Papilledema	49%
Motor or sensory deficit	34%
Seizures	37%
Drowsiness, confusion, coma	30%
Dysphasia	12%
Multiple cranial nerve palsies	12%
Bilateral or alternating cortical signs	3%
Cerebellar incoordination	3%
Nystagmus	2%
Hearing loss	2%

Cerebral thrombosis may occur in various single or multiple venous sites. About one fourth of CVT occurs in one sinus only (24%) with superior sagittal sinus and lateral sinus being the most frequent sites (13% and 9%, respectively). Occurrence in deep veins only or isolated cortical veins represent about 1% each. More often, there are multiple thrombi involving superior sagittal sinus (72% of cases), lateral sinus (70%), right or left side (each one 26% and both 18%), cerebral veins (38%), and straight sinus (14.5%) [1–3].

The incidence of CVT is unknown at large. In autopsy series the calculated incidence rates are about 3–4 cases per million adults and 7 cases per million children, while in clinical series the incidence rates are ten times greater [2]. There are reports of 1.3% of sagittal sinus thrombosis in 12,500 autopsies, as early as 1936 by Ehlers and Courville [6]. Kalbag and Woolf have noticed that about 22 deaths per year in England & Wales in the decade of 1952–1961 were due to CVT [7]. In recent decades, there is an increased rate of CVT diagnosis since the advent of brain scan techniques such as CT, MRI and DS angiography [1–3].

There seems to be a difference in incidence due to gender, with a male/female ratio of 1.29/1 [8]. While the age distribution for males is rather uniform, for females there is an over-representation in the age group of 20–35 years. About two-thirds of younger women present CVT, most probably as a result of contraceptive pills [1–3, 8].

2 Etiology, Diagnosis and Treatment of Cerebral Thrombosis

CVT is a multifactorial disease, caused by idiopathic, infective or non infective factors [1–3]. More specifically:

a. *Idiopathic causes* mostly include genetic predisposing mutations. There are several genetic disorders involving CVT occurrence (Table 2). For example, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is the most common genetic degenerative disorder of small cerebral vessels characterized by ischemic strokes and progressive vascular dementia before the age of 40 years [9, 10].

Table 2 Genetic disorders characterized by CVT occurrence

Disease (Inheritance)	Locus	Gene	Mechanism	Age at onset (years)
CADASIL: Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (AD)	19p13.1-13.2	*Notch3*	Cerebral small vessel disease Multiple ischemic strokes	25–35
CARASIL: Cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (AR)	10q25.3-26.2	*Hrt1*	Cerebral small vessel disease Multiple ischemic strokes	25–35
Marfan syndrome (AD)	15q21.1	*Fibrillin 1*	Cardiac embolism ischemic stroke aneurysm	<15
Ehlers-Danlos syndrome (AD)	2q31	*Collagen IV*	Ischemic stroke aneurysm	<15

b. *Infective causes* include either brain region infection (local direct septic trauma, intracranial infection) or general causes (septicemia, encephalitis, measles, HIV, CMV, malaria etc).
c. *Non infective causes* include either local (head injury, brain tumors, neurosurgery etc) or general causes (postoperative, pregnancy/postpartum, thrombophilia, dehydration, connective tissue disease, malignancy etc).

Diagnostic investigations for cerebral thrombosis include CT-scan that identifies infarctions in nonarterial distribution (often hemorrhagic), as well as empty delta, dense triangle and cord signs, DS angiography and MRI/V. In early CVT incidents MRI may reveal absence of flow and iso-intense for occluded vessel on T1, or hypo-intense on T2 [1–3]. In late incidents MRI may reveal hyper-intense thrombus on T1 and T2. Other diagnostic investigations may include EEG, CSF, and isotope brain scanning [1–3].

Etiologic investigations include (a) biochemical tests FBE, ANA, antiphospholipid antibodies, antithrombin, protein C, protein S, homocysteine, APC resistance, (b) genetic testing for factor V Leiden gene mutation, factor II (prothrombin) G20210A gene mutation, MTHFR C677T gene mutation. Biochemical tests are repeated every 4–6 months. Genetic tests are performed once in a lifetime.

Treatment for CVT depends on the etiology (e.g. infective cause) and the symptoms (e.g. increased intracranial pressure). It usually involves anticoagulation therapy; initially heparin, warfarin for longer duration, or even direct urokinase infusion [1–3].

Regarding prognosis, the mortality rate in case of untreated CVT is up to 50% [1–3, 11]. When treated, the mortality drops to 10% if the cause of thrombosis is non-septic, or to 30% if the cause is septic. For survivors, prognosis is rather good, as many studies have shown (including a followup study of 8 years). In 77% of the cases there are no aftereffects, while for 20% there is at least one more intra/extracerebral incident of thrombosis in the following years (1–3.11).

In summary, CVT is an uncommon disease that mimic many benign conditions, such as headache. It nevertheless has a life threatening potential and carries 50% mortality if it remains untreated. If CVT is treated then most patients (>75%) do not have any long-term disability. An underlying cause of CVT should always be sought in order to better treat the disease. Accumulating evidence has revealed that a major etiological factor seems to be thrombophilia [1–3, 12].

3 Thrombophilia and Cerebral Venous Thrombosis

Thrombophilia (from Greek "thrombos" meaning clot and "philia" meaning friendship) is a multifactorial condition of increased susceptibility to thrombosis, due to deregulation of the hemostatic system by hereditary and lifestyle-related factors [13–15]. Increased risk for thrombosis is a common underlying pathological mechanism of venous thrombosis, ischemic stroke (including transient stroke), and ischemic heart disorder. All three diseases are associated with a major global burden with important consequences in health status of 15–25% of the population, while thrombosis is the cause of one in four deaths worldwide [16, 17]. Thrombophilia manifestations also include pulmonary embolism and miscarriage, involving about 60% of spontaneous abortions in first trimester of pregnancy [12]. Furthermore, CVT in pregnancy and post-partum may occur probably due to the existing hypercoagulable state during gravidity, often exacerbated by dehydration and iron deficiency anemia that result from inappropriate perinatal care [18].

There is accumulating evidence from genetic association studies that indicate a correlation between DNA variations (mutations and polymorphisms) in genes encoding factors of the hemostatic system and thrombosis-related disease [12, 19, 20]. Taken together, these findings indicate that about 1 in 6–7 of Europeans have a high risk-related genotype [21].

It follows that as a multifactorial predisposition for thrombosis with a strong genetic component, thrombophilia seems to be the most common inherited cause of CVT. Evidently, about one fifth of patients have APC resistance, while one fourth have a detectable thrombophilia-related genetic mutation (Table 3). Among them 95% have the factor V Leiden mutation and 72% have a second contributing factor, such as another thrombophilia-associated mutation (e.g. prothrombin G20210A) or systematic consumption of oral contraceptive pill.

The relative risk for developing CVT is increased four times on the average by thrombophilia-predisposing mutations and up to 13 times by oral contraceptives [22]. Studies have observed that the combined effect of thrombophilia-related mutations and oral contraceptives increases 30 times the relative risk for CVT [22, 23].

Table 3 Meta-analyses and prospective studies that have shown significant correlation of hereditary thrombophilia and ischemic stroke

Population	Coagulation factor	Reference	Outcome	Pooled OR (95% CI)
Children	Protein C	Strater et al. (2002)	R	3.50 (1.1–10.9)
Children	Protein C	Haywood et al. (2005)	F	6.49 (2.96–14.27)
Children	Factor V Leiden	Juul et al. (2002)	F	4.79 (3.26–7.03)
Adults	Factor V Leiden	Casas et al. (2004)	F	1.33 (1.12–1.58)
Adults	Prothrombin G20210A	Casas et al. (2004)	F	1.44 (1.11–1.86)

F first-ever stroke, *R* recurrent stroke, *OR* odds ratio, *CI* confidence interval

4 Genetic and Lifestyle Causes of Thrombophilia

The etiology of thrombophilia include both not genetic and genetic causes. Causes that are not inherited include lifestyle-related factors such as poor diet (low uptake of vegetables and fruits in combination to increased consumption of carbohydrates and lipids), obesity, and extreme bedtime rest. They also include health-related conditions, such as hyper-thyreoidism, hormonal therapies, neoplasias, inflammations, surgical operations and pregnancy.

Thrombophilia is mostly caused by inherited mutations or DNA polymorphisms in genes that encode factors involved in coagulation, platelet function, fibrinogenesis and fibrinolysis, as well as endothelium activation by the complement system and inflammation factors. Genetic variations that may lead to thrombosis-related alterations of the above mechanisms have been associated with thrombophilia [20]. The most significant and best studied genetic factors include factor V Leiden, factor II G20210A, *MTHFR* C677T, antithrombin, protein C deficiency, protein S deficiency, *ACE* D/I, and PAI-1 4G/5G [20, 21].

The individual risk for thrombosis is influenced by genetically-influenced susceptibility in combination to lifestyle and environmental factors. For example, a 40 years old man who smokes 20 cigarettes a day and has inherited thrombophilia associated mutation(s), increased blood cholesterol levels, hypertension, and constant stress has >10 times probability of a thrombotic episode than a man of the same age with only one of these factors. The morbidity and mortality burden of cerebral and heart thrombosis increases significantly in periods of socio-economic crisis, as shown by recent epidemiological observations in Argentina and in Greece [24, 25]. In times of crisis, quality of diet deteriorates and there is increase of stress, depression, smoking and alcohol consumption.

5 Prevention of Thrombophilia Including Cerebral Venous Thrombosis

Although a family history of cerebral venous thrombosis is not usually observed, there is abundant evidence that there are multitudes of pedigrees in which thrombophilia seems to be inherited as an autosomal dominant trait. In such pedigrees,

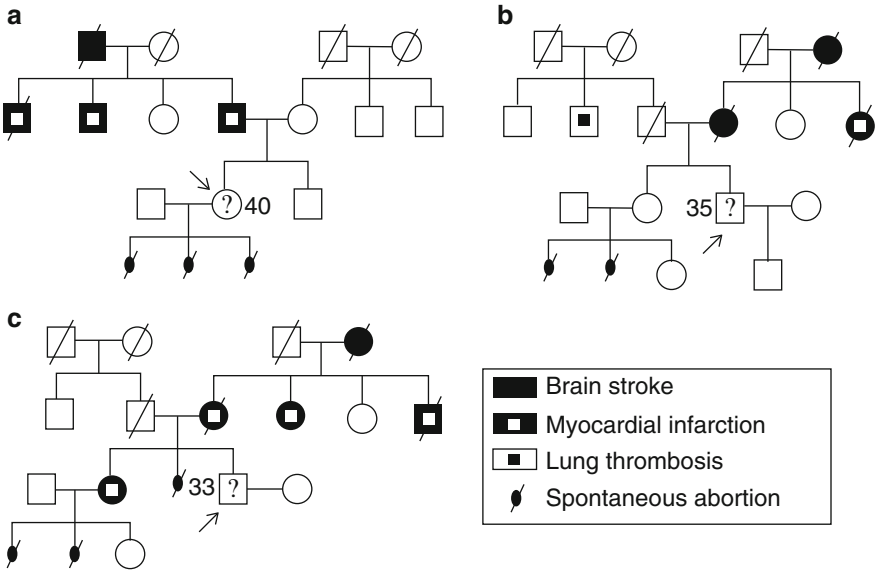

Fig. 1 Characteristic thrombophilia-related pedigrees from Yapijakis et al. [34] with permission. *Arrow-marked* Healthy individuals with unknown predisposition for thrombosis (index cases) asked for genetic counseling: (**a**) a 40 year old woman who had three miscarriages at first trimester; (**b**) a 35 year old father of a child with cri-du-chat syndrome; (**c**) a 33 year old man with oligospermia

there are individuals in each generation that manifest deep venous thrombosis, ischemic stroke, myocardial infarction, pulmonary embolism, recurrent spontaneous abortion, or even cerebrovascular aneurysm etc (Fig. 1) [12, 13, 21, 26].

Prevention of thrombotic disease is imperative, since it is very common and life-threatening [27]. Susceptibility to thrombosis may be identified through a combination of genetic counseling and presymptomatic DNA testing in individuals with a positive family history of thrombophilia. Pretest genetic counseling may be very helpful in reducing anxiety and confusion about thrombophilia facts [28, 29]. Geneticists may play a significant role in the prevention of such a common condition like thrombophilia [20, 29]. During counseling for other diseases and family history taking, geneticists may recognize that some individuals are at risk for thrombosis and inform them about available molecular tests and preventive measures.

Detection of the inherited genetic susceptibility for thrombosis may contribute to early intervention and prevention of thrombotic incidents, including cerebral venous thrombosis. Hematologists may prescribe preventive anticoagulant therapy for some individuals at risk for thrombosis, at least in high-risk periods such as during surgery or pregnancy [27, 30–32].

It is true that there are many thrombophilia-contributing genetic variations, but some mutations seem to be more common and important in pathogenesis of thrombotic disease. For example, FV Leiden and FII G20210A appear to be

major susceptibility factors for thromboembolic incidents in Southern and Eastern European populations [21, 33]. A Greek study indicated that the combination of genetic counseling and molecular testing for these two common thrombophilia-associated variations may result in fivefold increased identification of at risk people compared to general population screening and thus it may contribute significantly to prevention of thrombotic incidents [34]. Among 96 unrelated individuals with positive family history for thrombosis 53% had either FV Leiden and/or FII G20210A while in 100 age and gender matched controls from the general population only 10% had either one of the two mutations [34].

Prevention of thrombophilia through DNA testing of blood or saliva samples may save lives. It follows that genetic testing combined with healthy lifestyle choices may prevent about half of CVT cases. In the future it is expected that molecular genetic testing will become a routine approach, applied both in hospital and community medicine. It may be also envisaged that thrombophilia, a major current burden worldwide, will be mostly preventable in the future by the combination of genetic testing and anticoagulant preventive treatment.

6 Conclusions

Cerebral vascular thrombosis is a multifactorial condition of unknown incidence. There is association of CVT with certain inherited disorders but there is great heterogeneity in inheritance pattern, incidence and phenotype. The mortality rate in case of untreated CVT is up to 50%, but the mortality drops to 10% when CVT is treated.

Prevention of CVT is feasible through healthy lifestyle, genetic counseling, molecular genetic analysis for common thrombophilia mutations, and prophylactic anticoagulative medication. As the father of clinical medicine Hippocrates noticed about two and a half millenia ago “preventing is better than treating” [35].

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References

1. Bousser, M.G., and J.M. Ferro. 2007. Cerebral Venous Thrombosis: An Update. *Lancet Neurology* 6: 162–170.
2. Guenther, G., and A. Arauz. 2011. Cerebral Venous Thrombosis: A Diagnostic and Treatment Update. *Neurología* 26: 488–498.
3. Ferro, J.M., and P. Canhão. 2014. Cerebral Venous Sinus Thrombosis: Update on Diagnosis and Management. *Current Cardiology Reports* 16: 523.

4. Ribes, M.F. 1825. Des recherches faites sur la phlébite. *Revue Médicale Francaise et Etrangère et Journal de Clinique de l'Hôtel-Dieu et de la Charité de Paris* 3: 5–41.
5. Abercrombie, J. 1828. *Pathological and Practical Researches on the Diseases of the Brain and Spinal Cord*, 83–85. Edinburgh: Waugh and Innes.
6. Ehlers, H., and C.B. Courville. 1936. Thrombosis of Internal Cerebral Veins in Infancy and Childhood. *The Journal of Pediatrics* 8: 600–623.
7. Kalbag, R., and A. Woolf. 1967. *Cerebral Venous Thrombosis*. Oxford: Oxford University Press.
8. Agnelli, G., and M. Verso. 2008. Epidemiology of Cerebral Vein and Sinus Thrombosis. *Frontiers of Neurology and Neuroscience* 23: 16–22.
9. Choi, J.C. 2015. Genetics of Cerebral Small Vessel Disease. *Journal of Stroke* 17: 7–16.
10. Paraskevas, G.P., V.C. Constantinides, A. Bougea, E. Gerakoulis, C. Yapijakis, E. Kararizou, and E. Kapaki. 2016. Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leucoancepahlopathy Presenting with Postpartum Psychosis and Late-Onset Stroke. *Future Neurology* 11: 207–213.
11. Coutinho, J.M., S.M. Zuurbier, and J. Stam. 2014. Declining Mortality in Cerebral Venous Thrombosis: A Systematic Review. *Stroke* 45: 1338–1341.
12. Reitsma, P.H. 2015. Genetics in Thrombophilia: An Update. *Haemostaseol* 35: 47–51.
13. Bick, R.L. 2006. Hereditary and Acquired Thrombophilic Disorders. *Clinical and Applied Thrombosis/Hemostasis* 12: 125–135.
14. Albisetti, M., A. Moeller, K. Waldvogel, V. Bernet-Buettiker, V. Cannizzaro, A. Anagnostopoulos, C. Balmer, and M. Schmugge. 2007. Congenital Prothrombotic Disorders in Children with Peripheral Venous and Arterial Thromboses. *Acta Haematologica* 117: 149–155.
15. Gary, T., F. Hafner, H. Froehlich, T. Stojakovic, H. Scharnagl, E. Pilger, and M. Brodmann. 2010. High Factor VIII Activity, High Plasminogen Activator Inhibitor 1 Antigen Levels and Low Factor XII Activity Contribute to a Thrombophilic Tendency in Elderly Venous Thromboembolism Patients. *Acta Haematologica* 124: 214–217.
16. Lozano, R., M. Naghavi, K. Foreman, et al. 2012. Global and Regional Mortality from 235 Cases of Death for 20 Age Groups in 1990 and 2010. *Lancet* 380: 2095–2128.
17. Raskob, G.E., P. Angchaisuksiri, A.N. Blanco, H. Buller, A. Gallus, B.J. Hunt, E.M. Hylek, A. Kakkar, S.V. Konstantinides, M. McCumber, Y. Ozaki, A. Wendelboe, and J.I. Weitz. 2014. Thrombosis: A Major Contributor to the Global Disease Burden. *Arteriosclerosis, Thrombosis, and Vascular Biology* 34: 2363–2371.
18. Dentali, F., M. Crowther, and W. Ageno. 2006. Thrombophilic Abnormalities, Oral Contraceptives, and Risk of Cerebral Vein Thrombosis: A Meta-Analysis. *Blood* 107: 2766–2673.
19. Lane, D.A., and P.J. Grant. 2000. Role of Hemostatic Gene Polymorphisms in Venous and Arterial Thrombotic Disease. *Blood* 95: 1517–1532.
20. Yapijakis, C., Z. Serefoglou, and C. Voumvourakis. 2015. Common Gene Polymorphisms Associated with Thrombophilia. In *Thrombosis, Atherosclerosis and Atherothrombosis - New Insights and Experimental Protocols*, ed. Mojca Bozic-Mijovski. InTech Publications, Rijeka, Croatia.
21. Yapijakis, C., Z. Serefoglou, A.M. Nixon, A. Vylliotis, V. Ragos, and E. Vairaktaris. 2012. Prevalence of Thrombosis-Related DNA Polymorphisms in a Healthy Greek Population. *In Vivo* 26: 1095–1102.
22. de Bruijn, S.F., J. Stam, M.M. Koopman, and J.P. Vandenbroucke. 1998. Case Control Study of Risk of Cerebral Sinus Thrombosis in Oral Contraceptive Users who are Carriers of Hereditary Prothrombotic Conditions. *British Medical Journal* 316: 589–592.
23. Amoozegar, F., P.E. Ronksley, R. Sauve, and B.K. Menon. 2015. Hormonal Contraceptives and Cerebral Venous Thrombosis Risk: A Systematic Review and Meta-Analysis. *Frontiers in Neurology* 6: 7.
24. Gurfinkel, E.P., G.E. Bozovich, O. Dabbous, B. Mautner, and F. Anderson. 2005. Socio-Economic Crisis and Mortality. Epidemiological Testimony of the Financial Collapse of Argentina. *Thrombosis Journal* 3: 22.

25. Makaris, E., G. Michas, R. Micha, D. Gkotsis, C. Panotopoulos, I. Pisimisis, D. Svoronos, I. Tsichlis, G. Koudounis, G. Asimakis, and S. Zobolos. 2013. Greek Socio-Economic Crisis and Incidence of Acute Myocardial Infarction. *International Journal of Cardiology* 168: 4886–4887.
26. Andreou, A., C. Papapetrou, K. Papadimitriou, D. Avgoustidis, and C. Yapijakis. 2015. Cerebrovascular Aneurysms may be Associated with Thrombophilia-Predisposing Mutations in Patients with Familial Risk. *In Vivo* 29: 395–398.
27. Cardiovascular Disease Educational and Research Trust, Cyprus Cardiovascular Disease Educational and Research Trust, European Venous Forum, International Surgical Thrombosis Forum, International Union of Angiology, Union Internationale de Phlébologie. 2006. Prevention and Treatment of Venous Thromboembolism: International Consensus Statement (Guidelines According to Scientific Evidence). *International Angiology* 25: 101–161.
28. Federici, C., J. Gianetti, and M.G. Andreassi. 2006. Genomic Medicine and Thrombotic Risk: Who, When, How and Why? *International Journal of Cardiology* 106: 3–9.
29. Reich, L.M., M. Bower, and N.S. Keys. 2003. Role of the Geneticist in Testing and Counseling for Inherited Thrombophilia. *Genetics in Medicine* 5: 133–143.
30. Langlois, N.J., and P.S. Wells. 2003. Risk of Venous Thromboembolism in Relatives of Symptomatic Probands with Thrombophilia: A Systematic Review. *Thrombosis and Haemostasis* 90: 17–26.
31. Selby, R., W. Geerts, F.A. Ofosu, S. Craven, L. Dewar, A. Phillips, and J.P. Szalai. 2009. Hypercoagulability After Trauma: Hemostatic Changes and Relationship to Venous Thromboembolism. *Thrombosis Research* 124: 281–287.
32. Taniguchi, S., I. Fukuda, K. Daitoku, M. Minakawa, S. Odagiri, Y. Suzuki, K. Fukui, K. Asano, and H. Ohkuma. 2009. Prevalence of Venous Thromboembolism in Neurosurgical Patients. *Heart and Vessels* 24: 425–428.
33. De Stefano, V., I. Martinelli, P.M. Mannucci, K. Paciaroni, P. Chiusolo, I. Casorelli, E. Rossi, and G. Leone. 1999. The Risk of Recurrent Deep Venous Thrombosis among Heterozygous Carriers of Both Factor V Leiden and the G20210A Prothrombin Mutation. *The New England Journal of Medicine* 341: 801–806.
34. Yapijakis, C., T. Antoniadi, K. Salavoura, C. Voumvourakis, and E. Vairaktaris. 2012. Potential Prevention of Thromboembolism by Genetic Counseling and Testing for Two Common Thrombophilia Mutations. *In Vivo* 26: 165–172.
35. Yapijakis, C. 2009. Hippocrates of Kos, the Father of Clinical Medicine, and Asclepiades of Bithynia, the Father of Molecular Medicine. *In Vivo* 23: 507–514.

Correlations Between Nutrition Habits, Anxiety and Metabolic Parameters in Greek Healthy Adults

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Abstract Background: Anxiety combined with nervousness and apprehension consist a focal response to different life conditions. Lifestyle habits, anxiety and biochemical markers are in a constant interaction. **Aim:** To investigate the prevalence of anxiety in healthy adults and its possible association with biochemical factors—lipid profile, liver markers, thyroid hormones—and lifestyle habits. **Methods:** Quantitative descriptive correlation study. A total of 100 healthy adults participated in the research. A specially designed questionnaire and Hamilton's scale were used. Anthropometric and biochemical analyses were performed. **Findings:** Overall, 61% of the participants presented moderate to very serious anxiety. The average score on the Hamilton scale was 13.82 (±9.000), with men exhibiting less stress than women. For $p \leq 0.05$: Stress was positively correlated with impaired thyroid and hepatic function. Hepatic function was affected by both sugar products and water melon, which were positively correlated with total bilirubin and AST/SGOT respectively. Tomato, peppers and legumes were negatively correlated with AST/SGOT. Deep fried food was positively correlated with GGT and triglycerides. Legumes and fish were negatively correlated with CPK. Regarding the lipid metabolism, it was found that food cooked with oil

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was positively associated with uric acid, but non-cooked olive oil was negatively correlated with the risk for CAD. Thyroid function was negatively correlated with non-homemade food and pasta consumption and positively correlated with consumption of whole grains and green tea. Participants with subclinical hypothyroidism seemed to consume less vitamin B12, folic acid and vegetables. **Conclusion**: No direct correlation between lifestyle habits and anxiety was found. Nevertheless, eating habits influenced biochemical markers—especially the thyroid hormones—which may be indirectly responsible for anxiety and related moods.

Keywords Anxiety • Eating habits • Lipid profile • Liver enzymes • Thyroid function

Abbreviations

ALT/SGPT	Alanine aminotransferase
AST/SGOT	Aspartate aminotransferase
BMI	Body mass index
CAD	Cholesterol/LDL
CPK	Creatine phosphokinase
CPK-MB	Creatine kinase myocardial band
fT3	Free triiodothyronine
fT4	Free thyroxine
GABA	Gamma-aminobutyric acid
GGT	Gamma-glutamyltransferase
HDL	High density lipoprotein
T3	Triiodothyronine
T4	Thyroxine
TSH	Thyroid-stimulating hormone

1 Introduction

Anxiety, which can be described as a combination of nervousness and apprehension, is a natural response to different life conditions [1]. This unpleasant emotional state is associated with several adaptive behavioral responses, such as increased blood pressure and heart rate and changes in lifestyle—including eating, sleep and physical activity habits [2]. The feeling of anxiety is considered normal in a chasing environment, provided that it is not so intense so it negatively affects an individual's daily life [3].

The current demands at a workplace necessitate increasing load and long working hours. When these are combined with nonflexible work timetable, time pressure,

critical managers, and the probable sidelining of private life, professionals can be affected by the widespread phenomenon, called "work stress" [4, 5].

High stress levels have a close association with adverse health effects. Not only high stress levels have a direct impact on the immune system and other physiological functions, but they can also drastically reduce healthy behaviors that the individual might exhibit, such as balanced nutrition, adequate hours of night sleep and frequent physical activity [6].

Thyroid function may be influenced during a stressful period of life. Specifically, it usually gets down-regulated as T3 and T4 levels decrease with anxiety. Stress suspends the secretion of TSH through the action of glucocorticoids on the central nervous system [7]. The thyroid hormones are directly associated with the regulation of several neurotransmitters. Both synthesis and secretion of GABA, serotonin and norepinephrine are influenced by the thyroid function. When the thyroid is underactive, the neurotransmitters are getting destabilized, leading to anxiety [8].

Non-autoimmune subclinical hypothyroidism is a frequently observed biochemical disorder that is characterized by elevated TSH concentrations in the presence of normal T4 and T3, absence of autoantibodies and a normal thyroid gland function [9]. The upper normal limit of serum TSH level is 2.5–3 mIU/L, which can be associated with clinical thyroid disease [10].

Lifestyle habits play a key role in stress prevention and treatment. Specifically, balanced nutrition has proved to beneficiate mood, as it triggers the central nervous system to secret hormones of satisfaction and relaxation [11]. Regarding sleep habits, it is already known that the secretion of mood hormones, such as serotonin and cortisol is regulated by the circadian rhythm. Therefore, inadequate hours of night sleep are responsible for anxiety, drowsiness and reduced productivity [12].

The purpose of this study was to investigate the prevalence of anxiety in healthy adults and its possible association with biochemical factors, environmental conditions, dietary preferences and sleep habits.

2 Material and Methods

2.1 Subjects and Ethics

Convenience sampling was performed after ensuring the permission for the research conduction. Participants gave their full consent and their anonymity was assured. No personal information was collected or used.

2.2 Lifestyle and Hamilton Questionnaire

A standardized questionnaire, regarding lifestyle habits and family medical history, constructed by the researchers (crombach a = 0.78) and consisting of a total of 43 questions, was used [13].

Additionally, the Hamilton anxiety scale was utilized (cronbach a = 0.836). Specifically, there were 13 questions regarding anxiety symptoms and each question was scored from 0–4. The total score of 0–17 was considered mild, 18–25 mild to moderate, and 26–30 moderate to severe. A total score exceeding 30 points was an indication of severe anxiety [14].

2.3 Anthropometric Measurements

Anthropometric measurements consisted of height, weight, waist circumference, hip circumference and Body Mass Index (BMI in kg/m^2) for all the participants was performed according to previous studies [15].

2.4 Blood Samples and Serum Assays

Peripheral blood samples were collected from 100 fasting healthy participants. Complete biochemical tests were performed. Specifically, lipid profile, glucose, markers of liver and thyroid function were measured. All serum analyses were performed at the same laboratory following the same procedure.

2.5 Statistical Analysis

Continuous variables are presented as mean (±SD). To evaluate the effect of independent on dependent variables, the parametric t-test for two independent samples and analysis of variance (ANOVA) were used. In case of non-normality, the Mann-Whitney and the Kruskal-Wallis tests were used. Pearson's correlation was used to evaluate the relation between quantitative variables, while odds ratios were calculated to measure the association between categorical variables. The data were analyzed with the use of the statistical package 20.0 SPSS for windows. The predetermined significance level in all cases was <0.05.

3 Results

3.1 Descriptive Statistics

A total of 100 apparently-healthy adults voluntarily agreed to participate in the study; 82 of them were females and 18 were males. Overall, 81.4% of the participants were living in Laconia, Greece.

3.1.1 Anthropometric Characteristics

Participants' mean age was 39.61 (± 10.710) years old. Their average weight was 68.20 (± 12.564) kg, average height 1.66 (± 0.078) meters, average BMI 24.62 (± 3.768) and waist circumference 85.81 (± 11.888) cm.

3.1.2 Lifestyle Habits

The frequencies of the consumption of the main food groups in a weekly basis are shown in Table 1.

Overall, 70.0% of the participants stated that they sleep earlier than 24:00 while the average duration of their night sleep was 6.82 (± 1.220) hours.

Moreover, it was observed that 52.0% of the participants were either active or passive smokers. Interestingly, 19.0% of them were exposed to smoke every day.

Table 1 Percentage distribution of fruit, vegetable, meat, fish, legumes, grain products, olive oil/olives, dairy products, and junk/fast/non-homemade food consumption in a weekly basis, for the total of the sample (n = 100)

Distribution of food consumption (%)	None	Once	Twice	3 times	4 times	5 times	6 times	Daily
Fruits	7.0	12.0	10.0	23.0	12.0	15.0	3.0	18.0
Vegetables	2.0	7.0	13.0	14.0	26.0	10.0	17.0	11.0
Meat	7.0	26.0	28.0	24.0	9.0	4.0	2.0	0.0
Fish	14.0	52.0	24.0	4.0	4.0	2.0	0.0	0.0
Legumes	5.0	38.0	37.0	10.0	5.0	1.0	4.0	0.0
Grain products	35.0	15.0	12.0	8.0	5.0	4.0	8.0	13.0
Olive oil/olives	7.0	9.0	4.0	8.0	15.0	12.0	9.0	36.0
Dairy products	3.0	6.0	6.0	11.0	10.0	8.0	11.0	45.0
Junk/fast/non-homemade food	22.0	49.0	17.0	4.0	4.0	1.0	2.0	1.0

Table 2 Distribution of the prevalence of hypertension, diabetes, stroke, myocardial disease, cancer, and obesity in 1st and/or 2nd degree relatives for N = 100

Disease entity	Percentage distribution of the appearance of the characteristic (%)
Stroke case	24.0
Cardiovascular disease	33.0
Cancer disease	34.0
Diabetes mellitus	2.0
Thyroid disease	26.0
Other chronic disease	12.0

3.1.3 Medical and Family History

Table 2 depicts the family history for close (1st and 2nd degree) relatives.

3.2 Quantitative Evaluation of Stress

From the participants, 61% presented moderate to very serious anxiety, 39% suffered from insomnia and 28% showed depressed mood in a daily basis. The average score on the anxiety scale was 13.82 (± 9.000). Men seemed to have less stress (7.83 ± 5.102) than women (15.13 ± 9.155) ($p = 0.001$) (Table 3).

3.3 Correlations Between Stress Levels, Demographic Characteristics and Biochemical Markers

Hamilton's anxiety scale score was greater in females ($p < 0.001$) and it was negatively correlated with the participants' age ($p = 0.010$). Place of residence did not significantly influence the score ($p = 0.141$).

Subjects with family medical history of stroke ($p = 0.004$) and cardiovascular disease ($p = 0.020$) also exhibited greater anxiety. Anxiety scale's score was negatively correlated with fT3 ($p = 0.051$).

3.4 Correlations Between Stress Levels and Lifestyle Habits

Hamilton's anxiety scale score was not correlated neither with sleep nor with eating habits.

Table 3 Hamilton anxiety scale

		None (%)	Mild (%)	Moderate (%)	Serious (%)	Very serious (%)
Hamilton scale	Anxiety mood	9.0	30.0	33.0	16.0	10.0
	Tension	15.0	28.0	33.0	16.0	8.0
	Phobias	54.0	23.0	14.0	5.0	4.0
	Insomnia	31.0	30.0	22.0	14.0	3.0
	Cognitive	38.0	28.0	25.0	8.0	1.0
	Depressive mood	36.0	36.0	21.0	4.0	3.0
	Physical symptoms (muscular symptoms)	36.0	18.0	28.0	13.0	5.0
	Physical symptoms (sensorial symptoms)	48.0	21.0	19.0	9.0	3.0
	Cardiovascular symptoms	44.0	31.0	16.0	6.0	3.0
	Respiratory symptoms	53.0	25.0	16.0	3.0	3.0
	Gastrointestinal symptoms	59.0	16.0	15.0	5.0	5.0
	Urogenital symptoms	78.0	10.0	9.0	2.0	1.0
	Symptoms from the autonomic nervous system	49.0	33.0	9.0	6.0	3.0
Hamilton score	Total	13.82 (± 9.000)				
	Males	7.83 (± 5.102)				
	Females	15.13 (± 9.155)				

Mean value (± standard variation)

3.4.1 Eating Habits and Hepatic Function

Moderate consumption (2–4 times per week) of sugar products was correlated with increased total ($p = 0.009$) and direct bilirubin ($p = 0.016$). Consumption of tomatoes was negatively correlated with AST/SGOT ($p = 0.009$). Red and orange pepper consumption seemed to be negatively correlated with ALT/SGPT ($p = 0.029$) and AST/SGOT ($p = 0.012$). Moderate consumption of watermelon was positively correlated with total bilirubin ($p = 0.039$) and AST/SGOT ($p = 0.003$). Regarding the consumption of food cooked with oil, it seemed to be positively associated with ALT/SGPT ($p = 0.037$), GGT ($p = 0.035$), and alkaline phosphatase ($p = 0.028$).

Legumes were negatively correlated with AST/SGOT ($p = 0.001$). Deep fried food consumption was positively correlated with GGT ($p = 0.019$).

3.4.2 Eating Habits and Lipid Profile

Moderate consumption of sugar products was positively correlated with uric acid ($p = 0.050$) and triglycerides ($p = 0.019$). Red meat consumption seemed to be positively associated with total protein ($p = 0.002$). Moderate consumption of watermelon was correlated with increased glucose level ($p = 0.010$). Pomegranate consumption was negatively correlated with CPK ($p = 0.003$) and CK-MB ($p = 0.014$). High consumption of vegetables cooked with olive oil was associated with increased uric acid ($p = 0.022$). However, non-cooked olive oil decreased the risk for CAD ($p = 0.037$). Legumes ($p = 0.023$) and fish ($p = 0.046$) were negatively correlated with CPK. Green tea was negatively correlated with CAD ($p = 0.027$). Nuts were positively correlated with HDL ($p = 0.050$). Finally deep fried food consumption increased triglycerides level ($p = 0.016$).

3.4.3 Eating Habits and Thyroid Hormones

The frequency of non-home-made meal consumption [16] was positively correlated with T4 ($p = 0.037$). T3 level seemed to be positively correlated with consumption of vegetables cooked with olive oil ($p = 0.026$), while it was negatively correlated with whole grain consumption ($p = 0.021$). Pasta consumption was positively correlated with FT4 ($p = 0.045$) and green tea consumption was negatively correlated with T3 ($p = 0.021$).

Overall, 12% of the participants showed predisposition for subclinical hypothyroidism, with TSH < 2.5 mIU/l (average 3.09 ± 0.45) and normal values for T3 and T4. The participants were separated in two categories depending on the TSH level. Correlations between thyroid function and lifestyle habits were also made in these categories.

TSH levels seemed to be influenced by the consumption of nutrients rich in vitamin B12 ($p = 0.044$), folic acid ($p = 0.05$) and vegetables ($p = 0.005$). Subjects with TSH levels >2.5 mIU/l were found to consume more of these products ($p = 0.05$).

No statistical important correlations between subclinical hypothyroidism, sleep habits and stress levels were found.

4 Discussion

The aim of this study was to investigate the correlation of anxiety with biochemical factors, environmental conditions, dietary preferences and sleep habits.

This study was based mainly on residents of Laconia, which were characterized by normal weight, height and waist circumference. A total of 35% of the participants exhibited moderate stress levels (with women being more stressful than men), as demonstrated by the Hamilton Anxiety Scale. The greater level of stress in women can be explained by the hormonal fluctuations and particular biochemistry of brain [17].

Age was negatively correlated with levels of anxiety, according to the Hamilton Anxiety Scale score. Moreover, a positive family medical history for cardiovascular disease and stroke seemed to affect subjects' stress levels. It has already been noted that age is inversely associated with the everyday negative affect, neuroticism and daily stress. A possible explanation is that humans over 30 tend to be more calm and mature as they gain more experience, which help them deal easier with difficult situations [18]. Regarding the family history, we can hypothesize that participants are getting anxious for their health progress, as they are aware for their predisposition for a certain disease.

Concerning the correlation of dietary habits with the biochemical markers in the current study 64% of the participants reported consumption of sugar products, which was positively correlated with uric acid, and total and direct bilirubin. The high consumption of sweets has shown to increase cholesterol, triglycerides and blood sugar levels. The transport of these lipids to the liver can cause oxidation and liver dysfunction [19].

Regarding fruit consumption, we found correlations between pomegranate, watermelon and biochemical markers. More precisely, we showed that pomegranate consumption was negatively correlated with CPK, while watermelon consumption was positively correlated with glucose and total bilirubin. A possible explanation for the beneficial role of pomegranate is, that it is an important source of vitamin C, which is crucial for heart function [20]. Watermelon consumption was found to increase glucose levels, which may happen due to its high glycemic index [21].

The current study found that vegetables and specifically tomatoes, red and orange pepper have a beneficial effect on the liver function. Literature has already shown that tomato has an important role in body's detoxification. Tomato's content in chlorine and sulfur contributes to liver's stimulation and function through filtering and detoxifying body waste [22]. Pepper and particularly chili peppers have proved to reduce the occurrence of liver fibrosis and liver damage, due to their antioxidant action and content of capsaicin [23].

Additionally, it was found that nuts, fish, legumes and uncooked olive (as opposed to cooked oil) have beneficial effects for heart and liver function. Specifically, it is already known that omega-3 and omega-6 fats contribute in the prevention of atherosclerotic plaque development, which can be deposited in arteries and cause coronary heart disease [24]. However, the way in which olive oil is cooked may reverse its effective role; for example fried food is able to increase the advanced glycation end-product (AGEs) causing diabetic vasculature and development of atherosclerosis [25].

The current study has shown that the frequent consumption of non-homemade food and pasta was positively correlated with T4, as opposed to the negative effect that consumption of green tea had in T3 levels. Participants with TSH levels > 2.5 mIU seemed to consume more vitamin B12, folic acid and vegetables. Literature has already indicated that fast food and snacks impede the proper function of the thyroid gland, due to their low content of iodine, selenium, magnesium and vitamin D. Additionally, they often increase normal weight and insulin levels, consequently destabilizing the entire hormonal system and preventing the conversion of T4 into T3 [26]. One of the main ingredients of green tea is catechins which have antioxidative and anticarcinogenic action. However, catechins seem to also have antithyroid activity, through their influence in thyroid peroxidase, 5′-deiodinase I (5′-DI), and Na(+), K(+)-ATPase, as evidenced from both in vivo and in vitro studies show [27, 28] Another very interesting point that literature has highlighted is a high prevalence of B12 deficiency in hypothyroid patients compared to healthy individuals. Vitamin B12 deficiency may occur as a result of autoimmune pernicious anemia, malabsorption, malnutrition or use of drugs including proton-pump inhibitors [29]. In the current study, we found that healthy participants with TSH levels > 2.5 mIU prefer to consume foods high in vitamin B12.

The confounding role of dietary habits and lifestyle variables on the tested parameters deserves further attention in future research.

5 Conclusion

To sum up, the present study did not find a direct correlation between lifestyle habits and anxiety. However, eating habits seemed to influence the biochemical markers and especially the thyroid function which indirectly may be responsible for humans' mood. Adopting a balanced diet in line with the model of the Mediterranean diet, has a protective effect for both the metabolism and the function of the thyroid gland. Beyond this, it is essential to ensure good mental health.

6 Limitations

The research was conducted chiefly in a specific geographic area of Greece, Laconia, which entails that the results mentioned above are relevant only to this region. Additionally, the sample was mainly comprised of women since they were more willing to participate in the survey. Hence further research entailing an equally distributed between the two sexes number of participants can further elucidate the relationship between anxiety, dietary habits and gender.

References

1. Marmor, J. 1962. Anxiety and Worry as Aspects of Normal Behavior. *California Medical* 97 (4): 212–215.
2. Rosen, J.B., and J. Schulkin. 1998. From Normal Fear to Pathological Anxiety. *Psychological Review* 105 (2): 325–350.
3. Hansell, J., and L. Damour. 2008. *Abnormal Physology*. 2nd ed. Hoboken, NJ: Wiley.
4. Cooper, C.L., R.D. Cooper, and L.H. Eaker. 1988. *Living with Stress*. Harmonsworth: Penguin.
5. Michie, S. 2002. Causes and Management of Stress at Work. *Occupational and Environmental Medicine* 59: 67–72.
6. Ronald, G., and K. Janice. 2005. Stress-Induced Immune Dysfunction: Implications for Health. *Nature Reviews Immunology* 5: 243–251.
7. Helmreich, D.L., D.B. Parfitt, Lu XY, H. Akil, and S.J. Watson. 2005. Relation Between the Hypothalamic-Pituitary-Thyroid (HPT) Axis and the Hypothalamic-Pituitary-Adrenal (HPA) Axis During Repeated Stress. *Neuroendocrinology* 81: 183–192.
8. Engum, A., T. Bjøro, A. Mykletun, and A.A. Dahl. 2002. An Association Between Depression, Anxiety and Thyroid Function, a Clinical Fact or an Artefact? *Acta psychiatrica Scandinavica* 106 (1): 27–34.
9. Gharib, H., R.M. Tuttle, H.J. Baskin, L.H. Fish, P.A. Singer, and M.T. McDermott. 2005. Subclinical Thyroid Dysfunction: A Joint Statement on Management from the American Association of Clinical Endocrinologists, the American Thyroid Association, and the Endocrine Society. *The Journal of Clinical Endocrinology and Metabolism* 90: 581–585.
10. Fatourechi, V. 2009. Subclinical Hypothyroidism: An Update for Primary Care Physicians. *Mayo Clinic Proceedings* 84 (1): 65–71.
11. Parker, G., I. Parker, and H. Brotchie. 2006. *Journal of Affective Disorders* 92 (2): 149–159.
12. Wells, M., and B. Vaughn. 2012. Poor Sleep Challenging the Health of a Nation. *Neurodiagnostic Journal* 52 (3): 233–249.
13. Kargakou, A., A. Sachlas, N.G. Lyrakos, S. Zyga, M. Tsironi, and A.P. Rojas Gil. 2015. Association Between Optimism, Dietary Habits, Lifestyle and General Health Self-Assessment: A Pilot Study. *International Journal of Health and Psychology Research* 1 (3): 13–28.
14. Hamilton, M. 1959. The Assessment of Anxiety States by Rating. *The British Journal of Medical Psychology* 32: 50–55.
15. Duren, D.L., R.J. Sherwood, S.A. Czerwinski, M. Lee, A.C. Choh, R.M. Siervogel, and W. Cameron Chumlea. 2008. Body Composition Methods: Comparisons and Interpretation. *Journal of Diabetes Science and Technology (Online)* 2 (6): 1139–1146.
16. Lee, K.W., W.O. Song, and M.S. Cho. 2016. Dietary Quality Differs by Consumption of Meals Prepared at Home vs. Outside in Korean Adults. *Nutrition Research and Practice* 10 (3): 294–304.
17. World Health Organization. 2001. *The World Health Report 2001 – Mental Health: New Understanding*. Copenhagen: New Hope.
18. Mroczek, D.K., and D.M. Almeida. 2004. The Effect of Daily Stress, Personality, and Age on Daily Negative Affect. *Journal of Personality* 72 (2): 355–378.
19. Mikolajczyk, R.T., W.E. Ansari, and A.E. Maxwell. 2009. Food Consumption Frequency and Perceived Stress and Depressive Symptoms Among Students in Three European Countries. *Nutrition Journal* 8: 31–46.
20. Hamid, R., A. Mohammad, and N.O. Seyed. 2012. A Comprehensive Review of *Punica granatum* (Pomegranate) Properties in Toxicological, Pharmacological, Cellular and Molecular Biology Researches. *Iranian Journal of Pharmaceutical Research* 11 (2): 385–400.
21. Abdelwahab, S.I., L.E. Hassan, H.M. Sirat, S.M. Yagi, W.S. Koko, S. Mohan, M.M. Taha, S. Ahmad, C.S. Chuen, P. Narrima, M.M. Rais, and A.H. Hadi. 2011. Anti-Inflammatory Activities of Cucurbitacin E Isolated from *Citrullus lanatus var. citroides*: Role of Reactivenitrogen Species and Cyclooxygenase Enzyme Inhibition. *Fitoterapia* 82 (8): 1190–1197.

22. Debjit, B., K.P. Sampath, P. Shravan, and S. Shweta. 2012. Tomato-A Natural Medicine and Its Health Benefits. *Journal of Pharmacognosy and Phytochemistry* 1 (1): 33–43.
23. European Association for the Study of the Liver. 2015. Chilli Peppers Hold Promise of Preventing Liver Damage and Progression. ScienceDaily. www.sciencedaily.com/releases/2015/04/150423085442.htm.
24. Orosco, M., C. Rouch, and V. Daugé. 2002. Behavioral Responses to Ingestion of Different Sources of Fat. Involvement of Serotonin? *Behavioural Brain Research* 132 (1): 103–109.
25. Goldin, A., J.A. Beckman, A.M. Schmidt, and M.A. Creager. 2015. Advanced Glycation End Products Sparking the Development of Diabetic Vascular Injury. *Circulation* 131 (24): 535.
26. Cheryl, H. 2012. Thyroid Disease and Diet – Nutrition Plays a Part in Maintaining Thyroid Health. *Today's Dietitian* 14 (7): 40.
27. Chandra, A.K., and N. De. 2013. Catechin Induced Modulation in the Activities of Thyroid Hormone Synthesizing Enzymes Leading to Hypothyroidism. *Molecular and Cellular Biochemistry* 374 (1–2): 37–48.
28. Chandra, A.K., N. De, and S.R. Choudhury. 2011. Effect of Different Doses of Un-Fractionated Green and Black Tea Extracts on Thyroid Physiology. *Human and Experimental Toxicology* 30 (8): 884–896.
29. Jabbar, A., A. Yawar, S. Wasim, N. Islam, N.U. Haque, N. Zuberi, A. Ataullah Khan, and J. Akhter. 2008. Vitamin B12 Deficiency Common in Primary Hypothyroidism. *JPMA* 58: 258–261.

Neuronal Correlation Parameter and the Idea of Thermodynamic Entropy of an *N*-Body Gravitationally Bounded System

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Abstract Understanding how the brain encodes information and performs computation requires statistical and functional analysis. Given the complexity of the human brain, simple methods that facilitate the interpretation of statistical correlations among different brain regions can be very useful. In this report we introduce a numerical correlation measure that may serve the interpretation of correlational neuronal data, and may assist in the evaluation of different brain states. The description of the dynamical brain system, through a global numerical measure may indicate the presence of an action principle which may facilitate a application of physics principles in the study of the human brain and cognition.

Keywords Network • Connectivity • Neurons • Statistical • Lambert • Cluster • Entropy • Galactic • Bekenstein

1 Introduction

Theoretical analysis of brain dynamics may provide insights into cognitive, psychiatric and neurological disorders (Menon, 2011) and brain network changes, and may aid the early detection of pathophysiology in patients, and therefore it may have a very relevant significance. Different brain regions have been identified that relate to different functions and there is a reach literature of structural-anatomical and theoretical models that have been proposed to integrate brain areas and functions.

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It remains however, a very challenging problem to accurately describe the dynamics of the brain due to its high anatomical, physiological and functional complexity. For example, Cerebrovascular alterations include vascular density, vascular plasticity, and vascular reactivity to acute metabolic changes [1] with important consequences. Moreover, certain anatomical regions such as the Posterior Cingulate Cortex (PCC) that have high rate of metabolism (associated with normal conscious state) may play a role in the tuning of metastability of intra- and inter- network connectivity (Leech, 2014). Alterations in functional properties or anatomical disconnection between brain regions may be caused by white matter loss or demyelination ("disconnection" hypothesis") [2]. Of course, there exist vast literature related to studies that discuss healthy, diseased and aging conditions of the human brain, and modeling of such different states is desirable. In general some examples of the functional and anatomical changes that would require a non-linear and most likely stochastic modeling may be (a) metabolic changes within regions of brain networks, (b) vascular and myelin abnormalities in white matter connections, (c) abnormalities in other brain regions with which they interact (Leech and Sharp, 2013), (d) alterations in brain cells such as neuroglial cells (Taylor and Francis, 2007), (e) changes in brain volume and neurotransmission, (e) network alterations through influences by agent-independent connections between environment and observer, (f) functional compensation. A characteristic example is the aging brain where dopaminergic receptors decline, structures volumetrically shrink [3, 4], (Good et al., 2001) and white matter becomes less dense [5, 6] which points to a less efficient information transmission system. However, the brain continuously engages in functional reorganization and functional repair for self-generated support [3] and to meet extrinsically imposed as well as intrinsic biochemical and cognitive challenge.

The brain may be thought of as a biochemical and bioelectric system with neuronal chemical, and electrical discharges that form the substrate for the encoding and processing of neuronal network information that facilitates sensory as well as motor events. The brain coordinates information crucial to genetic, chemical, and physiological processes largely of a nonlocal "entanglement" character that is likely to be operating in a non-algorithmic way. Therefore, it may be useful as a first approach to describe the coding and processing of information in the brain in terms of a simple parameter. Perhaps a single number, a "determinant" of the "matrix" of electrophysiological and biochemical processes, that form a substrate for semantic processing of neuronal information. It seems natural that such a numerical description of this hyper-complex system should include the memory of previous configurations of the system as a dynamic parameter, and from the physics point of view it may be thought of as some kind of an action principle. A selection of attributes describing the brain dynamics may lead to practical and applicable conclusions, however the brain as a collection of neurons and other cells may require a practically intractable amount of degrees of freedom to fully describe its different states, and a global functional numerical measure may be useful as a binding bio index of its state.

Understanding information encoding and neuronal processing requires study of correlations between neurons. How the populations of neurons encode information

and control human behavior is major point of interest of today's neuroscience. Neurons respond with variable strength to stimulation [7, 8]. This variability may be shared among different neurons, indicating a form of correlation. This responsiveness can result to a substantial effect on the amount of information encoded by a neuronal population [9]. In computational and cognitive neuroscience, it is important to determine how correlations are affected by stimulus drive, experience, learning or various changes in behavioral context, as well as how human brain connectivity reflects higher level network organization of the human brain [10]. Neuronal interconnections cannot be directly observed, and therefore the construction of brain networks is usually an inference problem. Furthermore, there are various different approaches for the construction of brain networks. These methods are usually based upon image modality and the type of connectivity. The connectome to be the comprehensive map of all these connections Sporns et al. [10]. In a recent paper by Prasad et al. [11] the authors present a method for studying brain connectivity by simulating a dynamical evolution of the nodes of the network [11]. The nodes are treated as particles which are evolved under a simulated force analogous to the gravitational acceleration in a well-known N-body problem, where the particle nodes correspond to regions of the cortex, and where the locations of particles are defined as the centers of respective regions on the cortex and their masses are proportional to each region's volume. Furthermore, the attractive force is modeled on the gravitational force, and is explicitly made proportional to the elements of the connectivity matrix derived from imaging data. The authors also present experimental results of simulations on a population of 110 subjects from the Alzheimer's Disease Neuroimaging Initiative (ADNI), that consists of healthy elderly controls, early mild cognitively impaired (eMCI), late MCI (LMCI), and Alzheimer's disease (AD) patients. In healthy controls, the results demonstrate a significant difference in the dynamical properties of connectivity networks when compared to eMCI as well as AD patients. Inspired by the idea that nodes can be treated as particle in an N-body scenario as in [11], where the particle nodes correspond to regions of the cortex, we attempt to calculate neuronal correlation, by implementing the ideas of thermodynamic galaxy clustering theory. In particular, galactic clustering theory predicts that a two-particle correlation function contains much information about large scale clustering, for it evolves self consistently with all the higher order correlation functions [12]. The problem is to extract information about higher order correlations from a two-particle function without having to solve the BBGKY (Bogoliubov–Born–Green–Kirkwood–Yvon) hierarchy, that sometimes is called Bogoliubov hierarchy [12] (This is a system of coupled equations describing the dynamics of interacting particles where a particle distribution function involves with $n + 1$ particle distribution function). Assuming that the neurons are non extended structures (REF) the entropy of such a system can be written in terms of the temperature of the system T and the correlation parameter b. Finally, considering that brain has measurable physical parameters (such as energy and radius in a spherical approximation), we will use Bekenstein entropy bound to calculate the upper entropy limit of the brain. Thus, equating the two entropies (Bekenstein and N-Body) we calculate the neuronal correlation

parameter b, expressed as a function of the brain parameters, thus providing possible a numerical correlation measure of the entire brain network activity, in the form of a single number.

2 Galactic Cluster Entropy and Bekenstein Bound

Thermodynamics and statistical mechanics is the tool for the description of the entropy of a system. The entropy of a system is a non conserved state function that is a very important in science and scientific research. Following [12] we can write the entropy of an N-body system to be:

$$S = S_0 - \left(3b + T^{3/2} \ln b\right) \tag{1}$$

where b is the correlation parameter between two particles in the given system, and T is the temperature of the system. Because our system of interest (the brain) has finite dimensions, we use the Bekenstein bound in the estimation of entropy S. This is an upper limit of the entropy S, or information N, contained within a given finite region of space, of finite amount of energy content E that corresponds to a total amount of mass m The Bekenstein bound can also be thought as the maximum amount of information required to completely describe a given physical system down to the quantum level. Therefore in relation to the brain the Bekenstein bound relation for the entropy S can be written as:

$$S_B \leq \frac{2\pi\ k_B R E}{\hbar c} \leq \frac{2\pi k_B R_b m_b c}{\hbar}. \tag{2}$$

where S is the entropy, k_B is Boltzmann's constant, R is the radius of a sphere that can enclose the given system, $E = mc^2$ is the total mass–energy including any rest masses, which in the case of the brain is equal to $E_b = m_b c^2$, $\hbar$ is the reduced Planck constant, and c is the speed of light. Note that while gravity plays a significant role in its enforcement, the expression for the bound does not contain the gravitational constant G.

To clarify our approach we simply state that in this paper we will treat neurons as an N-body particle scenario in which clustering capabilities are possible. Thus we can say that correlation coefficient b, measures the influence of the "gravitational correlation energy W" between neurons. In an N-body scenario the correlation coefficient b depends in principle on the form of the two-particle correlation function ξ as it is given in Saslaw [12]. Moreover, and in relation to neurons we can say that the correlation coefficient b is a parameter that contains information about their clustering on all scales through the dependence of ξ. If we treat the brain as theoretically infinite (involving various length scales and large brain size relative to the neurons), as well as a thermodynamic homogeneous system, the only characteristic length scale that enters the potential energy is the average neuron

separation given by the relation $\bar{r} = n^{-1/3}$. So b should depend just on the ratio of the potential and kinetic energies of two typical neurons. Our main assumption is that b is the same for each level of any possible clustering hierarchy on scales larger than the two-particle correlation at that level (Our understanding is that this implies more randomness in speed and direction of information transmission among clusters of low hierarchies. And b may be a binding parameter that may provide a link between neurophysiology and cognition.). We also assume that the entropy of the neuron system in the brain is in equilibrium and does not depend on the path via which the system reaches it's state. Equating Eqs. (1) and (2) and solving for the neuron correlation coefficient b we find that:

$$b = \frac{1}{3} T_b^{3/2} W\left[\frac{3}{T_b^{3/2}} e^{-\frac{2\pi k_B c R_b m_b}{\hbar T_b^{3/2}}}\right]. \tag{3}$$

where W is the Lambert function of the indicated argument. So we have obtained an expression for the correlation coefficient between two neurons in an N-body scenario equating the upper entropy limit as it is calculated via the Bekenstein relation to that predicted by N-body correlation scenario as given in Saslaw [12] and also [13]. We have found that the correlation coefficient b can be written as the Lambert function of three measurable brain parameters indicated namely: temperature T_b, radius R_b and mass m_b. Similarly we calculate that the rate of change of the correlation coefficient w.r.t the brain temperature T_b, mass m_b and radius R_b, are given by:

$$\frac{\mathrm{d}b}{\mathrm{d}T_b} = \frac{T_b^{3/2} W(\Phi) + \frac{2}{\hbar}\left[\pi k_B c m_b R_b - \hbar T_b^{3/2} + \frac{\left(\hbar T_b^{3/2} - \pi c k_B m_b R_b\right)}{1+W(\Phi)}\right]}{2T_b}, \tag{4}$$

$$\frac{\mathrm{d}b}{\mathrm{d}m_b} = -\frac{2\pi c k_B R_b\left[1 - \frac{1}{1+W(\Phi)}\right]}{3\hbar}, \tag{5}$$

$$\frac{\mathrm{d}b}{\mathrm{d}R_b} = -\frac{2\pi c k_B m_b\left[1 - \frac{1}{1+W(\Phi)}\right]}{3\hbar}, \tag{6}$$

and where Φ is given by:

$$\Phi = e^{-\frac{2\pi k_B c R_b m_b}{\hbar T_b^{3/2}}}. \tag{7}$$

3 Discussion and Numerical Results

Before we numerically evaluate our results let us look at the at the exponential term within the Lambert function $W(\Phi)$. The exponential involves the terms:

$$e^{-\frac{2\pi k_B c R_b m_b}{\hbar T_b^{3/2}}}. \tag{8}$$

Looking at the numerator, the term cm_bR_b has units of kg m^2 s^{-1} = J.s. Therefore we conclude that this term represents some form of action, that reads:

$$\hbar_b = cm_bR_b, \tag{9}$$

as a *brain quantum of action* $\hbar_b$. Using the following values for the mass of a male and female brains i.e. $m_b = 1.3$ kg, female $m_b = 1.5$ kg, [14] to a spherical approximation and using a volume brain $V_b = 1350$ cm^3 [15] we find that $R_b = 0.0686$ m. Substituting in Eq. (13) we obtain a first estimate for the brain quantum of action $\hbar_n$ to be:

$$\hbar_{b_{female}} = cm_bR_b = 2.675 \times 10^7 \text{ Js}, \tag{10}$$

$$\hbar_{b_{male}} = cm_bR_b = 3.087 \times 10^7 \text{ Js}. \tag{11}$$

Therefore we can define a Compton wavelength λ_C that is equal to:

$$\lambda_{C_{male}} = \frac{\hbar_b}{m_bc} = 0.0686 \text{ m}, \tag{12}$$

$$\lambda_{C_{male}} = \frac{\hbar_b}{m_bc} = 0.0685897 \text{ m}. \tag{13}$$

This is in principle the physical dimension of an object which if it becomes equal to the Compton wavelength quantum effects are predominant. The numerical values of Eqs. (12) and (13) represent the physical radius of the brain to a spherical approximation. Thus this may be an indication that quantum phenomena might operate in certain brain functions. Therefore Eq. (7) can be written as follows:

$$b = \frac{1}{3}T_b^{3/2}W\left[\frac{3}{T_b^{3/2}}e^{-\frac{2\pi k_B}{T_b^{3/2}}\left(\frac{\hbar_b}{\hbar}\right)}\right]. \tag{14}$$

We now find that the neuron correlation coefficient b appears to be related to the brain temperature $T_b^{3/2}$ and the ratio of the brain defined action $\hbar_b$ over $\hbar$ i.e. the one defined by quantum mechanics. Numerically the correlation coefficient between neurons falls in the range $0 \leq b \leq 1$. If $b = 1$ neuron sub-clusters formed are in

virial equilibrium, and $b_n = 0$ no correlation exists. As $\hbar_b \to \hbar$ then $b \to \alpha$ where is a number less than 1. In the case where the correlation coefficient $\hbar_b$ is equal to $\hbar$ the correlation coefficient obtained is the limiting value given by the equation:

$$b_n = \frac{1}{3} T_b^{3/2} W \left[\frac{3}{T_b^{3/2}} e^{-\frac{2\pi k_B}{T_b^{3/2}}} \right]. \tag{15}$$

In this case the rate of the neuron correlation coefficient b w.r.t the brain temperature T_b

$$\frac{\mathrm{d}b_b}{\mathrm{d}T_b} = \frac{2\pi k_B W(\Phi') + T_b^{3/2} W^2(\Phi')}{2T_b(1 + W(\Phi'))} \tag{16}$$

Where $\Phi' = e^{-\frac{2\pi k_B}{T_b^{3/2}}}$. Using Eq. (17) and a brain temperature $T_b = 36.9°\mathrm{C}$ [16] and converting it to an absolute temperature we find the following numerical value of the correlation coefficient to be $b = 0.999450$. Using Eq. (14) and a temperature range $T_b = 36.9, 37.5, 38.2°\mathrm{C}$ we obtain that the rate of change of the neuron correlation coefficient w.r. t the temperature becomes:

Absolute Brain Temperature T_b [K]	$\mathrm{d}b_n/\mathrm{d}T_b$ [K^{-1}]
310.06	2.65389×10^{-6}
310.66	2.64103×10^{-6}
311.36	2.6263×10^{-6}

In the table below we give correlation coefficient values for a range of brain temperatures (Fig. 1):

Absolute Brain Temperature T_b [K]	Neuron Correlation Coefficient b_n
308.25	0.999446
308.65	0.999447
309.05	0.999448
309.15	0.999449
309.75	0.999450
310.05	0.999451
310.85	0.999453

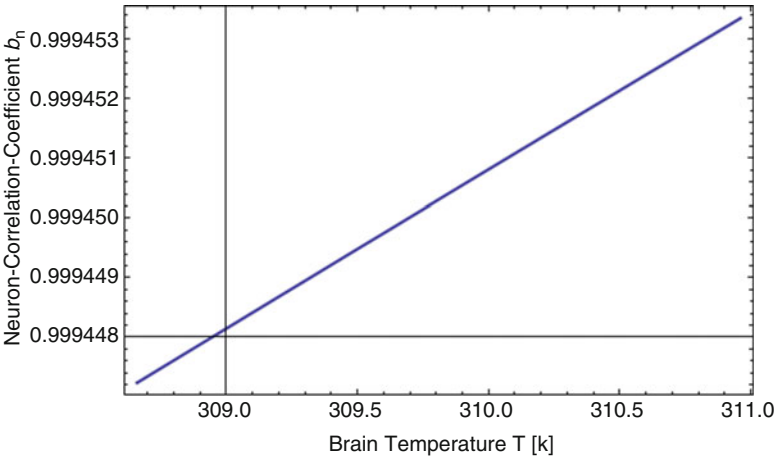

Fig. 1 Plot of brain correlation coefficient as a function of brain temperature in the case where the brain quantum of action is equal to the Planck's $\hbar$

4 Conclusions

Network estimation through functional connectivity between network nodes is believed to have profound clinical implications, and the fusion of network analytical and statistical methods may revolutionize the understanding of brain function [17]. Multiple network metrics have been used in an effort to understand network structure mainly describing complex topologies, multiple variables of interest (disease status, age, race) and local network features (nodal clustering, nodal centrality, etc.). The application of statistical and network science tools for analyzing brain network data leads to the development of complexity theory. Network estimation proceeds through linear association measures (Sean et al., 2013) that include correlation and coherence, nonlinear measures including mutual information, generalized synchronization, functional segregation and integration is estimated by measures such as clustering coefficient and transitivity, characteristic path length, global efficiency, etc. However, the development of informative descriptive metrics and the resolution of computational issues due to dimensionality remain important problems requiring statistical input in network analysis, and propagation of error from network estimation may lead to divergence between the brain activity and statistical interpretation.

At the same time, computational neuroscience converges to an imitation of theoretical physics, in an effort to discover mathematical laws that capture the fundamental laws that govern the operation of neural systems [18] and to understand the brain to a similar degree as we now understand the material world. An ultimate goal of such an approach is to quantitatively predict complicated cognitive behaviours and provide insights into cognitive and affective, psychiatric and neurological disorders, network changes that may relate to early detection

of pathophysiology. However, higher level brain functions involve processing of information by a variety of specialized brain areas. Simple elements and complex architectures used to investigate the richness of the brain, results in an incredible complexity in the modeling brain neuronal hardware due to neuronal phenomena such as neuromodulation, synaptic adaptation on various spatial and time scales, diversity of neuron types, and the role of glia cells, etc. (ibid, 2008). Therefore the existing challenges may be either resolved or avoided by the use of simple methods. The highly complex structure of the brain involves many distinct neurotransmitters and receptors, cell types, and a variety of wiring patterns [19], based on small-scale dynamics that may not be successfully mathematically modeled and should also not be ignored. Ideally, one would look for one variable, a control parameter that would govern the macroscopic phase of the system [20]. This seems to be compatible and in parallel with the anatomical and functional brain approach that large numbers of neurons collectively interact to produce emergent properties like cognition and consciousness. Our work is an effort to describe the dynamics of the human brain through a single parameter such as the entropy of the brain or the correlation parameter that encodes information about the brain as a dynamical system. The clinical significance of the this theoretical approach would require an elaborate experimentation that would classify the different numerical values of these parameters with brain states and health conditions of human subjects. However, the approach in this report indicates a simple unifying binding principle of all scales of events in the brain that we conjecture that maybe a link between neurobiology and cognition.

References

1. Riddle, D.R., ed. 2007. *Brain Aging: Models, Methods, and Mechanisms. Chapter 12*. Boca Raton, FL: CRC Press/Taylor & Francis.
2. O'Sullivan, M., D.K. Jones, P.E. Summers, R.G. Morris, S.C. Williams, and H.S. Markus. 2001. Evidence for Cortical "Disconnection" as a Mechanism of Age-Related Cognitive Decline. *Neurology* 57: 632–638.
3. Park, D.C., and P. Reuter-Lorenz. 2009. The Adaptive Brain: Aging and Neurocognitive Scaffolding. *Annual Review of Psychology* 60: 173–196.
4. Raz, N., U. Lindenberger, K.M. Rodrigue, K.M. Kennedy, D. Head, et al. 2005. Regional Brain Changes in Aging Healthy Adults: General Trends, Individual Differences and Modifiers. *Cerebral Cortex* 15 (11): 1676–1689.
5. Head, D., R.L. Buckner, J.S. Shimony, L.E. Williams, and E. Akbudak. 2004. Differential Vulnerability of Anterior White Matter in Nondemented Aging with Minimal Acceleration in Dementia of the Alzheimer Type: Evidence from Diffusion Tensor Imaging. *Cerebral Cortex* 14 (4): 410–423.
6. Wen, W., and P. Sachdev. 2004. The Topography of White Matter Hyperintensities on Brain MRI in Healthy 60- to 64-Year-Old Individuals. *NeuroImage* 22 (1): 144–154.
7. Shadlen, M.N., and W.T. Newsome. 1998. The Variable Discharge of Cortical Neurons: Implications for Connectivity, Computation, and Information Coding. *The Journal of Neuroscience* 18: 3870–3896.

8. Tolhurst, D.J., J.A. Movshon, and A.F. Dean. 1983. The Statistical Reliability of Signals in Single Neurons in Cat and Monkey Visual Cortex. *Vision Research* 23: 775–785.
9. Averbeck, B.B., P.E. Latham, and A. Pouget. 2006. Neural Correlations, Population Coding and Computation. *Nature Reviews Neuroscience* 7: 358–366.
10. Sporns, O., G. Tononi, and R. Kötter. 2005. The Human Connectome: A Structural Description of the Human Brain. *PLoS Computational Biology* 1 (4): 1–42.
11. Prasad, P., Burkart, J., Joshi, S.H., Talia, M.N., Toga, W.A., and Thompson, P.M.. 2013 Multimodal Brain Image Anal. 8159, 129–137, 2013. Physics Letters A, 28 (19), 2013.
12. Saslaw, W.C. 1987. Gravitational Physics of Stellar and Galactic Systems. *Cambridge Monographs on Mathematical Physics*: 245–248.
13. Iqbal, N., M.S. Khan, and T. Masood. 2011. Entropy Changes in the Clustering of Galaxies in an Expanding Universe. *Natural Science* 3 (1): 65–68.
14. Shoshani, J., W.J. Kupsky, and G.H. Marchant. 2006. Elephant Brain. Part I: Gross Morphology Functions, Comparative Anatomy, and Evolution. *Brain Research Bulletin* 70: 124–157.
15. Cosgrove, K.P., M.M. Calrolyne, and J.K. Staley. 2007. Evolving Knowledge of Sex Differences in Brain Structure, Function, and Chemistry. *Biological Psychiatry* 62 (8): 847–855.
16. Wang, H., B. Wang, K.P. Normoyle, K. Jackson, K. Spitler, F.M. Sharrock, M.C. Miller, C. Best, D. Llano, and R. Du. 2014. Brain Temperature and Its Fundamental Properties: A Review for Clinical Neuroscientists. *Frontiers in Neuroscience* 8: 307.
17. Simpson, S.L., F. DuBois, F. Bowman, and P.J. Laurienti. 2013. Analyzing Complex Functional Brain Networks: Fusing Statistics and Network Science to Understand the Brain. *Statistics Surveys* 7: 1–36.
18. Tsodyks, M. 2008. Computational Neuroscience Grand Challenges - A Humble Attempt at Future Forecast. *Frontiers in Neuroscience* 2 (1): 17–18.
19. Beggs, J. 2015. Can There Be a Physics of the Brain? *Physical Review Letters* 114: 220001.
20. Beggs, M.J. 2008. The Criticality Hypothesis: How Local Cortical Networks Might Optimize Information Processing. *Philosophical Transactions of the Royal Society A* 366: 329.
21. Brain Jan. 2014. 137 (1) 12–32; doi:10.1093/brain/awt162.
22. Brain Temperature and Its Fundamental Properties: A Review for Clinical Neuroscientists, Frontiers in Neuroscience 8: 307, 2014.
23. Haranas, I., and I. Gkigkitzis. 2013a. Bekestein Bound of Information Number N and Its Relation to Cosmological Parameters in a Universe with and without Cosmological Constant. *Modern Modern Physics Letters A* 62 (8): 1350077.
24. ———. 2013b. Number of Information and Its Relation to the Cosmological Constant Resulting from Landauer's Principle. *Astrophysics and Space Science* 348 (2): 553–557.
25. Oligodendrocytes, Astrocytes, and Microglial Cells, NG2+ Cells. Reference: Riddle DR, editor. *Brain Aging: Models, Methods, and Mechanisms*. Chapter 5, Boca Raton (FL): CRC Press/Taylor & Francis; 2007.
26. The role of the posterior cingulate cortex in cognition and disease, Robert Leech, David J. Sharp

Determination of Soft Tissue Breakpoint Based on Its Temperature Enhancement Pattern: In Vivo and In Vitro Experiments

C. Austerlitz, I. Gkigkitzis, A.L.S. Barros, J. Melo, I. Haranas, and D. Campos

Abstract The breakpoint of fresh commercial meats and in vivo mice has been assessed using tissue temperature enhancement pattern. A 1 cm length and 0.1 cm diameter gold rod was implanted in fresh chicken breast, beef, fish, and in vivo *Mus musculus* white mice and was insonated with ultrasound. The temperature enhancement of gold rods was measured with a needle type thermistor over a temperature range from 35 to 50 °C. From these results the breakpoints were determined by plotting the gold rod temperature versus ultrasound exposure duration using the interception point of two curves fitted by a linear regression equations of thermal response above and below 43 °C. The linear correlation coefficients for all fitted curves lie within 0.985 and 0.997. The breakpoints were found to be 42.1 ± 1.1, 42.3 ± 0.9, 42.6 ± 0.8 and 43.5 ± 0.6 for fish, chicken breast, beef and in vivo *Mus musculus* white mice, respectively. The interception of the thermal response curves above and below 43 °C. Soft tissue temperature enhancement pattern has demonstrated to be a fast method to determine breakpoint. It denotes the temperature where cells may start to be destroyed and may be used to spot the startup point in dosimetry of hyperthermia cancer therapy.

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Keywords Breakpoint • Fresh meat • *Mus musculus* white mice • Temperature enhancement pattern • Ultrasound • Gold rods

1 Introduction

Numerous in vitro studies shown that the rate of cell killing during exposure to heat is exponential and dependent on the temperature and exposure lengths [1, 2]. The temperature at which the inactivation of proteins and enzymes starts has been derived from such in vitro studies using cell survival curve and Arrhenius plot [3] which has a typically biphasic slope or a breakpoint. A method to treat cancer tumor with gold seeds and ultrasound has been described elsewhere [4–7]. In vitro [4] and in vivo experiments [5] as well as analytical calculations [6, 7] have been performed and developed to test the feasibility and have provided subsidies to such methodology.

Based on such a method, a single 1-cm high and 0.1-cm diameter 24 K gold seed implanted in a cancer tumor exposed to ultrasound may be enough to destroy a 1-cm diameter tumor. It may happen only if the temperature at the boundary between normal tissue and tumor is enough to start the breakdown of connective tissues and length of exposure(s) be sufficient for a complete tumor regression. Therefore, the application of this method may requires high ablative hyperthermia in the tissue close to the gold seed and the knowledge of the breakpoint where ablative hyperthermia occurs in the boundary of the tumor to be treated.

The determination of the breakpoint derived from cell survival curve is laborious task and may not be determined in practical cancer treatment with hyperthermia. This work has had the aim to verify the feasibility to determine the breakpoint of fresh commercial meat and in vivo *Mus musculus* white mice using tissue temperature enhancement pattern.

2 Material and Methods

2.1 Mus musculus *White Mice*

Two female *Mus musculus* white mice (5 week-old) were acquired from the Department of Antibiotics of the Federal University of Pernambuco, Brazil, and maintained and bred under conventional laboratory conditions at the Bioassay Laboratory for Drug Research from the same university. Before the implant, the mice were anesthetized using Xylazine and Ketamine (5 mg/kg and 25 mg/kg body wt, respectively) [8], their hair around the medial dorsal region was shaved and a 1-cm length and 0.1-cm diameter 24 k gold rod was implanted in the medial dorsal region of the mice. Ultrasound gel was spread on the implanted area and insonated using a using a 4 cm diameter transducer connected to an ultrasound equipment [9] under a nominal frequency of 1 MHz and power up to 75 W. The

temperature enhancement of gold rods was measured with a needle type thermistor connected to a FLUKE thermometer over a temperature range from 35 to 50 °C. To avoid any recovering from the anesthetic procedure during the experiments, in vivo measurements were performed with the maximum intensity allowed by the GS8.2E equipment. In vitro measurements were performed with about half of the maximum intensity.

2.2 Fresh Meats

Pads of about 4 cm diameter and 1 cm thick of fresh; chicken breast, beef and fish were used in this work to evaluate the breakpoints. A 1-cm length and 0.1-cm diameter 24 k gold rod was implanted in the center and about 0.3 cm depth of these pads The pads were imbibed with ultrasound gel, placed on the surface of a 4 cm diameter, one at a time, and exposed to the ultrasound beam described above. The temperature enhancement of gold rods was measured with a needle type thermistor over a temperature range from 35 to 50 °C. It was assumed that the temperature of tissue around the gold rod is the same of the gold rod.

The breakpoints for in vivo and in vitro measurements were determined by plotting the gold rod temperature versus ultrasound exposure duration using the interception point of two curves fitted by linear regression equations of thermal response above and below 43 °C. The linear regression correlation coefficients were calculated by means of the SigmaPlot computer program and the measurements were repeated three times for each sample and the mean value of the breakpoints was used to express the results presented in this work.

3 Results and Discussion

Examples of plots of gold seeds temperature enhancement implanted in tissues and exposed to ultrasound energy in fresh beef, fish, chicken breast, and in vivo *Mus musculus* mice are shown in Figs. 1–4, respectively. As may be seen from these figures, independently from in vivo or in vitro measurements, the curves present a biphasic slope of thermal response or a breakpoint above and below 43 °C. For in vitro experiments, the breakpoints lies within 42.1 ± 1.1 (fish) and 42.6 ± 0.8 (beef). In vivo experiment with *Mus musculus* white mice the breakpoint was found to be 43.3 ± 0.6. Because in the living normal tissue the vessels dilate when physiological or artificially heated, which causes increase in blood flow through the region and decreases its temperature [10], as expected, the breakpoint for in vivo measurement is higher than those for in vitro measurements. However, these breakpoint values lie within the statistically uncertainty of the measurements performed in this work. Nevertheless, they agree with the standardized value (e.g. equivalent minutes at 43 °C for comparison of thermal treatments [2]. Although the change in slope below and above the breakpoint at 43 °C has been suggested to be a result of thermotolerance during heating [11], many studies on protein

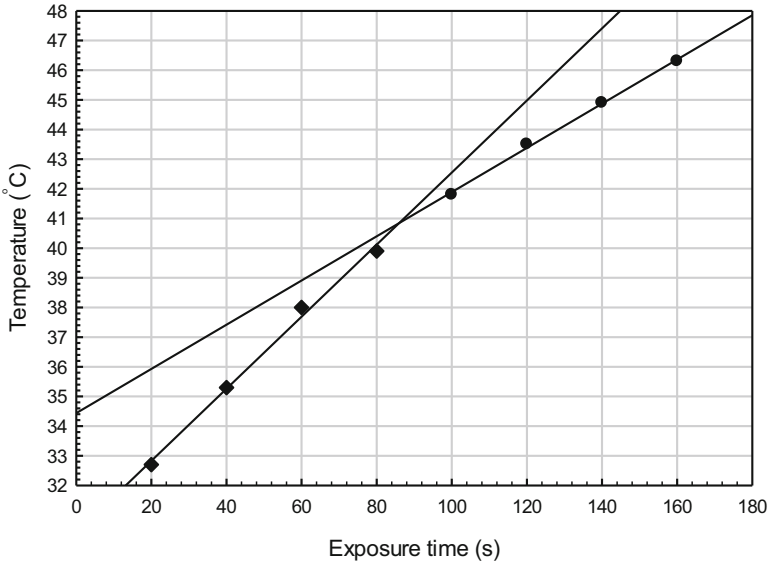

Fig. 1 Temperature increase patter around a gold rod incrusted in beef during ultrasound expose

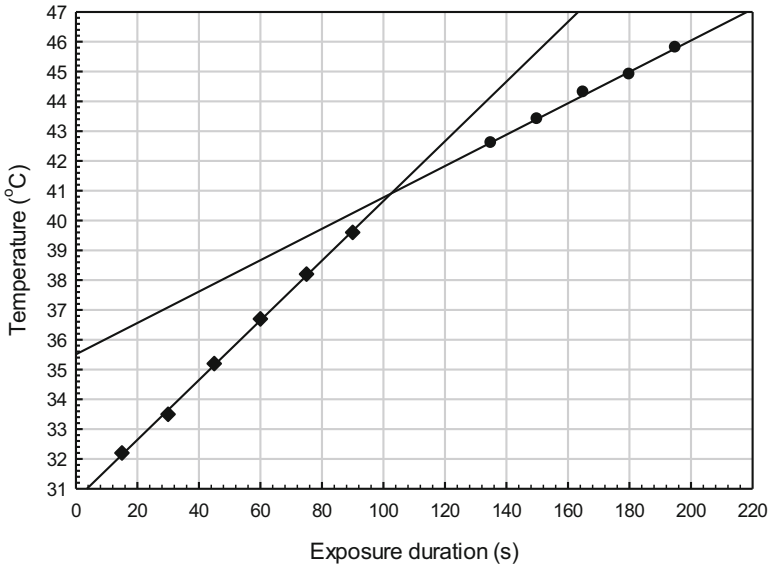

Fig. 2 Temperature increase patter around a gold rod incrusted in fish during ultrasound expose

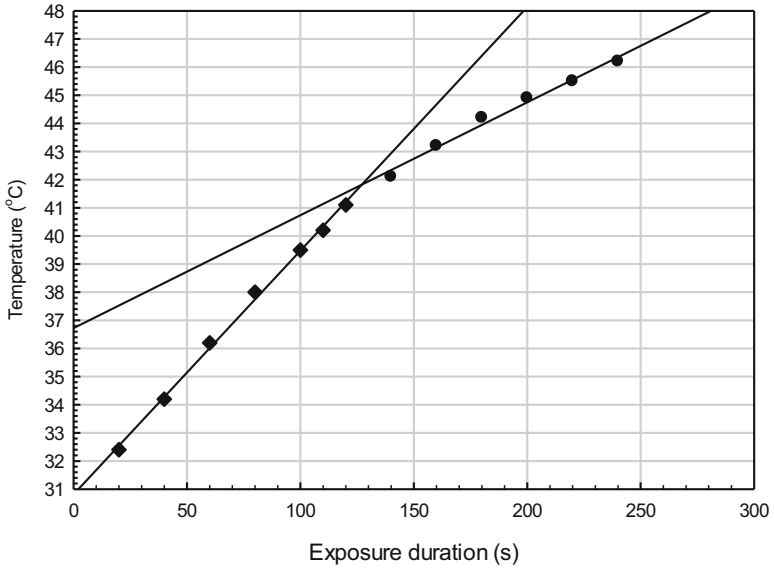

Fig. 3 Temperature increase patter around a gold rod incrusted in fresh breast chicken during ultrasound expose

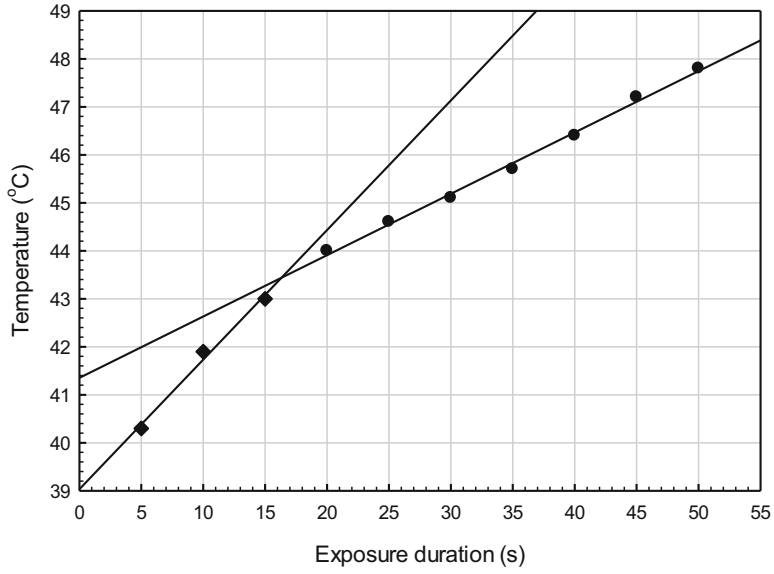

Fig. 4 Temperature increase patter around a gold rod implanted in *Mus musculus* white mice during ultrasound expose

denaturation have shown that alterations to lipid mainly occur below ∼45 °C while major protein denaturation starts from 40 to 45 °C [12]. Also, major cell injury occurs at temperatures above 45 °C and protein denaturation has been proposed to play a more important role in defining cell injury than the change in lipids. Additionally, temperature break points below and above which the activation energy changes significantly are mainly located at ∼43 and ∼50 °C [12].

The linear correlation coefficients for all fitted curves below and above the breakpoint and the breakpoint temperatures for all the measurements are shown in Table 1. As can be seen in this table, the linear correlation coefficients lie within 0.985 and 0.997. Such correlation coefficient represents the percent of the data that is closest to the line of best fit, and the 0.985 value means that 98.5% of the total variation in tissue temperature may be explained by the relation between tissue temperature and ultrasound exposure duration. Therefore, for both in vitro and in vivo experiments there is a quite strong correlation between tissue temperature and ultrasound exposure duration. A slightly less steep slope below the break point that may exist for human cells compared with rodent cells using Arrhenius plot has been reported by Mark Dewhirst et al. [13]. The slope below the break point determined in this work may not be compared. The slices of meat had different masses and physical dimensions. This may be seen in the x-axis of Figs. 1–4, wire the exposure lengths ranged from 55 s (*Mus musculus* white mice, Fig. 4) to 300 s (chicken breast, Fig. 3). However, the slopes from in vitro and in vivo measurements determined in this work are virtually identical and in agreement with those derived from in vitro and in vivo studies using Arrhenius plots derived from in vivo studies are virtually identical to those that are derived from in vitro studies reported by Field and Morris [14]. The ratio between the slopes below and above the breakpoint lies within 1.94 ± 0.28 and 2.15 ± 0.05 and the percent difference between in vivo (2.15 ± 0.05) and the mean value of in vitro measurements (2.07 ± 0.44) is in order of 4%. As mentioned above, in living normal tissue the vessels dilate when heated and increase in blood flow decreasing its temperature so that the it may be expected that the ratio between the slopes below and above the breakpoint lies in vivo be higher than in vitro. However, this deviation in these ratios lies within the uncertainty of the measurements and such prediction could not be confirmed.

The measurements performed in this work have shown strong correlation between gold seeds temperature enhancement and ultrasound exposure duration using just first order linear regression. Therefore, non-invasive tissue temperature monitoring using magnetic resonance [15], diffuse optical spectroscopic imaging [16], or invasive temperature measurement by means of biomedical fiber optic thermometers [17] may be tested and used as a guidance to determine the breakpoint of tumor and normal tissue (thresholds for tissue damage) in cancer hyperthermia treatments.

Hyperthermia has been be defined as raising the temperature of a part of or the whole body above normal for a defined period of time. The extent of temperature elevation associated with hyperthermia is on the order of a few degrees (41–45 °C) and the extent of thermal damage to tissue depends on tissue sensitivity, temperature and exposure time [18]. A cumulative equivalent minutes at 43 °C (CEM 43 °C) has been proposed as a model to calculate a thermal isoeffect dose [11]. Even

Table 1 Linear correlation coefficients (r^2), slope of curves below and above the breakpoint (b), ratio of slopes below and above the breakpoint and, the breakpoint values of biphasic slopes induced around the implanted gold rods

Medium	r^2 below the breakpoint	r^2 above the breakpoint	b below the breakpoint	b above the breakpoint	$\frac{b \text{ below the breakpoint}}{b \text{ above the breakpoint}}$	Breakpoint (°C)
Mice	0.989	0.997	0.320	0.165	2.15 ± 0.05	43.5 ± 0.6
Chicken	0.997	0.985	0.087	0.040	2.25 ± 0.14	42.3 ± 0.9
Beef	0.991	0.998	0.108	0.075	1.94 ± 0.28	42.6 ± 0.8
Fish	0.995	0.997	0.103	0.057	2.02 ± 0.31	42.1 ± 1.1
[a]Mean & standard deviation			0.103 ± 0.011	0.057 ± 0.018	2.07 ± 0.44	42.3 ± 1.6

[a]In vitro measurements

though, thermal damage to tissue depends on tissue sensitivity and above and below the breakpoint a change of 1 °C is equivalent to a change in heating time by a factor of two and six, respectively [14], the CEM 43 °C has been extensively used as a thresholds of thermal damage [19–23]. Therefore, besides that these relationships provide a means of monitoring the temperature of a hyperthermia during a treatment, the tissue temperature enhancement recorded may be used to determine the breakpoint and the quantity the CEM 43 °C be replaced by a "$CEM_{breakpoint}$" so that accuracy may be improved in hyperthermia dosimetry.

4 Conclusions

Temperature enhancement pattern has demonstrated to be a fast method to determine breakpoint in soft tissues. It denotes the temperature where cells may start to be destroyed and may be used to spot the startup point in dosimetry of hyperthermia cancer therapy.

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References

1. Dewey, W.C. 2009. Arrhenius Relationships from the Molecule and Cell to the Clinic. *International Journal of Hyperthermia* 25 (1): 3–20. doi:10.1080/02656730902747919.
2. Dewhirst, M., B. Vigilanti, M. Lora-Michiels, P. Hoopes, and M. Hanson. 2003. Thermal Dose Requirements for Tissue Effect: Experimental and Clinical Data. *Proceedings of SPIE The International Society for Optical Engineering* 37. doi:10.14440/jbm.2015.54.A.
3. Kufe, D.W., R.E. Pollock, R.R. Weichselbaum, R.C. Bast Jr., T.S. Gansler, J.F. Holland, and FEI. 2003. In *Cancer Medicine*, ed. H.-F.C. Medicine, 6th ed. Hamilton, ON: BC Decker.
4. Austerlitz, C., I. Gkigkitzis, D. Campos, and I. Haranas. 2014. An Experimental Study on the Heat Enhancement and Bio-Heat Transfer Using Gold Macro Rod and Ultrasound: A Potential Alternative to Kill Cancer Cells. *Physics International* 5 (2): 132–135. doi:10.3844/pisp.2014.132.135.
5. Austerlitz, C., A. Barros, I. Gkigkitzis, D. Campos, T. Silva, S. Nascimento, and I. Haranas. 2015. On the Use of Gold Macro-Rods and Ultrasound as a Hyperthermia Cancer Treatment: Experimental Results on Erlich Tumor in *Mus musculus* Mice. In *Patents Eb Ser Top Anti-Cancer Res*., vol. 4. Benthan Science Publishers. doi:10.2174/9781681080765115040009.
6. Gkigkitzis, I., I. Haranas, and C. Austerlitz. 2015. Analytic Considerations and Axiomatic Approaches to the Concept Cell Death and Cell Survival Functions in Biology and Cancer Treatment. *Advances in Experimental Medicine and Biology* 822: 85–105. doi:10.1007/978-3-319-08927-0_11.
7. Gkigkitzis, I., C. Austerlitz, I. Haranas, and D. Campos. 2015. The Effect of the Shape and Size of Gold Seeds Irradiated with Ultrasound on the Bio-Heat Transfer in Tissue. *Advances in Experimental Medicine and Biology* 820: 103–124. doi:10.1007/978-3-319-09012-2_8.

8. Welberg, L.A., B. Kinkead, K. Thrivikraman, M.J. Huerkamp, C.B. Nemeroff, and P.M. Plotsky. 2006. Ketamine-Xylazine-Acepromazine Anesthesia and Postoperative Recovery in Rats. *Journal of the American Association for Laboratory Animal Science* 45 (2): 13–20. http://www.ncbi.nlm.nih.gov/pubmed/16542037.
9. 9 Ultrasonic Cavitation Fast Slimming Equipment Mod. GS8.2E, Guangzhou Cosmetology Equipment Limited Company, Ltd, Guangzhou/Guangdong, Guizhou, China.
10. Chicheł, A., J. Skowronek, M. Kubaszewska, and M. Kanikowski. 2007. Hyperthermia – Description of a Method and a Review of Clinical Applications. *Reports of Practical Oncology & Radiotherapy* 12 (5): 267–275. doi:10.1016/S1507-1367(10)60065-X.
11. Sapareto, S.A., and W.C. Dewey. 1984. Thermal Dose Determination in Cancer Therapy. *International Journal of Radiation Oncology, Biology, Physics* 10 (6): 787–800. doi:10.1016/0360-3016(84)90379-1.
12. He, X. 2011. Thermostability of Biological Systems: Fundamentals, Challenges, and Quantification. *Open Biomedical Engineering Journal* 5: 47–73.
13. Dewhirst, M.W., E. Jones, T. Samulski, S. Vujaskovic, C. Li, and L. Prosnitz. 2004. Hiperthermia. In Cancer Medicine, eds. Kufe DW, Polock, RE, Weichselbaum RR, Bast Jr RC, Gansler TS, Holland JF, Frei E, 6 627–636. New York: Dekker.
14. Field, S.B., and C.C. Morris. 1983. The Relationship Between Heating Time and Temperature: Its Relevance to Clinical Hyperthermia. *Radiotherapy and Oncology* 1 (2): 179–186. doi:10.1016/S0167-8140(83)80020-6.
15. Hirsch, L.R., R.J. Stafford, J.A. Bankson, et al. 2003. Nanoshell-Mediated Near-Infrared Thermal Therapy of Tumors under Magnetic Resonance Guidance. *Proceedings of the National Academy of Sciences of the United States of America* 100 (23): 13549–13554. doi:10.1073/pnas.2232479100.
16. Chung, S.H., R. Mehta, B.J. Tromberg, and A.G. Yodh. 2011. Non-Invasive Measurement of Deep Tissue Temperature Changes Caused by Apoptosis during Breast Cancer Neoadjuvant Chemotherapy: A Case Study. *Journal of Innovative Optical Health Sciences* 4 (4): 361–372. doi:10.1142/S1793545811001708.
17. Crouzier, D., L. Selek, B.A. Martz, V. Dabouis, R. Arnaud, and J.C. Debouzy. 2012. Risk Assessment of Electromagnetic Fields Exposure with Metallic Orthopedic Implants: A Cadaveric Study. *Orthopaedics & Traumatology, Surgery & Research* 98 (1): 90–96. doi:10.1016/j.otsr.2011.08.012.
18. van Rhoon, Gerard C., Theodoros Samaras, Pavel S. Yarmolenko, Mark W. Dewhirst, Esra Neufeld, and N.K. Kuster. 2013. CEM43 °C Thermal Dose Thresholds: A Potential Guide for Magnetic Resonance Radiofrequency Exposure Levels? *European Radiology* 23 (8): 2215–2227. doi:10.1007/s00330-013-2825-y.
19. TS, A. 2015. Thresholds of thermal damage. In: World Heath Organizations 26-28 May 2015, ed. Thermal Dose Thresholds for MRI University of Thessaloniki. Istanbul.
20. Murbach, M., E. Neufeld, K.P. Pruessmann, N. Kuster. 2012. Safe MR Scan Times Based on CEM43 Tissue Damage Thresholds, Using Electromagnetic and Thermal Simulations with Anatomically Correct Human Models and Considering Local Thermoregulation. *ISMRM 20th Annual Meeting & Exhibition: Adapting MR in a Changing World, Melbourne Australia*, 31, 313.
21. Thrall, D., and G. Rosner. 2000. Using Units of CEM 43 C T90, Local Hyperthermia Thermal Dose Can be Delivered as Prescribed. *International Journal of Hyperthermia* 16 (5): 415–428. doi:10.1080/026567300416712.
22. Field, S.B.R.G. 1990. Thermal Dose. In *An Introduction to the Practical Aspects of Clinical Hyperthermia*, ed. S.B. Field and J.W. Hand. London: Taylor and Francis.
23. Weininger, S., J. Pfefer, and I. Chang. 2005. Factors to Consider in a Risk Analysis for Safe Surface Temperature. 2005 IEEE Symposium on Product Safety Engineering, vol. 2005, 83–91. doi:10.1109/PSES.2005.1529527.

Huntington Disease: Genetics, Prevention, and Therapy Approaches

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Abstract Huntington's chorea or Huntington disease (HD) is a late-onset autosomal dominant neurodegenerative disorder caused by a trinucleotide repeat expansion. The multidisciplinary study of HD has been the focus of an international collaborating effort of basic and applied research for several decades. HD was the first human genetic disease mapped using linkage analysis of DNA polymorphisms and became a paradigm for scores of genes mapped in the same manner. Presymptomatic and prenatal testing have been available for HD families in the last 30 years, following genetic counseling and careful bioethical guidelines. Nevertheless, with the cure for the disease still elusive the uptake of predictive testing by at risk individuals is low. Current treatment of HD is mostly symptomatic, but ongoing observational studies, clinical trials and development of new gene silencing technologies have provided hopeful results.

Keywords Hereditary chorea • Late-onset disorder • Neurodegenerative disease • Prenatal testing • presymptomatic testing

1 Introduction

Huntington's chorea or Huntington disease (HD) is a late-onset hereditary neurodegenerative disorder inherited in an autosomal dominant fashion. It was first described as a clinical entity in 1872 by George Huntington (1850–1916) immediately after he received his medical training [1, 2]. Reports on HD were published for about a century, until 50 years ago Myrianthopoulos wrote a very comprehensive review on HD in which he stated: "It is somewhat disappointing that this review has to end on such a pessimistic note. The prospects for resolving the prognostic (and

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Table 1 A short history of Huntington disease

1872	First detailed clinical description by G. Huntington
1966	First comprehensive review by N. Myrianthopoulos and call for interdisciplinary collaboration
1967	All HD researchers gathered at the 2nd International Congress of Neurogenetics at Montreal (host A. Barbeau)—Establishment of International Research Group on HD
1983	A polymorphic DNA marker genetically linked to HD on 4p16.3 by J. Gusella et al.
1988	Predictive DNA testing in Canada, UK and USA
1993	The novel huntingtin (*HTT*) gene found by the HD Collaborative Research Group (59 coauthors)
1993–2002	Genetic and protein studies by several groups
1993–2016	Clinical and imaging assessment of patients and at risk individuals (Huntington Study Group)
2008	First approved drug after clinical trials (tetrabenazine for hypekinesis, USA)

See text for corresponding references

hence, genetic) and therapeutic problems of Huntington's chorea do not appear to be very good and they are not likely to get better unless efforts become concerted and systematic and include integrated team work in the field, the clinic, and the laboratory" [3].

That statement prompted the beginning of a remarkable international collaboration (see Table 1 for a brief summary of the HD history). In 1967 all clinicians and researchers involved with HD gathered at the 2nd International Congress of Neurogenetics at Montreal invited by Barbeau and established the International Research Group on HD [4]. The purpose of this International Group was to meet every couple of years and discuss all issues regarding HD and promoting international and interdisciplinary collaboration in order to solve the many neurological, genetic, psychological and social issues of this devastating disease. The International Research Group on HD kept on these meetings on a regular basis, until about 15 years later the gene responsible for HD was mapped on a specific locus of chromosome 4 (4p16.3), with the use of linkage analysis with a polymorphic DNA marker [5]. HD became the first human genetic disease that was mapped using that new (at the time) molecular technology and became a paradigm for scores of genes mapped in the same manner, thereafter and for two decades until the Human Genome project was completed.

It took a decade to identify and sequence the gene for HD through several attempts to finemap the HD locus by linkage analysis results of particular families [6, 7]. In the mean time, both presymptomatic and prenatal testing became available by linkage analysis at an accuracy rate of 96–99%, based on the closest informative polymorphic marker [8].

Finally, the HD-responsible gene was identified in 1993 by a collaborative effort of six groups [9]. The gene contains a polymorphic CAG triplet repeat, which is translated in a variable size polyglutamine sequence in the encoded protein named

huntingtin [10]. HD is caused by expanded CAG repeats, which results in a protein with an abnormally long polyglutamine sequence. Despite many efforts of basic and applied research over the years, there is still no cure or definite treatment that may slow or reverse the progression of HD.

2 Clinical Characteristics of Huntington Disease

HD is an autosomal dominant neurogenetic disease with estimated prevalence of 1 in 10,000 individuals of European origin and much rarer in individuals of Asian or African origin [11]. The disease is characterized by a late onset in middle age (35–45 years), but 10% of cases have an onset in the first two decades of life, representing the juvenile form of HD [11].

Clinical diagnosis of HD is based on the observation of involuntary (choreiform) movements and insidious onset of mood disturbance in individuals with family history of the disease [12]. The symptoms include motor, psychological and cognitive abnormalities that aggravate progressively. Pathological findings include progressive degeneration of basal ganglia mainly but also of other brain regions, such as the substantia nigra, cerebral cortex, hippocampus, lateral tuberal nuclei of the hypothalamus and parts of the thalamus [10–12].

Movement symptoms include chorea, dystonia, bradykinesia, rigidity and loss of postural reflexes. Cognitive symptoms include impulsivity, perceptual distortions, lack of insight, distractability, difficulties in learning as well as in planning and organizing thoughts, activities and communication. Psychiatric symptoms include sleep disturbance, depression, anxiety, irritability, apathy, obsessive-compulsive disorder, hypersexuality and psychosis [13]. Some symptoms may fluctuate in severity during the progression of HD, while others may worsen in a steady manner. In the earliest stages of HD, prodromal cognitive and behavioral changes are observed in 40% of individuals up to 15 years prior to the development of motor symptoms [10].

The age of onset ranges considerably, with HD cases recorded as early as 3 years on the one extreme and 75 years at the other. On average, the age of greatest risk for developing the disease spans between 35 and 40 years, while that risk is considerably smaller under 20 and over 60 years [10–12]. The mean duration of HD is about 15 years, with a little longer time period in female patients. The onset of symptoms and their severity is unique to each person with HD, but a typical life with the disease may be delineated [14]. A typical person with HD learns about the disease at the average age of 14 years when her parent is diagnosed with HD and discusses or attempts suicide. At the average age of 16 years she becomes aware of her own personal risk of 50% to have inherited the neurodegenerative disease. At the average age of 25 years (usually already married) she undertakes predictive DNA testing and learns that she has inherited the mutant HD gene. Two years later she may have her first child born, after a prenatal test result that is negative for HD. At the average age of 35 years she may be diagnosed with onset of cognitive and

motor signs of the disease. In the next 5 years she may be dysfunctional at work, progressively she may need supervision in her daily living activities, while she will experience the death of her affected parent. At the average age of 45 she may be placed in long-term care facility because she needs 24-h assistance. The typical person with HD dies in her early 50s [14].

Among the exceptional characteristics of the disease is the fact that *de novo* HD-causing mutations are extremely rarely observed, homozygotes for HD mutant genes display the same phenotype as heterozygotes, and the phenomenon of "anticipation" [10–12, 14]. Observed in other neurogenetic disorders caused by trinucleotide repeat expansion, anticipation signifies the often observed earlier and more severe manifestation of disease in posterior generations.

3 Genetics of Huntington Disease

The autosomal dominant mode of inheritance of HD was noticed by Huntington in his 1872 article: "Unstable and whimsical as the disease may be in other respects, in this it is firm, it never skips a generation to manifest itself in another" [1]. Every child of a HD patient has 50% chance of inheriting and developing the disease. The fact that the mean time of HD onset is after 35 years of age suggests that most patients have already acquired offspring before the manifestation of the disease [14]. It follows that prevention of HD may be effectively accomplished only with predictive genetic testing.

Presymptomatic and prenatal testing for HD using restriction fragment length polymorphism (RFLP) analysis were initiated in Canada, UK and USA as early as 1987 [8, 15–18]. A world-wide survey published in 1993, after the identification of the HD mutant gene, reported a total of 1479 presymptomatic tests using RFLP analysis in 19 countries [8]. According to that survey, UK, USA and Canada were the first three countries, ranked by total number of performed predictive tests. The first three countries that were ranked by number of tests performed per center were the Netherlands, Greece and Denmark; all of them had a national centralized facility respectively in Leiden, Athens and Copenhagen [8, 19, 20].

The mutation responsible for HD is an expanded trinucleotide CAG repeat in the first of 67 exons of the huntingtin gene (*HTT*) that spans 200 kb on chromosome 4 [9–11]. The CAG repeat encodes a polyglutamine repeat in the huntingtin protein that may be up to a certain normal length without having deleterious effects. The length of the trinucleotide repeat is polymorphic with 7–35 repeats found in normal individuals, 36–39 repeats observed in individuals with reduced penetrance and very late onset of HD, 40–59 repeats observed in patients with typical HD, and 60–95 repeats in patients with juvenile onset of HD [10, 11, 21]. The expanded trinucleotide repeat results in a toxic gain of function in the mutant huntingtin protein, that causes neurodegeneration and the characteristic HD symptoms [10, 11]. The length of the CAG repeat may be studied using polymerase chain reaction

(PCR) amplification, normal or long-range, in combination with gel electrophoretic analysis or capillary electrophoresis, or even direct DNA sequencing.

Huntingtin associates to cytoplasmic membranous structures such as the endoplasmic reticulum, Golgi and mitochondria through a normal cytoplasmic retention signal [10, 13]. During cellular stress huntingtin becomes double-phosphorylated targeting it to subregions of the nucleus. Increased nuclear aggregation of double-phosphorylated huntingtin may be avoided though its clearance by the proteasome and lysosome. In disease, the expanded polyglutamine region of the mutant huntingtin acquires a β-sheet conformation that results in its misfolding and aggregation both in the cytoplasm and the nucleus [22]. In addition, many studies performed in cell cultures and animal models have revealed the mutant RNA toxicity that is triggered by aberrant interactions between cellular proteins and the expanded CAG repeat and alters several cellular processes including aberrant alternative splicing, RNA interference, transcript nuclear transport and export, as well as nucleolar stress related apoptosis [22].

Since 1993 presymptomatic and prenatal testing have been possible by direct mutation detection with CAG repeat length analysis [21, 23]. In cases that an individual at risk for HD does not want her status to be identified but at the same time she desires to have children who will be free of the disease, there are two possible options. One option is in vitro fertilization of an embryo that was previously tested genetically and was found to be HD-free. Another possible option is to perform prenatally an exclusion testing using linkage analysis so that the chromosome 4 at 50% risk for HD to be identified as present or not in the fetus. This approach leads to termination of pregnancies at 50% risk corresponding possibly to unaffected fetuses that have not inherited the disease [24].

The international clinical and genetic community has established guidelines for the appropriate molecular genetic testing of HD [25, 26]. They include the standard approach that presymptomatic testing should be offered only to individuals over 18 years old after genetic counseling and psychological evaluation. Genetic testing of children under 18 years old may be justified only for confirmation of clinical diagnosis. Genetic testing to individuals with psychological imbalance and mental retardation should be refused. Psychological support should be provided to all tested individuals before and after genetic testing regardless of the result.

Despite its clinical and scientific availability, the uptake of predictive DNA testing by at risk individuals has been low. A European Union-funded Consortium of six countries (UK, Netherlands, Belgium, France, Italy and Greece) has gathered information on 451 presymptomatic and 305 prenatal tests of HD during a period of 6 years between 1993 and 1998 [27]. Presymptomatic testing was only asked from less than 15% of estimated people with a HD parent in all six countries, while prenatal testing was less than 10% of the predictive tests in the same period [23, 24, 27]. About two decades later, and with a cure for the disease still elusive, predictive testing was asked by 5% of 6000 people with a HD parent [28] and prenatal testing was asked by 17% of 354 people with HD gene [29]. Interestingly, about 40% of healthy carriers with HD-related expanded repeats have premanifest either neuropsychiatric symptoms or cognitive impairment [30].

4 Therapy of Huntington Disease

The aims of treatment for HD include reduction of severe symptoms, maximization of everyday function and optimization of life quality. A care team of various health professionals may assist the patient over time, since her symptoms progress through the course of the disease. A typical care team of health professionals at a Center of excellent treatment for HD should include primary experts (neurologist, psychiatrist or psychologist, neuropsychiatrist or neuropsychologist, geneticist, social worker, clinic coordinator, nurse), and ancillary experts (physical therapist, occupational therapist, speech therapist, dietician) [14]. Other possibly needed skills might include those of an experienced research coordinator and even a clergyman [14].

Current treatment of HD is mostly symptomatic and focuses in decreasing dystonia, dysarthria, swallowing difficulty, incontinence as well as psychological problems and irritability. Therapy for chorea and co-morbid psychiatric symptoms (psychosis or bipolar disorder, episodic aggression and agitation not managed by behavioral interventions) may be better treated by neuroleptic medications. Some patients need increasing doses of anti-chorea medications over time, therefore annual re-evaluation of therapy is advised [14]. Some other patients may develop increasing dystonia and rigidity in parallel with HD progression, thus a decrease of anti-chorea medicines might be necessary.

In 2008 tetrabenazine became the first drug for HD drug that was approved in USA because double-blind placebo controlled TETRA-HD study showed significant reduction of chorea [31]. Its mechanism of action is depletion of dopamine release by presynaptic striatal neurons. There are some side effects though (Table 2) that patients and their caregivers should know about, before treatment begins [14].

Additional clinical trials for symptomatic therapies of HD include studies of latrepirdine, pridopidine, riluzole, phenylbutyrate, ethyl-EPA, minocycline, RP103 (delayed released cysteamine) and ACR-16 (32). In 2009 phase 2 trial of latrepirdine to treat cognitive symptoms in HD (DIMOND) established its safety and tolerability, and some improvement in cognition. Currently, a larger phase 3 study (HORIZON)

Table 2 Current treatment for symptoms of Huntington disease

Medication	Initial dose	Maximal dose	Side effects
Tetrabenazine	12.5 mg	50 mg/day	Depression, Akathisia, Worsening of voluntary motor control, Sedation
Haloperidol	0.5–1 mg	10–15 mg/day	Extrapyramidal syndrome (abnormal involuntary movements): akathisia, dystonia, bradykinesia, Sedation
Fluphenazine	1–2 mg	10 mg/day	Extrapyramidal syndrome, Sedation
Risperidone	0.5–1 mg	5–10 mg/day	Extrapyramidal syndrome at higher doses, Sedation
Olanzapine	1.25–2.5 mg	10–15 mg/day	Extrapyramidal syndrome, Sedation, Weight gain and metabolic syndrome

of safety and efficacy of latrepirdine co-directed by the Huntington Study Group and the EURO-HD Network [32]. Additional clinical trials sponsored by the HSG include trials for neuroprotection and disease-modifying studies to slow HD progression, such as the NIH-funded study of coenzyme Q10 [32].

5 Observational Studies for Huntington Disease

All previously mentioned clinical trials were based on a parallel course of observational studies of cohorts of HD patients in order to develop a clinical research platform that increases likelihood of later success. The aims was to select patients that are closer to core HD biology, to document as precisely as possible certain pharmacodynamic biomarkers and to associate biomarker readouts with clinically meaningful changes.

Since 1993 the Huntington Study Group that includes more than 500 clinical investigators from USA, Canada, Europe, Oceania, and South America has completed 22 clinical trials and several observational studies that have collected clinical information from >8000 patients in a natural history database. Some major observational studies include PHAROS long-term study of people at 50% risk for HD who chose not to know their gene status, PREDICT-HD that prospectively enrolls people who know their HD gene status and are not yet diagnosed with HD, and COHORT that enrolls people who are presymptomatic, people diagnosed with HD, their at-risk family members, and not at-risk control subjects (spouses and caregivers).

There has been considerable progress in development of new clinical assessment scales that are specific for HD, as well as of new sensitive imaging technologies. Assessment scales with proven utility include Q-Motor Score (speed of finger tapping in correlation with brain volume) and Cognitive Assessment Battery for HD (CAB-HD) [33, 34]. Best current imaging technologies that assess brain volume and brain connectivity changes in HD patients include Magnetic Resonance Imaging (MRI), Positron Emission Tomography (PET), High Resolution Functional Magnetic Resonance (HR-fMR) and Diffusion Tensor Imaging (DTI). MRI provides information about structural and functional organization of brain regions, while PET may detect changes in brain tissue [35]. HR-fMR may fine map cortex architecture and reveal structures in cortical laminar and subcortical regions [36]. DTI may assess mean longitudinal diffusivity changes in certain brain regions (e.g. neostriatum) in HD mutation carriers before disease onset [37, 38]. These advances in clinical assessment and imaging have improved identification of subtle phenotypic changes in individuals with presymptomatic and prodromal HD and correlation of these alterations to cortical atrophy. Nevertheless, there is still need for a low-cost and widely available, more comfortable and easier for patients, but at the same time high resolution and noninvasive analysis method for objectively and quantitatively studying the neurophysiology of HD. In this regard, Electroencephalography (EEG) may be proven to be useful and easier method, as compared to neuroimaging techniques [39].

6 Future Prospects

The HD-causing expansion of the CAG repeat results in a toxic gain of function in the abnormal huntingtin protein, that causes neurodegeneration and the characteristic signs of the disorder. It follows that a key therapeutic strategy would be to develop a treatment that decreases the amount of mutant huntingtin protein. In this end, recent approaches of personalized gene therapy for inherited diseases seem to be promising, including "allele-specific" genetic treatment and "gene silencing" techniques using antisense RNA molecules [40]. Allele-specific targeting of entire huntingtin gene mRNA or some of its regions (CAG repeat, junction sites or polymorphisms within transcript) may be used for oligonucleotide design, synthesis and in vitro testing for selectivity and effectiveness in lowering amount of produced huntingtin [40]. Gene silencing that involves viral delivery of small interfering RNA molecules (siRNA) into the nucleus may induce expression of pre-miRNA. RNA-induced silencing complex (RISC) is a multiprotein complex (ribonucleoprotein) that incorporates one strand of a single stranded RNA (ssRNA), such as a microRNA. RISC uses RNAs as templates to recognize complementary mRNA and interfere with its proper translation. Nevertheless, many little methodological details have to be addressed and be resolved before a possible future gene therapy becomes routinely available for HD.

There is a trend for personalized medicine that may shift emphasis from reaction to prevention of disease, and may favor selection of optimal therapy and reduce trial-and-error prescription. Optimal personalized treatment is expected to increase patient adherence to therapy, decrease adverse drug reactions, and improve quality of life. In addition, additional or alternative activities of drugs may be revealed during personalized treatment.

There are still challenges for HD clinical research. They mostly include identification of important disease-modifying parameters as well as development of validation criteria for clinical trials. The long hoped-for cure treatment for HD is still evasive but there are potential advances with some new drugs and new gene silencing methodologies in various phases of development.

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References

1. Huntington, G. 1872. On Chorea. *Medical Surgical Report* 26: 317–321.
2. Durbach, N., and M.R. Hayden. 1993. George Huntington: The Man Behind the Eponym. *Journal of Medical Genetics* 30 (5): 406–409.
3. Myrianthopoulos, N.C. 1966. Huntington's Chorea. *Journal of Medical Genetics* 3 (4): 298–314.
4. Wexler, A. 1996. *Mapping Fate: A Memoir of Family, Risk, and Genetic Research*. California: University of California Press.
5. Gusella, J.F., N.S. Wexler, P.M. Conneally, S.L. Naylor, M.A. Anderson, R.E. Tanzi, P.C. Watkins, K. Ottina, M.R. Wallace, A.Y. Sakaguchi, et al. 1983. A Polymorphic DNA Marker Genetically Linked to Huntington's Disease. *Nature* 306: 234–238.
6. MacDonald, M.E., S.V. Cheng, M. Zimmer, J.L. Hains, A.M. Poustka, B.A. Allitto, B. Smith, et al. 1989. Clustering of Multiallele DNA Markers Near the Huntington's Disease Gene. *The Journal of Clinical Investigation* 84: 1013–1016.
7. Snell, R.G., L.M. Thompson, D.A. Tagle, T.L. Holloway, G. Barnes, H.G. Harley, L.A. Sandkuijl, et al. 1992. A Recombination Event That Redefines the Huntington Disease Region. *American Journal of Human Genetics* 51: 357–362.
8. The World Federation of Neurology Research Group on Huntington's Disease. 1993. Presymptomatic Testing for Huntington's Disease: A World Wide Survey. *Journal of Medical Genetics* 30: 1020–1022.
9. The Huntington's Disease Collaborative Research Group. 1993. A Novel Gene (HTT) Containing a Trinucleotide Repeat That Is Expanded and Unstable on Huntington's Disease Chromosomes. *Cell* 72: 971–983.
10. Ross, C.A., E.H. Aylward, E.J. Wild, D.R. Langbehn, J.D. Long, J.H. Warner, R.I. Scahill, B.R. Leavitt, J.C. Stout, J.S. Paulsen, R. Reilmann, P.G. Unschuld, A. Wexler, R.L. Margolis, and S.J. Tabrizi. 2014. Huntington Disease: Natural History, Biomarkers and Prospects for Therapeutics. *Nature Review Neurology* 10: 204–216.
11. Harper, P.S., ed. 1996. *Huntington's Disease*. 2nd ed. London, UK: Saunders Publications.
12. Reilmann, R., B.R. Leavitt, and C.A. Ross. 2014. Diagnostic Criteria for Huntington's Disease Based on Natural History. *Movement Disorders* 29: 1335–1341.
13. Novak, M.J., and S.J. Tabrizi. 2010. Huntington's Disease. *British Medical Journal* 340: c3109.
14. Nance, M., J.S. Paulsen, A. Rosenblatt, and V. Wheelock. 2011. *A Physician's Guide to the Management of Huntington's Disease*. 3rd ed. USA: Huntington's Disease Society of America.
15. Quarrell, O.W., A.L. Meredith, A. Tyler, S. Youngman, M. Upadhyaya, and P.S. Harper. 1987. Exclusion Testing for Huntington's Disease in Pregnancy with a Closely Linked DNA Marker. *Lancet* 1 (8545): 1281–1283.
16. Hayden, M.R., J. Hewitt, J.J. Kastelein, S. Langlois, R.D. Wilson, S. Fox, C. Hilbert, and M. Bloch. 1987. First-Trimester Prenatal Diagnosis for Huntington's Disease with DNA Probes. *Lancet* 1 (8545): 1284–1285.
17. Farrer, L.A., R.H. Myers, L.A. Cupples, and P.M. Conneally. 1988. Considerations in Using Linkage Analysis as a Presymptomatic Test for Huntington's Disease. *Journal of Medical Genetics* 25: 577–588.
18. Harper, P.S., M.J. Morris, and A. Tyler. 1990. Genetic Testing for Huntington's Disease. *British Medical Journal* 300: 1089–1090.
19. Yapijakis C. Molecular Genetic Study of Huntington's Chorea and Myotonic Dystrophy in Greek Families. Doctorate Dissertation Thesis, Department of Biology, University of Athens, 1993.

20. Yapijakis, C., F. Laccone, and S.A. Sorensen. 2002. Predictive and Prenatal Testing for Huntington's Disease in Greece, Germany, Austria, Switzerland and Denmark. In *Prenatal Testing for Late Onset Neurogenetic Diseases*, ed. G. Evers-Kiebooms, M. Zoeteweij, and P.S. Harper. Oxford, UK: BIOS Scientific Publishers.
21. Yapijakis, C., D. Vassilopoulos, M. Tzagournisakis, T. Maris, C. Fesdjian, C. Papageorgiou, and A. Plaitakis. 1995. Linkage Disequilibrium Between the Expanded (CAG)n Repeat and an Allele of the Adjacent (CCG)n Repeat in Huntington's Disease Patients of Greek Origin. *European Journal of Human Genetics* 3: 228–234.
22. Fiszer, A., and W.J. Krzyzosiak. 2013. RNA Toxicity in Polyglutamine Disorders: Concepts, Models, and Progress of Research. *Journal of Molecular Medicine* 91: 683–691.
23. Evers-Kiebooms, G., K. Nys, P. Harper, M. Zoeteweij, A. Durr, G. Jacopini, C. Yapijakis, and S. Simpson. 2002. Predictive DNA Testing for Huntington's Disease and Reproductive Decision Making: A European Collaborative Study. *European Journal of Human Genetics* 10: 167–176.
24. Simpson, S., M. Zoeteweij, K. Nys, P. Harper, A. Durr, G. Jacopini, C. Yapijakis, and G. Evers-Kiebooms. 2002. Prenatal Testing for Huntington's Disease: A European Collaborative Study. *European Journal of Human Genetics* 10: 689–693.
25. International Huntington Association (IHA) and the World Federation of Neurology (WFN) Research Group on Huntington's Chorea. 1994. Guidelines for the Molecular Genetics Predictive test in Huntington's Disease. *Neurology* 44: 1533–1536.
26. MacLeod, R., A. Tibben, M. Frontali, G. Evers-Kiebooms, A. Jones, A. Martinez-Descales, and R.A. Roos. 2013. Editorial Committee and Working Group 'Genetic Testing Counselling' of the European Huntington Disease Network. Recommendations for the Predictive Genetic Test in Huntington's Disease. *Clinical Genetics* 83: 221–231.
27. Evers-Kiebooms, G., and M. Zoeteweij. 2002. In *Prenatal Testing for Late Onset Neurogenetic Diseases*, ed. P.S. Harper. Oxford, UK: BIOS.
28. Walker, F.O. 2007. Huntington's Disease. *Lancet* 369: 218–228.
29. Bouchghoul, H., S.F. Clément, D. Vauthier, C. Cazeneuve, S. Noel, M. Dommergues, D. Héron, J. Nizard, M. Gargiulo, and A. Durr. 2016. Prenatal Testing in Huntington Disease: After the Test, Choices Recommence. *European Journal of Human Genetics* 24: 1535–1540.
30. Vinther-Jensen, T., I.U. Larsen, L.E. Hjermind, E. Budtz-Jørgensen, T.T. Nielsen, A. Nørremølle, J.E. Nielsen, and A. Vogel. 2014. A Clinical Classification Acknowledging Neuropsychiatric and Cognitive Impairment in Huntington's Disease. *Orphanet Journal of Rare Diseases* 9: 114.
31. Dorsey, R., K. Biglan, S. Eberly, P. Auinger, A. Brocht, C.C. Umeh, D. Oakes, K. Clarence-Smith, F. Marshall, I. Shoulson, and S. Frank. 2011. Use of Tetrabenazine in Huntington Disease Patients on Antidepressants or with Advanced Disease: Results from the TETRA-HD Study. *PLoS Curr* 3: RRN1283.
32. www.huntington-study-group.org/ClinicalResearch/.
33. Tabrizi, S.J., R.I. Scahill, G. Owen, A. Durr, B.R. Leavitt, R.A. Roos, B. Borowsky, B. Landwehrmeyer, C. Frost, H. Johnson, D. Craufurd, R. Reilmann, J.C. Stout, and D.R. Langbehn. 2013. TRACK-HD Investigators. Predictors of Phenotypic Progression and Disease Onset in Premanifest and Early-Stage Huntington's Disease in the TRACK-HD Study: Analysis of 36-Month Observational Data. *Lancet Neurology* 12: 637–649.
34. Stout, J.C., S. Queller, K.N. Baker, S. Cowlishaw, C. Sampaio, C. Fitzer-Attas, B. Borowsky, and HD-CAB Investigators. 2014. HD-CAB: A Cognitive Assessment Battery for Clinical Trials in Huntington's Disease 1,2,3. *Movement Disorders* 29: 1281–1288.
35. Niccolini, F., and M. Politis. 2014. Neuroimaging in Huntington's Disease. *World Journal of Radiology* 6: 301–312.
36. Cheng, K. 2011. Recent Progress in High-Resolution Functional MRI. *Current Opinion in Neurology* 24: 401–408.

37. Sritharan, A., G.F. Egan, L. Johnston, M. Horne, J.L. Bradshaw, I. Bohanna, H. Asadi, R. Cunnington, A.J. Churchyard, P. Chua, M. Farrow, and N. Georgiou-Karistianis. 2010. A Longitudinal Diffusion Tensor Imaging Study in Symptomatic Huntington's Disease. *Journal of Neurology Neurosurgery and Psychiatry* 81: 257–262.
38. Mandelli, M.L., M. Savoiardo, L. Minati, C. Mariotti, D. Aquino, A. Erbetta, S. Genitrini, S. Di Donato, M.G. Bruzzone, and M. Grisoli. 2010. Decreased Diffusivity in the Caudate Nucleus of Presymptomatic Huntington Disease Gene Carriers: Which Explanation? *American Journal of Neuroradiology* 31: 706–710.
39. Plerou A, Bobori C, Vlamos P. Molecular Basis of Huntington's Disease and Brain Imaging Evidence. The 15th IEEE International Symposium on Signal Processing and Information Technology, Abu Dhabi, December 2015. doi:10.1109/ISSPIT.2015.7394365.
40. Sah, D.W., and N. Aronin. 2011. Oligonucleotide Therapeutic Approaches for Huntington Disease. *The Journal of Clinical Investigation* 121: 500–507.

A Paris System-Based Implant Approach to Hyperthermia Cancer Tumor with Gold Seeds and Ultrasound

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Abstract A Paris system-based implant approach has been used to improve the bio-heat distribution from implanted gold rods in insonated tissues. Experiments with single-plane implants using parallel equidistant 1.018 ± 0.015 cm height and 0.136 ± 0.001 cm diameter 24-K gold rods) arranged in triangular and square shapes were performed in *Mus musculus* white mice (medial dorsal region). The mice were anesthetized and gold rods were implanted by means of a trocar needle and the implanted region was insonated with a 4-cm diameter transducer oscillating with a nominal frequency of 1 MHz and power of about 75 W. Intramuscular tissue temperature measurements were recorded using implantable needle type thermocouples affixed to a portable Fluke thermometer. Superficial tissue temperature profile was also measured with a FLIR infrared camera and thermographic analysis was performed using the ImageJ computer software. In both cases, the central implant planes have been assigned to that approximately bisects all the implanted rods. Measured with the needle type thermistor, for the triangular implant, the percentage deviation between the maximum and minimum temperature within the triangular plane was 5%. For a square shape, this percentage deviation was 6%. The thermographic analysis have shown a deviation of 3 and 5% for the

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triangular and square shapes, respectively. The Paris system-based implant approach for gold rods implanted in tissue and exposed to ultrasound may greatly improve the bio-heat propagation and sustain a constant temperature profile inside triangular and square patterns formed by gold rods implants. Additionally, the Paris system may minimize ablations areas and treatment length in hyperthermia if used in cancer tumor treatment with gold seeds and ultrasound.

Keywords Ultrasound • Gold seeds • Paris system • Implant • Hyperthermia • *Mus musculus* mice • Bio-heat transfer • Ablation

1 Introduction

A new method to treat cancer tumor with gold seed and ultrasound has been proposed in the scientific literature. In vitro [1] and in vivo [2] experiments as well as analytical calculations [3, 4] have been performed to test the feasibility and provide subsidies to such methodology, respectively.

In vitro experiments have been performed to determine the bio-heat propagation to surrounding tissue from a gold rod implanted in fresh chicken breast and to determine the temperature at which the breakdown of cells starts and to validate analytical data. In vivo experiments with Ehrlich tumor derived from a mouse adenocarcinoma, has been used to kill cancer cells in *Mus musculus* mice Analytical modeling based on stationary bio-heat diffusion equations has been developed to calculate the temperature elevation in the gold rods and in the tissue surrounding it to compare calculated data against experimental observations and predicting pattern in experimental studies.

All the above-mentioned experiments and modeling were based on the use of a single gold rod of about 1-cm height and 0.1-cm diameter. From both, analytical calculations and measurements it was predicted that such a single gold rod would may be enough to induce hyperthermia in a tissue up to, about 0.5 cm radial distance from the gold rod. For points equidistant from implant plane, temperature is lower, so that the temperature of the rod should be high enough to provide ablation temperature in the boundary of the tumor to be treated. Besides, this process may requires long ultrasound exposure duration since hyperthermia treatment depends both on temperature and heating time.

In the branch of radiation brachytherapy several methods to uniform the dose delivered to tumor by mean of several radiation seeds have been proposed. The most commonly used are Quimby system, Paterson-Parker system and Paris system [5, 6]. The Quimby system is based on a uniform distribution of source strength, accepting a non-uniform delivery of dose. Usually, the dose in the centre of the treatment volume is higher than the dose near the periphery. The Patterson–Parker system is an implant planning system, using crossing needles, designed to deliver uniform dose within $\pm 10\%$ to a plane or volume to be treated. The sources are distributed non-uniformly following certain rules, based on the size of the target volume, with more source strength concentrated in the periphery. Usually the prescribed dose

is about 10% higher than the minimum dose within the treated volume. The Paris system is used primarily for single and double plane implants and does not address the other types of volume implant. It is necessary to follow a set of general rules for the selection and placement of the sources in order to achieve the desired dose distributions. The general rules are as follows; (a) sources must be linear and their placement parallel, (b) the centers of all sources must be located in the same plane, (c) the linear source strength must be uniform and identical for all sources, (d) adjacent sources must be equidistant from each other, and (e) the spacing between sources should be wider when using long sources.

A successfully and desired treatment with implanted gold seeds and ultrasound may requires uniform heat distribution inside the tissue to be treated, minimum treatment length and sharp heat fall-off outside the target volume. This work has aimed to test the feasibility of a Paris system-based implant for a uniform distribution of heat from implanted gold rods in tissue, in a single plane, exposed to ultrasound.

2 Material and Methods

2.1 Mus musculus *White Mice*

Two female *Mus musculus* white mice (5 week-old) were acquired from the Department of Antibiotics of the Federal University of Pernambuco, Brazil, and maintained and bred under conventional laboratory conditions at the Bioassay Laboratory for Drug Research from the same university. Before the treatment, the mice were anesthetized using Xylazine and Ketamine (5 mg/kg and 25 mg/kg body wt, respectively) [7], their hair around the medial dorsal region was shaved and by mean of a skin marker and a triangular and square template shape a 1 cm edge triangular shape was drawn in one mouse and a 1 cm square shape in the other.

2.2 Paris System-Based Implant

Four 24-K gold rods 1.018 ($\pm$0.015) cm heights and 0.136 ($\pm$0.001) cm diameters manufactured by Piatto DOro Ltda [8], were used as the implantable seeds. Single-plane implants using parallel equidistant gold rods arranged in triangular and square shapes were performed in the demarcated triangular and square areas in the medial dorsal region of the *Mus musculus* mice described above. The rods were inserted between the dermis and hypodermis (about 0.3 cm underneath the skin) by means of a trocar needle and a stylette (wire) to thrust the seed forward into the demarked edges of the triangular and square shapes. The rods were parallel arranged so that their centers were on the same plane. The central implants plane has been assigned

to that approximately bisects all the implanted rods. The positioning of the seeds was checked by visual inspection and their displacement was measured with a digital caliper.

2.3 Temperature Monitoring after Ultrasound Exposure

Ultrasound gel was spread on the shaved mice's skin as contact media gel and exposed for 30 min to a 4 cm transducer connected to a GS8 2E ultrasound under a nominal frequency of 1 MHz and a nominal power of 75 W for 30 min. After the exposure, a needle type thermistor connected to a FLUKE thermometer was inserted in the central implant plane, and in the tissue closest to gold rods to recorded the temperature in these points, Because of the drop of tissue's temperature when ultrasound exposure ceased, the temperature of the tissue closest to gold rods was measured twice; before and after that of the central implant plane. The comparison between the temperatures of these two points was based on the mean value of the temperatures in the central implant against that of the tissue closest to gold rods.

Infrared images of the implanted areas in the dorsal areas of the mice after the ultrasound exposure were also taken using an infrared camera and analyzed with the ImageJ software computer program. Plot profiles (gray value versus number of pixels) were built from a freehand line running through the location of both triangular and square central planes. Gray value, which denotes temperature was normalized to 100% at the point corresponding to the central implant plans. A device consisting of a 3 cm ring wire assembled to a plastic ring and connected to a 12 V power source (Fig. 1) was built and used in this work to convert distance in pixels

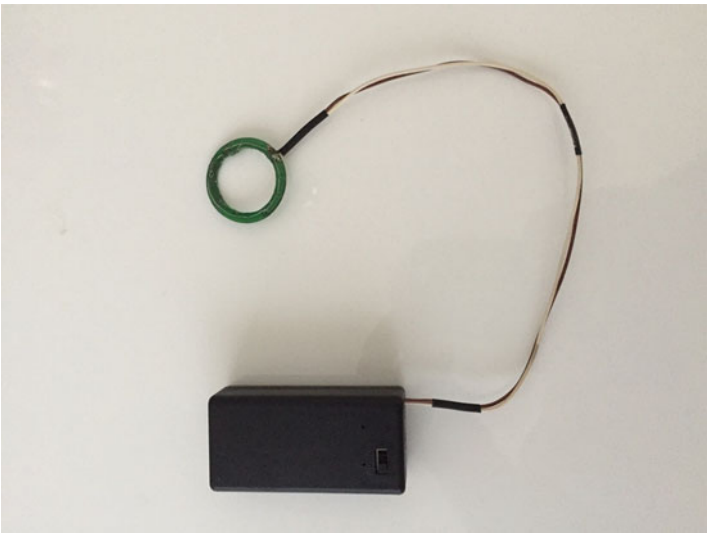

Fig. 1 Device to calibrate the infrared images in terms of distance in pixels to distance in cm

to distance in cm. The calibration of infrared image in terms of distance in pixels to distance in cm was performed by taking a infrared image of the ring device placed on the surface of the mice skin centered in the ultrasonic exposed area. The known diameter of the ring device as seen by infrared picture was used to convert the number the distance in pixels shown in the ImageJ program.

3 Results and Discussion

Figure 2 shows a living *Mus musculus* female mice after been anesthetized with their hair around the area to be irradiated shaved and with gold rods implanted in a triangular shape. The protrusion on the skin caused by the implanted rods may be seen in the shaved areas together with the triangular draw.

Figure 3 show an infrared pictures of the triangular implant pattern of the dorsal region of the mice after 30 min exposure. The temperature profile of a line running through the central plane implant of the square shape is shown in Fig. 4. A thermographic image of the device used to convert distance in pixels to distance in cm is shown in Fig. 5.

Based on the thermographic images analysis, the percent deviation between the maximum and minimum peak heat in a line running through the location of the triangular central plane within the central plane was about $\pm 4\%$ for square implant (Fig. 4). In the case of the triangular configuration such deviation was $\pm 3\%$. Measured with the needle type thermistor, for the triangular implant, the percentage

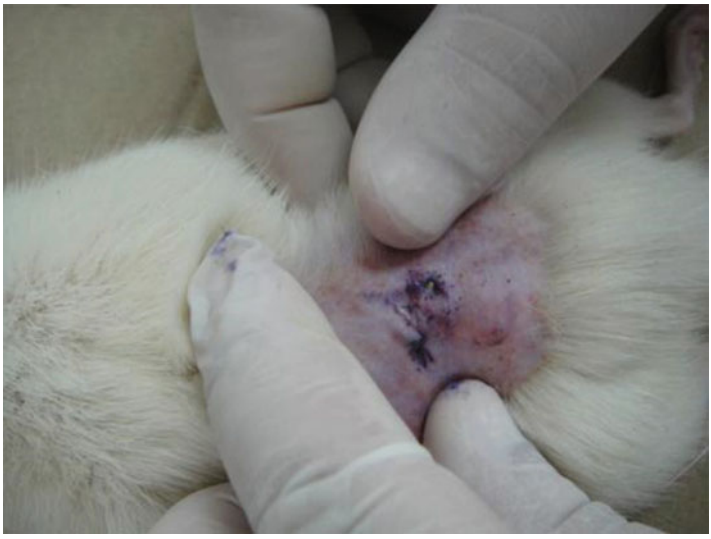

Fig. 2 Gold seeds implanted in a triangular shape in a *Mus musculus* mice

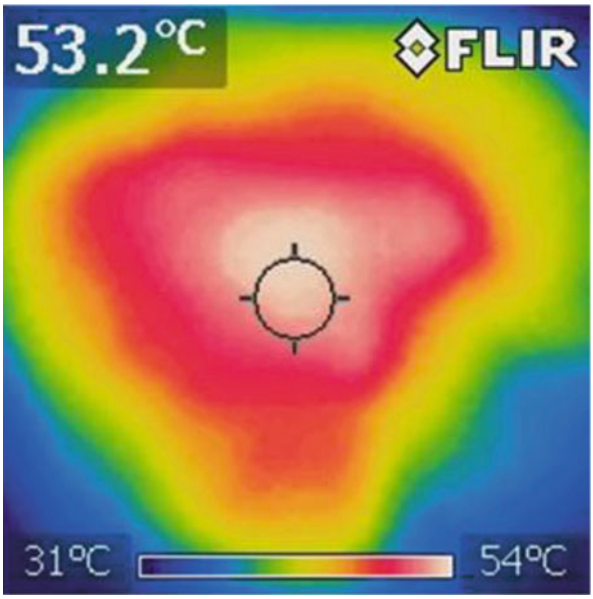

Fig. 3 Infrared pictures of the triangular and square pattern after 30 min exposure

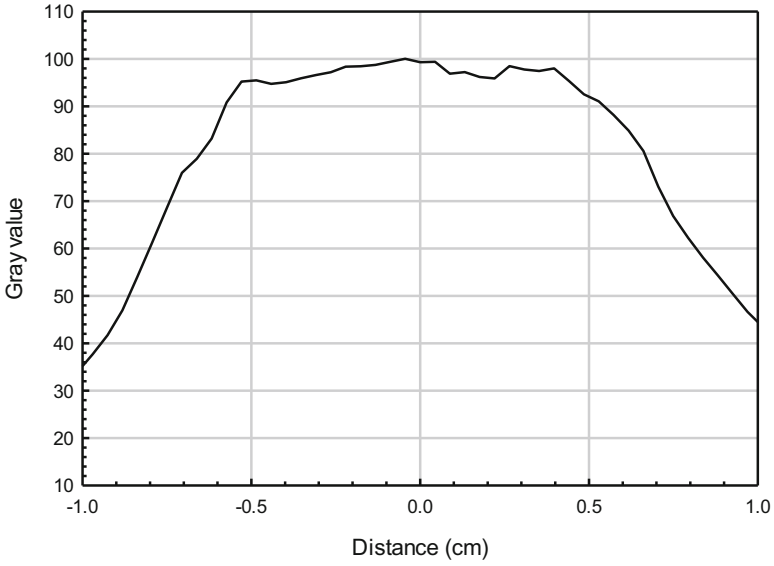

Fig. 4 Temperature profile of a line running through the location of the triangular central plane

deviation between the peak heat in the center of the implant's plane and that closest to the gold rod was 5%. For a square shape, this percentage deviation was 6%. Environmental temperature during all measurements performed in this study was (20 °C ± 1 °C).

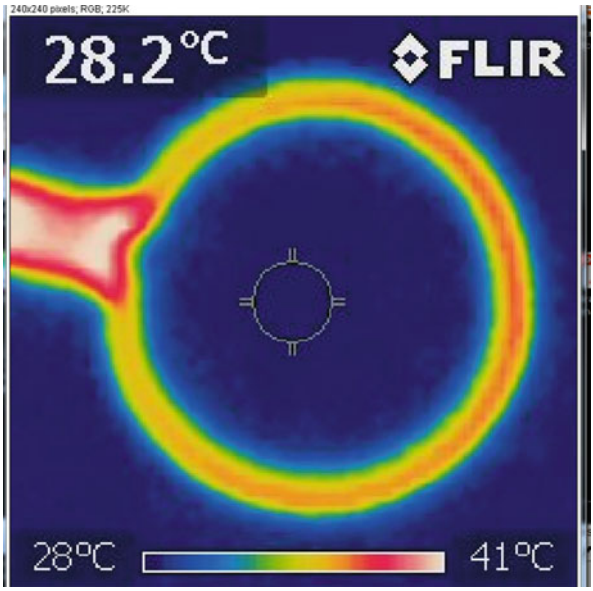

Fig. 5 Infrared image of the device used to convert distance in pixels to distance in cm

A single 0.1 cm diameter and 1 cm height gold rod implanted in cancer tumor, and irradiated with ultrasound may be enough to destroy a 1 cm diameter tumor [1], However, because the temperature enhancement of tissue exposed to ultrasound shows a biphasic slope of thermal response above and below around 43 °C [9, 10], this statement may involve high ablation temperature in tissues surrounding the gold rod so that the temperature in the boundary of the tumor be above the break point(43 °C) to starts to "cook" the tissue by thermal ablation [11]. Therefore, the Paris system-based implant approach using gold rods for either triangular or square shapes have greatly improved the isothermals inside these shapes while may avoid high ablation temperature around the gold seed and exposure time [9].

Compared with the triangle shape, the central implants plane for a square shape is further away from the gold rods so that its percentage deviation between the highest and lowest peak temperature within these central planes is a little bit higher (about 2%). This also means that the protrusion on the skin caused by the implanted gold rods did not affect the energy transferred from the transducer to gold rods and the bio-heat transfer from gold rods to surround tissue. The weight of the transducer kept at a horizontal position during the ultrasound exposure may be the reason to flattener the entire irradiated area and eliminate undesired non-uniform energy transferred to tissues and gold rods as well. In addition, uniform of heat distribution avoid sharp heat fall-off in the target and long treatment length. The heat fall-off outside the targets (triangular and square implant) and the influence of the environmental temperature on the tissue temperature does not make part of this work.

In general, mouse epidermis is <25 μm in thickness whereas human epidermis is >50 μm thick. The short inter-follicular regions in mouse skin do not contain

any rete ridges, while human dermis is substantially thicker than mouse dermis and mice contain an entire cutaneous muscle layer [12]. The convective heat transfer depends on the rate of perfusion and the vascular anatomy, which vary widely among the different tissues, organs of the body, and pathology (e.g., inflammation and cancer) [13]. Therefore, the results on the bioheat transfer from implanted gold rods irradiated with ultrasound to surrounding tissue from this experiment may not be applicable for other living species. Also, the measurements were performed a single implant plane which may not be applied to a volume. Nevertheless, the use of implanted gold rods greatly uniform the heat inside the target and may serve to validate analytical calculations. Infrared image has presented a simple, rapid and inexpensive method for digitalized visualization of temperature profile in superficial tissue. However, for absolute values of temperature and distance, gray and pixel values require calibrations.

The principles of the Paris system which was designed originally for brachytherapy, and was used primarily for single-and double-plane implants, and for specification of spacing between planes and spacing between sources. Brachytherapy quantities are defined for water. The dose rate at the point of interest in brachytherapy is defined in water [14], which turns easily the standardization of this quantity. Gold seeds may be used for more than one implant plane. Conversely, thermal dose depletion may depends on the physiological and anatomical components of the bioheat transfer which may vary widely among the different tissues, and therefore much more data is required before any specification of spacing between planes and spacing between seeds is completely determined. Such a determination that may lead to a protocol for future potential alternative cancer treatment will most likely be facilitated by the use of mathematical and computational methods that will enable the calculation of the effect of differences in shapes and sizes of triangular, square and other (for example, spiral) rod gold target configurations activated with ultrasonic energy on the heat enhancement and bio-heat transfer in tissue. Computer codes, based on bio-heat diffusion differential equations and appropriate boundary conditions may be used to calculate the temperature elevation in the gold rods and in the tissue surrounding it as a result of time exposure, ultrasonic power and other parameters, such as the mass of the gold targets. The development of such software is under preparation by our group.

4 Conclusions

Based on the temperature measurements with needle type thermistor and thermographic analysis, the Paris system-based implant approach for gold rods implanted in tissue and exposed to ultrasound may greatly improve the bio-heat propagation and sustain a constant temperature profile inside triangular and square patterns formed by gold rod implants. Additionally, the Paris system may minimize ablations areas and treatment length in hyperthermia if used in cancer tumor treatment with gold seeds and ultrasound.

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References

1. Austerlitz, C., I. Gkigkitzis, D. Campos, and I. Haranas. 2014. An Experimental Study on the Heat Enhancement and Bio-Heat Transfer Using Gold Macro Rod and Ultrasound: A Potential Alternative to Kill Cancer Cells. *Physics International* 5 (2): 132–135. doi:10.3844/pisp.2014.132.135.
2. Austerlitz, C., A. Barros, I. Gkigkitzis, D. Campos, T. Silva, S. Nascimento, and I. Haranas. 2015. On the Use of Gold Macro-Rods and Ultrasound as a Hyperthermia Cancer Treatment: Experimental Results on Erlich Tumor in *Mus musculus* Mice. In *Patents Eb Ser Top Anti-Cancer Res*, vol. 4, Benthan Science Publishers. doi:10.2174/978168108076511504009.
3. Gkigkitzis, I., C. Austerlitz, I. Haranas, and D. Campos. 2015. The Effect of the Shape and Size of Gold Seeds Irradiated with Ultrasound on the Bio-Heat Transfer in Tissue. *Advances in Experimental Medicine and Biology* 820: 103–124. doi:10.1007/978-3-319-09012-2_8.
4. Gkigkitzis, I., I. Haranas, and C. Austerlitz. 2015. Analytic Considerations and Axiomatic Approaches to the Concept Cell Death and Cell Survival Functions in Biology and Cancer Treatment. *Advances in Experimental Medicine and Biology* 822: 85–105. doi:10.1007/978-3-319-08927-0_11.
5. Suntharalingam, N., E.B. Podgorska, and H. Tölli. 2005. *Brachytherapy: Physical and Clinical Aspects*. Vienna: International Atomic Energy Agency.
6. Levitt, S.H., J.A. Purdy, C.A. Perez, and S. Vijayakumar. 2006. *Technical Basis of Radiation Therapy*. 4th ed. Berlin: Springer.
7. Welberg, L.A., B. Kinkead, K. Thrivikraman, M.J. Huerkamp, C.B. Nemeroff, and P.M. Plotsky. 2006. Ketamine-Xylazine-Acepromazine Anesthesia and Postoperative Recovery in Rats. *Journal of the American Association for Laboratory Animal* 45 (2): 13–20. http://www.ncbi.nlm.nih.gov/pubmed/16542037.
8. Piatto, D. 2015. Oro Ltda.
9. Dewhirst, M., B. Vigilanti, M. Lora-Michiels, P. Hoopes, and M. Hanson. 2003. Thermal Dose Requirements for Tissue Effect: Experimental and Clinical Data. *Proceedings of SPIE The International Society for Optical Engineering* 37. doi:10.14440/jbm.2015.54.A.
10. Sinden, D., and G. ter Haar. 2014. Dosimetry Implications for Correct Ultrasound Dose Deposition: Uncertainties in Descriptors, Planning and Treatment Delivery. *Translational Cancer Research* 3 (5): 459–471.
11. Ahmed, M., and S. Goldberg. 2011. Basic Science Research in Thermal Ablation. *Surgical Oncology Clinics of North America* 20 (2): 237–258.
12. Gudjonsson, J.E., A. Johnston, M. Dyson, H. Valdimarsson, and J.T. Elder. 2007. Mouse Models of Psoriasis. *The Journal of Investigative Dermatology* 127 (6): 1292–1308. doi:10.1038/sj.jid.5700807.
13. JW, V. 2005. *Encyclopedia of Medical and Instrumentation -Bioheat Transfer*. 2 ed. Texas TU of ed. Texas: Austin.
14. Rivard, M.J., B.M. Coursey, L.A. DeWerd, et al. 2004. Update of AAPM Task Group No. 43 Report: A Revised AAPM Protocol for Brachytherapy Dose Calculations. *Medical Physics* 31 (3): 633–674. doi:10.1118/1.1905824.

Clinical, Social and Demographics Factors Associated with Spiritual Wellbeing in End Stage Renal Disease

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Abstract Spiritual health is one of the important aspects of health status that is often neglected. Aim: the present study aims to evaluate spiritual wellbeing in end stage renal disease patients undergoing hemodialysis and its relation to sociodemographic and clinical variables. Methods: A convenience sample of 183 individuals undergoing hemodialysis was recruited. Measurements were conducted with the following instruments: (a) a sheet containing demographic data and clinical information such as duration of dialysis e.t.c (b) Facit Spiritual Wellbeing

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Scale (Facit-Sp12). Statistical analysis was contacted with SPSS v.22. Descriptive statistics were initially generated for sample characteristics. Parametric and non-parametric statistics were used for searching the relations between the variables. P values <0.05 were defined as reflecting the acceptable level of statistical significance. Results: From the total of the 183 participants of the study the 69.9% were male and 30.1% female. The age range was from 26 to 88 years old, with mean 61.39 ± 14.11. The subscale "peace" is associated to gender ($t = 2.150$, $p = 0.033$), educational level ($F = 2.698$, $p = 0.047$) and duration of dialysis ($F = 2.969$, $p = 0.033$) and religious beliefs ($t = -2.059$, $p = 0.041$). The subscale "faith" is associated to gender ($t = -3.428$, $p = 0.001$), age ($p = 0.006$), number of children ($F = 4.347$, $p = 0.014$). Moreover, the subscale "meaning" is associated to age ($p = 0.001$). Finally its worth to be mentioned that comorbidity is associated to subscales "meaning" ($t = -2.071$, $p = 0.040$), "peace" ($t = -2.377$, $p = 0.018$) and the overall spiritual wellbeing ($t = -1.988$, $p = 0.048$). Conclusions: Social, demographic factors as well as clinical variables such duration of dialysis and comorbidities are affecting spiritual wellbeing in end stage renal disease.

Keywords Spiritual wellbeing • Spirituality • End stage renal disease

1 Introduction

Chronic kidney disease (CKD) is a serious and life-threaten condition. It is a complex, often slow and progressive syndrome that is characterized by the inability of kidneys to remove the products of metabolism and to perform their regulatory functions. CKD is a major public health problem of increasing importance, that tends to become an epidemic, it is estimated that every year 120 patients per 1,000,000 of population are entering in end stage renal disease (ESRD). In Greece the number of patients that are in kidney replacement treatment via hemodialysis are almost 10,000, leading Greece in the 8th place in the prevalence of kidney replacement treatment [1–3].

Patients with chronic diseases such as ESRD are facing a number of difficulties and stress factors, such as pain, feelings of uncertainty and change of body image. These kinds of stressors can lead to changes of their wellbeing, spiritual distress and raise existential questions such as "What is the meaning of life?". This search for the meaning and purpose of life, can bring into conflict with other people and doubts for their choices in life. Like every other chronic disease, ESRD is inducing the patients in a new "reality" surrounded by medical examination, management of the disease (medication, dietary restrictions) and a complicated treatment plan. Thus, patients suffering by ESRD are obligated to adjust to new data and experience enormous changes in their daily habits. The negative effect that those changes on daily life have in patients not only in physical and mental health as well as in quality of life in general, is well document over the years. Individuals that are suffering by ESRD are facing many psychosocial issues related to loss of health, hopelessness, and increasing mortality [4–6].

Symptoms that patients with ESRD are facing can be similar to those of patients suffering from cancer and are receiving palliative care. In ESRD, several components of palliative care are particularly applicable, including pain and symptom management, advance care planning, psychosocial and spiritual support [7]. To be diagnosed with ESRD, is a crucial turning point in the patient's life and spirituality can contribute positively by giving strength and hope. According to McSherry and Ross [8], spiritual wellbeing is positive related to quality of life and the wiliness to cope with the disease [8].

The term "spirituality" is a concept clearly undefined, which contains many meanings beyond religious limits [9] and as result it is difficult to be determined. According to Harrison and Bernard there are as many definitions of spirituality as the people who are trying to define it and they argue that words and concepts are unable to describe the nature of spirituality. Spirituality can be defined as a structure that contains beliefs of faith and sense. The element of faith in spirituality is often associated with religion and religious faith, while spirituality is a broader concept and it can be found to religious individuals as well as to individuals without any religious preferences [10]. According to Maugans, spirituality is "a belief system which focuses on intangible elements that transmit vitality and events that give meaning to life [11]. Furthermore, Carson and Green support that the process of spiritual growth is the same regardless of whether someone believes or not [12].

The researcher, who seeks to determine a generalized, widely accepted definition of religion or spirituality, has to face many challenges. The most likely is that whatever definition will be given to spirituality, it will differ from what this term means for each believer. This happens because on one hand it is very difficult to be described what is experienced as spiritual and on the other hand, spirituality focuses on the intangible characteristics of life that they are not perceived by the senses [13, 14]. Firstly, is the value of giving sense to the most intense and vibrant quality of life, often described as "giving life and energy" to the most essential human elements of an individual. Secondly, spirituality focuses on the intangible characteristics of life that are not perceived by the senses. The main religions use similar terminology to refer to what is considered as sacred, holy or divine. However, some characteristics of spirituality are observable as "Spiritual techniques" [15, 16]. Some people understand spirituality as a transcendental relationship with what is considered sacred in life or as something divine that exceeds oneself [17]. Spiritual factors have been associated with better than the average outcomes in mental and physical health [13]. Among the key findings, there are researches that shown the association between spiritual and religious factors with low rates of arterial disease, emphysema, liver cirrhosis, suicide, low pressure, decreased levels of pain in patients with cancer, and, in general, reduced mortality, increased healthy habits and longevity [18–21].

According to Ramirez et al. [22], hemodialysis patients rely on their religion to cope with the disease. Greater use of positive religious coping that patients had found to be associated with better overall, mental health and social relations HRQoL. Similar results had the study of Saisunantararom et al. [23] were spirituality was found to be positively associated with QOL, and negatively associated with depression. Spirituality was found to be more associated with mental health than

physical health and depression seem to play an important role in the association, suggesting that healthcare professionals should be concerned with the spirituality of CKD patients, since understanding spirituality might lead to less depression and prolonging of patient lives. Although the beneficial role that spiritual wellbeing may have in health and recovery, and that ESRD patient have many unmet spiritual needs, spirituality of those patients is often neglected [24].

In Greece, few studies have been conducted to investigate the spiritual needs of Greek patients and specially to investigate the spirituality of Greek ESRD patients. Thus, the purpose of this study is to assess spiritual wellbeing of ESRD patients undergoing hemodialysis and to evaluate clinical, social and demographic factors that are associated with spiritual wellbeing of those patients.

2 Material and Methods

A cross sectional study was conducted using a structured questionnaire on a convenience sample of 183 patients undergoing hemodialysis. The inclusion criteria were: (1) >18 years old (2) ability to speak and read Greek (3) To have been diagnosed with ESRD (4) Hemodialysis (HD) treatment for at least 6 months (5) adequate time and space orientation. The exclusion criteria were: (1) patients suffering from psychiatric or cognitive disorders, (2) patients with functional disabilities, visual or hearing disorders. Written approval to conduct the study was obtained from the Scientific Council of the hospitals. According to the ethical standards of the Helsinki Declaration all patients were informed about their rights to refuse or stop the participation in the study. To respect the rules of research ethics, the patients were asked to complete anonymous questionnaires.

Data Collection: Data were obtained by anonymous self- administrated questionnaires consisting of the following instruments.

A semi-structured questionnaire concerning clinical, social and demographic data (age, gender, employment, education, comorbidities, duration of dialisis).

The Functional Assessment of Chronic Illness Therapy-Spiritual Well-Being Scale (FACIT-Sp-12). It is a five-level Likert scale range from 0 to 4, created by Cella et al. [25] and has been widely used for the assessment of spirituality in chronic patients. It constitutes a part of a bigger evaluation tool that measures important factors of functionality in patients with chronic illness. Specifically, it includes three subscales: the meaning of life, peace and sense of support and strength that is drawn from faith. Greater scores represent greater spiritual well-being. The total sum of all answers gives information about the general spiritual well-being [25, 26]. The Greek version of Facit sp.-12 is a valid tool with a high reliability index Cronbach's alpha 0.77 [13].

Statistical analysis: Statistics of the research's empirical data were processed with IBM SPSS v. 22.0 for Windows. Descriptive and inferential statistical methods were generated. More specifically, the frequency distribution of the variables was estimated, as well as the position and dispersion parameters (mean, standard deviation, minimum and maximum value) of the quantitative variables. Pearson's

correlation coefficient, independent samples t-test and one-way analysis of variance (ANOVA) were used for the assessment of possible correlations between the variables. The FACIT – Sp 12 score was used as an outcome of the under research correlations. Statistical significance was set at $p < 0.050$.

3 Results

Descriptive statistical measures for characteristics of 183 hemodialysis patients, as well as for the FACIT – Sp 12 scale are presenting in Tables 1 and 2.

From the total of the 183 participants of the study the 69.9% were male and 30.1% female. The age range was from 26 to 88 years old, with mean 61.39 (sd = 14.112). The majority of them were living in urban or suburban environment (61.2%). The 67.8% of the patients were married and 52.0% had 1 to 2 children. Regarding the educational status of the sample 45.9% had attended primary school, 36.6% high school and 17.5% had an undergraduate degree. The majority of them were pensioners 44.3%, household or unemployed were the 26.2%, private or public sector 14.8% and the 14.8% were freelancers. Regarding the health related parameters of the sample, 5 or less years on hemodialysis stated the 68.3% of them while 6–10 years the 23.0%. The 53.0% of the participants stated that they dealing with an additional health problem while 47.0% didn't. Regarding their current activity level most of them, 49.2% had a normal activity without any symptoms or some symptoms, but do not require bed rest during waking day ("good level"). The 31.7% of the patients stated that they require bed rest for less than 50% of the day ("moderate level") and finally 19.1% of them require bed rest for more than 50% of waking day ("bad level") (Table 1).

For the total FACIT – Sp 12 scale (12 items) the score ranged from 6 to 48 and the mean was 30.62 (sd = 7.215). For the FACIT – Sp 12 subscales the mean of score was 12.49 (sd = 2.865) for the "Meaning" (4 items), 8.64 (sd = 3.345) for the "Peace" (4 items) and 9.49 (sd = 4.319) for the "Faith" (4 items). The internal consistency reliability of the total FACIT – Sp 12 scale, as was determined by the Cronbach's Alpha coefficient (α), was 0.77. Chronbach's α for the FACIT – Sp 12 subscales was 0.70 for the "Meaning", 0.73 for the "Peace" and 0.87 for the "Faith". The scale and the subscales had a $\geq$0.70 fact that supports that the scale has a satisfactory internal consistency (Table 2).

Bivariate analysis (Table 3) was generated to investigate the relation between the FACIT – Sp 12 score (dependent variable) and the patients' characteristics (independent variables).

Male had higher mean score than female in the "Peace" (8.98 ± 3.231 vs 7.84 ± 3.495, $p = 0.033$) while female higher mean score than male in the "Faith" (11.11 ± 3.578 vs 8.79 ± 4.434, $p = 0.001$). The increase in age of patients was statistically significant associated with a decrease in "Meaning" score ($r = -0.245$, $p = 0.001$) and an increase in "Faith" score ($r = 0.202$, $p = 0.006$). Residents in urban or suburban region showed higher mean score in the "Peace" than residents in rural (9.09 ± 3.357 vs 7.93 ± 3.222, $p = 0.022$). Divorced and

Table 1 Sample characteristics (n = 183)

Characteristics		n	(%)
Gender	Male	128	(69.9%)
	Female	55	(30.1%)
Age (years)	Mean ± St. Dev.	61.39 ± 14.112	
	Min–Max	26–88	
Place of residence	Rural	71	(38.8%)
	Suburban	31	(16.9%)
	Urban	81	(44.3%)
Marital Status	Single	39	(21.3%)
	Married	124	(67.8%)
	Divorced	9	(4.9%)
	Widowed	11	(6.0%)
Number of children	0	49	(26.8%)
	1	25	(13.7%)
	2	70	(38.3%)
	≥3	39	(21.3%)
Educational level	Attend primary school	13	(7.1%)
	Finished primary school	71	(38.8%)
	High school	67	(36.6%)
	University degree	32	(17.5%)
Occupation	Unemployed	16	(8.7%)
	Household	32	(17.5%)
	Freelancer	27	(14.8%)
	Private sector	14	(7.7%)
	Public sector	13	(7.1%)
	Pensioner	81	(44.3%)
Duration of hemodialysis (years)	<1	33	(18.0%)
	1–5	92	(50.3%)
	6–10	42	(23.0%)
	≥11	16	(8.7%)
Additional health problem	Yes	97	(53.0%)
	No	86	(47.0%)
Current activity	Bad level	35	(19.1%)
	Moderate level	58	(31.7%)
	Good level	90	(49.2%)

widowed patients had a lower mean score in the "Meaning" than single patients (10.90 ± 3.932 vs 12.64 ± 2.497, p = 0.026) and married patients (10.90 ± 3.932 vs 12.70 ± 2.714, p = 0.009). The increase in number of children who had the patients was statistically significant associated with an increase in "Faith" score (r = 0.168, p = 0.023). Regarding the educational level, it was found statistically significant positive correlation with in "Peace" score (r = 0.165, p = 0.026) and negative correlation with in "Faith" score (r = −0.295, p = 0.000). Also there

Table 2 Reliability and descriptive statistical measures of the FACIT – Sp 12 Scale (n = 183)

FACIT – Sp 12 Scale	Mean ± St. Dev.	Min–Max	Cronbach's α
Subscale "Meaning"	12.49 ± 2.865	3–16	0.70
Subscale "Peace"	8.64 ± 3.345	0–16	0.73
Subscale "Faith"	9.49 ± 4.319	1–16	0.87
Total Scale	30.62 ± 7.215	6–48	0.77

were statistically significant correlations between the occupation and "Peace" score ($F = 2.729$, $p = 0.021$) and between the occupation and "Faith" score ($F = 2.481$, $p = 0.034$) (Table 3).

Concerning the health related parameters of the sample, patients with 1–5 years duration of hemodialysis showed higher mean score in the "Peace" than patients with shorter duration of hemodialysis (9.24 ± 3.014 vs 7.27 ± 4.018, $p = 0.004$). Other statistically significant correlations between the FACIT – Sp 12 scale and duration of hemodialysis were not found. Patients who did not state an additional health problem had higher mean score in the "Meaning" (12.95 ± 2.670 vs 12.08 ± 2.981, $p = 0.040$), in the "Peace" (9.26 ± 3.174 vs 8.09 ± 3.413, $p = 0.018$) and in the total scale of FACIT – Sp 12 (31.72 ± 6.322 vs 29.64 ± 7.826, $p = 0.048$) than patients who did it. Also, there were statistically significant correlations between the FACIT – Sp 12 scale and current activity level. The increase in current activity level of patients was statistically significant associated with an increase in "Meaning" score ($r = 0.469$, $p = 0.000$), an increase in "Peace" score ($r = 0.261$, $p = 0.000$), a decrease in "Faith" score ($r = -0.250$, $p = 0.001$) and an increase in the total scale score ($r = 0.158$, $p = 0.033$) (Table 3).

4 Discussion

This study was conducted to assess spiritual wellbeing among Greek hemodialysis patients and to explore its association with clinical, social and demographic variables. According to the results of the present study, Greek hemodialysis patients had poorer spiritual wellbeing compared to other studies contacted in hemodialysis patients [27], as well as in women suffering by breast cancer [28, 29], but the were similar to others finding from patients suffering by diabetes 2 [30]. Results from the present study revealed that subscales of spiritual wellbeing sp. 12, are associated with gender, age, duration of dialysis and comorbidities among others.

There are several studies supporting that women tend to be more spiritual and religious than male hemodialysis patients [26, 27, 31, 32] our results are in accordance to those studies and women scored higher than men in the facit sp. subscale of faith. On the other hand, and in construct with the above studies, men showed higher scores in the subscale "Peace" compared to the women of the sample.

According to the results of the present study, higher scores of spiritual well-being were reported among participants that were older and with better educational status. Spirituality tendency increases with age, tendency to spirituality is considered as a

Table 3 Relation between FACIT – Sp 12 score and patients'characteristics

Characteristics	"Meaning"	"Peace"	"Faith"	Total scale
Gender				
Male	12.65 ± 2.882	8.98 ± 3.231	8.79 ± 4.434	30.42 ± 7.282
Female	12.13 ± 2.816	7.84 ± 3.495	11.11 ± 3.578	31.07 ± 7.102
t	1.129	2.150	−3.428	−0.558
p	0.260	**0.033**	**0.001**	0.577
Age (years)				
r	−0.245	0.005	0.202	0.026
p	**0.001**	0.942	**0.006**	0.723
Place of residence				
Rural	12.41 ± 3.050	7.93 ± 3.222	9.83 ± 4.216	30.17 ± 7.458
Urban/Suburban	12.54 ± 2.754	9.09 ± 3.357	9.27 ± 4.389	30.90 ± 7.076
t	−0.313	−2.313	0.859	−0.668
p	0.755	**0.022**	0.392	0.505
Marital status				
Single (1)	12.64 ± 2.497	8.90 ± 2.909	8.74 ± 4.511	30.28 ± 6.236
Married (2)	12.70 ± 2.714	8.59 ± 3.340	9.67 ± 4.276	30.96 ± 6.992
Divorced/Widowed (3)	10.90 ± 3.932	8.45 ± 4.224	9.80 ± 4.262	29.15 ± 10.06
F	3.571	0.161	0.738	0.593
p	**0.030**	0.852	0.479	0.554
Post Hoc Test (LSD)	(3)< (1) p = 0.026 (3)< (2) p = 0.009			
Number of children				
r	−0.001	0.001	0.168	0.101
p	0.987	0.989	**0.023**	0.175
Educational level				
r	0.074	0.165	−0.295	−0.071
p	0.318	**0.026**	**0.000**	0.343

Occupation				
Unemployed (1)	11.50 ± 3.502	7.94 ± 3.193	9.38 ± 4.801	28.81 ± 6.765
Household (2)	12.19 ± 2.788	7.53 ± 3.389	11.56 ± 3.784	31.28 ± 6.985
Freelancer (3)	12.52 ± 2.901	7.44 ± 3.846	8.93 ± 4.497	28.89 ± 6.583
Private sector (4)	13.07 ± 2.731	9.71 ± 1.490	8.36 ± 4.050	31.14 ± 6.298
Public sector (5)	11.92 ± 3.402	9.15 ± 3.913	7.38 ± 3.686	28.46 ± 8.161
Pensioner (6)	12.79 ± 2.691	9.35 ± 3.131	9.41 ± 4.303	31.54 ± 7.545
F	0.845	2.729	2.481	1.080
p	0.519	**0.021**	**0.034**	0.373
Post Hoc Test (LSD)		(2)< (4) p = 0.039 (2)< (6) p = 0.009 (3)< (4) p = 0.036 (3)< (6) p = 0.010	(2)> (3) p = 0.018 (2)> (4) p = 0.019 (2)> (5) p = 0.003 (2)> (6) p = 0.016	
Duration of hemodialysis				
<1 year (1)	11.88 ± 3.655	7.27 ± 4.018	10.18 ± 4.673	29.33 ± 8.908
1–5 years (2)	12.59 ± 2.619	9.24 ± 3.014	9.46 ± 4.194	31.28 ± 6.849
≥6 years (3)	12.69 ± 2.735	8.47 ± 3.235	9.14 ± 4.339	30.29 ± 6.710
F	0.944	4.477	0.616	0.972
p	0.391	**0.013**	0.541	0.380
Post Hoc Test (LSD)		(1)< (2) p = 0.004		
Additional health problem				
Yes	12.08 ± 2.981	8.09 ± 3.413	9.46 ± 4.449	29.64 ± 7.826
No	12.95 ± 2.670	9.26 ± 3.174	9.51 ± 4.195	31.72 ± 6.322
t	−2.071	−2.377	−0.074	−1.988
p	**0.040**	**0.018**	0.941	**0.048**
Current activity level				
r	0.469	0.261	−0.250	0.158
p	**0.000**	**0.000**	**0.001**	**0.033**

function of aging, because it is a way by which a person faces with the reality of death and adjusts with that [33]. Furthermore. factors such as age and educational status have been associated with reports of higher level of spiritual well-being in different previous studies. Furthermore, it is widely agreed that in times of crises, such as being diagnosed with a chronic, life threating or terminal disease, people get closer to the divine and they trying to find a meaning to their suffering [26, 27, 34–36].

In addition, duration of dialysis and co-morbidities found to be associated with spiritual wellbeing in our study, fact that contrasts with other studies done in hemodialysis patients where those clinical factors are no related to spiritual wellbeing [24]. This may be due to that the Greek people are deeply religious and studies which have been in other chronic diseases in Greece argue that fact [37].

Limitations in our study warrant mention. The study was carried out in a clinical setting during dialysis. Additionally, in the sample of this study were included the patients who were available at that time and as such, we cannot generalize the results to the entire population concerned.

5 Conclusions

For many people, spirituality and religion are important dimensions of their existence, and there are used as a source of support that contributes to their well-being and helps them coping with life's difficulties. For many patients, the integration of their spiritual beliefs in the healing process is vital and it has been found to be associated with positive outcomes for their mental and physical health as well. The positive influence that spirituality may have in the perception of patients about their health, their individual treatment and their adjustment to a serious illness has been documented by many studies. To be diagnosed with ESRD is a crucial point for every patient. Social and demographic factors such as gender, educational level and number of children as well as clinical variables such duration of dialysis and comorbidities are affecting spiritual wellbeing in end stage renal disease. Many patients undergoing hemodialysis are reporting unmet spiritual needs, addressing those needs and facilitating ESRD patients to find meaning and propose to their life and suffering must be a priority by healthcare professionals and should not be neglected.

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References

1. Agrogiannis, B. 2002. *Pathology*. Vol. I, 689–695. Athens: Paschalides Medical Publications.
2. Jha, V., et al. 2013. Chronic Kidney Disease: Global Dimension and Perspectives. *Lancet* 382 (9888): 260–272.
3. Kafkia, Th., Z. Dimosthenis, and M. Kourakos. 2008. Exercise Protocols in Patients with Chronic Kidney Disease (CKD) and on Renal Replacement Therapy: A Literature Review. *The Scientific Journal of the Hellenic Regulatory Body of Nurses* 1 (2): 55–59.
4. Finkelstein, F.O., D. Wuerth, and S.H. Finkelstein. 2009. Health Related Quality of life and the CKD Patient: Challenges for the Nephrology Community. *Kidney International* 76 (9): 946–952. doi:10.1038/ki.2009.307.
5. Levey, A.S., et al. 2005. Definition and Classification of Chronic Kidney Disease: A Position Statement from Kidney Disease: Improving Global Outcomes (KDIGO). *Kidney International* 67 (6): 2089–2100.
6. Temimovic, R., S. Rasic, and A. Muslimovic. 2015. High Prevalence of Early Chronic Kidney Disease in High Risk Outpatients. *Materia Socio-Medica* 27 (2): 79–82. doi:10.5455/msm.2015.27.79-82.
7. Ottavian, A.C., et al. 2014. Hope and Spirituality Among Patients with Chronic Kidney Disease Undergoing Hemodialysis: A Correlational Study. *Revista Latino-Americana de Enfermagem* 22 (2): 248–254.
8. McSherry, W., and L. Ross. 2010. *Spiritual Assessment in Healthcare Practice*. Keswick: M&K Update Ltd.
9. Fontana, D., and D. Fonatana. 2003. *Psychology, Religion, and Spirituality*. Malden, MA: Wiley.
10. Karasu, B.T. 1999. Spiritual Psychotherapy. *American Journal of Psychotherapy* 53: 143–162.
11. Maugans, T.A. 1996. The Spiritual History. *Archives of Family Medicine* 5 (1): 11–16. doi:10.1001/archfami.5.1.11.
12. Carson, V.B., and H. Green. 1992. Spiritual Well-Being: A Predictor of Hardiness in Patients with Acquired Immunodeficiency Syndrome. *Journal of Professional Nursing* 8 (4): 209–220. doi:10.1016/8755-7223(92)90082-a.
13. Fradelos, E.C., F. Tzavella, E. Koukia, et al. 2016. The Translation, Validation and Cultural Adaptation of Functional Assessment of Chronic Illness Therapy-Spiritual Well-Being 12 (facit-sp12) Scale in Greek Language. *Mater Sociomed* 28 (3): 229–234. doi:10.5455/msm.2016.28.229-234.
14. Zyga S. (2016) Assessing Patients Spirituality: A New Age Holistic Approach or. Available at: http://www.hsj.gr/medicine/assessing-patients-spirituality-a-new-age-holistic-approach-or-a-forgotten-nursing-practice.php?aid=6528. Accessed 7 June 2016.
15. Miller, W.R., and C.E. Thoresen. 2003. Spirituality, Religion, and Health: An Emerging Research Field. *American Psychologist* 58 (1): 24–35. doi:10.1037/0003-066x.58.1.24.
16. Simpson, J., and E. Weiner, eds. 1989. *The Oxford English Dictionary*. 2nd ed. New York: Oxford University Press.
17. Zinnbauer, B.J., and K.I. Pargament. 2005. Religiousness and Spirituality. *Handbook of the Psychology of Religion and Spirituality*: 21–42.
18. Coyle, J. 2002. Spirituality and Health: Towards a Framework for Exploring the Relationship Between Spirituality and Health. *Journal of Advanced Nursing* 37 (6): 589–597. doi:10.1046/j.1365-2648.2002.02133.x.
19. King, M.B., and H.G. Koenig. 2009. Conceptualising Spirituality for Medical Research and Health Service Provision. *BMC Health Services Research* 9 (1): 116. doi:10.1186/1472-6963-9-116.
20. Rippentrop, E.A., E.M. Altmaier, J.J. Chen, et al. 2005. The Relationship Between Religion/Spirituality and Physical Health, Mental Health, and Pain in a Chronic Pain Population. *Pain* 116 (3): 311–321. doi:10.1016/j.pain.2005.05.008.
21. Thoresen, C.E., and A.H.S. Harris. 2002. Spirituality and Health: What's the Evidence and What's Needed? *Annals of Behavioral Medicine* 24 (1): 3–13. doi:10.1207/s15324796abm2401_02.

22. Ramirez, S.P., D.S. Macêdo, P.M.G. Sales, et al. 2012. The Relationship Between Religious Coping, Psychological Distress and Quality of Life in Hemodialysis Patients. *Journal of Psychosomatic Research* 72 (2): 129–135.
23. Saisunantararom, W., A. Cheawchanwattana, T. Kanjanabuch, et al. 2015. Associations Among Spirituality, Health-Related Quality of Life, and Depression in Pre-Dialysis Chronic Kidney Disease Patients: An Exploratory Analysis in Thai Buddhist Patients. *Religions* 6 (4): 1249–1262.
24. Davison, S.N., and G.S. Jhangri. 2010. Existential and Supportive Care Needs Among Patients with Chronic Kidney Disease. *Journal of Pain and Symptom Management* 40 (6): 838–843. doi:10.1016/j.jpainsymman.2010.03.015.
25. Cella, D.F., D.S. Tulsky, et al. 1993. The Functional Assessment of Cancer Therapy Scale: Development and Validation of the General Measure. *Journal of Clinical Oncology* 11 (3): 570–579.
26. Peterman, A.H., G. Fitchett, et al. 2002. Measuring Spiritual Well-Being in People with Cancer: The Functional Assessment of Chronic Illness Therapy Spiritual Well-Being Scale (FACIT-Sp). *Annals Behaviroal Medicine* 24 (1): 49–58.
27. Cheawchanwattana, A., D. Chunlertrith, W. Saisunantararom, and N.P. Johns. 2014. Does the spiritual well-being of chronic hemodialysis patients differ from that of pre-dialysis chronic kidney disease patients? *Religions* 6 (1): 14–23.
28. Jafari, N., A. Zamani, M. Lazenby, et al. 2012. Translation and Validation of the Persian Version of the Functional Assessment of Chronic Illness Therapy—Spiritual Well-Being Scale (FACIT-Sp) Among Muslim Iranians in Treatment for Cancer. *Palliative and Supportive Care* 11 (01): 29–35. doi:10.1017/s1478951512000727.
29. Raja Lexshimi, R.G., E. Mohd Fahmi, S.C. Lee, et al. 2014. Spirituality and Mental Adjustment as Coping Strategies Among Women with Breast Cancer Malaysian. *Journal of Public Health Medicine* 14 (1): 1–9.
30. Jafari, N., Z. Farajzadegan, A. Loghmani, and M. Majlesi. 2014. Spiritual Well-Being and Quality of life of Iranian Adults with Type 2 Diabetes. *Evidence-Based Complementary and Alternative Medicine* 2014: 1–8. doi:10.1155/2014/619028.
31. Ferraro, K.F., and J.A. Kelley-Moore. 2000. Religious Consolation Among Men and Women: Do Health Problems Spur Seeking? *Journal for the Scientific Study of Religion* 39 (2): 220–234. doi:10.1111/0021-8294.00017.
32. Reig-Ferrer, A.M., D. Arenas, R. Ferrer-Cascales, et al. 2012. Evaluation of Spiritual Well-Being in Haemodialysis Patients. *Nefrologia* 32: 731–742.
33. Allahbakhshian, M., M. Jafarpour, and S. Parvizi. 2011. Spiritual Well-Being of Patients with Multiple Sclerosis. *Iranian Journal of Nursing and Midwifery Research* 16 (3): 202–206.
34. Abu-El-Noor, N.I., and A.S. Radwan. 2015. Assessing Spiritual Well-Being of Arab Muslim Prostate Cancer Survivors: A Reflection for a New Spiritual Health Care Policy. *International Journal of Research in Applied Natural and Social Sciences* 3 (8): 1–14.
35. Clay, K.S., C. Talley, and K.B. Young. 2010. Exploring Spiritual Well-Being Among Survivors of Colorectal and Lung Cancer. *Journal of Religion and Spirituality in Social Work: Social Thought* 29 (1): 14–32.
36. Mystakidou, K., E. Tsilika, E. Parpa, et al. 2008. Demographic and Clinical Predictors of Spirituality in Advanced Cancer Patients: A Randomized Control Study. *Journal of Clinical Nursing* 17 (13): 1779–1785.
37. Tzounis, E., T. Kerenidi, Z. Daniil, et al. 2016. A Qualitative Content Analysis of Spirituality and Religiosity Amongst Greek COPD Patients. *Religions* 7 (3): 22. doi:10.3390/rel7030022.

Cognitive Enhancement for Elderly Facing Dementia with the Use of Cognitive Rehabilitation Therapy Techniques and Psychological Treatment. A Case Study

Georgia Dim Stratakou and Antonia Plerou

Abstract Psychological therapies in order to provide cognitive enhancement have gained some momentum the last decades. The goal of this case study was to evaluate the effects of a cognitive enhancement training program on daily living activities, cognition, and depression in a demented elderly participant. A 6-month training program was proposed for the participant, whose overall evaluation results suggest significant deficits impairment but whose response rate to the proposed tasks of the treatment was interestingly high. However, additional research is needed to overall evaluate the efficacy of the proposed method to elderly adults.

Keywords BCoS • Cognitive enhancement • Cognitive rehabilitation • Depression • Early dementia

1 Introduction

The patient was referred for cognitive assessment, and for cognitive rehabilitation and counseling, due to her cognitive difficulties and emotional disturbance. The medicating participant was suggested to follow a cognitive rehabilitation program for 6 months, which would focus on attention retraining, with the use of paper and pencil and computerized tasks, teaching of compensatory strategies, metacognitive skills training, and counseling therapy. The principle of errorless learning [1] was applied in order to teach the participant new information/skills with fewer mistakes and better learning [2].

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2 Participant Medical History

The participant named P.C. was a 57 years old woman who has received 12 years of education. After a major stressful event in her family, major depressive symptoms arose alongside with mild cognitive issues. The MRI scan imaging revealed foci in the subcortical white matter of the left frontal area and of the right occipital area as well as in the supraventricular white matter of the right frontal area, findings attributed to ischemic microvascular deteriorations, suggesting degenerative white matter change and indicating early stages of the dementia syndrome. Her pharmacological treatment at the time proposed treatment began, was rivastigmine (Exelon), piracetam (Nootropic drug), fluoxetine (Ladose) and for about 10–15 years long she used to take bromazepam (lexotanil) before bedtime.

3 Material

The primary goal of the proposed treatment was to enhance attention (attending, selecting and processing stimuli) in order to improve the quality of her everyday life. For the cognitive assessment, two neuropsychological batteries were administered in two sessions of one and a half hours long for each session, with intervals. The first assessment was the Birmingham Cognitive Screen [3] which provides a sensitive and informative analysis of the cognitive profile of brain-lesioned individuals in a time efficient manner and with maximal inclusions of examinees. The second one, the Boston Diagnostic Aphasia Examination [4] is used to evaluate adults suspected of having aphasia. The Boston Diagnostic Aphasia Examination was administered in order to evaluate the language difficulties as a guide to the cognitive rehabilitation process. During the examination, the patient was kind and cooperative. Instructions were often repeated due to impulsivity, poor comprehension, and confusion.

As the trainee showed signs of mild to moderate aphasia according to the BcoS and further suggested by the BDAE results, it was proposed that the material should be simple, including small phrases, high-frequency words, and objects in order to reduce cognitive load and improve cognitive function [5, 6]. P.C was highly emotionally disturbed, therefore counseling therapy was applied and focused on reducing anxiety with the use of not Cognitive Behavioral Therapy techniques but behavioral techniques Cognitive Behavioral Therapy (CBT) techniques, such as breathing relaxation and progressive muscle relaxation. Cognitive Behavioral Therapy (CBT) principles were taken into consideration, namely principles of recognizing and altering negative thoughts (i.e. I will never do it) and cognitions (i.e. I am a failure) which result in high levels of anxiety and frustration [7]. The objective of this approach was to improve mood and emotional symptoms, in accordance with previous studies that suggest this method to be highly effective in dementia population [8]. This treatment was offered for approximately 30 min every day, especially when there were mood swings. The objective of this approach was to support the trainee to adjust to the current state, enhance the perception of her strengths and weaknesses as well as to establish a therapeutic alliance.

The exact material used for cognitive treatment was paper and pencil exercises and computerized cognitive training exercises. All the paper and pencil exercises mainly involved attention training and were selected from the Attention Workbook from Brainwave R series [9]. Each one of them was repeated for 5 days, in order to stabilize her performance. At the end of each exercise, she had to rate her performance on a scale from 0–5, in order to enhance her awareness [10].

Computerized training exercises were retrieved from Rehacom's software exercises and focused on sustained attention, on selective attention, on working memory, on vigilance, on auditory and visual response, on saccadic training, and on visual exploration. Rehacom is a system of software for computerized cognitive rehabilitation. It is suggested to be effective in several cognitive disorders like stroke, TBI, dementia, ADHD, schizophrenia and other [11, 12].

4 Methods

4.1 Paper and Pencil Process Training

After the initial assessment, the therapist focused on identifying P.C's individual needs, strengths, weaknesses, and goals. During the initial treatment period, participant's attention disorder's symptoms were noticed to be severe due to increased psychological stress. The computerized cognitive training exercises were noted as a source of anxiety, therefore a short training time once or twice a week for approximately 5–10 min was administered in order to ease participant's stress levels.

During this initial period, only paper and pencil exercises were used. In particular, during the first 2 months of therapy, the exercises focused on training (auditory) sustained attention, with the use of numbers and words. During the first days, the participant was asked to respond and write down on a blank sheet of paper a target stimulus provided. While the training was in progress, the participant was asked to circle the target stimulus on the page that the target stimuli were provided. Each exercise lasted approximately 5 min. The number of stimuli was adjusted in respect of the participant's psychological condition. The nine exercises to follow were targeted to (auditory) sustained attention as well as to selective attention and working memory. In particular, the trainee was guided to listen to stimuli with similar characteristics and thereafter to choose (by circling) the target stimulus to the given sheet. Moreover, she was asked to memorize a different target-stimulus iteratively and circle it in the given sheet. In addition, she had to memorize a specific stimulus in order to recognize a specific sequence and perform simple calculations. The time needed for this exercise reached a 5 min period. In the case that calculations were needed, 10 min were additionally provided.

As the treatment was progressing perseveration to the previous exercise and impulsivity (she continued to count or circle without the presence of stimulus) were noted as her main difficulties. For the above-mentioned difficulties, the instructions

were repeated until the time the participant was able to repeat them out loud and rehearse them mentally. In addition, the visual material was used to teach the trainee to encode a group of letters such as vowels and numbers such as zero. In the case of the working memory training, initially, the target stimulus was given visually until it was encoded. The time needed for each exercise was approximately 10 min whereas the practices were limitless.

During the 3rd and 4th month of treatment, the aim of the treatment approach was set to sustain the participant's concentration up to 60 min, through reading and copying exercises. Additionally, participant's sustained attention in a noisy background was trained. The participant had to listen to classic music or the news for 15 min in a medium loud volume and to attend to the therapist who was producing letters or numbers at the same time. The task that she had to perform was to circle in her sheet the target letter or number.

In relation to the copying exercises, the goal was to produce spontaneous writing and keep track of what the therapist was dictating for 1 h. Her main difficulty was to convert phoneme to grapheme, therefore, the therapist would give the alphabet in a visual format so that she could see the letters and find the one she was searching for.

4.2 Computerized Cognitive Training

In reference to the computerized cognitive training, four procedures were followed during the fourth and fifth month of the training period: the attention and concentration, the saccadic training, the response behavior and the calculations. Attention and concentration procedure was designed to train selective attention deficits, sustained attention and to ignore irrelevant stimuli. Each session's time length was approximately 15 min. The participant completed 35 sessions in a 5 month period. The Reaction Behavior procedure was designed to train response speed in visual stimuli. The time length of each session was 15 min. She completed 27 sessions over a 4 month period. The Saccadic training was designed to train visual exploration. The time length of each session was 15 min over a 4 month period and she completed 21 sessions overall. There were no related sessions for the aforementioned procedures in the middle of the training period for 30 days. The Calculations procedure was designed to train arithmetical cognitive skills. The training involves a variety of tasks and the participant completed 22 sessions of 15 min time length.

The participant, due to family reasons, withdrew on the fifth month of treatment, therefore the proposed 6-month period of treatment was not completed and reassessment didn't take place. It should be noted that during the training period, P.C. was highly cooperative and had a strong will to make it.

5 Results

5.1 Overall Assessment Performance

The initial results are referred both to cognitive and behavioral assessment and the detailed description is to follow. The examinee showed dysfunction in several domains of cognition (BcoS). In terms of memory assessment, the results indicated impairment. Short-term memory for verbal information was impaired both for immediate encoding and retrieval as well as for immediate recognition. Delayed verbal memory using free recall response choice was impaired whereas delayed verbal memory using delayed recognition was slightly better (7/15, n.13, 6/10, n.9).

In the case of attention performance, the spatial attention results suggested a significant impairment in terms of controlled attention, namely in concentration, impulsivity and working memory. Auditory controlled attention and relative components were mainly impaired (working memory, sustained attention, and selective attention). The rule finding and concept switching task results suggested impairment, mainly in terms of the comprehension and perseveration aspect.

In respect of praxis evaluation, the trainee presented poor skills in figure organization (5/47, n 42). In the case of using multiple objects, she presented a significant impairment in terms of performing a sequence of actions with multiple objects (0/12, n.11). However, she could recognize without naming the target object and its use ("in the dark"). In gesture production, she presented a significant impairment for both transitive and intransitive ones. There were spatial and semantic errors, however, in the intransitive gestures, she was able to describe the concept of use of the objects but not to imitate the appropriate action. In gesture recognition of intransitive actions, semantic errors were noticed. Nevertheless, in recognition of the transitive ones her performance was intact. In imitation, her performance was poor due to impulsivity, poor working memory, and perseveration in relative previous tasks.

In respect to language performance, the examinee showed semantic difficulties, difficulties in naming (4/14, n.13), difficulties in recognizing and distinguishing different concepts. Her speech was fluent but non-specific rather than descriptive, sometimes with no relevancy to the subject and with impaired syntactic processes. Instructions should be repeated several times to be understood (2/3, n.3). In writing words and non-words she presented a slow rate and she had difficulties in generating the exception words correctly. In the case of reading words and non-words was noticed to be intact. In terms of number perception, the examinee presented a significant impaired performance in reading complex numbers, prices and perceiving quantities. Nevertheless, she presented difficulties in performing simple calculations. The reading time was noticed to be intact in the case that time was represented digitally. The BDAE examination suggested that she showed mild to moderate aphasia, reinforcing the BcoS language impairment results.

5.2 Paper and Pencil Task Performance

According to the cognitive training with paper and pencil tasks, in the first pool of exercises, in three out of five exercises, the trainee presented an improvement in her performance as well as in the awareness of her performance. For the five of the nine exercises that followed, her performance was improved and stabilized. In the rest four tasks, a variance in her performance or inability to complete the given task was noticed. Additionally, for the two exercises that were used to train sustained attention in a noisy background a slight improvement was noticed. Nevertheless, her performance was not stabilized, while impulsivity (keep circling on the absence of target stimulus) and fatigue for the auditory stimuli was noticed.

In the copying task, in the beginning of the training period, she had difficulties to write one sentence, but as the training was progressing she would write up to 20 sentences within 60 min. In the reading task, she became able to read gradually for 60 min, by pointing the text with her finger. In the very first days, she needed three intervals and she would lose track of the text at least 10–15 times. There were variances in her performance, regarding her mood or fatigue. As the training was progressing, she needed less help (3–4 times). However, there was 1 day at the end of the training that she managed to read without any help.

5.3 Computerized Training Procedure Performance

For the Attention and Concentration procedure, results are presented in Figs. 1 and 2. Especially, Fig. 1 shows the median reaction time for all session's trials (in the x-axis and y-axis the task number and the median reaction time is

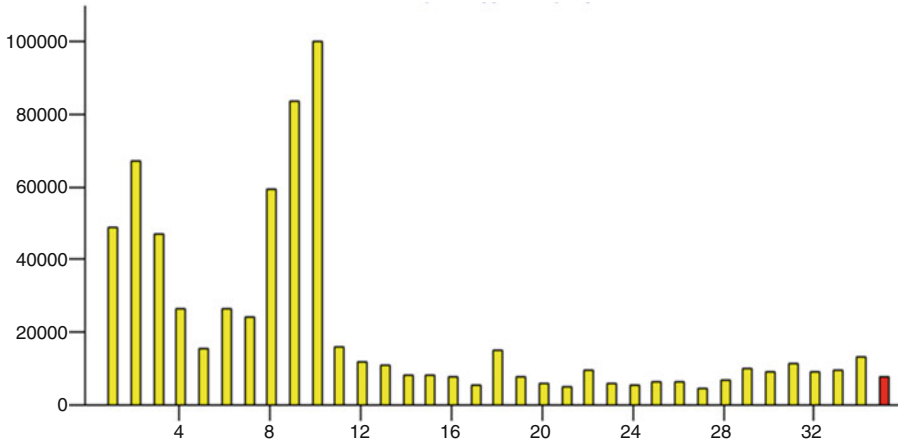

Fig. 1 Median reaction time for all session's trials for the Attention and Concentration procedure

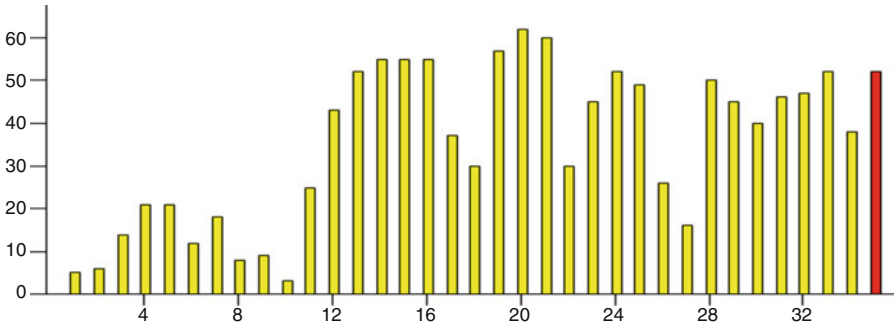

Fig. 2 Number of completed trials for each session for the Attention and Concentration procedure

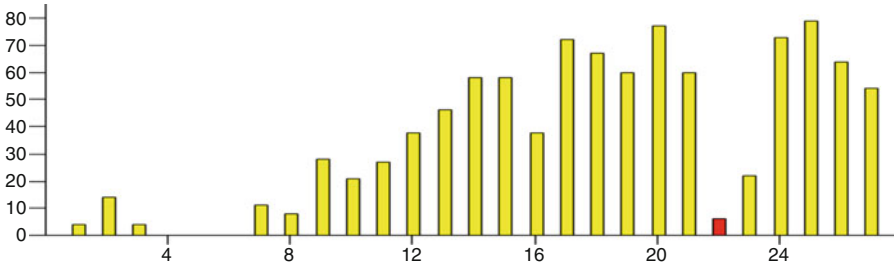

Fig. 3 Number of correct reactions in each session for the Reaction Behavior procedure

presented respectively) and the Fig. 2 shows the number of trials she completed in each session (in the x-axis and y-axis the task number and the number of the participant efforts are presented respectively). The results suggest that the participant's ability to concentrate and select the appropriate stimuli was improving. Namely, the median reaction time gradually decreases and the number of complete trials in each session gradually increases. Nevertheless, significant performance variances are observed.

For the Reaction Behaviour, procedure results are shown in Figs. 3 and 4. In Fig. 3 the number of correct reactions in each session is presented (in the x-axis and y-axis the task number and the number of the provided correct reactions are presented) and in Fig. 4 the median reaction time for all session's trials is visualized (in the x-axis and y-axis the task number and the median reaction time is presented). The participant reaction was decreasing gradually (except from 21–23, level 3 was reached) and correct reactions were increasing but with variances.

In the case of the Saccadic training, results are shown in Figs. 5 and 6. Figure 5 presents the median reaction time on the left side of the screen (in the x-axis and in y-axis the task number and the median reaction time is presented) while in Fig. 6 the median reaction time on the right side of the screen is visualized (In the x-axis and in y-axis the task number and the median reaction time is presented). Her

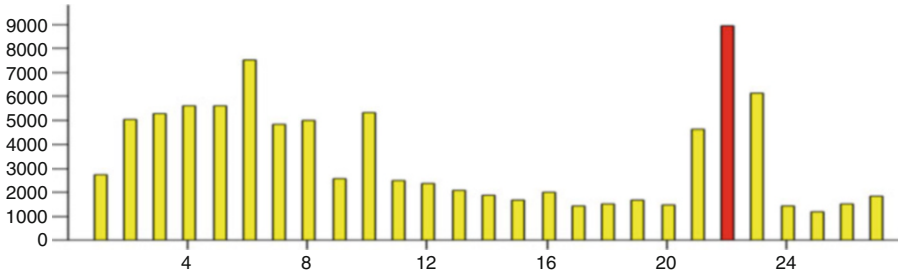

Fig. 4 Median reaction time for all session's trials for the Reaction Behavior procedure

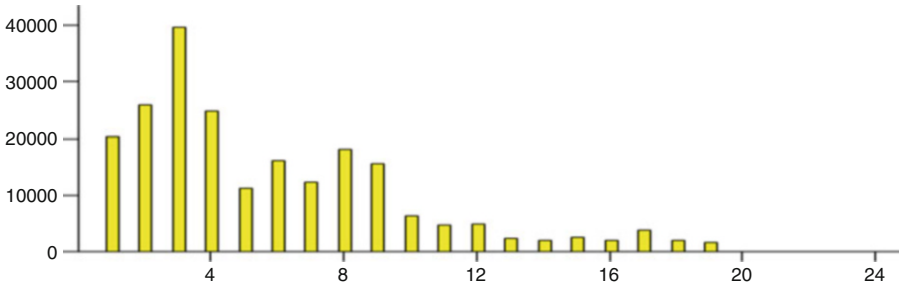

Fig. 5 Median reaction time on the left side of the screen for the Saccadic training procedure

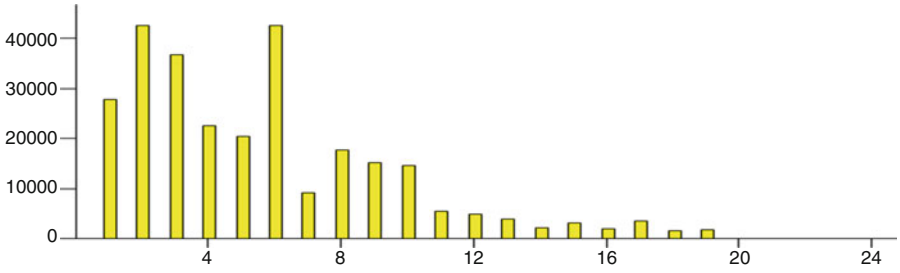

Fig. 6 Median reaction time on the right side of the screen for the Saccadic training procedure

saccadic movements presented gradually an increscent, while her mild to moderate representation disorder was decreased.

For the Calculations procedure, results are shown in Figs. 7 and 8. Figure 7 shows the difficulty level she reached (9/42) (In the x-axis and y-axis the task number and the difficulty level is presented) while Fig. 8 presents the number of correct solutions for all the exercises of each session (in the x-axis and y-axis the task number and the correct responses is presented). As the difficulty level increases she needs more time to solve the task while her performance varies.

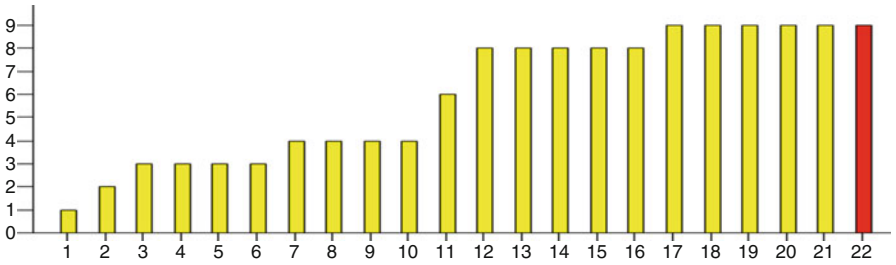

Fig. 7 The level of exercises for the Calculations procedure

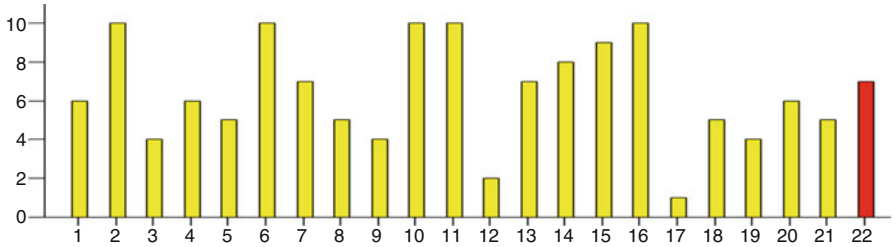

Fig. 8 The number of correct calculations for all the exercises of each session for the Calculations procedure

6 Discussion and Conclusions

The paper objective is to provide an overview of the way that multi-tasked psychological therapies could be essential in older people. Additionally insights on the efficiency of modifications of therapeutic procedure and content while working with this age are provided. Research findings suggest that the proposed approach was highly effective in reference to the participant deficits impairment. This study was a pilot approach of a cognitive enhancement training program of a single participant over a short period of time. Although these results are not generalized, they shed light into several insights within these research implementation. A discrete approach in order to encourage to caregivers on supporting patient's everyday effort for cognitive enhancement under psychologist guidance by means of specified daily activities is ongoing. Authors future suggestions lean towards the implementation of an equivalent rehabilitation program on a large number of subjects from various institutions to obtain a generalizable assessment of its effects on cognitive function, emotional state, and daily living activities of elderly individuals with dementia.

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References

1. Baddeley, A., and B.A. Wilson. 1994. When Implicit Learning Fails: Amnesia and the Problem of Error Elimination. *Neuropsychologia* 32 (1): 53–68.
2. de Werd, M.M.E., D. Boelen, M.G.M.O. Rikkert, and R.P.C. Kessels. 2013. Errorless Learning of Everyday Tasks in People with Dementia. *Clinical Interventions in Aging* 8: 1177–1190. doi:10.2147/CIA.S46809.
3. Humphreys, G.W., W.L. Bickerton, D. Samson, and M.J. Riddoch. 2012. *BCoS Cognitive Screen*. London: Psychology Press.
4. Goodglass, H., and E. Kaplan. 1972. *The Assessment of Aphasia and Related Disorders*. Philadelphia: Lea and Febiger.
5. Lavie, N. 2005. Distracted and Confused?: Selective Attention Under Load. *Trends in Cognitive Sciences* 9 (2): 75–82. doi:10.1016/j.tics.2004.12.004.
6. Belke, E., G.W. Humphreys, D.G. Watson, A.S. Meyer, and A.L. Telling. 2008. Top-Down Effects of Semantic Knowledge in Visual Search are Modulated by Cognitive But Not Perceptual Load. *Perception and Psychophysics* 70 (8): 1444–1458. http://doi.org/10.3758/PP.70.8.1444.
7. Beck, A.T. 1976. *Cognitive Therapy and the Emotional Disorders*. New York: International Universities Press.
8. Paukert, A.L., J. Calleo, C. Kraus-Schuman, L. Snow, N. Wilson, N.J. Petersen, M.E. Kunik, and M.A. Stanley. 2010. Peaceful Mind: An Open Trial of Cognitive-Behavioral Therapy for Anxiety in Persons with Dementia. *International Psychogeriatrics/IPA* 22 (6): 1012–1021. doi:10.1017/S1041610210000694.
9. Malia, K.B., K.C. Bewick, K.J. Raymond, and T.L. Bennett. 1997. *Brainwave-R: Cognitive Strategies and Techniques for Brain Injury Rehabilitation*. Texas: ProEd Inc.
10. Lincoln, N., M. Majid, and N. Weyman. 2000. Cognitive Rehabilitation for Attention Deficits Following Stroke. In *Cochrane Database of Systematic Reviews*, ed. N. Lincoln. Chichester, UK: Wiley.
11. Rohling, M.L., M.E. Faust, B. Beverly, and G. Demakis. 2009. Effectiveness of Cognitive Rehabilitation Following Acquired Brain Injury: A Meta-Analytic Re-Examination of Cicerone et al.'s (2000, 2005) Systematic Reviews. *Neuropsychology* 23 (1): 20–39. doi:10.1037/a0013659.
12. Tárraga, L., M. Boada, G. Modinos, A. Espinosa, S. Diego, A. Morera, M. Guitart, J. Balcells, O.L. López, and J.T. Becker. 2006. A Randomised Pilot Study to Assess the Efficacy of an Interactive, Multimedia Tool of Cognitive Stimulation in Alzheimer's Disease. *Journal of Neurology Neurosurgery and Psychiatry* 77 (10): 1116–1121. doi:10.1136/jnnp.2005.086074.

Biobibliometrics (UGDH-TP53–BRCA1) Genes Connections in the Possible Relationship Between Breast Cancer and EEG

Yannis Martzoukos, Sozon Papavlasopoulos, Marios Poulos, and Maria Syrrou

Abstract In recent years there has been an increasingly amount of data stored in biomedical Databases due to the breakthroughs in biology and bioinformatics, biomedical information is growing exponentially making efficient information retrieval from scientist more and more challenging. New Scientific fields as Bioinformatics seem to be the tool needed to extract scientifically important data based on experimental results and information provided by papers and journals. In this paper we are going to implement a custom made IT system in order to find connections between genes in the breast cancer pathways such the BRCA1 with the electrical energy in the human brain with UGDH gene via the TP53 tumor gene. The proposed system will be able to identify the appearance of each gene ID and compare the coexistence of two genes in PubMed articles/papers. The final system could become a useful tool against the struggle of scientists and medical professionals in the near future.

Keywords MedLine • Bioinformatics • Co-citation coupling • Bibliometrics • PubMed • Unattended system • Gene

1 Introduction

Following the breakthroughs in biology and bioinformatics, biomedical information is growing exponentially making efficient information retrieval from scientist more and more challenging. New technologies, such as new generation sequencing [1] are producing vast amounts of data which cannot be categorized or used "as is". Across the scientific community there have been a number of tools and methods for

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scientists to use in order to validate data and results produced by experiments or projects. Specifically, a vast amount of techniques, combining biomedical data and computing science have been used to facilitate scientific research, giving birth to a newly created field called Bioinformatics [2]. Bioinformatics is a scientific field whose main purpose is to analyze statistically and categorize the information flow produced by scientific experiments or laboratory work.

With the help of Bioinformatics scientist are able to analyze scientific data and evaluate large data sets, a task which was immensely difficult during the past decades because of the lack of computational power and the lack of an inter-disciplinary field which could be used, like Bioinformatics. As mentioned above, during the last few years vast amount of biomedical data is starting to accumulate from the systematic and non-systematic DNA sequencing. It is imperative to find a technique in order to utilize the data required for scientific research in the future. In order to do so several attempts have been made to extract information from scientific papers and/or journals freely available online. A major attempt was made some years back by Stapley et al. [3]. The group of researchers have introduced term "biobibliometrics" to describe the use of bibliometric techniques on biological related papers. Stapley's implementation could verify the bibliometrical connection between biological data (genes, proteins etc.) based solely on the rate of their common appearances in the abstracts of scientific papers and journals.

Another attempt on tackling the problem created by the vast amount of biological data accumulating in online databases was made by Martzoukos et al. [4] where the proposed solution is an unattended platform which combines a number of computing programs, bioinformatics and biobibliometrics. The system is able to create connections between genes and proteins by using and analyzing the vast numbers of papers freely available via PubMed [5].

Based on an investigated issue [medical Hypothesis] Electroencephalography (EEG) is considered one of the most important research areas related to how information is supplied by the brain. The partial decoding of EEG findings has shown that it can be used for diagnostic and prognostic purposes [6]. In recent studies some brain activities recorded via EEG have been shown to be associated with particular genes and proteins [6] that play a crucial role in the normal (or, conversely, the abnormal) development of breast cells [7]. Other studies have determined that the human electroencephalogram is influenced by the genome and also by a significant number of gene types (SGIP1, ST6GALNAC3, and UGDH) [8]. Moreover, it has been shown that specific hereditary genes such as BRCA1 and BRCA2 and proteins such as 14-3-3 [9] affect neuronal reactivity. Genetic studies have demonstrated that the aforementioned genes and proteins are associated directly with two types of breast cancer [10–14]: hereditary and non-hereditary. Unfortunately there has been no data proving the direct connection between genes that affect EEG and BRCA genes.

In this paper, we are going to implement a custom made IT system in order to find connections between genes in the breast cancer pathways such as BRCA1 and BRCA2 [14], with EEG which consist of a significant medical issue. Thus, as first step we implement a search in the free full-text archive of biomedical and

life sciences journal literature at the U.S. in order to be ascertained the possible association between the selected genes. Thereinafter, we detect a third common associated gene in order to apply the basic bibliometric rule [15]. This is the TP53 gene which provides instructions for making a protein called tumor protein p53 (or p53). This protein acts as a tumor suppressor, which means that it regulates cell division by keeping cells from growing and dividing too fast or in an uncontrolled way. In this way this gene could be explained in a bibliometric foundation the possible activity between aforementioned genes.

Thus, the paper is divided in the following three parts: The "methodology" in which is explained the methodology of this study. The second part concerns the "results" and the third part includes some discussion and the final conclusion.

2 Methodology

The primary and basic operational capacity of the system will be the ability to search for a specific gene supplied by the user and identify any connections or interactions with other genes based on how frequently they are met together in several papers stored in PubMed central database [4]. Based on the principals of bibliometrics and statistics the system will take into account a series of parameters in order to create a weight-graph between genes. The basic parameters will be:

- Frequency of the co-appearance of two genes in the abstracts of papers, freely available online with no restrictions
- Gene Co-citation coupling [15]
- Analysis of related genes in pairs
- Analysis of the probability of relation between genes that co-exist in several papers based on the PubMed Central Database

2.1 High-Level Design

The system will constantly poll for human genes and analyze their appearance in papers stored in PubMed [4]. It will then store and link this information when it is requested by the user. For example when Gen1 is analyzed the system will store the PID (Paper ID) of PubMed for each paper that contains Gen1. Then the same procedure is going to be followed for Gen2, Gen3 ... GenN. The system based on the user input will construct relations between genes following the basic principles mentioned above. This routine will be running in real time and will update the information of each gene since the amount of papers being submitted every day could change the final graphs dramatically.

As seen on Table 1 we could construct a relation node between Gen1 and Gen2 with the weight of 3.

Table 1 Constructing relations between genes

Gene ID		Gene ID
Gen1		Gen2
PID		PID
0001	→	**0001**
0003		**0002**
0005	→	**0005**
0006	→	**0006**
0010		**0011**

Table 2 Co-appearances between genes

	SGIP1	ST6GALNAC3	UGDH	BRCA1	BRCA2
SGIP176 papers		1 (1.3%)	2 (2.6%)	4 (5.2%)	2 (2.6%)
ST6GALNAC373 papers	1 (1.36%)		1 (1.36%)	3 (4.1%)	4 (5.4%)
UGDH270 papers	2 (0.74%)	1 (0.37%)		10 (3.7%)	10 (3.7%)
BRCA126,566 papers	4 (0.01%)	3 (0.01%)	10 (0.037%)		11,656 (43%)
BRCA214,864 papers	2 (0.01%)	4 (0.005%)	10 (0.06%)	11,656 (78%)	

3 Experimental Results

We are going to use the findings of the previous study [medical hypothesis] to identify possible bibliographic relationship between the genes SGIP1, ST6GALNAC3, UGDH which are associated with the EEG and the genes BRCA1 and BRCA2 which are associated with the Breast Cancer [6] and the brain [7]. In order to do so, we have applied a searching mechanism via PCM of PubMed services and the results are presented in Table 2.

3.1 Investigating the Relations Between Genes

In this step we correlate the possible connection between the BRCA1 and UGDH genes with TP53. By analyzing the data from Table 1 and analyzing the connections between the above mentioned genes we obtained the following results which are depicted in Fig. 1.

According to the above results now it is possible to apply the co-citation normalization procedure [16] which is based on the following equation

$$norm = \frac{|in\,(\mathbf{BRCA1}) \cap in\,(\mathbf{UGDH})|}{in\,(\mathbf{BRCA1}) \cup in\,(\mathbf{UGDH})} = \frac{in(TP53)}{10} = \frac{4}{10} = 0.25$$

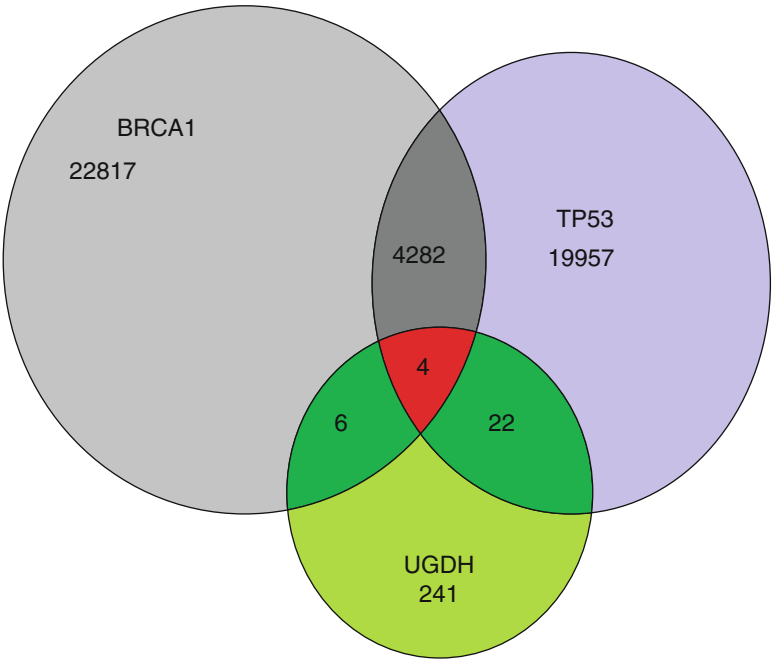

Fig. 1 The intersection between BRCA1 and UGDH genes with TP53

The interpretation of this result indicates that the value 0.25 gives a possible bringing between BRCA1 and UGDH genes at 25% and this lead the ascertainment that a possible research in this issue obtains a higher successful rate than previous attempts.

3.2 Observing the Relation Between Genes Over Time

In order to visualize the relation between genes BRCA1, UGDH and TP53 we are going to investigate the appearance of each gene, in PubMed Central Database, over the past 2 years with 1 month interval (Fig. 2). We have also determined the relation between TP53 and BRCA1, and between BRCA1 and UGDH over the period of 2 years, again with 1 month interval (Fig. 3). All the data used to construct the graphs in Figs. 2 and 3 can be observed in detail in Table 3.

As we can observe in Figs. 2 and 3 there is a noticeable increase in TP53 appearances in journals, BRCA1 appearance rates seem to be stable and there is a slight increase in the appearances of UGDH. We can also notice that there is a slight increase of the biobibliometric relation between TP53 and BRCA1. Finally we identify an almost stable relation between BRCA1 and UGDH.

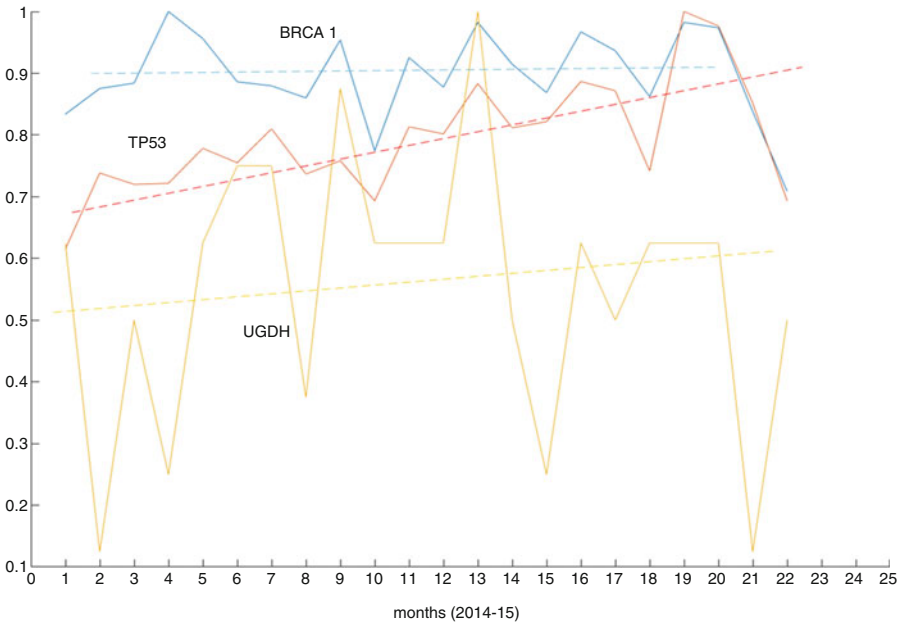

Fig. 2 Appearances of BRCA1, TP53 and UGDH genes in PubMed Central over the past 2 years

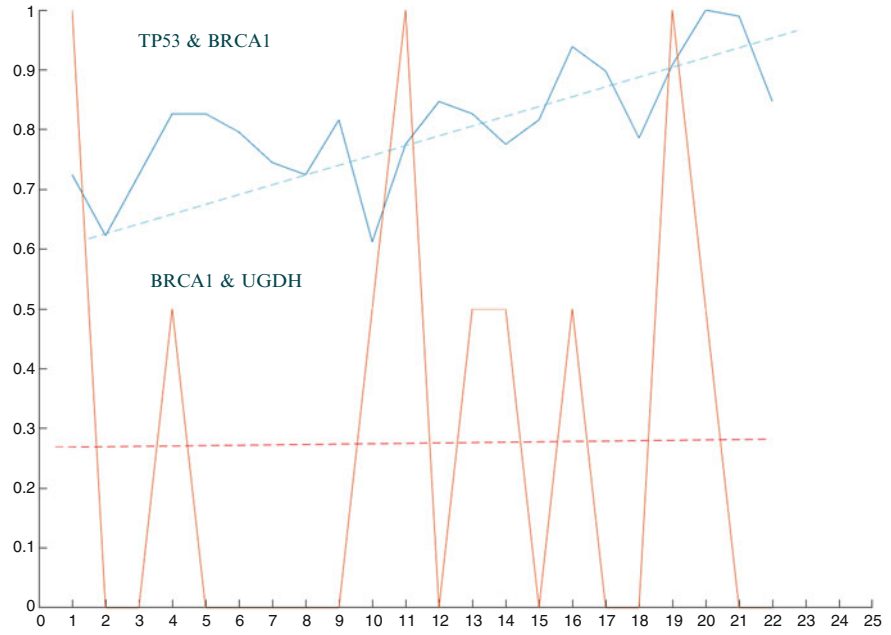

Fig. 3 Number of co-appearances between TP53 and BRCA1 and between BRCA1 and UGDH genes in PubMed Central over the past 2 years

Table 3 Appearance and co-appearances of BRCA1, TP53 and UGDH genes

	BRCA1	TP53	UGDH	TP53 & BRCA1	TP53 & UGDH
Jan-14	1576	1745	24	314	2
Feb-14	381	369	5	71	2
Mar-14	400	443	1	61	0
Apr-14	404	432	4	71	0
May-14	457	433	2	81	1
Jun-14	437	467	5	81	0
Jul-14	405	453	6	78	0
Aug-14	402	486	6	73	0
Sep-14	393	442	3	71	0
Oct-14	436	455	7	80	0
Nov-14	354	416	5	60	1
Dec-14	423	488	5	76	2
Jan-15	1725	1984	31	378	7
Feb-15	401	481	5	83	0
Mar-15	449	530	8	81	1
Apr-15	418	487	4	76	1
May-15	397	493	2	80	0
Jun-15	442	532	5	92	1
Jul-15	428	523	4	88	0
Aug-15	394	445	5	77	0
Sep-15	449	600	5	89	2
Oct-15	445	586	5	98	1
Nov-15	383	511	1	97	0
Dec-15	324	416	4	83	0

4 Discussion and Conclusion

The novel approach (Breast Cancer-EEG) described herein could provide a ground breaking tool for the scientific community, improving breast cancer prognosis and treatment. We have managed to identify the crucial part which the TP53 gene plays regarding the identification of possible relation between EEG and breast cancer occurrences. Based on the graphs provided we are fairly sure that TP53 could be the guide and the common link between genes affecting EEG and Cancer growth such as UGDH and BRCA1. For this reason we believe that further pursue of this work could be made by taking into account the crucial role of the TP53 gene.

Having identified a gap between the results given by scientific experiments and the amount of scientific data stored in large Biomedical databases we have introduced a statistical and computational system that will be able to fill the void. The goal of this research is to provide the medical field with an alternative yet promising use of a well-known, reliable prognostic tool. Until now, EEG has not been fully explored as a possible prognostic tool for cancer. The outcomes of this

study may prove to be widely beneficial and could pioneer developments in several fields, including medicine, biology, biochemistry, pharmacology, computer science, etc. Although this research may be perceived as a multi-dynamic opportunity for science evolution, it also places emphasis on the patient's quality of life, clearly manifesting the humanitarian dimension of science.

We believe that the statistical system will become a useful tool in the hands of researchers around the world. The implementation will provide a mean to connect, seemingly unconnected human genes. Even though, several human genes are already connected based on experiments held in laboratories around the world, there is no tool which is able to use the large datasets of information contained in papers published online in order to extract crucial information and form connections between any human genes, the proposed system could become a useful tool for scientist in the future. Additionally, based on the graphical representation of gene connections scientists may be able to identify relations between diseases that might seem unrelated before. This assumption could possibly be extremely crucial since the statistical system could be a useful tool for Doctors and physicians in the future.

References

1. Metzker, M.L. 2010. Sequencing Technologies—The Next Generation. *Nature Reviews Genetics* 11: 31–46.
2. Cohen, Jacques. 2004. Bioinformatics—An Introduction for Computer Scientists. *ACM Computing Surveys* 36 (2): 122–158.
3. Stapley, B.J., et al. 2000. Biobibliometrics: Information Retrieval and Visualization from Co-occurrences of Genenames in Medline Abstracts. *Pacific Symposium on Biocomputing* 5: 526–537.
4. Martzoukos, Y., et al. Biobibliometrics & Gene Connections, IISA2015.
5. http://www.ncbi.nlm.nih.gov/pmc/ (10/04/2015).
6. Poulos, Marios, Theodoros Felekis, and Angelos Evangelou. 2012. Is it Possible to Extract a Fingerprint for Early Breast Cancer Via EEG Analysis? *Medical Hypotheses* 78 (6): 711–716.
7. Nielsen, Finn Cilius, Thomas van Overeem Hansen, and Claus Storgaard Sørensen. 2016. Hereditary Breast and Ovarian Cancer: New Genes in Confined Pathways. *Nature Reviews Cancer*. http://www.nature.com/nrc/journal/vaop/ncurrent/full/nrc.2016.72.html.
8. Hodgkinson, Colin A., Mary-Anne Enoch, Vibhuti Srivastava, Justine S. Cummins-Oman, Cherisse Ferrier, Polina Iarikova, Sriram Sankararaman, et al. 2010. Genome-Wide Association Identifies Candidate Genes that Influence the Human Electroencephalogram. *Proceedings of the National Academy of Sciences* 107 (19): 8695–8700.
9. Yoshida, Kiyotsugu, and Yoshio Miki. 2004. Role of BRCA1 and BRCA2 as Regulators of DNA Repair, Transcription, and Cell Cycle in Response to DNA Damage. *Cancer Science* 95 (11): 866–871.
10. Sommer, B.J., J.J. Barycki, and M.A. Simpson. 2004. Characterization of Human UDP-Glucose Dehydrogenase. *Journal of Biological Chemistry* 279: 23590.
11. Benzinger, A., N. Muster, H.B. Koch, J.R. Yates, and H. Hermeking. 2005. Targeted Proteomic Analysis of 14-3-3 Sigma, a p53 Effector Commonly Silenced in Cancer. *Molecular & Cellular Proteomics* 4: 785–795.
12. Dougherty, M.K., and D.K. Morrison. 2004. Unlocking the Code of 14-3-3. *Journal of Cell Science* 117: 1875–1884.

13. Umbricht, C.B., E. Evron, E. Gabrielson, A. Ferguson, J. Marks, and S. Sukuma. 2001. 14-3-3r (Stratifin) is an Early Event in Breast Cancer. *Oncogene* 20 (26): 3348–3353.
14. Gail, M.H., W.F. Anderson, M. Garcia-Closas, and M.E. Sherman. 2007. Absolute Risk Models for Subtypes of Breast Cancer. *Journal of the National Cancer Institute* 99 (22): 1657–1659.
15. Small, Henry. 1973. Co-citation in the Scientific Literature: A New Measure of the Relationship Between Two Documents. *Journal of the Association for Information Science and Technology* 24 (4): 265–269.
16. Li, Fang, et al. 2009. Applying Association Rule Analysis in Bibliometric Analysis-A Case Study in Data Mining. Proceedings of the Second Symposium International Computer Science and Computational Technology (ISCSCT '09), Huangshan, P. R. China, 26–28 December, pp. 431–434.

Diagnostic Evaluation of Huntington's Disease Within the Frame of Bioinformatics

Catherine Bobori

Abstract Huntington's disease is an autosomal dominant neurodegenerative disorder characterized by motor and cognitive impairment. At the early stages of the disease scientists have observed psychological changes and differences in patients' alpha, beta and theta powers. The early diagnosis of Huntington's disease is very important in order to administrate the correct medication to the patients and slow down the progress of the disease. This paper aims to suggest a protocol in which experiments could be based on, in an attempt to make an early diagnosis of the disease by taking into consideration the patients' electroencephalographic recordings during the early stages of the disease, as well as the methods of processing and classifying those EEG signals.

Keywords Huntington's disease • Early diagnosis • Bioinformatics • EEG • ERP

1 Introduction

Huntington' disease (also known as Huntington's chorea) is an autosomal-dominant, neurodegenerative, monogenic, fully penetrant [1] and inherited disorder of the brain. The disease is caused by the mutation of the IT15 gene that produces a protein called huntingtin (htt) on chromosome 4p16.52 [2]. The gene was mapped in 1983 and cloned in 1993. The mutant protein (m-htt) results from the expanded repetition of the trinucleotide CAG leading to a polyglutamine strand toxic for the brain. As a result, the produced protein causes chorea, dystonia, mental disabilities such as irritability, anxiety, depression, reduced thinking ability etc., cell death and eventually death [2, 3].

In 1872 George Huntington first published a paper describing the disease and its inheritance pattern. In early 1980s gene mappers found the location of the gene

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using blood samples of the families with Huntington's disease. In 1993 the gene was cloned and the presymptomatic diagnosis of the disease became possible [3]. Formal diagnosis is based on chorea, dystonia and bradykinesia [1].

2 Huntington's Disease

2.1 Pathogenesis

Huntington's disease is an autosomal dominant disease, thus one mutated gene is sufficient to cause it [4]. Huntington's disease gene is primarily expressed in the brain, though moderate amounts are present in the liver, heart and lungs. Findings suggest that the amount of the gene that exists in the testicles can be a cause of the mutation. In cases where the expansion is between 28 and 35 repeats, the number of the expansions can be increased during spermatogenesis. As a result, when the defective genes are passed through generations by males, the number of repetitions increases. Therefore, the age of onset of symptoms is dramatically reduced. This is known as genetic anticipation [5]. If the mutation happens during spermatogenesis then the mutation is considered as a new one (de novo mutation) and is usually in the borderline (28–35 CAG repeats) [3, 6].

It is proven that the number of CAG repeats varies from person to person; as a result variations in the genotype and the phenotype have been observed. Sometimes the variations between the genotype and the phenotype are linked and others have no relation at all. One common variation is the age of onset of the disease. The expansion of CAG repeats is related at a 60% with the onset of symptoms and how severe the disease would be. The more repetitions results in a faster and more severe disease [5].

2.2 Symptoms

In Huntington's disease symptoms can emerge at any time since birth and after, though they primarily occur between the ages of 35–50 years (40–50 CAG repeats). In juvenile cases the symptoms arise before the age of 20 and usually have a larger number of CAG repeats (>55) [6]. Before the onset of symptoms the patients are healthy.

It is estimated that 5–7 individuals per 100,000 are affected by the disease. Most of them are white people (higher frequency of 28–35 repeats); while the percentage in Asia and Africa is much lower (higher percentage of dentatorubral pallidoluysian atrophy (DRPLA)).

Even though one may inherit the identical genes from his parents, there are several phenotypic changes that make him different. As a result, genetic diseases are characterized by great complexity. At first, the symptoms of the disease are usually

chorea, abnormal and involuntary movements (facial as well as motor), reduced thinking ability, memory loss, irritability, anxiety, depression, obsessive behavior and other mood changes. The disease destroys nerve cells (neurons) in certain parts of the brain [2]. Even though, in many cases the patients initially show emotional or cognitive changes, the onset of the disease is determined based on the progress of chorea. This occurs due to the acknowledgement of chorea to be the determinant factor of the disease [4, 6].

Huntington's disease mainly affects the basal ganglia region (usually there is a loss of about 50–60% [4]). The basal ganglia are in the forebrain (forebrain) and consists of the striatum and globus pallidus (this is the area of the brain organizes muscle movement and emotional functions in the brain) [2].

2.3 Early Diagnosis

As Huntington's disease is an incurable hereditary disease with autosomal character, the early diagnosis, the confirmation of the disease and the rapid administration of the appropriate medication is of great importance. One of the most common diagnostic techniques is prenatal diagnosis, as well as pre-symptomatic testing [2]. Furthermore, psychological tests that can evaluate the patients' behavior are suggested. In addition, doctors perform MRI, PET and CT scans in order to have an overall clinical evaluation of the patients' condition. Recently, with the use of an electrical microscope and statistical analysis doctors were able to estimate the number of neurons [7].

2.4 EEG in Huntington's Disease

The electroencephalogram (EEG) is a process of making a recording of the brain waves or otherwise the imaging of brain function. The waveforms are of different frequency and amplitude and are measured in mV [8]. These waves are four, "delta" that extends from 0,1 to 4 Hz, "theta" in the frequencies of 4–7 Hz, "alpha" in the frequencies 7–13 Hz and "beta" in the frequencies 13–60 Hz.

The reason why the electroencephalograms are selected for the diagnosis of diseases is that they are a non-invasive and non-destructive test procedure. The registration is simple and cheap in cost and provides data on the evolution of the Central Nervous System (CNS). Additionally, it provides information which helps in the diagnosis and prognosis of diseases as well as spatial accuracy in brain points near the skull and temporal resolution which gives a very detailed and accurate sample. Generally, an electroencephalogram can detect and localize brain lesions [9].

3 Diagnostic Protocol

This paper aims to suggest a model that is based on brain activity measurements proposing a way for early diagnosis in people who are likely to develop the disease. The validity of the protocol will be tested using data from patients as well as control subjects.

In particular, patients that will participate, should have a confirmation that they carry the mutant gene and that they are in the first stages of the disease. The control subjects will be of the same age as the patients. The number of the patients and the control subjects will be equal.

The aim is to observe the brain activity of patients with Huntington in the early stages of the disease and any anomalies which may appear during the execution of some procedures. The machine to be used to record the brain activity of patients is the MP150 BIOPAC namely Dual Wireless EEG BioNomadix Pair, carrying the AcqKnowledge software which is capable of recording, saving and processing EEG signals. Also, the machine has a transceiver which has electrodes to be placed in suitable positions in order to take the right measurements.

The placement of the electrodes will be made at specific points related to the activities that the examinee has to carry out during the experiment. The brain points to be examined are the frontal lobe whose functions are related to problem solving, speech, personality and emotions, attention, concentration and judgment. The temporal lobe, which is associated with memory, hearing, thinking and organizing. Finally, the side lobe which is related to reading. All of these areas have specific names and can be represented by the Global System 10–20 for the description and application of electrode placement on the human head (Fig. 1). The exact names of areas in which the electrodes will be applied for the experiment are Fp1, Fp2, F7, F8, Fz, T3, T4, Pz [10].

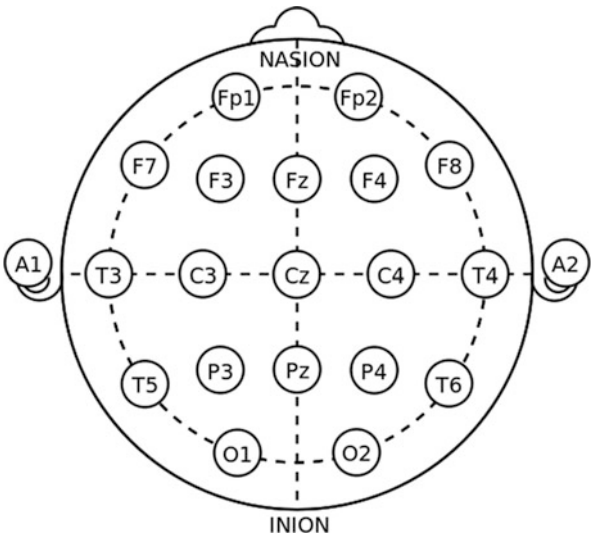

Fig. 1 International 10–20 system for EEG

The data will be saved and processed to remove noise and soothing. Using MATLAB and specifically its toolbox, EEGLAB, the data will be processed and classified in order to get the results that will lead to the early diagnosis of the disease based on the encephalographic recordings of patients and control subjects. Three algorithms are suggested to be used for classification, Event—Related Potential Algorithm (ERP), Linear Discriminant Analysis (LDA) and Time Series Analysis (TSA). At the same time, importance will be given to the examinees psychological tests results as well as their behavior and appearance. The suggested tests for this protocol are Raven's Test, MMPI and California's Verbal Learning Test [9–12].

3.1 Bioinformatics Algorithms

Particularly, the algorithms to be used for processing the EEG recordings are Event Related potential (ERP), Linear Discriminant Analysis (LDA), Time Series Analysis (TSA). These algorithms will be used for the collection, classification and analysis of data of brain function.

3.1.1 Event Related Potential

An ERP is a non—invasive electrophysiological examination, in which the brain responses are observed after stimulus. The ERP exploits the temporal information of the signals, using the time values from non-processed electroencephalograms as data [13]. The ERP is temporally constant in eliciting an event. The latency of each ERP element must remain constant in all tests that are used to calculate the average signal. The two most common ERP measurement approaches are the identification of peaks and edges that have been observed in terms of polarity and the order of appearance in the waveform [13].

The ERP waveforms consist of a series of positive and negative voltage changes which are dependent on a number of factors. The ERPs provide a continuous measurement process between a stimulus and a response (even when there is no reaction), making it possible to determine which response was affected. Therefore, ERPs are suitable for research on the speed of neuronal activity [11].

One of the most popular ERP is P3. P3 wave is a potential which is related to the decision-making process. Its characteristic is that corresponds to reactions that occur with a delay of 300 ms from the moment after the stimulus [14].

3.1.2 Linear Discriminant Analysis

Linear discriminant analysis is a method of statistical analysis that seeks to express a variable as a linear combination of features. The dependent variable belongs to a class and the algorithm requires that the independent variables have a normal

distribution. The purpose of the process is to develop a series of distinctive functions which maximize the variance between categories in relation, using as a learning sample a set of alternative activities, the classification of which is known.

Linear Discriminant Analysis (LDA) will be used for the creation of classes. In medicine, this algorithm helps in assessing the severity of the patient's condition and prognosis of disease's outcome. This discriminant analysis assumes that different classes generate data based on Gaussian distributions [15].

3.1.3 Time Series Analysis

Time Series is a sequence or data points related to time interval (stochastic system). It is used in statistics, signal processing, pattern recognition as well as electroencephalography. Time series analysis uses various methods in order to analyze the data and extract meaningful information. There are two basic methods for the analysis, frequency—domain and time—domain. It could also be parametrical and non—parametrical [14]. Regarding this suggested protocol, Time Series Analysis is used as a tool for clustering and classification of the received data. The aim is to use time series analysis to separate the signal of brain function in small blocks in which the values of the signals would be observed if they exceed the normal range or if during a specific process, their values are abnormally low [16].

3.2 Psychological Tests

There are many factors to be considered while trying to make a correct diagnosis of a disease. Those factors might affect the patients' clinical image. Huntington's disease is known to have psychological changes in the first stages of the disease thus there will be specific psychological tests within the frame of this suggested diagnostic protocol. These tests will help obtain a more comprehensive and integrated clinical image of the patients' and volunteers' condition.

3.2.1 Raven Test

One of the psychological tests that are suggested is Raven Test. Raven's Progressive Matrixes are used for measuring abstract reasoning and intelligence. The test consists of a series of 6×6 or 9×9 matrixes with a visual geometric design and a missing piece (Fig. 2). Then, the examinee is given six or nine pieces from which he/she is supposed to choose the one that misses and complete the matrix. Moreover, there is a time limitation which is 45 min for completing the process [17].

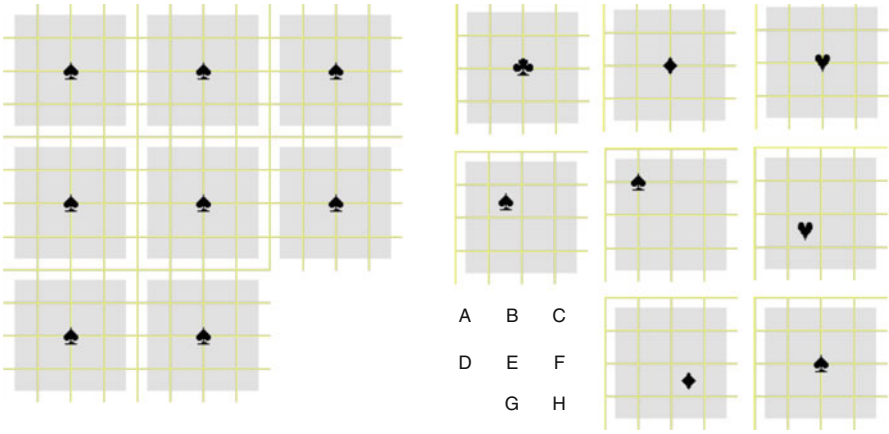

Fig. 2 Example of Raven's test

◉ Male ○ Female
◉ Long Form ○ Short Form
◉ Fancy Charts ○ Plain Charts

MMPI-2 Questions

○ False ○ True 1. I like mechanics magazines.
○ False ○ True 2. I have a good appetite.
○ False ○ True 3. I wake up fresh and rested most mornings.
○ False ○ True 4. I think I would enjoy the work of a librarian.
○ False ○ True 5. I am easily awakened by noise.
○ False ○ True 6. My father is a good man (or if your father is dead) my father was a good man.
○ False ○ True 7. I like to read newspaper articles on crime.
○ False ○ True 8. My hands and feet are usually warm enough.
○ False ○ True 9. My daily life is full of things that keep me interested.
○ False ○ True 10. I am about as able to work as I ever was.
○ False ○ True 11. There seems to be a lump in my throat much of the time.

Fig. 3 Example of MMPI—2 questionnaire

3.2.2 MMPI

The Minnesota Multiphasic Personality Inventory (MMPI) is the most widely used and researched standardized psychometric test of adult personality and psychopathology. It is a structured—general questionnaire (Fig. 3) since it attempts a comprehensive description of personality characteristics compared to other more specialized tests referred to individual characteristics. Among those characteristics there are depression, anxiety, hostility, aggression, psychosis, paranoia and other, some of which we encounter in Huntington's chorea.

The test consists of the total of 10 clinical scales as well as some additional scales that evaluate the validity of the test. For the purpose of this paper, Scale 2 (the Depression Scale), Scale 6 (the Paranoia Scale) and lastly, Variable Response Inconsistency Scale (VRIN) as the validity scale are suggested to be used in order to help the scientists form a fuller opinion about the patient's psychopathology [18].

3.2.3 California Verbal Learning Test

The California Verbal Learning Test (CVLT) is one of the most widely used neuropsychological tests. It is a measure of episodic verbal learning and memory.

This test is designed to calculate not only how much information has been learned by the examinee but also the manner in which it was learned and thus what mistakes were made during learning. The CVLT measures the free and cued recall, serial position effects, semantic clustering, intrusions, interference and recognition.

This test is conducted by the examiners who read a list of 16 nouns in a specific order with 1 s delay between the words. This list is repeated five times. After each trial, participants were asked to recall as many words as they can in any order (free recall). The words which are used belong to four semantic categories (Fig. 4).

Then, a further list (list B) is used. The patient is examined for free recall and recall after a signal, directly (short delay) and with a delay of 20 min (long delay). Finally, there is the process of recognition where the examinee is asked to recognize what the target and distractor words are in a list of 44 words in total [19].

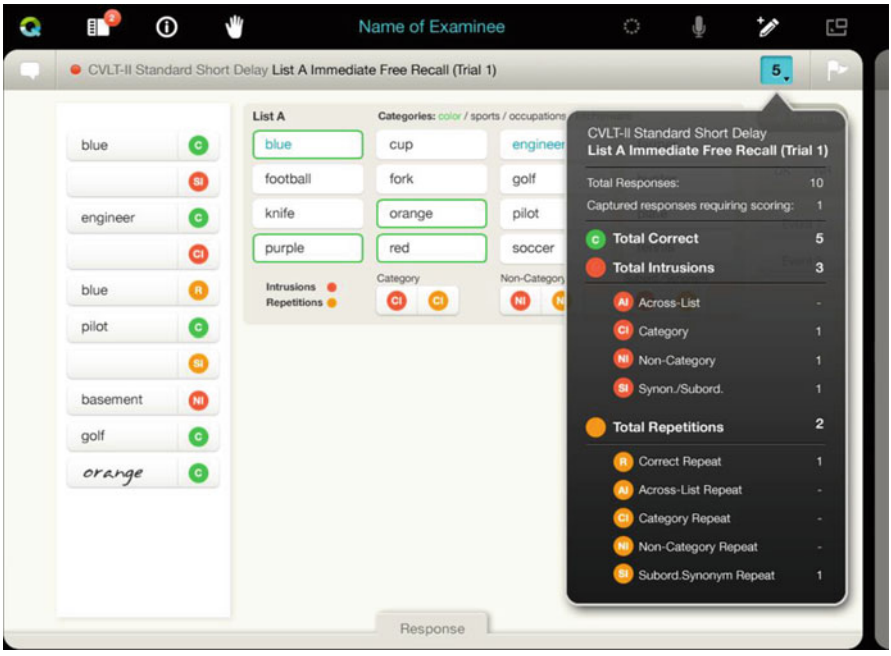

Fig. 4 Example of a California verbal learning test environment

4 Conclusion

In conclusion, the aim of this research is to suggest an experimental protocol which will use EEG recordings of patients in the first stages of the disease and it will process and evaluate the recordings using three bioinformatics algorithms. Those algorithms are Event Related Potential, Linear Discriminant Analysis and Time Series Analysis. At the same time, the patients' mental health will be examined by a set of psychological tests including MMPI, Raven's Test and California Verbal Learning Test.

In the future, the research will focus on testing and verifying the effectiveness of the suggested protocol in order to achieve an early diagnosis of Huntington's disease based on patients' brain activity.

Apart from that, the research will be directed to a different theory which will be tested. It is given that the mutation in HD gene exists in the genome since birth of each person, as the disease is hereditary, even though in most cases symptoms begin to show between 30 and 50 years of age. Over the years, the amount of misfolded proteins that molecular chaperones can destroy is reduced due to the reduction of chaperone concentrations in cells; there is "chaperone overload" in the first years of life.

Hence, is there any correlation between the onset of symptoms and the protein concentration? Is it correct to assume that the reduction of protein increases the rates of the mutant protein causing the initial symptoms or is there any other reason for causing the onset of symptoms?

Nevertheless, we know that there are cases where the symptoms show up during childhood. In these cases the expansion of CAG repetition is maximum (>90). Consequently, does the number of CAG repeats affect the functioning of molecular chaperones?

References

1. Ross, Ch.A., and S.J. Tabrizi. "*Huntington's Disease: from Molecular Pathogenesis to Clinical Treatment*". Departments of Psychiatry, Neurology, Pharmacology, and Neuroscience, and Program in Cellular and Molecular Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA (C A Ross MD); and Department of Neurodegenerative Disease, UCL Institute of Neurology, London, UK (S J Tabrizi FRCP).
2. Bobori, C. "*Molecular Genetics of Huntington's Disease*". 7 Tsirigoti Square, Corfu, Greece: Department of Informatics, Ionian University.
3. Walker, F.O. "*Huntington's Disease*". Medical Center Blvd, Winston Salem, NC 27157, USA: Department of Neurology, Wake Forest University.
4. Rubinsztein, D.C. "*The Molecular Pathology of Huntington's Disease(HD)*". Wellcome Trust/MRC Building, Addenbrooke's Hospital, Hills Road, Cambridge, CB2 2XY, UK: Department of Medical Genetics, Cambridge Institute for Medical Research.
5. Bay, J., A. Graves, H. Mora, M. Dragunow, P. Narayan, and R. Faull. "*Huntington's Disease: Understanding a mutation*", LENScience Connect Senior Biology Seminar Series. The University of Auckland.

6. Foroud, T., J. Gray, J. Ivashina, and P.M. Conneally. "*Differences in Duration of Huntington's Disease Based on Age at Onset*". Indianapolis, In, USA: Department of Medical and Molecular Genetics, Indiana University School of Medicine.
7. Fox, L. "Mapping the Anatomy of HD: A whole – Brain History". http://en.hdbuzz.net/205.
8. Plerou, A., C. Bobori, and P. Vlamos. 2015. Molecular Basis of Huntington's Disease and Brain Imaging Evidence. In 15th IEEE International Symposium on Signal Processing and Information Technology (ISSPIT 2015).
9. Κόλλιας, Χ. "Καταγραφή και Ανάλυση Βιοδυναμικών Εγκεφάλου με χρήση του Συστήματος BIOPAC", Πανεπιστήμιο Πατρών, Διατμηματικό Πρόγραμεμα μεταπτυχιακών Σπουδών στην Ιατρική Φυσική, Πάτρα 2012.
10. Dr. Chow, F.L. "The 10 – 20 International System of Electrode Placement Basic EEG", http://www.hksccm.org/files/Presentations/CCM_-_Basic_EEG.pdf.
11. Picton, T.W., S. Bentin, P. Berg, E. Donchin, S.A. Hillyard, R. Johnson Jr., G.A. Miller, W. Ritter, D.S. Ruchkin, M.D. Rugg, and M.J. Taylor. 2000. Guidelines for Using Human Event-Related Potentials to Study Cognition: Recording Standards and Publication Criteria. *Psychophysiology* 37: 127–152. Printed in the USA.Copyright © 2000 Society for Psychophysiological Research.
12. Bishop, Ch.M. 2006. *Pattern Recognition and Machine Learning (Information Science and Statistics)*. New York: Springer.
13. Lotte, F. 2014. A Tutorial on EEG Signal Processing Techniques for Mental State Recognition Inn Brain – Computer Interfaces. In *Guide to Brain – Computer Music Interfaces*, eds. Eduardo Reck Miranda, Castet, Springer. HAL Id: hal-01055103, https://hal.inria.fr/hal-01055103.
14. Hruby, T., P. Marsalek. "Event-Related Potentials - The P3 Wave", Charles University Prague, Department of Psychiatry, First Medical Faculty, Ke Karlovu 11, Praha 2, CZ-128 08, Department of Pathological Physiology, First Medical Faculty, U nemocnice 5, Praha 2, CZ-128 53, Czech Republic.
15. Welling, Max. "*Fisher Linear Discriminant Analysis*". 10 King's College Road, Toronto, M5S 3G5, Canada: Department of Computer Science, University of Toronto.
16. Γ.Ε. Κοκολάκης, "Σημειώσεις Ανάλυσης Χρονοσειρών".
17. Raven, J.C., et al. 1998. *Raven's Progressive Matrices and Vocabulary Scales*. Oxford: Oxford Psychologists Press.
18. Butcher, James Neal. 1990 *Butcher James Neal, "The MMPI-2 in Psychological Treatment*". New York, NY: Oxford University Press. The MMPI-2 in psychological treatment. ix 195 pp.
19. Delis, D.C., J.H. Kramer, E. Kaplan, and B.A. Ober. 1987. *Verbal Learning Test: Adult Version: Manual*. CVLT, California: Psychological Corporation.

Gold Rods Irradiated with Ultrasound for Combination of Hyperthermia and Cancer Chemotherapy

Andre Barros, Carlos Austerlitz, Ioannis Gkigkitzis, Diana Campos, Jeyce Andrade, Christina Peixoto, Jaciana Aguiar, Silene Nascimento, Teresinha G. Silva, and Ioannis Haranas

Abstract *Purpose:* The aim of this study was to analyze feasibility (in vitro and in vivo) the use of hyperthermia produced by gold rods irradiated with ultrasound and their combination with chemotherapy with doxorubicin. *Materials and Methods:* initially was determined the cell viability and Hsp70 levels after treatment by gold rods irradiated with ultrasound (GR+U) in cell culture. The pretreatment with GR+U combined with doxorubicin (DOX) was evaluated from IC_{50}, caspase-3 expression and mechanisms of cell death by electron microscopy. For evaluate the in vivo effects was used solid Ehrlich carcinoma (SEC) Tumor. The animals received three treatments with the combination of GR+U+DOX over 16 days. *Results:* The cell viability was completely inhibited after 40 min of treatment with GR+U and significant increases the expression of HSP70 was only observed after 10 min of treatment. GR+U+DOX presented significant reduction of IC_{50} representing 50.7%, 76.5% 45.2% and 46.6% for cell lines K562, NCI-H292, Hep-2 and MCF-7 respectively. GR+U+DOX presented significant reduction of IC_{50} representing

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50.7%, 76.5% 45.2% and 46.6% for cell lines K562, NCI-H292, Hep-2 and MCF-7 respectively. The caspase-3 level and ultraestructural analysis showed that treatment with GR+U+DOX enhances induction of apoptosis. Pretreatment with GR+U combined with doxorubicin (1 mg) showed 87% inhibition against SEC. and no showed cardiotoxic effect. *Conclusions:* The combined treatment of GR+U and DOX exhibit synergistic characteristics observed by increasing the efficiency of doxorubicin.

Keywords Cancer • Hyperthermia • Gold rods • Ultrasound • Doxorubicin

1 Introduction

Hyperthermia therapy can be defined as a treatment approach in which the temperature of a particular area of the body or the whole body is heated above normal temperatures to achieve therapeutic effects. An advantage of using hyperthermia to kill cancer cells is that usually normal tissues or cells are not as susceptible to high temperature as are cancerous tissues [1]. A variety of changes have been observed in cells after hyperthermia treatment including changes in cell membrane, metabolism, nuclear and cytoskeletal structures, macromolecular synthesis, expression of the heat shock genes and intracellular signal transduction. However, it is generally believed that protein denaturing and cell membrane damage is the most direct effect of hyperthermia toxicity [2–5].

Hyperthermia combination therapy refers to the simultaneous or continuous administration of thermal therapy with radiotherapy and/or chemotherapy for tumor treatment. Many preclinical and clinical trials for advanced and intractable tumor types, showed that thermal combination therapy can serve as an effective adjuvant to radiotherapy and/or chemotherapy as powerful enhancer [6–8].

Numerous studies have shown out that hyperthermia can increase tumor sensitivity to chemotherapeutic drugs and the uptake rates of drugs, and also increases drug accumulation in tumor tissues, which enhances the therapeutic effect of chemotherapy [9, 10]. This synergism is observed as a continuous change with increasing temperatures of the rate at which cells are killed by the drug. The associated mechanisms to thermal enhancement include increased rate constants of alkylation, increased drug uptake and inhibition of repair of drug-induced lethal or sublethal damage [7, 11].

The irradiation of gold macro rod (GR) with ultrasound is a method for inducing hyperthermia described by [12]. This method has been demonstrated by means of analytical considerations about bio-heat transfer in tissue, infrared pictures of GR exposed to ultrasound and most recently the viability in Preclinical Model [13]. Based on the above, the aim of this study was to analyze the use of gold rods irradiated with ultrasound for combination of hyperthermia and chemotherapy with doxorubicin.

2 Materials and Methods

2.1 Cell Culture Conditions

The cell lines used to evaluate the effects of hyperthermia alone and combined with chemotherapy were K562 (human erythroleukemia), NCI-H292 (human lung mucoepidermoid carcinoma), HEp-2 (human laryngeal epidermoid carcinoma) and MCF-7 (human breast adenocarcinoma). The cells were grown in DMEM supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 μg/ml Strep and 100 U/ml Pen, and incubated at 37 °C in a 5% CO2 atmosphere. Human cells were obtained from the Adolfo Lutz Institute, São Paulo, Brazil.

2.2 Animals

In this study, 48 BALB/c mice (male, 25–30 g), were obtained from the Laboratory of Immunopathology Keizo-Asami (LIKA) of the Federal University of Pernambuco (UFPE), Brazil. The animals were kept in cages with free access to food and water, under 12 h light-dark cycles. The animals were treated in accordance with the International Council for Laboratory Animal Science (ICLAS), and following the ethical principles of the Brazilian Society of Science in Laboratory Animals (SBCAL). All experiments were approved by the Ethics Committee for Animal Experimentation of the Biological Sciences Center of the Federal University of Pernambuco, Brazil, number 23076.013243/2012-04.

2.3 Cell Viability After Heat Treatment by Gold Rods Irradiated with Ultrasound

The cells were seeded in 24-well plates (10^5 cells/ml for adherent cells or 3×10^5 cells/ml for leukemias). After 24 h were performed four different experimental models as follows:

Control: untreated cells.

GR: gold macro-rods inserted on plate not irradiated with ultrasound.

U: plates irradiated with ultrasound only.

GR+U: gold macro-rods inserted on plate and irradiated with ultrasound.

In GR+U model, gold rods(24 K, 0.155 cm diameter and 0.54 cm height) were plated in the wells and stimulated by ultrasound generated by a 4 cm diameter transducer oscillating with a nominal frequency of 1 MHz and power of about 75 W. increasing the temperature in each well was measured using a Precision Infrared Thermometer (Fluke®). All experimental models were tested at 10, 20, 30 and 40 min. Six hours after treatments, the cell survival was quantified

by the mitochondrial oxidation of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT assay) [14, 15]. Was added 25 μl of MTT (5 mg/ml), 3 h later, the MTT formazan product was dissolved in 100 μl of DMSO, and absorbance was measured at 450 nm in plate spectrophotometer.

2.4 Quantification of HSP70

To the quantification of HSP70 from GR+U-treated cancer cells a commercially available kit HSP70 ELISA (Sigma) was used. The supernatants of GR+U-treated were collected at different post-GR+U times for ELISA analysis by adding to the microtiter wells coated with human anti-HSP70 monoclonal antibody. The captured HSP70 was detected with a HSP70-specific biotinylated rabbit polyclonal antibody that was subsequently bound by an horseradish peroxidase conjugate. The color development was stopped with acid stop solution which converts the endpoint color to yellow. The intensity of the color was measured in a microplate reader at 450 nm.

2.5 Effect of Pretreatment with GR+U on Cytotoxic Activity of Doxorubicin

For these experiments, the cells were plated in 24-well plates (10^5 cells/ml for adherent cells or 3×10^5 cells/ml for leukemias) and incubated for 24 h. The dose-response curve of doxorubicin (alone and pre-treated with GR+U 10 min) was obtained by the dissolution in culture medium to final concentrations of 0–50 μM, incubated for 24 h. After 72 h, the cytotoxicity was quantified by MTT assay (as described in Sect. 2.3). The IC_{50} values and their 95% confidence intervals for three different experiments were obtained by nonlinear regression using Graphpad Prism version 6.0 for Windows (GraphPad Software, San Diego, California USA).

2.6 Caspase-3 Assay

The effect of doxorubicin alone and combined with hyperthermia on caspase-3 activity was determined using a commercially available caspase-3 (active) ELISA kit (Invitrogen Corporation, Camarillo, CA). The cells was treated with DMSO (control), GR+U (10 min), DOX (1 μM) and GR+U (10 min) + DOX (0.5 μM) and incubated for 24 h. Later 24 h, the cells were collected and lysed in cell extraction buffer and 100 μl of cell lysates were incubated in the microplate wells provided in the kit and incubated at room temperature for 2 h. The samples were aspirated and washed 4 times with washing buffer and incubated with 100 μl of

detection antibody (caspase-3) for 1 h at room temperature. After removal of the antibody solution, the wells were washed again and incubated with 100 μl of HRP anti-rabbit antibody for 30 min at room temperature. After the aspiration of the anti-rabbit antibody, blue color was developed by adding 100 μl of stabilized chromogen solution for 15–20 min at room temperature. The reaction was stopped adding 100 μl of stopping solution and the yellow color developed was read using a microplate reader at 450 nm. The results were expressed as ng of caspase per mg of total proteins of the treated cells relative to control.

2.7 Electron Microscopy Analysis of Cell Death

For morphological assessment of cell death, transmission electron microscopy was used for K562, NCI-H292, HEp-2 and MCF-7 cells. The cells was treated with DMSO (control), GR+U (10 min), DOX (1 μM) and GR+U (10 min) + DOX (0.5 μM) and incubated for 24 h, after this period, were fixed with 2.5% glutaraldehyde (Sigma) and 4% paraformaldehyde (Sigma) in 0.1 M cacodylate (Sigma) buffer. After fixation, the samples were washed twice in the same buffer and post-fixed in 0.1 M cacodylate buffer (pH 7.2) containing 1% osmium tetroxide (Sigma), 2 mM calcium chloride and 0.8% potassium ferricyanide. Next, cells were dehydrated using acetone and embedded in SPIN-PON resin (Embed 812). Polymerization was performed at 60 °C for 3 days. Ultrathin sections were collected on nickel 300-mesh grids, counterstained with 5% uranyl acetate and lead citrate and examined with an FEI Morgani 268D transmission electron microscope. A minimum of 100 cells per sample were observed from three independent experiments to evaluate any cellular morphological alterations [16].

2.8 Solid Ehrlich Carcinoma (SEC) Tumor Model

Ehrlich ascites carcinoma (EAC) cells were derived from a spontaneous murine mammary adenocarcinoma. EAC cells were maintained in the undifferentiated form by passaging in syngeneic mice by transplanting 2.5×10^6 cells/ml (i.p.) each week. The ascitic fluid was removed by opening the belly and collecting all of the fluid with a sterile syringe. Ascitic tumor cell counts were carried out using the trypan blue dye exclusion method with a neubauer hemocytometer. Animals received 200 μl of a suspension containing 5×10^6 cells/ml (i.p.). Tumor volume was measured using a electronic caliper, assuming a fairly constant relationship of mass to volume, tumor development can be expressed in terms of volume increment. In order to determine tumor volume by external caliper, the greatest longitudinal diameter (length) and the greatest transverse diameter (width) were determined.

Tumor volume based on caliper measurements were calculated by the modified ellipsoidal formula [17, 18]:

$$Tumor\ volume\ \left(mm^3\right) = 1/2\left(length \times width^2\right)$$

The inhibition rate (%) was calculated as follows [19]:

$$Inhibition\ rate\ (\%) = [(A–B)\ /A] \times 100$$

where A is the average weight of the non treated group and B is the average tumor weight of the treated group.

2.9 In Vivo Antitumor Activity

After 5 days of tumor implantation (Tumor volume = 300 ± 18 mm^3), mice were randomly assigned into four groups (6 mice/group). In this experiment were evaluated the treatments with NaCl 0.9% (control), GR+U (10 min), DOX (3 mg/kg) and GR+U (10 min) + DOX (1 mg/kg). The treatment with GR+U was carried out by insertion of the gold rod (0.155 cm diameter and 0.54 cm height) in the center of tumor using of a trocar needle. A layer of ultrasound transmission gel was spread on the shaved mice's skin as contact media gel and exposed for 10 min with a 1 cm transducer connected to a GS8 2E ultrasound under a nominal frequency of 1 MHz and about 75 W. The temperature increase was measured every minute in the central and peripheral region of the tumor with needle type thermistor connected to a thermometer FLUKE. Changes in body temperature were valued by rectal temperature.

The experiment lasted 16 days, during this period the groups were treated 3 times (5th, 10th and 15th day). Tumor volume was measured from the 5th to the 16th day after implantation of SEC. On 16th day, the mice were sacrificed and the tumors were dissected and weighed, additionally blood samples were collected from each group for evaluation of adverse effects.

2.10 Hematological and Biochemical Analysis

Hematological analysis was carried out using an automatic cell counter (ABX-MICROS-60 cell counter Horiba, Inc). The samples were evaluated for the following hematological parameters: number of erythrocytes, concentration of hemoglobin, number of platelets and total count and differential of leukocytes. Part of the collected blood was poured into heparinized tubes for analysis of blood levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and creatine phosphokinase (CPK).

2.11 Statistical Analysis

The results are presented as the mean ± standard deviation (SD) or confidence intervals. One-way ANOVA followed by the Newman-Keuls test was used to evaluate the differences among the treatments. P values <0.05 were considered to be statistically significant.

3 Results

3.1 Cell Viability After Heat Treatment by Gold Rods Irradiated with Ultrasound

In this assay was observed the heat generated by the gold rods irradiated with ultrasound in each well of the plate. Increasing the temperature as a function of time can be seen in Fig. 1, the heat rate produced increased about 0.72 °C/min from 37 to 66 °C during of assessment 40 min.

The effect of the temperature elevation induced by GR+U varied according to the tested cell lines (Fig. 2). The cell viability, in all cell lines, was completely inhibited after 40 min of treatment with GR+U.

3.2 The Effect of GR+U in HSP70 Levels on Cell Culture

HSP70 levels were assessed into culture supernatants after treatment with GR+U by 10, 20 and 30 min (Fig. 3). The GR+U treatment by 10 min don't showed alterations compared with control in any tested cells types, however, in the treatments of 20 and 30 min there was significant increases the expression of HSP70.

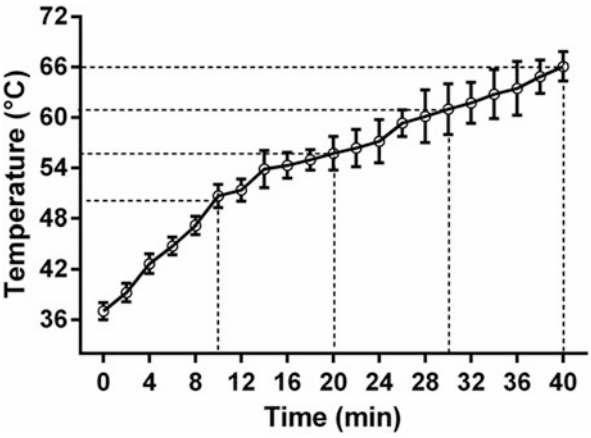

Fig. 1 The heat enhancement on cell culture during the irradiation of the gold rod with ultrasound

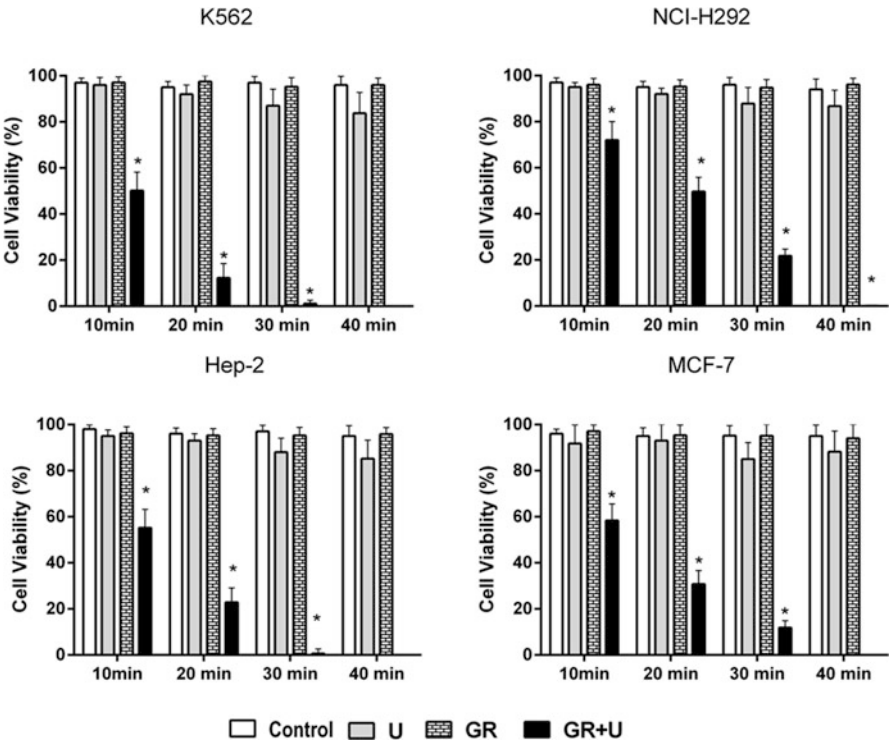

Fig. 2 The effect of gold rods irradiated with ultrasound for 10, 20, 30 and 40 min, in K562, NCI-H292, Hep-2 and MCF-7 cell lines. Results represent mean ± standard deviation of two experiments. * $p < 0.05$ compared to control by ANOVA followed by Newman-Keuls test

3.3 Effect of Pretreatment with GR+U on Cytotoxic Activity of Doxorubicin

The cytotoxic activity of doxorubicin alone and combined with GR+U pretreatment on the human tumor cell lines were evaluated after 24 h using MTT, and the results are presented in Fig. 4. Pretreatment with GR+U increased the cytotoxic effect of doxorubicin in all tested cell lines. Was observed significant reduction of IC_{50} representing 50.7%, 76.5% 45.2% and 46.6% for cell lines K562, NCI-H292, Hep-2 and MCF-7 respectively.

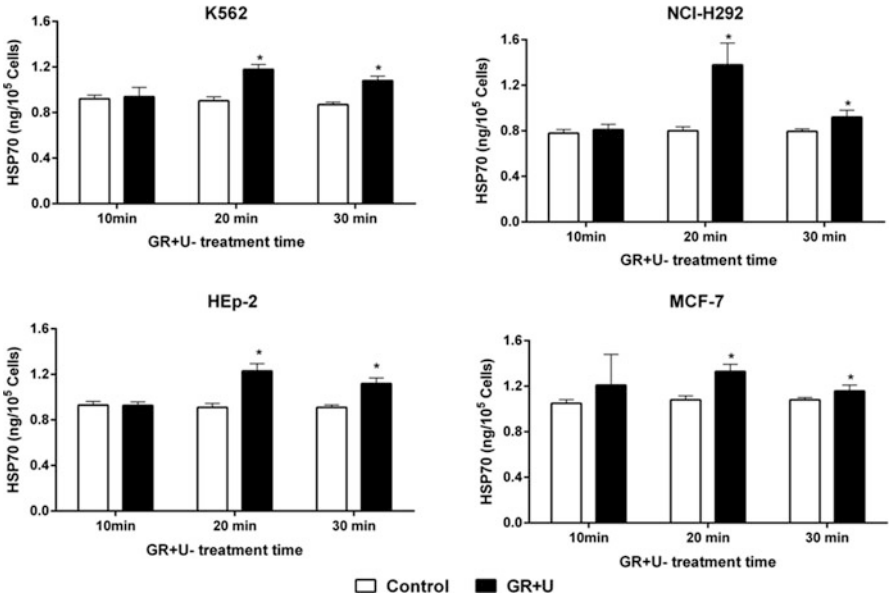

Fig. 3 HSP70 level into culture supernatants from K562, NCI-H292, Hep-2 and MCF-7 cells treated by gold rods irradiated with ultrasound for 10, 20 and 30 min. Results represent mean ± standard deviation of two experiments. * $p < 0.05$ compared to control by ANOVA followed by bonferroni post-hoc correction

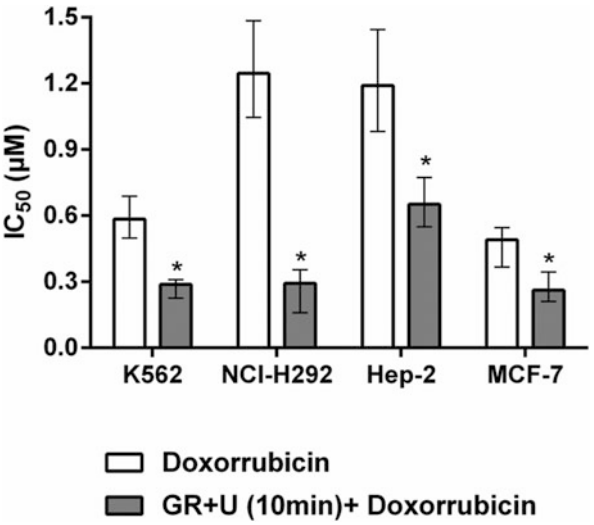

Fig. 4 Effect of pretreatment with GR+U on cytotoxic activity of doxorubicin. Results represent IC_{50} values and confidence intervals of three different experiments obtained by nonlinear regression. * $p < 0.05$ compared to DOX alone treatment by ANOVA followed by Newman-Keuls test

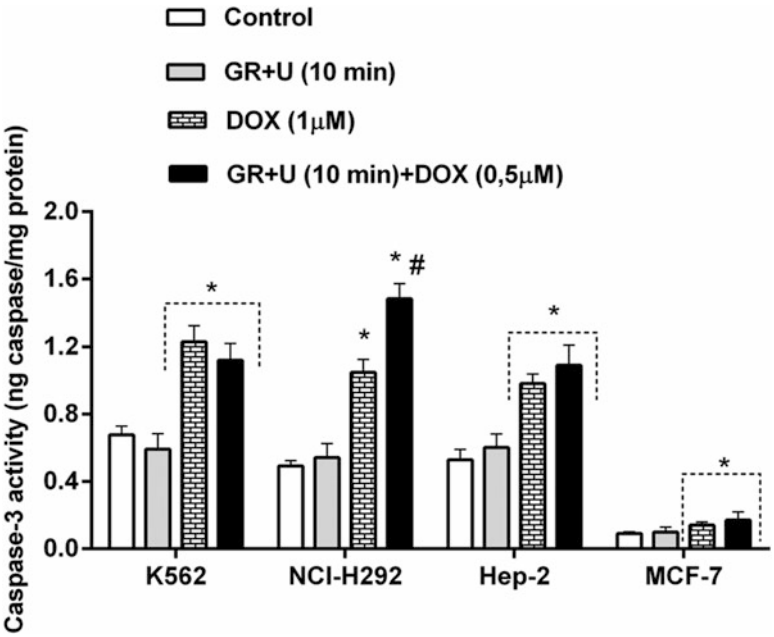

Fig. 5 Effects of GR+U combined with doxorubicin treatment on caspase-3 activity after 24 h incubation. Data are presented as mean ± standard deviation of two independently performed experiments. * $p < 0.05$ compared to DMSO-treated and # $p < 0.05$ compared to all treatments by ANOVA followed by Newman-Keuls post-test

3.4 Effect of GR+U Combined with Doxorubicin Treatment on Caspase-3 Activation

The effect of treatment with DMSO (control), GR+U (10 min), DOX (1 μM) and GR+U (10 min)+DOX (0.5 μM) in caspase-3 activity is showed in Fig. 5. There were no statistically significant differences between treatment with DOX (1 μM) and GR+U (10 min)+DOX (0.5 μM) in K562, Hep-2 and MCF-7 cell lines. The caspase-3 activity in NCI-H292 cells was 42% higher in treatment with GR+U (10 min)+DOX (0.5 μM) compared to cells treated with DOX (1 μM). These results may indicate that pretreatment with GR+U enhances the efficacy of doxorubicin.

3.5 Cell Death Analysis by Electron Microscopy

The specific morphological parameters used to classify apoptosis were: chromatin condensation, nuclear fragmentation, cell shrinkage, cell retraction, nuclear

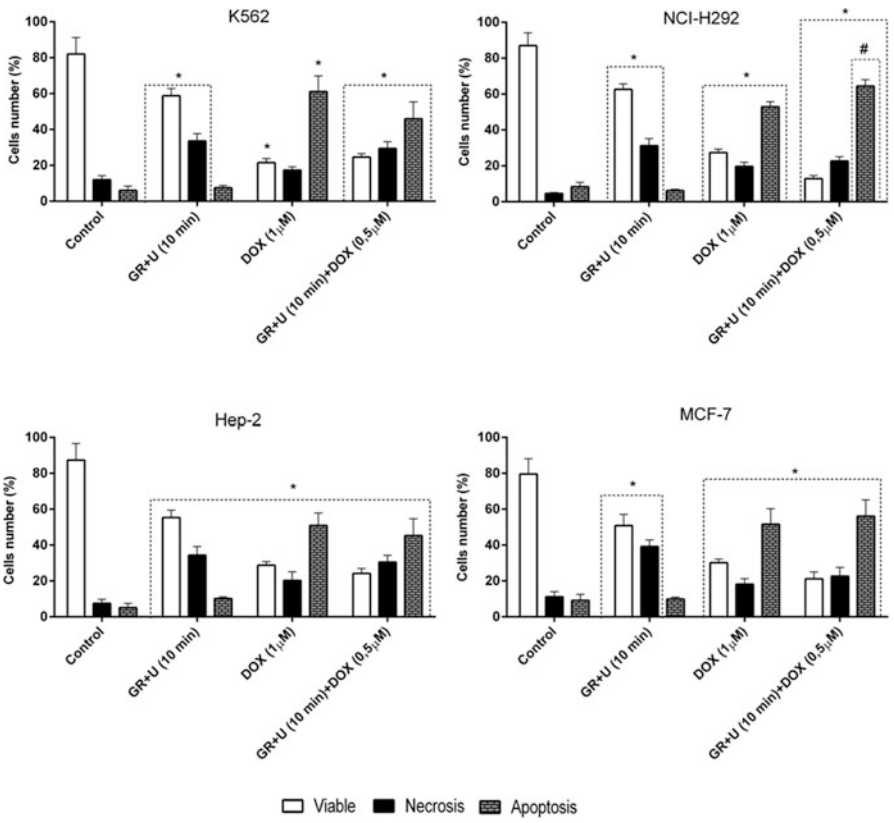

Fig. 6 Ultrastructural analyses of cell death after treatment with DMSO (control), GR+U (10 min), DOX (1 μM) and GR+U (10 min) + DOX (0.5 μM). Results represent mean ± standard deviation of three experiments for each cell line tested. * $p < 0.05$ compared to DMSO-treated and # $p < 0.05$ compared to all treatments by ANOVA followed by Newman-Keuls post-test

blebbing and presence of apoptotic bodies. For the identification of necrosis morphological parameters used were: cell swelling, lack of an intact cell membrane and disintegration or ruptures of the intracellular organelles. The ultrastructural analyses of the cell lines treated with DMSO (control), GR+U (10 min), DOX (1 μM) and GR+U (10 min)+DOX (0.5 μM) can be observed in Fig. 6. The treatment with GR+U (10 min)+DOX (0.5 μM) produced the same effects of 1 μM doxorubicin in K562, Hep-2 and MCF-7 cells. The combination of GR+U and doxorubicin increased the number of apoptotic cells (64.5%) compared to treatment with doxorubicin alone (53%) in NCI-H292 cell line. Cell morphology of NCI-H292 on transmission electron microscopy after aforementioned treatments is shown in Fig. 7.

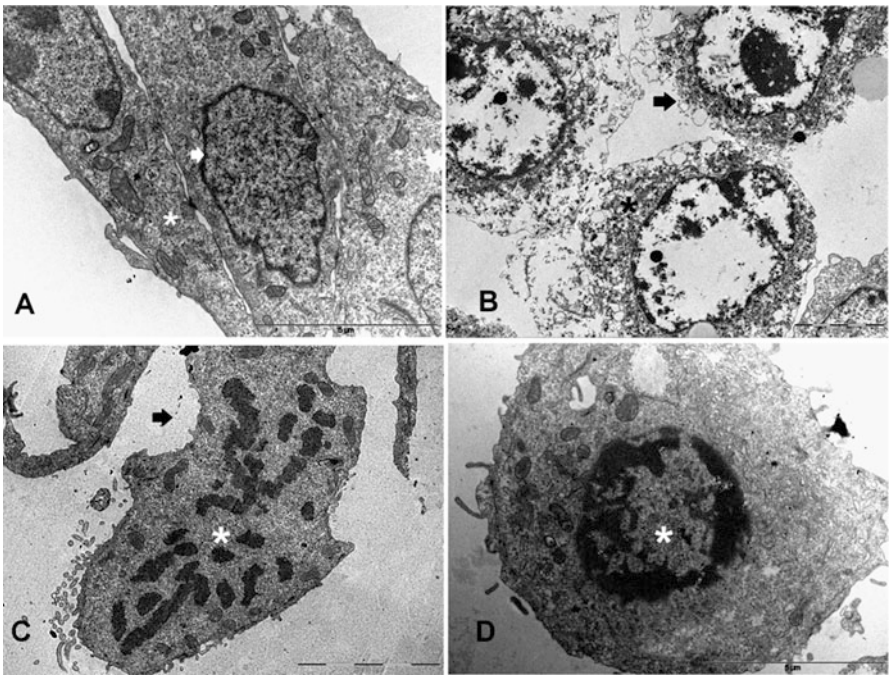

Fig. 7 Electron micrograph of NCI-H292 cells. (**a**) DMSO control: normal cells morphology showing intact nuclear membrane (*white arrow*) and cytoplasmic organelles preserved (*white asterisk*); (**b**) GR+U (10 min): Necrotic cells showing plasmatic membrane disintegration (*black arrow*) and ruptures of the intracellular organelles (*black asterisk*); (**c**) DOX (1 μM): apoptotic cell with fragmentation of the nucleus and chromatin condensation (*white asterisk*) and cell retraction (*black arrow*); (D) GR+U (10 min) + DOX (0,5 μM): apoptotic cell with perinuclear condensation of chromatin (*white asterisk*). All images are shown at the same magnification (3500×)

3.6 In vivo Antitumor Activity

In this study was observed the influence of pretreatment with gold rods irradiated with ultrasound on the efficacy of doxorubicin. The effect of GR+U (10 min), DOX (3 mg/kg) and GR+U (10 min) + DOX (1 mg/kg) against solid Ehrlich carcinoma is showed in Fig. 8. Tumor volumes were evaluated for 16 days, in this period, treatment with the combination of GR+U and doxorubicin induced significant reductions in developing SEC as of the 11th day. The tumor volume of the group GR+U (10 min) + DOX (1 mg/kg) observed at the final of assessment was 178 mm^3, whereas the groups treated with GR+U and DOX alone showed 486 and 388 mm^3 respectively.

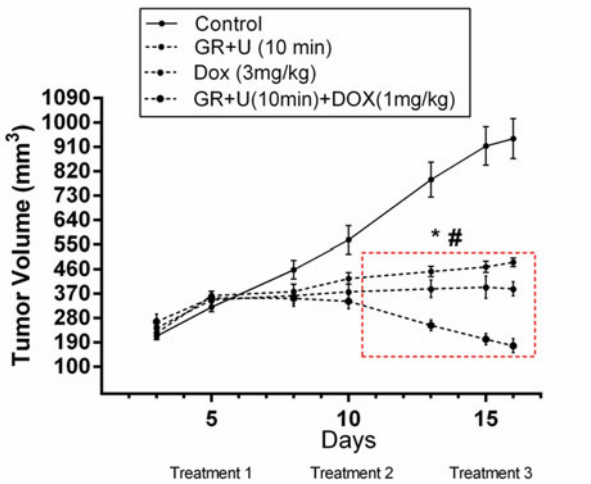

Fig. 8 The effect of treatment with GR+U (10 min), DOX (3 mg/kg) and GR+U (10 min)+DOX (1 mg/kg) on the growth of Ehrlich tumor. Data are presented as mean ± standard deviation. * $p < 0.05$ compared to control by ANOVA followed by Newman-Keuls test. # $p < 0.05$ compared to all treatments by ANOVA followed by Newman-Keuls post-test

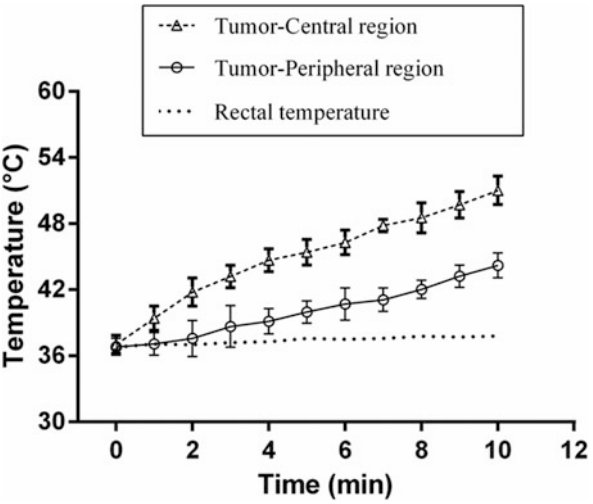

Fig. 9 The heat enhancement on tumor during the irradiation of the gold rod with ultrasound for 10 min

The temperature in the tumor was measured with the FLUKE thermistor in two regions defined in accordance with the proximity to the GR inserted. In central region, the heat rate produced increased about 1.5 °C/min from 36 to 51 °C and in peripheral region 0.78 °C/min from 36.4 to 44.2 °C (Fig. 9). Significatives changes in rectal temperature not were observed.

The antitumor activity of treatments with GR+U, DOX alone and the combination GR+U+DOX is described in Fig. 10. The three treatments tested inhibited the tumor development over the trial period, however, the GR+U+DOX group had higher rate of inhibition (87%) in relation to other treatments.

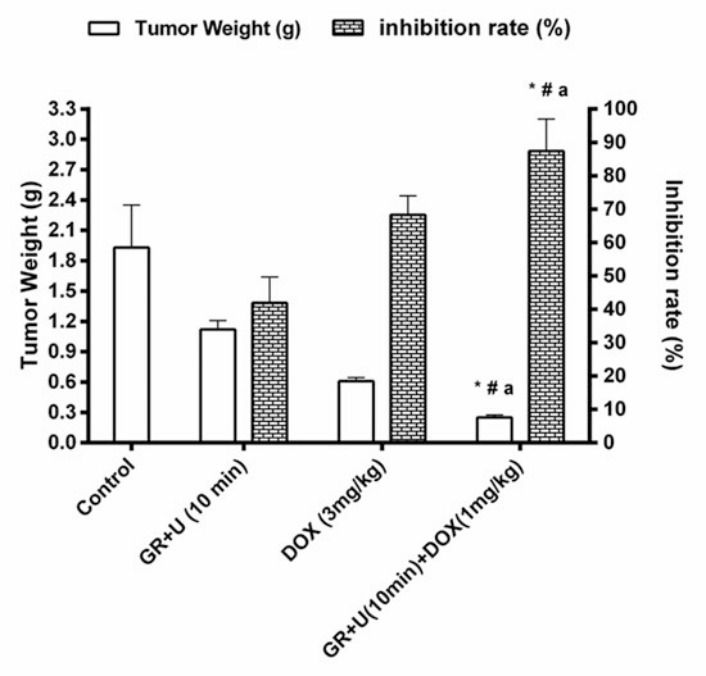

Fig. 10 Antitumor activity of treatment with GR+U (10 min), DOX (3 mg/kg) and GR+U (10 min)+DOX (1 mg/kg) in mice transplanted with SEC. Results are presented as mean ± standard deviation. * $p < 0.05$ compared to control by ANOVA followed by Newman-Keuls test. # $p < 0.05$ compared to GR+U (10 min) by ANOVA followed by Newman-Keuls test. [a] $p < 0.05$ compared to DOX (3 mg/kg) by ANOVA followed by Newman-Keuls test

3.7 Toxicological Analysis

In animals treated with the combination GR+U+DOX, the doxorubicin dose used was approximately 33% of the dose received by animals treated with doxorubicin only. The use of a lower dose reduced the toxic effects of doxorubicin on hematological parameters as shown in Table 1. Furthermore, it was also observed significant inhibition of the cardiotoxic and hepatotoxic effects of doxorubicin, measured by reduction in levels of alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase and creatine phosphokinase (Fig. 11).

4 Discussion

The cancer treatment by hyperthermia using macro of gold rods irradiated with ultrasound was described initially by [12]. The dimensions of the gold rod and the thermal energy produced under irradiation with ultrasound was described from

Table 1 Effect of treatment with three doses of GR+U (10 min)+DOX (1 mg/kg) on hematological parameters of mice 16 days after implantation of Erhlich carcinoma

Parameter	Control	GR+U	DOX	GR+U+DOX
Leukocytes (10^3/mm^3)	8.73 ± 0.68	9.67 ± 0.88	3.22 ± 0.47	5.3 ± 1.03$^{*\,\#}$
Neutrophil (%)	9.4	11.7	13.7	17.6
Monocyte (%)	15.5	24.5	12.4	13.6
Lymphocyte (%)	73.6	62.2	61.2	67.8
Red blood cells (10^6/mm^3)	9.13 ± 0.94	10.2 ± 1.12	4.03 ± 0.65	8.82 ± 0.99$^{\#}$
Platelets (10^3/mm^3)	483 ± 37.5	437 ± 64.8	223 ± 57.8	419 ± 69.8$^{\#}$

The values are presented as the average ± the standard deviation. * ($p < 0.05$) compared to the control and # ($p < 0.05$) compared to DOX (3 mg/kg) treatment by ANOVA followed by Newman-Keuls post-test

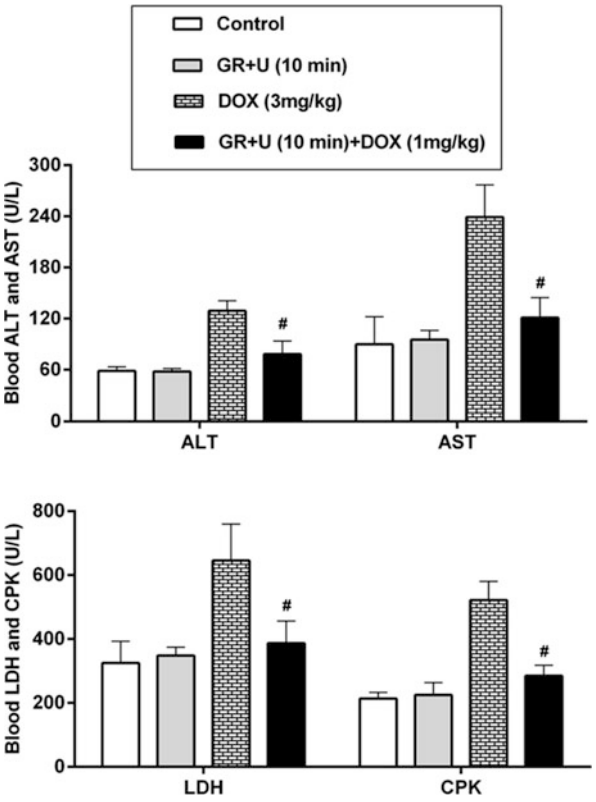

Fig. 11 Effect of treatment with three doses of GR+U (10 min) + DOX (1 mg/kg) on biochemical parameters parameters of mice 16 days after implantation of Erhlich carcinoma. *ALT* alanine aminotransferase; *AST* aspartate aminotransferase; *LDH* lactate dehydrogenase; *CPK* creatine phosphokinase. # ($p < 0.05$) compared to DOX (3 mg/kg) treatment by ANOVA followed by Newman-Keuls post-test

theoretical observations and computer simulations by [13] and all the experimental setup involving hyperthermia by GR+U as well as the development of surgical technique for insertion of gold rods in tumors and the application of the technique, were based on these results.

In this study, the temperature increase produced by the gold rods irradiated with ultrasound induced decrease in cell viability in direct proportion to the irradiation time. After 40 min nearly all the cells died. The Hyperthermia has cytotoxic effects on tumor cells via several mechanisms. The first phase of direct killing is characterized by linear growth arrest, which is typified by decreased RNA (brief) and DNA (prolonged) synthesis specifically at the S phase but also by a slowed M phase of the cell cycle [11]. Deficiencies in DNA repair mechanisms become evident as early as 40 °C and above 43 °C, occurs cell membrane disruption, transmembrane proteins denaturation, cellular architectural distortion, and ultimately, necrotic and apoptotic pathway activation [20, 21].

To combine hyperthermia produced by GR+U with a conventional chemotherapeutic agent was considered the interference of heat shock proteins in apoptosis mechanisms. Heat-shock proteins (HSPs) are expressed constitutively and are further induced under stress conditions, including temperature increase. Hsp70 blocks several steps of the apoptotic cascade: upstream from mitochondria, release of cytochrome C and apoptosis-inducing factor (AIF), nuclear import of AIF, activation of procaspases-9 and -3, and even downstream of active caspase-3 [22–27]. In vitro results presented in our work showed that cells treated with GR+U for 10 min don't showed significant changes in hsp70 level. For this reason, was used this irradiation time in the combined treatment of GR+U+DOX.

The advantages of combined hyperthermia and chemotherapy have been explained in clinical trial reports [28, 29]. In this study, pretreatment with GR+U (10 min) increased the cytotoxicity of doxorubicin about 65% compared to treatment with DOX alone. Increased efficiency of drug on combination therapy with hyperthermia can be correlated to the plasma membrane fluidity changes at hyperthermic range of temperature [30].

The anthracyclin drug doxorubicin is one of the most effective antineoplastic agents, and widely used to treat a number of malignancies, despite extensive clinical utilization, the mechanisms of action of anthracyclines in cancer cells remain a matter of controversy. The main mechanisms considered are: (1) intercalation into DNA, leading to inhibited synthesis of macromolecules; (2) generation of free radicals, leading to DNA damage or lipid peroxidation; (3) DNA binding and alkylation; (4) DNA cross-linking; (5) interference with DNA unwinding or DNA strand separation and helicase activity; (6) direct membrane effects; (7) initiation of DNA damage via inhibition of topoisomerase II [31–33]. For this reason, in order to quantify apoptosis, was used in this study the levels of caspase-3.

Caspase-3, an executioner caspase, can be activated by mitochondrial or intrinsic pathway involving caspase-9 or a death receptor/extrinsic pathway involving caspase-8 [34]. Activation of caspase-3 is thought to be a fundamental biochemical event marking the induction of apoptosis, followed by the cell death in a systematic fashion in such a manner that surrounding cells and tissues are unaffected [35]. Caspase-3 levels induced by pretreatment with GR+U (10 min) + DOX (0.5 μM) were statistically equivalent to treatment with DOX (1uM) in K562, Hep-2 and MCF-7. In NCI-H292 cell line, the amount of caspase-3 in the combined treatment was 36% higher compared to DOX alone. The increase of caspase-3 in NCI-H292 was confirmed by ultrastructural analysis.

The increase in the number of apoptotic cells shown by treatment with GR+U+DOX against the NCI-H292 line cells can be explained by the activation stress-activated protein kinases (SAPKs). Under stress, activation of SAPKs appears to be important in promoting apoptosis in many cell types, including NCI-H292 cells in culture. SAPKs, and especially the JNK pathway, contribute is important in the activation of the mitochondria-dependent apoptotic pathway (also known as the intrinsic pathway) but dispensable for apoptosis induced by the activation of death receptors (the extrinsic pathway) [36]. JNK- and p38-mediated phosphorylation of p53, which augments the p53 response, may also play a role in their pro-apoptotic actions [37, 38].

In the current study we investigated the effect of pretreatment with GR+U (10 min) on the cytotoxic activity of DOX against the growth of solid Ehrlich carcinoma in mice. SEC is an undifferentiated carcinoma that has high transplantable capability and rapid proliferation. After the inoculation the number of cells increases rapidly and the host animal died due to the pressure exerted by the tumor volume and/or the damage that resulted from the tumor [39, 40].

According to the analysis of tumor growth per 16 days, the difference between the antitumor effects by the treatment with GR+U(10 min)+DOX(1 mg/kg) compared to treatment with doxorubicin(3 mg/kg) was observed only from 10th day. The tumor inhibition, at the end of the experiment was 20% more in GR+U+DOX compared with DOX only. Our results showed that pretreatment with hyperthermia increased the efficiency of doxorubicin enabling the use of smaller doses without compromising the effect of the drug.

Doxorubicin is highly effective in treating of leukemias and many solid tumors [41]. However, this drug presents dose-dependent adverse effects on bone marrow, heart and other organs. Like other members of the anthracycline class, its usage is greatly limited especially by the risk of severe cardiotoxicity leading to potentially lethal congestive heart failure [42, 43]. Due to the great importance of DOX in chemotherapy for the treatment of many types of cancer, researchers have exerted great efforts to attenuate their side effects. In this study, the pretreatment GR+U combined with low doses of doxorubicin reduced toxic effects in bone marrow. Furthermore, animals treated with DOX+GR+U not show cardiotoxic effects in accordance with the observed biochemical parameters.

5 Conclusion

Significant effects are observed by use of gold rods irradiated with ultrasound and doxorubicin. The combined treatment of hyperthermia and DOX exhibit synergistic characteristics observed by increasing the efficiency of doxorubicin. The combined treatment accelerated the DOX passive permeation and therefore increasing intracellular DOX concentration and cytotoxicity. In the evaluation of antitumor effects in vivo, the pretreatment with GR+U showed similar results to

those observed on in vitro tests. However from the observations in vivo, our study showed that in combination with hyperthermia the toxic effects of doxorubicin were minimized.

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Conflicts of Interest The authors declare no conflict of interest.

References

1. Van der Zee, J. 2002. Heating the Patient: A Promising Approach? *Annals of Oncology* 13: 1173–1184.
2. Hokland, S.L., M. Pedersen, R. Salomir, B. Quesson, H. Stødkilde-Jørgensen, and C.T.W. Moonen. 2006. MRI-Guided Focused Ultrasound: Methodology and Applications. *IEEE Transactions on Medical Imaging* 25: 723–731.
3. Hegyi, G., G.P. Szigeti, and A. Szász. 2013. Hyperthermia Versus Oncothermia: Cellular Effects in Complementary Cancer Therapy. *Evidence-Based Complementary and Alternative Medicine*. doi:10.1155/2013/672873.
4. Vertree, R.A., A. Leeth, M. Girouard, J.D. Roach, and J.B. Zwischenberger. 2002. Whole-Body Hyperthermia: A Review of Theory, Design and Application. *Perfusion* 17: 279–290.
5. Day, E.S., J.G. Morton, and J.L. West. 2009. Nanoparticles for Thermal Cancer Therapy. *Journal of Biomechanical Engineering* 131: 074001.
6. Horsman, M.R., and J. Overgaard. Hyperthermia: A Potent Enhancer of Radiotherapy. Clinical Oncology (R Coll Radiol) [Internet]. 2007 Aug [cited 2014 Nov 30];19(6): 418–426. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17493790.
7. Issels, R.D. 2008. Hyperthermia Adds to Chemotherapy. *European Journal of Cancer* 44: 2546–2554.
8. Wust, P., B. Hildebrandt, G. Sreenivasa, B. Rau, J. Gellermann, H. Riess, et al. 2002. Hyperthermia in Combined Treatment of Cancer. *The Lancet Oncology* 3: 487–497.
9. Bergs, J.W.J., N.A.P. Franken, J. Haveman, E.D. Geijsen, J. Crezee, and C. van Bree. 2007. Hyperthermia, Cisplatin and Radiation Trimodality Treatment: A Promising Cancer Treatment? A Review from Preclinical Studies to Clinical Application. *International Journal of Hyperthermia* 23: 329–341.
10. Oyama, T., M. Kawamura, T. Abiko, Y. Izumi, M. Watanabe, E. Kumazawa, et al. 2007. Hyperthermia-Enhanced Tumor Accumulation and Antitumor Efficacy of a Doxorubicin-Conjugate with a Novel Macromolecular Carrier System in Mice with Non-Small Cell Lung Cancer. *Oncology Reports* 17: 653–659.
11. Hildebrandt, B., P. Wust, O. Ahlers, A. Dieing, G. Sreenivasa, T. Kerner, et al. 2002. The Cellular and Molecular Basis of Hyperthermia. *Critical Reviews in Oncology/Hematology* 43: 33–56.
12. Gkigkitzis, I., D. Campos, and I. Haranas. 2014. An Experimental Study on the Heat Enhancement and the Bio-Heat Transfer Using Gold Macro Rod and Ultrasound: A Potential Alternative to Kill Cancer Cells. *Physics International* 5 (2): 132–135.
13. Gkigkitzis, I., C. Austerlitz, I. Haranas, and D. Campos. 2015. The Effect of the Shape and Size of Gold Seeds Irradiated with Ultrasound on the Bio-Heat Transfer in Tissue. *Advances in Experimental Medicine and Biology* 820: 103–124.
14. Mosmann, T. 1983. Rapid Colorimetric Assay for Cellular Growth and Survival: Application to Proliferation and Cytotoxicity Assays. *Journal of Immunological Methods* 65: 55–63.

15. Alley, M.C., D.A. Scudiero, A. Monks, M.L.M.J. Hursey Czerwinski, D.L. Fine, B.J. Abbott, et al. 1988. Feasibility of Drug Screening with Panels of Human Tumor Cell Lines Using a Microculture Tetrazolium Assay. *Cancer Research* 48: 589–601.
16. Krysko, D.V., T. Vanden Berghe, K. D'Herde, and P. Vandenabeele. 2008. Apoptosis and Necrosis: Detection, Discrimination and Phagocytosis. *Methods* 44: 205–221.
17. Euhus, D.M., C. Hudd, M.C. LaRegina, and F.E. Johnson. 1986. Tumor Measurement in the Nude Mouse. *Journal of Surgical Oncology* 31: 229–234.
18. Tomayko, M.M., and C.P. Reynolds. 1989. Determination of Subcutaneous Tumor Size in Athymic (Nude) Mice. *Cancer Chemotherapy and Pharmacology* 24: 148–154.
19. Bezerra, D.P., F.O. De Castro, A.P.N.N. Alves, C. Pessoa, M.O. De Moraes, E.R. Silveira, et al. 2008. In vitro and In vivo Antitumor Effect of 5-FU Combined with Piplartine and Piperine. *Journal of Applied Toxicology* 28: 156–163.
20. Eppink, B., P.M. Krawczyk, J. Stap, and R. Kanaar. 2012. Hyperthermia-Induced DNA Repair Deficiency Suggests Novel Therapeutic Anti-Cancer Strategies. *International Journal of Hyperthermia* 28: 509–517.
21. Purschke, M., H.-J. Laubach, R.R. Anderson, and D. Manstein. 2010. Thermal Injury Causes DNA Damage and Lethality in Unheated Surrounding Cells: Active Thermal Bystander Effect. *The Journal of Investigative Dermatology* 130: 86–92.
22. Yenari, M.A., J. Liu, Z. Zheng, Z.S. Vexler, J.E. Lee, and R.G. Giffard. 2005. Antiapoptotic and Anti-Inflammatory Mechanisms of Heat-Shock Protein Protection. *Annals of the New York Academy of Sciences* 1053: 74–83.
23. Guo, F., C. Sigua, P. Bali, P. George, W. Fiskus, A. Scuto, et al. 2005. Mechanistic Role of Heat Shock Protein 70 in Bcr-Abl-Mediated Resistance to Apoptosis in Human Acute Leukemia Cells. *Blood* 105: 1246–1255.
24. Mosser, D.D., A.W. Caron, L. Bourget, C. Denis-Larose, and B. Massie. 1997. Role of the Human Heat Shock Protein hsp70 in Protection Against Stress-Induced Apoptosis. *Molecular and Cellular Biology [Internet]* 17: 5317–5327. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=232382&tool=pmcentrez&rendertype=abstract.
25. Lui, J.C.K., and S.K. Kong. 2007. Heat Shock Protein 70 Inhibits the Nuclear Import of Apoptosis-Inducing Factor to Avoid DNA Fragmentation in TF-1 Cells During Erythropoiesis. *FEBS Letters* 581: 109–117.
26. Beere, H.M., B.B. Wolf, K. Cain, D.D. Mosser, A. Mahboubi, T. Kuwana, et al. 2000. Heat-Shock Protein 70 Inhibits Apoptosis by Preventing Recruitment of Procaspase-9 to the Apaf-1 Apoptosome. *Nature Cell Biology* 2: 469–475.
27. Jäättelä, M., D. Wissing, K. Kokholm, T. Kallunki, and M. Egeblad. 1998. Hsp7O Exerts Its Anti-Apoptotic Function Downstream of Caspase-3-Like Proteases. *The EMBO Journal* 17: 6124–6134.
28. Li, T.J., C.C. Huang, P.W. Ruan, K.Y. Chuang, K.J. Huang, D.B. Shieh, et al. 2013. In vivo Anti-Cancer Efficacy of Magnetite Nanocrystal - Based System Using Locoregional Hyperthermia Combined with 5-Fluorouracil Chemotherapy. *Biomaterials* 34 (32): 7873–7883. Available from: doi:10.1016/j.biomaterials.2013.07.012.
29. Roviello, F., S. Caruso, D. Marrelli, C. Pedrazzani, A. Neri, A. De Stefano, et al. 2011. Treatment of Peritoneal Carcinomatosis with Cytoreductive Surgery and Hyperthermic Intraperitoneal chemotherapy: State of the Art and Future Developments. *Surgical Oncology* 20 (1): e38–e54.
30. Preetha, A., R. Banerjee, and N. Huilgol. 2007. Effect of Temperature on Surface Properties of Cervical Tissue Homogenate and Organic Phase Monolayers. *Colloids Surfaces B Biointerfaces* 60: 12–18.
31. Gewirtz, D.A. 1999. A Critical Evaluation of the Mechanisms of Action Proposed for the Antitumor Effects of the Anthracycline Antibiotics Adriamycin and Daunorubicin. *Biochemical Pharmacology* 15: 727–741.
32. Thorn, C.F., C. Oshiro, S. Marsh, T. Hernandez-Boussard, H. McLeod, T.E. Klein, et al. 2011. Doxorubicin Pathways: Pharmacodynamics and Adverse Effects. *Pharmacogenetics and Genomics* 21: 440–446.

33. Tacar, O., P. Sriamornsak, and C.R. Dass. 2013. Doxorubicin: An Update on Anticancer Molecular Action, Toxicity and Novel Drug Delivery Systems. *Journal of Pharmacy and Pharmacology* 65: 157–170.
34. Rupinder, S.K., A.K. Gurpreet, and S. Manjeet. 2007. Cell Suicide and Caspases. *Vascular Pharmacology* 46: 383–393.
35. Fan, T.J., L.H. Han, R.S. Cong, and J. Liang. 2005. Caspase Family Proteases and Apoptosis. *Acta Biochimica et Biophysica Sinica (Shanghai)* 37: 719–727.
36. Tournier, C., P. Hess, D.D. Yang, J. Xu, T.K. Turner, A. Nimnual, et al. 2000. Requirement of JNK for Stress-Induced Activation of the Cytochrome c-Mediated Death Pathway. *Science* 288: 870–874.
37. Duch, A., E. De Nadal, and F. Posas. 2012. The p38 and Hog1 SAPKs Control Cell Cycle Progression in Response to Environmental Stresses. *FEBS Letters* 586: 2925–2931.
38. Sauter, K.A.D., E.A. Magun, M.S. Iordanov, and B.E. Magun. 2010. ZAK is Required for Doxorubicin, a Novel Ribotoxic Stressor, to Induce SAPK Activation and Apoptosis in HaCaT Cells. *Cancer Biology and Therapy* 10: 258–266.
39. Kabel, A.M., M.N. Abdel-Rahman, A.E.D.E. El-Sisi, M.S. Haleem, N.M. Ezzat, and M.A. El Rashidy. 2013. Effect of Atorvastatin and Methotrexate on Solid Ehrlich Tumor. *European Journal of Pharmacology* 713: 47–53.
40. Vendramini-Costa, D.B., de Castro IBD, A.L.T.G. Ruiz, C. Marquissolo, R.A. Pilli, and J.E. de Carvalho. 2010. Effect of Goniothalamin on the Development of Ehrlich Solid Tumor in Mice. *Bioorganic and Medicinal Chemistry* 18: 6742–6747.
41. Lüpertz, R., W. Wätjen, R. Kahl, and Y. Chovolou. 2010. Dose- and Time-Dependent Effects of Doxorubicin on Cytotoxicity, Cell Cycle and Apoptotic Cell Death in Human Colon Cancer Cells. *Toxicology* 271: 115–121.
42. Chatterjee, K., J. Zhang, N. Honbo, and J.S. Karliner. 2010. Doxorubicin Cardiomyopathy. *Cardiology* 115: 155–162.
43. Zhang, S., X. Liu, T. Bawa-Khalfe, L.-S. Lu, Y.L. Lyu, L.F. Liu, et al. 2012. Identification of the Molecular Basis of Doxorubicin-Induced Cardiotoxicity. *Nature Medicine* 18 (11): 1639–1642.

Assessment of Depression in Elderly. Is Perceived Social Support Related? A Nursing Home Study

Depression and Social Support in Elderly

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Abstract Geriatric depression is more common in nursing homes and social support is a mechanism that mitigates the stressors of life factors and simultaneously promotes wellness and health. The purpose of the study was to assess the levels of depression and social support among elderly in nursing homes. During the period February 2016–March 2016 170 elderly residents in nursing homes completed the Geriatric Depression Scale-15 (GDS-15) and the Multidimensional Scale of

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Perceived Social Support (MSPSS). Statistical analysis was conducted with IBM SPSS Statistics 23. 37, 1% of the sample had depressive symptoms. Depression is statistically correlated with age and it is affected by the years of education ($p = 0.003$), the number of the children ($p = 0.006$), whether the elderly person is bedridden or not ($p < 0.001$), the frequency of visits by family members ($p < 0.001$) and whether the elderly performs activities outside the nursing home (0.001). Higher GDS score had those who were illiterate (6.41), those with one or no children (6.82 and 6.59 respectively), the bedridden (6.70), people without visits from relatives (7.69) and without activities outside (5.64). Also, social support is affected by the family status ($p < 0.001$), the number of children ($p < 0.001$), the frequency of visits by relatives ($p < 0.001$) and whether the elderly performs activities outside the foundation ($p < 0.008$). Higher MSPSS score had those who were married (61.60), those who had four children (63.50), people who accept visits from relatives every day (64.58) and people who do activities outside the institution (58.07). The appearance of this increased rate of depression symptoms in this elderly population leads to the need for more aid social support.

Keywords Depression • Elderly • Nursing home • Social support

1 Introduction

In modern societies of industrial countries, the phenomenon of population aging is particularly intense [1, 2]. It is estimated that by 2040, 22% of the total population will belong to the age group of over 65. The rapidly increasing number of older people is due to several factors such as the migration, urbanization and, mainly, the reduction of the mortality rate and births [3].

Aging is a non-pathological biological process that affects the human body, but differs from one person to another. It is often difficult to separate the physical changes that occur due to aging from those caused by chronic diseases. Most diseases for the elderly are chronic and, apart from medical procedures, psychological and social support may be needed through a wide range of services, home care or long-term care in special units [4]. When elderly people admitted to hospitals or nursing homes, interactions with family and community are severely limited [5]. This sudden environmental change brings the elderly faced with several stress factors, such as treatment regimens, diagnostic tests and unknown nurses and doctors. This unknown routine does not allow the elderly to control and understand the new environmental conditions. Therefore, when the elderly are no longer able to look after themselves, nurses need to help them in activities which cannot be performed, to provide health education and emotional support [6].

1.1 Depression in Elderly People

In people over 65, depression is the most common mental disorder, which affects one in seven elderly [7, 8]. However, geriatric depression is one of the most underdiagnosed and inadequately treated diseases which have physical, social and psychological consequences. Geriatric depression is very common in hospitals and nursing homes [9, 10]. Depression robs the satisfaction of life and reduces life expectancy while loss of executive functions includes disturbances in the organization, the removal as well as in designing [11]. The elderly with a higher risk of developing depression are women, unmarried, those who live alone and those with a physical disability or illness. If depression in the elderly coexists with other diseases, the risk of early insertion into nursing homes is increased [9].

1.2 Effect of Social Support in Depression

The concept of aging is linked to the contempt and dislike. The elderly are marginalized while it is widely considered that they are unreliable and unable to learn due to the loss of their memory. Psychological distress such as depression or anxiety and stress are effectively reduced with the help of social support. This leads to a variety of physical health benefits and adaptation in diseases such as diabetes mellitus, heart disease, pulmonary disease, arthritis and cancer [12]. Even if social support cannot eliminate the stressful situation, it allows elderly people to be more optimistic. Therefore, social support helps elderly people to cope with difficult situations, creating new solutions and reducing their despair [13, 14].

2 Methods

2.1 Aim

The aim of the study was to evaluate depression in elderly as well as to assess the perceived social support.

2.1.1 Design

In this analytic study 170 elderly people from nursing homes in the broader area of Epirus were asked to take part. The inclusion criteria were: (a) aged >60 years, (b) ability to communicate in Greek language, (c) ability to write and read the Greek language. The exclusion criteria were: (a) elderly people with psychiatric illness. The study was conducted from February 2016 to March 2016.

2.1.2 Data Collection

Elderly residents who participated in the study were given two anonymous questionnaires. In the first part, questions related to sociodemographic data were contained followed by the Geriatric Depression Scale-15 (GDS-15) and the Multidimensional Scale of Perceived Social Support.

2.2 The Multidimensional Scale of Perceived Social Support (MSPSS)

This questionnaire was developed [15] to measure the perceived social support and it is consisted of 12 items referred to three sources of support: family, friends and a special person. Each group is consisted of four items. This questionnaire scores a Likert type scale ranging from 1 (absolutely disagree) to 7 (absolutely agree). The sum of each group gives the sub-scale score. To construct the total score of the scale, all the responses on 12 questions is required to be added. Therefore, the score ranges between 12 and 84. The higher the score, the higher the perceived social support. It takes 3 min to complete. This questionnaire has been translated and cultural adapted in Greek population with Cronbachs' a 0.804 [16].

2.3 The Geriatric Depression Scale-15 (GDS-15)

It is a valid and handy tool which has been developed by Yesevage et al. [17] and has been widely used [18, 19], for assessing elderly depression. It includes 15 closed questions where the elderly respond with "yes" or "no". It takes approximately 5 min to complete. The answer "yes" in items 2, 3, 4, 6, 8, 9, 10, 12 and 14 and the answer "no" in questions 1, 5, 7, 11 and 13 suggest depression. Answers "yes" are encoded with 1 while answers "no" are coded with 0. Therefore, the score ranges from 0 to 15 (0–5: no depression, 6–10: moderate depression, 11–15: severe depression). To calculate the total score of the scale, the score of the 15 responses is required to be summed after the coding of questions 1, 5, 7, 11, 13 has been reversed. The internal consistency has been tested in Greece by Fountoulakis et al. [20] with Cronbach's alpha = 0.94.

2.4 Statistic Analysis

To describe the demographic characteristics and questions about social support and depression in the elderly, the basic position and dispersion measures, frequencies

and relative frequencies were calculated. For the statistical association between social support and the onset of depressive symptoms, parametric correlation coefficient Pearson r was used. To compare the social support and the occurrence of depressive symptoms between groups, the parametric t test for two groups and the non-parametric tests Mann-Whitney and Kruskal-Wallis were used. P-value less than 0.05 were considered statistically significant while for statistical analysis the statistical package IBM SPSS Statistics 23 was used.

2.5 Ethics

The survey responded to the fundamental ethical principles governing the investigation. More in detail, permissions required for the use of the questionnaires were ensured. Permission of the administration of the nursing home was secured, also. Subjects were informed in order to complete the questionnaires. In respect of information related to the elderly, complete confidentiality was observed and the security of data was preserved. Finally, elderly people were informed that their anonymity will be guaranteed and that the results obtained will be used only for the purpose of the research.

3 Results

In this study, 170 elderly people participated. Of these, 33.5% were male while the mean age was 79.52 (±7.135). 11.8% of elderly people were married while 24.1% had no children. A percent of 74.7% was not bedridden, 31.2% had visits from relatives 5–10 times/month while 94.1% was not staying with relatives annually. At the same time, on the question about activities outside the nursing home 77.1% answered negatively (Table 1).

The basic descriptive measures of location and dispersion of depression and perceived social support are presented in Table 2.

Regarding the severity of geriatric depression, 107 elderly people (62.9%) had "no depression", 52 (30.6%) had "moderate depression" while 11 (6.5%) had severe depression. Therefore, in total 37.1% of the residents suffered by depression.

3.1 Correlations

The statistical analysis showed that the total GDS-15 score was significantly correlated with the total MSPSS score. In particular, it was revealed that there is a moderate negative correlation between the two scales (Pearson's $r = -0.552$; $p < 0.001$).

Table 1 Demographic and general characteristics of the participants

		Frequency	(%)
Gender	Male	57	33.5
	Female	113	66.5
Age	79.52 (±7.135)		
Years of education	0	39	22.9
	2–7	94	55.3
	8–13	23	13.5
	>13	14	8.2
Family status	Unmarried	23	13.5
	Married	20	11.8
	Divorced	21	12.4
	Widowed	106	62.4
Number of children	None	41	24.1
	1	22	12.9
	2	41	24.1
	3	39	22.9
	4	14	8.2
	>4	13	7.6
Job	Non manual work	26	15.3
	Manual work	28	16.5
	Farmer	77	45.3
	Household	39	22.9
Bedridden	No	127	74.7
	Yes	43	25.3
Visits by relatives	None	16	9.4
	<5 times/month	48	28.2
	5–10 times/month	53	31.2
	10–20 times/month	29	17.1
	Every day	24	14.1
Staying with relatives annually	Not at all	160	94.1
	Often	9	5.3
	Poorly	1	0.6
Activities outside the nursing home	No	131	77.1
	Yes	59	22.9

Table 2 Basic statistics measures about perceived social support and depression among elderly people

Score	Mean	SD	Minimum	Maximum
MSPSS	54.44	11.315	13.00	80.00
GDS-15	4.98	3.191	0.00	14.00

Also, it was revealed that there is a low positive correlation between GDS-15 score and age (Pearson's $r = 0.174$; $p < 0.023$). GDS-15 score was significantly affected by years of education ($p = 0.003$), number of children ($p = 0.006$),

frequency of visits from relatives ($p < 0.001$) and activities outside the nursing home ($p < 0.001$). Higher GDS-15 score was noted by subjects with 0 years of education, those with none or one child, the bedridden, those who had no visits from relatives and no activities outside the nursing home.

As far as the correlation of MSPSS score with demographic characteristics is concerned, it was revealed that the total MSPSS score is not statistically correlated with age (Pearson's $r = -0.074$; $p = 0.337$). In contrast, the total MSPSS score was significantly affected by family status ($p < 0.001$), number of children ($p < 0.001$), frequency of visits from relatives ($p < 0.001$) and activities outside the nursing home ($p < 0.008$). Higher MSPSS score was noted by married, those with four children, those who had daily visits from relatives and those with activities outside the nursing home (Table 3).

4 Discussion

This study aimed to identify the perceived social support and depression levels among 170 elderly individuals who lived in nursing homes in the broad area of Epirus. In addition, the study aimed to explore correlations between social support and depression. The MSPSS was used in Greek elderly patients for first time. It was constructed in order to assess the perceived social support from family, friends and a special person. According to our findings, the presence of depression is common among elderly residents of nursing homes. In particular, social support can reduce depression levels in elderly.

This study showed that on average elderly persons experience depression. In particular, 30.6% and 6.5% of residents suffered from "moderate depression" and "severe depression" respectively. The reported levels of depression vary widely in elderly residents [21]. Many studies explore the frequency of depression among elderly. In the studies of Stylianopoulou et al. [22] and Argyropoulos et al. [23] 73.4% and 38.6% referred "moderate depression" while 26.6% and 9.5% "severe depression" respectively. In another study [24], 23.9% of residents referred "moderate depression" while the levels of severe depression was high (18.3%).

In current study, a major risk factor associated with depression was the advanced age. More specifically, the higher the age the higher the levels of depression. Similar results are mentioned in several studies [22, 25].

Important factors that affect depression levels are the number of children and the years of education. Argyropoulos et al. [23] found, also, that the low educational level might contribute to increased depression. Regarding the role of children it appears that as the number of children grows, the depression felt by elders is reduced. Stylianopoulou et al. [22] and Unsar et al. [2] highlight, also, the crucial role of children in the development of depression. Chao et al. [26] mention that children is the primary source of support even though the support is not the one they expect.

Table 3 Correlation of demographic characteristics with GDS-15 and MSPSS

		GDS-15				MSPSS		
		Frequency	Mean	SD	P	Mean	SD	P
Gender	Male	57	4.68	3.429	0.389[a]	53.63	12.785	0.512[a]
	Female	113	5.13	3.069		54.84	10.534	
Years of education	0	39	6.41	3.338	0.003[b]	53.72	12.092	0.879[b]
	2–7	94	4.79	2.976		54.20	10.862	
	8–13	23	3.48	3.013		54.70	9.068	
	>13	14	4.79	3.262		57.57	15.530	
Family status	Unmarried	23	6.00	3219	0.062[b]	48.30	13.620	<0.001[b]
	Married	20	3.70	2598		61.60	6.176	
	Divorced	21	6.10	3.986		48.29	11.727	
	Widowed	106	4.78	3.024		55.63	10.349	
Number of children	0	41	6,59	3263	0.006[b]	46.05	13.662	<0.001[b]
	1	22	6.82	3.647		49.82	9.490	
	2	41	4.29	2.866		56.46	7.820	
	3	39	3.54	2.522		57.69	7.226	
	4	14	3.71	2.367		63.50	9.501	
	>4	13	4.69	2.394		62.77	7.574	

Job	No manual work	26	4.15	3.107	0.079[b]	57.39	12.020	0.509[b]
	Manual work	28	5.04	4.096		52.21	11.911	
	Farmer	77	4.75	3.018		54.65	9.737	
	Household	39	5.95	2.695		53.64	13.176	
Bedridden	No	127	4.40	3.053	<0.001[a]	55.10	11.419	0.080[c]
	Yes	43	6.70	2.996		52.46	10.89	
Visits from relatives	Not at all	16	7.69	2.626	<0.001[b]	38.43	8.049	<0.001[b]
	<5 times/month	48	6.21	3.549		50.41	11.81	
	5–10 times/month	53	4.06	2.878		54.94	6.895	
	10–20 times/month	29	3.66	2.256		60.58	7.173	
	Daily	24	4.38	2.618		64.58	8.697	
Activities outside the nursing home	No	131	5.64	2.956	<0.001[c]	53.35	10.67	0.008[c]
	Yes	39	2.77	2.978		58.07	12.71	

[a]t-test
[b]Kruskal-Wallis test
[c]Mann-Whitney test

From this study, also, it was revealed that social support is not significantly correlated with age, but is affected significantly by marital status, the number of children, the frequency of visits received by the residents and the activities carried out by the elderly outside the nursing home. We found that there is risk of developing depression among unmarried or if the elderly subjects do not have visits from their family. This indicates that there is a strong association between lack of family support and depression. We recommend therefore the importance for elderly to ensure family support for preventing depression. Drageset et al. [27] argue that social support is positively correlated with depression while according to Han et al. [28] social support received by a depressed person can help in relieving depressive symptoms. Unsar et al. [2] emphasize to the importance of family support or living with a spouse. Simsek et al. [29] found that elderly who were living in nursing homes experienced worse quality of life than those living at their home with their children or a spouse. Loneliness and depression is strongly associated with a poor social network [30, 31]. This might be related to the emotional benefit of social support.

4.1 Limitations

As already mentioned, the study was conducted in the broad area of Epirus. Thus, we cannot generalize these results for all elderly residents of nursing homes. If the geographic area and the sample were larger, the findings would be more reliable. In addition, elderly completed the questionnaires with the presence of the rest residents and staff. However, the fact that the results agree with the major part of the literature limit the bias.

5 Conclusion

Through this study we found a significant negative correlation between GDS-15 score and MSPSS score. High GDS-score was noted by residents with advanced age, low educational level, those without children, without visits by relatives and, finally, those who long stay in bed. Higher MSPPS score was found among married elderly, those with children and visits by relatives and subjects who had activities outside the nursing home. Thus, these findings helps to better understand the needs of older people who have symptoms of depression. These elements can help to reinforce the social support which in turn can help elderly people cope with depression.

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References

1. Saraçlı, Ö., A.S.D. Akca, N. Atasoy, et al. 2015. The Relationship Between Quality of Life and Cognitive Functions, Anxiety and Depression among Hospitalized Elderly Patients. *Clinical Psychopharmacology and Neuroscience* 13: 194–200.
2. Unsar, S., I. Dindar, and S. Kurt. 2015. Activities of Daily Living, Quality of Life, Social Support and Depression Levels of Elderly Individuals in Turkish Society. *The Journal of the Pakistan Medical Association* 65: 642–646.
3. World Health Organization (WHO). 2015. Mental Health and Older Adults. Available from http://www.who.int/mediacentre/factsheets/fs381/en/.
4. Xiao, H., J.Y. Yoon, and B. Bowers. 2016. Quality of Life of Nursing Home Residents in China: A Mediation Analysis. *Nursing & Health Sciences*. doi:10.1111/nhs.12288.
5. Hung, W.W., J.S. S Ross, K. Boockvar, et al. 2011. Recent Trends in Chronic Disease, Impairment and Disability among Older Adults in the United States. *BMC Geriatrics* 11: 47.
6. Theofanidis, D., Th. Kapadohos, E. Kampisiouli, et al. 2007. Changes in Psychological State of Elder Patients During their Hospitalization. *Rostrum of Asclepius* 2: 1–9.
7. Borza, T., K. Engedal, S. Bergh, et al. 2015. The Course of Depression in Late Life as Measured by the Montgomery and Asberg Depression Rating Scale in an Observational Study of Hospitalized Patients. *BMC Psychiatry* 15: 191.
8. Lima, B.F.R., A.A. Alencar, D.M. Carneiro, et al. 2015. The Efficiency of Electroconvulsive Therapy in the Treatment of Depression in the Elderly Review. *International Archives of Medicine Section: Psychiatry and Mental Health* 8: 1–4.
9. Choi, N.G., S. Ransom, and R.J. Wyllie. 2008. Depression in Older Nursing Home Residents: The Influence of Nursing Home Environmental Stressors, Coping, and Acceptance of Group and Individual Therapy. *Aging & Mental Health* 12: 536–547.
10. Singh, A., and N. Misra. 2009. Loneliness, Depression and Sociability in Old Age. *Industrial Psychiatry Journal* 18: 51–55.
11. Ferreira, A.R., C.C. Dias, and L. Fernandes. 2016. Needs in Nursing Homes and Their Relation with Cognitive and Functional Decline, Behavioral and Psychological Symptoms. *Frontiers in Aging Neuroscience* 8: 72.
12. Kim, H.S., D.K. Sherman, and S.E. Taylor. 2008. Culture and Social Support. *American Psychological Association* 63: 518–526.
13. Erdem, K., and S.E. Apay. 2014. A Sectional Study: The Relationship Between Perceived Social Support and Depression in Turkish Infertile Women. *International Journal of Fertility & Sterility* 8: 303–314.
14. Tsai, H.H., and Y.F. Tsai. 2011. Changes in Depressive Symptoms, Social Support, and Loneliness Over 1 year After a Minimum 3-month Videoconference Program for Older Nursing Home Residents. *Journal of Medical Internet Research* 13 (4): e93. doi:10.2196/jmir.1678.
15. Zimet, E.G., N. Dahlem, S. Zimet, et al. 1988. The Multidimensional Scale of Perceived Social Support. *Journal of Personality Assessment* 52: 30–41.
16. Theofilou, P., S. Zyga, G. Tzitzikos, et al. 2013. Assessing Social Support in Greek Patients on Maintenance Hemodialysis: Psychometric Properties of the Multi-Dimensional Scale of Perceived Social Support. In *Chronic Kidney Disease: Signs/Symptoms, Management Options and Potential Complications*, ed. Rasheed A. Balogun, Emaad M. Abdel-Rahman, and Seki A. Balogun, 265–279. Hauppauge: Nova Publishers.
17. Yesavage, J.A., T.L. Brink, T.L. Rose, et al. 1982. Development and Validation of a Geriatric Depression Screening Scale: A Preliminary Report. *Journal of Psychiatric Research* 17 (1): 37–49.
18. Almeida, O.P., and S.A. Almeida. 1999. Confiabilidade da versão brasileira da escala de depressão em geriatria (GDS) versão reduzida. *Arquivos de Neuro-Psiquiatria* 57: 421–426.
19. Montorio, I., and M. Izal. 1996. The Geriatric Depression Scale: A Review of Its Development and Utility. *International Psychogeriatrics* 8: 103–112.

20. Fountoulakis, K.N., M. Tsolaki, A. Iacovides, et al. 1999. The Validation of the Short Form of the Geriatric Depression Scale (GDS) in Greece. *Aging (Milano)* 11: 367–372.
21. Paque, K., K. Goossens, M. Elseviers, et al. 2016. Autonomy and Social Functioning of Recently Admitted Nursing Home Residents. *Aging & Mental Health* 13: 1–7.
22. Stylianopoulou, C., G. Koulierakis, V. Karagianni, F. Babatsikou, and C. Koutis. 2010. Prevalence of Depression Among Elderly on Open Care Centers for Older People. *Rostrum of Asclepius* 9 (4): 490–504.
23. Argyropoulos, K., C. Bartsokas, and A. Argyropoulou. 2015. Depressive Symptoms in Late Life in Urban and Semi-Urban Areas of South-West Greece: An Undetected Disorder? *Indian Journal of Psychiatry* 57: 295–300.
24. Tika, Ch., M. Tsironi, P. Prezerakos, S. Zyga, S. Tziaferi, F. Babatsikou, and P. Kolovos. 2014. Prevalence of Depression among Elderly Population of a District Nursing Home and Their Satisfaction from the Nursing Care Provided. *Nursing Care & Research* 39: 13–13 1.
25. Koizumi, Y., S. Awata, and S. Kuriyama. 2005. Association Between Social Support and Depression Status in the Elderly: Results of a 1-Year Community-Based Prospective Cohort Study in Japan. *Psychiatry and Clinical Neurosciences* 59: 563–569.
26. Chao, J., L. Li, and H. Xu. 2013. Health Status and Associated Factors Among the Community-Dwelling Elderly in China. *Archives of Gerontology and Geriatrics* 56: 199–204.
27. Drageset, J., G.E. Eide, E. Dysvik, et al. 2015. Loneliness, Loss, and Social Support Among Cognitively Intact Older People with Cancer, Living in Nursing Homes – A Mixed-Methods Study. *Dovepress* 10: 1529–1536.
28. Han, B., B. Yan, J. Zhang, et al. 2014. The Influence of the Social Support on Symptoms of Anxiety and Depression among Patients with Silicosis. *ScientificWorldJournal* 2014: 724804. doi:10.1155/2014/724804.
29. Yümin, E.T., T.T. Şimşek, M. Sertel, A. Öztürk, M. Yümin. 2010. The Effect of Functional Mobility and Balance on Health-Related Quality of Life (HRQoL) Among Elderly People Living at Home and Those Living in Nursing Home. *Archives of Gerontology and Geriatrics* 52: e180–4. doi:10.1016/j.archger.2010.10.027.
30. Wilson, D.M., A. Marin, P. Bhardwaj, et al. 2010. A Hope Intervention Compared to Friendly Visitors as a Technique to Reduce Depression among Older Nursing Home Residents. *Nursing Research and Practice* 2010: 1–6. doi:10.1155/2010/676351.
31. Cohen-Mansfield, J., A. Parpura-Gill. 2007. Loneliness in Older Persons: A Theoretical Model and Empirical Findings. *International Psychogeriatrics* 19: 279–294.

Neurofibromatosis-Noonan Syndrome: A Possible Paradigm of the Combination of Genetic and Epigenetic Factors

Christos Yapijakis, Nikos Pachis, and Costas Voumvourakis

Abstract Neurofibromatosis-Noonan syndrome (NFNS) is a clinical entity possessing traits of autosomal dominant disorders neurofibromatosis type 1 (NF1) and Noonan syndrome (NS). Germline mutations that disrupt the RAS/MAPK pathway are involved in the pathogenesis of both NS and NF1. In light of a studied Greek family, a new theory for etiological pathogenesis of NFNS is suggested. The NFNS phenotype may be the final result of a combination of a genetic factor (a mutation in the NF1 gene) and an environmental factor with the epigenetic effects of muscle hypotonia (such as hydantoin in the reported Greek family), causing hypoplasia of the face and micrognathia.

Keywords Neurofibromatosis I • Von Recklinghausen disease • Noonan syndrome • RAS/MAPK pathway • Phenotype • New theory of pathogenesis • Epigenetics

1 Introduction

Neurofibromatosis-Noonan syndrome (NFNS) was recognized 30 years ago as a clinical entity possessing traits of both neurofibromatosis type 1 (NF1) and Noonan syndrome (NS), which are autosomal dominant disorders [1, 2]. Characteristic signs of NF1 include neurofibromas, café-au-lait spots, osseous lesions, and brain tumors

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such as gliomas [3]. NS includes characteristic facial appearance, hypertelorism, strabismus, broad nasal bridge, low-set ears, short stature, and low posterior hairline [4].

Molecular genetic research has revealed that the genes with NF1 or NS causing mutations are neither allelic nor contiguous. NF1 is caused by mutations in the tumor suppressor gene *NF1*, on chromosome 17p11.2 [5, 6]. *NF1* encodes neurofibromin, which is a large cytoplasmic protein functioning as a rat sarcoma oncogene homolog (RAS) GTPase-activating protein, thus regulating the initial stage of the RAS/mitogen activated protein kinase (MAPK) cascade [5–7].

NS is genetically heterogeneous and includes at least 10 genetically defined types [8, 9]. All known genes of NS types encode proteins that participate in the RAS/MAPK signal-transduction pathway [8, 9]. The most common type is autosomal dominant NS1 (>50% of cases), caused by mutations in gene *PTPN11* (chromosome 12q24), which encodes protein tyrosine phosphatase non-receptor type 11 [10, 11]. Most of the other types are inherited in an autosomal dominant way, except for NS2 which is autosomal recessive [8, 9, 12].

2 The RAS/MAPK Pathway

Extracellular signals such as growth factors activate the RAS/MAPK cascade by binding to a cell membrane-bound receptor tyrosine kinase (RTK), resulting in its dimerization and activation [8, 9]. As a result, activated RTK triggers activation of intracellular RAS GTPases. They in turn initiate activation of a series of protein kinases, which transmit a phosphorylation cascade signal to downstream targets, with each successive kinase serving as a substrate for the upstream one. As a result the RAS/MAPK signal transduction cascade leads to activation of extracellular-signal-regulated kinases that regulate the expression of genes involved in meiosis, mitosis, and postmitotic functions of differentiated cells [9].

The RAS/MAPK cascade is responsible for the pathophysiology of both NF1 and NS types. NF1 regulates RAS in the early stages of the RAS/MAPK cascade along with SHP2, which is encoded by the NS1-related *PTPN11* gene [5, 10]. SHP2 is a protein tyrosine phosphatase, which either inactivates pathway repressors (such as Sprouty family members), or prolongs their activation [9].

The SHP2 protein (NS1-type related) is important for the RTK binding of guanine nucleotide exchange factors Son of sevenless homolog 1 or 2 (SOS1, SOS2) which are NS4- related and NS9-related, respectively. Both SOS1 and SOS2 are able to replace RAS-1 bound GDP with GTP and therefore activate RAS [13, 14]. NF1 (NF1-related) on the other hand, inhibits the RAS/MAPK pathway by enhancing the GTPase activity of RAS proteins, such as the NS3-type related Kirsten rat sarcoma oncogene homolog (KRAS), the NS6-related neuroblastoma rat sarcoma oncogene homolog (NRAS), and the NS8-related RIT1 GTPase [15–18]. Subsequently, activated RAS-GTP triggers the NS5-related cytoplasmic kinase rapidly accelerated fibrosarcoma-1 proto-oncogene, serine/threonine kinase (RAF1)

and the NS7-related BRAF [19, 20]. A protein responsible for marking NF1 for ubiquitin-associated degradation is the NS10-type related leucine zipper-like transcription regulator 1 (LZTR1), which as a result hyperactivates the signal of RAS/MAPK [14]. Based on the above, it is obvious that germline mutations having an impact on function of the RAS/MAPK pathway are involved in the pathogenesis of both NS and NF1.

3 Pathogenesis of Neurofibromatosis-Noonan Syndrome (NFNS)

The rare NFNS shows combined characteristics of both genetic disorders and occurs either sporadically or in some families with typical NF1. Various theories regarding the etiological combination of the two phenotypes NF1 and NS have been proposed over a period of 30 years. These theories have included:

a) the accidental coexistence of the two disorders in some individuals,
b) the possibility of closely linked genes of the two disorders,
c) the possibility that NFNS is a distinct clinical entity,
d) the possibility that NFNS is a clinical variant of NF1.

The first theory, concerning the mere coincidence of two relatively frequent dominant conditions is not statistically justified, since there are far more observed cases than predicted by chance alone. For example, in a cohort of 94 NF1 patients, about 13% of them had NS signs [21]. On the other hand, the observation of mutations in both *NF1* and *PTPN11* genes in patients with NFNS is a rare phenomenon [22, 23].

The second theory, suggesting that NFNS phenotype results due to mutation(s) at closely linked loci has not been supported by linkage analysis and assignment of NS-type genes on various chromosomal regions, since none of them resides on or near the *NF1* gene locus on chromosome 17p11.2 [8, 24]. The third theory that NFNS is a separate entity was proposed by the first reports 30 years ago [1, 2], but since then there is not much evidence that supports it.

The fourth theory, suggesting that NFNS is a clinical variant of the NF1 spectrum of phenotypes, caused by mutations in the NF1 gene, seems plausible according to accumulated evidence. There are reports of families with some members presenting NF1 and other members NFNS, all linked to 17p11.2, while several mutations in the *NF1* gene have been found in NFNS patients [21, 25–30].

We have recently proposed a fifth probable theory explaining NFNS pathogenesis [31]. In light of a family with NF1 and NFNS members, it is possible to postulate that the NFNS phenotype is a result of combined effect of genetic and epigenetic factors.

4 Report of a Family: Clinical and Molecular Genetics

The detailed presentation of the reported Greek family has been previously published [31]. The extended pedigree and the nuclear family members are shown in Figs. 1 and 2, respectively. The father presented typical NF1 while the mother suffered from generalized epilepsy. Their two male children presented NFNS manifestations.

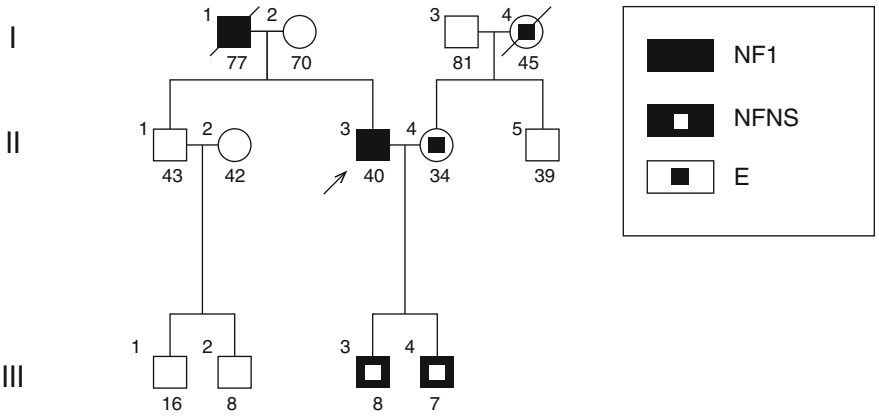

Fig. 1 Pedigree of the studied family. *NF1* Neurofibromatosis 1; *NFNS* neurofibromatosis-Noonan syndrome; *E* epilepsy ([31], with permission)

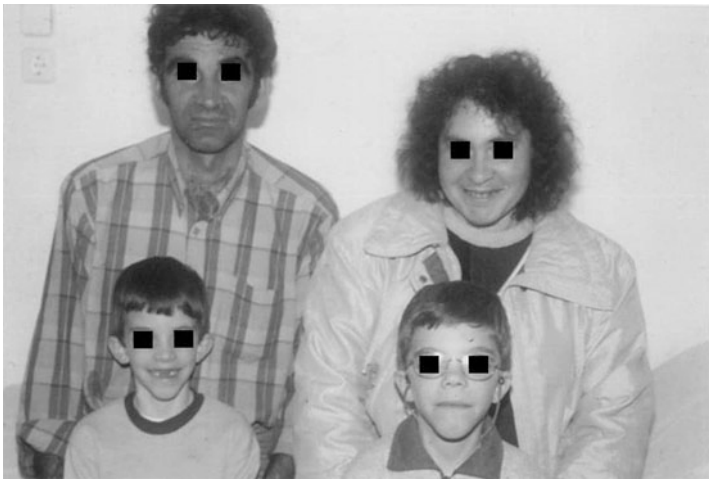

Fig. 2 The four nuclear family members ([31], with permission)

The father (40 years old) had a family history of NF1. He presented many neurofibromas and café-au-lait spots all over his body, an osseous lesion in left parietal bone, and hyperostosis of ulna in both hands.

The mother (34 years old) suffered from mild mental retardation. She first presented with generalized epilepsy at the age of 18 years and she had been under hydantoin treatment ever since. She did not have any clinical manifestations of NF1.

The first boy (8 years old) exhibited NFNS phenotype. He had short stature, weak constitution, moderate mental deficiency, ocular hypertelorism, myopia and strabismus, low nasal bridge, highly arched palate, increased width of mouth, low-set ears and slightly hypoplastic nails. He presented several neurofibromas and café-au-lait spots all over his body.

The second boy (7 years old) also exhibited NFNS phenotype. He had moderately short stature, weak constitution, moderate mental deficiency, hypertelorism, low nasal bridge, highly arched palate, increased width of mouth, low-set ears and hypoplastic distal phalanges and nails of the hands. He presented many neurofibromas and multiple café-au-lait spots.

Cytogenetic analysis of white blood cells revealed normal male karyotypes for both boys. DNA sequencing of *NF1* and *PTPN11* genes was conducted in the two boys and the father. No mutation was detected in the *PTPN11* gene in any of them. However, all three individuals shared mutation R1947X in exon 31 of the NF1 gene in heterozygosity with the normal allele. As a result, the father and his sons had a mutation causing autosomal dominant NF1.

The nonsense mutation R1947X is a C-to-T transition that changes arginine codon to a stop codon and results in protein synthesis of truncated NF1 protein. The mutation is quite common, being located in a CpG dinucleotide mutational hotspot, previously reported in multiple Caucasian and East Asian patients with NF1 [32–42]. As far as we know, it has not been observed in a NFNS case before.

Therefore, it seemed that the two boys presented NFNS as a phenotypic variant of NF1, since they both had inherited the NF1-causing mutation R1947X from their father. Both brothers had clinical signs of NF1, but they also displayed some NS features, such as short stature as well as characteristic facial appearance, including hypertelorism and strabismus, low nasal bridge, increased width of mouth, and low-set ears. At the same time, the two children did not present some other major NS signs, such as short or webbed neck.

The fact that their epileptic mother had received anti-convulsive treatment during both pregnancies in combination with the fact that both boys had hypoplastic nails led to the recognition that their Noonan-like phenotype was due to fetal hydantoin syndrome (FHS) [30]. FHS and NS share many phenotypic characteristics, but the presence of hypoplastic nails is a hallmark of FHS while it is not observed in NS [4, 8, 43]. Therefore, the NFNS phenotype in the two boys was caused by a combination of the inherited NF1-causing mutation and the exposure to the anti-convulsive hydantoin during fetal development.

5 A New Theory Explaining NFNS Pathogenesis

In the reported Greek family, the father clearly presented NF1 and transmitted the mutant *NF1* gene to his sons, while his epileptic wife was under hydantoin treatment throughout both pregnancies. This raises the possibility that the observed NFNS cases reported in the literature are in reality caused by a genetic and an epigenetic factor.

The NFNS phenotype may be the final result of the combined effect of a genetic factor (a mutation in the *NF1* gene) and an epigenetic factor (such as environmentally derived hydantoin). Signs of NS including short stature, mental retardation, and characteristic facial appearance may be observed in other syndromes with NS-like phenotype including XO/XY mosaicism, fetal hydantoin syndrome, fetal mysoline syndrome and fetal alcohol syndrome [4, 8, 44]. It seems that NS-like facial appearance may be produced by several genetic or environmental factors that cause weakness of fetal facial muscles and thus it may represent the end result of non-specific insult(s) during fetal development [1].

It has been suggested that the NS phenotype seen in some patients with NF1 may be the result of dysgenesis or other developmental alterations of the central nervous system resulting in muscular hypotonia [27]. If muscular hypotonia is present, development of craniofacial structures may be substantially altered, leading to midface hypoplasia and micrognathia. If these changes occur in combination with altered craniofacial features known to be common in NF1, such as prominent forehead, hypertelorism and broad nasal tip, the resulting NS-like facial phenotype is easy to envisage.

In conclusion, a new possibility for etiological pathogenesis of NFNS is suggested. The NFNS phenotype may be the final result of a combination of a genetic factor (a mutation in the NF1 gene) and an environmental factor with the epigenetic effects of muscle hypotonia (such as hydantoin in the reported Greek family), causing hypoplasia of the face and micrognathia.

References

1. Allanson, J.E., J.G. Hall, and M.I. Van Allen. 1985. Noonan Phenotype Associated with Neurofibromatosis. *American Journal of Medical Genetics* 21: 457–462.
2. Opitz, J.M., and D.D. Weaver. 1985. The Neurofibromatosis-Noonan Syndrome. *American Journal of Medical Genetics* 21: 477–490.
3. Ferner, R.E., S.M. Huson, N. Thomas, C. Moss, H. Willshaw, D.G. Evans, M. Upadhyaya, R. Towers, M. Gleeson, C. Steiger, and A. Kirby. 2007. Guidelines for the Diagnosis and Management of Individuals with Neurofibromatosis 1. *Journal of Medical Genetics*44: 81–88.
4. Allanson, J.E. 1987. Noonan Syndrome. *Journal of Medical Genetics* 24: 9–13.
5. Lu-Emerson, C., and S.R. Plotkin. 2009. The Neurofibromatoses. Part 1: NF1. *Reviews in Neurological Diseases* 6: E47–E53.
6. Williams, V.C., J. Lucas, M.A. Babcock, D.H. Gutmann, B. Korf, and B.L. Maria. 2009. Neurofibromatosis Type 1 Revisited. *Pediatrics* 123: 124–133.

7. Xu, G.F., P. O'Connell, D. Viskochil, R. Cawton, M. Robertson, M. Cilver, D. Dunn, J. Stevens, R. Gesteland, and R. White. 1990. The Neurofibromatosis Type 1 Gene Encodes a Protein Related to GAP. *Cell* 62: 599–608.
8. Jorge, A.A., A.C. Malaquias, I.J. Arnhold, and B.B. Mendonca. 2009. Noonan Syndrome and Related Disorders: A Review of Clinical Features and Mutations in Genes of the RAS/MAPK Pathway. *Hormone Research* 71: 185–193.
9. Viskochil, D.H. 2011. Disorders of the RAS Pathway: An Introduction. *American Journal of Medical Genetics. Part C, Seminars in Medical Genetics* 157C: 79–82.
10. Tartaglia, M., E.L. Mehler, R. Goldberg, G. Zampino, H.G. Brunner, H. Kremer, I. van der Burgt, A.H. Crosby, A. Ion, S. Jeffery, K. Kalidas, M.A. Patton, R.S. Kucherlapati, and B.D. Gelb. 2001. Mutations in PTPN11, Encoding the Protein Tyrosine Phosphatase SHP2, Cause Noonan Syndrome. *Nature Genetics* 29: 465–468.
11. Musante, L., H.G. Kehl, F. Majewski, P. Meinecke, S. Schweiger, G. Gillessen-Kaesbach, D. Wieczorek, G.K. Hinkel, S. Tinschert, M. Hoeltzenbein, H.-H. Ropers, and V.M. Kalscheuer. 2003. Spectrum of Mutations in PTPN11 and Genotype-Phenotype Correlation in 96 Patients with Noonan Syndrome and Five Patients with Cardiofacio-Cutaneous Syndrome. *European Journal of Human Genetics* 11: 201–206.
12. van der Burgt, I., and H. Brunner. 2000. Genetic Heterogeneity in Noonan Syndrome: Evidence for an Autosomal Recessive Form. *American Journal of Medical Genetics* 94: 46–51.
13. Roberts, A.E., T. Araki, K.D. Swanson, K.T. Montgomery, T.A. Schiripo, V.A. Joshi, L. Li, Y. Yassin, A.M. Tamburino, B.G. Neel, and R.S. Kucherlapati. 2007. Germline Gain-of-Function Mutations in SOS1 Cause Noonan Syndrome. *Nature Genetics* 39: 70–74.
14. Yamamoto, G.L., M. Aguena, M. Gos, C. Hung, J. Pilch, S. Fahiminiya, A. Abramowicz, I. Cristian, M. Buscarilli, M.S. Naslavsky, A.C. Malaquias, M. Zatz, O. Bodamer, J. Majewski, A.A.L. Jorge, A.C. Pereira, C.A. Kim, M.R. Passos-Bueno, and D.R. Bertola. 2015. Rare Variants in SOS2 and LZTR1 are Associated with Noonan Syndrome. *Journal of Medical Genetics* 52: 413–421.
15. Schubbert, S., M. Zenker, S.L. Rowe, S. Boll, C. Klein, G. Bollag, I. van der Burgt, L. Musante, V. Kalscheuer, L.-E. Wehner, H. Nguyen, B. West, K.Y.J. Zhang, E. Sistermans, A. Rauch, C.M. Niemeyer, K. Shannon, and C.P. Kratz. 2006. Germline KRAS Mutations Cause Noonan Syndrome. *Nature Genetics* 38: 331–336.
16. Ferrero, G.B., G. Baldassarre, A.G. Delmonaco, E. Biamino, E. Banaudi, C. Carta, C. Rossi, and M.C. Silengo. 2008. Clinical and Molecular Characterization of 40 Patients with Noonan Syndrome. *European Journal of Medical Genetics* 51: 566–572.
17. De Filippi, P., M. Zecca, D. Lisini, V. Rosti, C. Cagioni, C. Carlo-Stella, O. Radi, P. Veggiotti, A. Mastronuzzi, A. Acquaviva, A. D'Ambrosio, F. Locatelli, and C. Danesino. 2009. Germ-Line Mutation of the NRAS Gene may be Responsible for the Development of Juvenile Myelomonocytic Leukaemia. *British Journal of Haematology* 147: 706–709.
18. Aoki, Y., T. Niihori, T. Banjo, N. Okamoto, S. Mizuno, K. Kurosawa, T. Ogata, F. Takada, M. Yano, T. Ando, T. Hoshika, C. Barnett, H. Ohashi, H. Kawame, T. Hasegawa, T. Okutani, T. Nagashima, S. Hasegawa, R. Funayama, T. Nagashima, K. Nakayama, S. Inoue, Y. Watanabe, T. Ogura, and Y. Matsubara. 2013. Gain-Offunction Mutations in RIT1 Cause Noonan Syndrome, a RAS/MAPK Pathway Syndrome. *American Journal of Human Genetics* 93: 173–180.
19. Pandit, B., A. Sarkozy, L.A. Pennacchio, C. Carta, K. Oishi, S. Martinelli, E.A. Pogna, W. Schackwitz, A. Ustaszewska, A. Landstrom, J.M. Bos, S.R. Ommen, G. Esposito, F. Lepri, C. Faul, P. Mundel, J.P. López Siguero, R. Tenconi, A. Selicorni, C. Rossi, L. Mazzanti, I. Torrente, B. Marino, M.C. Digilio, G. Zampino, M.J. Ackerman, B. Dallapiccola, M. Tartaglia, and B.D. Gelb. 2007. Gain of-Function RAF1 Mutations Cause Noonan and LEOPARD Syndromes with Hypertrophic Cardiomyopathy. *Nature Genetics* 39: 1007–1012.

20. Sarkozy, A., C. Carta, S. Moretti, G. Zampino, M.C. Digilio, F. Pantaleoni, A.P. Scioletti, G. Esposito, V. Cordeddu, F. Lepri, V. Petrangeli, M.L. Dentici, G.M. Mancini, A. Selicorni, C. Rossi, L. Mazzanti, B. Marino, G.B. Ferrero, M.C. Silengo, L. Memo, F. Stanzial, F. Faravelli, L. Stuppia, E. Puxeddu, B.D. Gelb, B. Dallapiccola, and M. Tartaglia. 2009. Germline BRAF Mutations in Noonan, LEOPARD, and Cardiofaciocutaneous Syndromes: Molecular Diversity and Associated Phenotypic Spectrum. *Human Mutation* 30: 695–702.
21. Colley, A., D. Donnai, and D.G. Evans. 1996. Neurofibromatosis/Noonan Phenotype: A Variable Feature of Type 1 Neurofibromatosis. *Clinical Genetics* 49: 59–64.
22. Bertola, D.R., A.C. Pereira, F. Passetti, P.S. de Oliveira, L. Messiaen, B.D. Gelb, C.A. Kim, and J.E. Krieger. 2005. Neurofibromatosis–Noonan Syndrome: Molecular Evidence of the Concurrence of Both Disorders in a Patient. *American Journal of Medical Genetics. Part A* 136: 242–245.
23. Thiel, C., M. Wilken, M. Zenker, H. Sticht, R. Fahsold, G.-C. Gusek-Schneider, and A. Rauch. 2009. Independent NF1 and PTPN11 Mutations in a Family with Neurofibromatosis-Noonan Syndrome. *American Journal of Medical Genetics* 149A: 1263–1267.
24. Edman Ahlbom, B., N. Dahl, P. Zetterqvist, and G. Anneren. 1995. Noonan Syndrome with cafe-au-lait Spots and Multiple Lentigines Syndrome are not Linked to the Neurofibromatosis Type 1 Locus. *Clinical Genetics* 48: 85–89.
25. Nystrom, A.M., S. Ekvall, J. Allanson, C. Edeby, M. Elinder, G. Holmstrom, M.L. Bondeson, and G. Anneren. 2009. Noonan Syndrome and Neurofibromatosis Type I in a Family with a Novel Mutation in NF1. *Clinical Genetics* 76: 524–534.
26. Meinecke, P. 1987. Evidence that the Neurofibromatosis-Noonan Syndrome is a Variant of von Recklinghausen Neurofibromatosis. *American Journal of Medical Genetics* 26: 741–745.
27. Stern, H.J., H.M. Saal, J.S. Lee, P.R. Fain, D.E. Goldgar, K.N. Rosenbaum, and D.F. Barker. 1992. Clinical Variability of Type 1 Neurofibromatosis: Is there a Neurofibromatosis-Noonan Syndrome? *Journal of Medical Genetics* 29: 184–187.
28. Baralle, D., C. Mattocks, K. Kalidas, F. Elmslie, J. Whittaker, M. Lees, N. Ragge, M.A. Patton, R.M. Winter, and C. Ffrench-Constant. 2003. Different Mutations in the NF1 Gene are Associated with Neurofibromatosis–Noonan Syndrome (NFNS). *American Journal of Medical Genetics* 119A: 1–8.
29. De Luca, A., I. Bottillo, A. Sarkozy, C. Carta, C. Neri, E. Bellacchio, A. Schirinzi, E. Conti, G. Zampino, A. Battaglia, S. Majore, M.M. Rinaldi, M. Carella, B. Marino, A. Pizzuti, M.C. Digilio, M. Tartaglia, and B. Dallapiccola. 2005. NF1 Gene Mutations Represent the Major Molecular Event Underlying Neurofibromatosis–Noonan Syndrome. *American Journal of Human Genetics* 77: 1092–1101.
30. Stevenson, D.A., D.H. Viskochil, A.F. Rope, and J.C. Carey. 2006. Clinical and Molecular Aspects of an Informative Family with Neurofibromatosis Type 1 and Noonan Phenotype. *Clinical Genetics* 69: 246–253.
31. Yapijakis, C., N. Pachis, S. Natsis, and C. Voumvourakis. 2016. Is Neurofibromatosis Type 1-Noonan Syndrome a Phenotypic Result of Combined Genetic and Epigenetic Factors? *In Vivo* 30: 315–320.
32. Horiuchi, T., N. Hatta, M. Matsumoto, H. Ohtsuka, F.S. Collins, Y. Kobayashi, and S. Fujita. 1994. Nonsense Mutations at Arg-1947 in Two Cases of Familial Neurofibromatosis Type 1 in Japanese. *Human Genetics* 93: 81–83.
33. Andrews, J.D., D.N. Mancini, S.M. Singh, and D.I. Rodenhiser. 1996. Site and Sequence Specific DNA Methylation in the Neurofibromatosis (NF1) Gene Includes C5839T: The Site of the Recurrent Substitution Mutation in Exon 31. *Human Molecular Genetics* 5: 503–507.
34. Krkljus, S., C.R. Abernathy, J.S. Johnson, C.A. Williams, D.J. Driscoll, R. Zori, H.J. Stalker, S.A. RASmussen, F.S. Collins, B.G. Kousseff, L. Baumbach, and M.R. Wallace. 1998. Analysis of CpG C-to-T Mutations in Neurofibromatosis Type 1. Mutations in Brief no. 129. *Human Mutation* 11: 411.
35. Ainsworth, P.J., D.I. Rodenhiser, and M.T. Costa. 1993. Identification and Characterization of Sporadic and Inherited Mutations in Exon 31 of the Neurofibromatosis (NF1) Gene. *Human Genetics* 91: 151–156.

36. Estivill, X., C. Lazaro, T. Casals, and A. Ravella. 1991. Recurrence of a Nonsense Mutation in the NF1 Gene Causing Classical Neurofibromatosis Type 1. *Human Genetics* 88: 185–188.
37. Heim, R.A., L.M. Silverman, R.A. Farber, L.N.W. Kam-Morgan, and M.C. Luce. 1994. Screening for Truncated NF1 Proteins. *Nature Genetics* 8: 218–219.
38. Lazaro, C., H. Kruyer, A. Gaona, and X. Estivill. 1995. Two Further Cases of Mutation R1947X in the NF1 Gene: Screening for a Relatively Common Recurrent Mutation. *Human Genetics* 96: 361–363.
39. Klose, A., H. Peters, S. Hoffmeyer, A. Buske, A. Lüder, D. Hess, R. Lehmann, P. Nürnberg, and S. Tinschert. 1999. Two Independent Mutations in a Family with Neurofibromatosis Type 1 (NF1). *American Journal of Medical Genetics* 83: 6–12.
40. Park, K.C., H.O. Choi, K.H. Park, K.H. Kim, and H.C. Eun. 2000. A Nonsense Mutation at Arg-1947 in the NF1 Gene in a Case of Neurofibromatosis Type 1 in a Korean Patient. *Journal of Human Genetics* 45: 84–85.
41. Origone, P., A. De Luca, C. Bellini, A. Buccino, R. Mingarelli, S. Costabel, C. La Rosa, C. Garrè, D.A. Coviello, F. Ajmar, B. Dallapiccola, and E. Bonioli. 2002. Ten Novel Mutations in the Humanneurofibromatosis Type 1 (NF1) Gene in Italian Patients. *Human Mutation* 20: 74–75.
42. Yang, Q., C. Huang, X. Yang, Y. Feng, Q. Wang, and M. Liu. 2008. The R1947X Mutation of NF1 Causing Autosomal Dominant Neurofibromatosis Type 1 in a Chinese Family. *Journal of Genetics and Genomics* 35: 73–76.
43. Oguni, M., and M. Osawa. 2004. Epilepsy and Pregnancy. *Epilepsia* 45 (Suppl. 8): 37–41.
44. Rohrer, T. 2009. Noonan Syndrome: Introduction and Basic Clinical Features. *Hormone Research* 72 (Suppl. 2): 3–7.

Clinical Simulation Training in Geriatric Medicine: A Review of the Evidence and Lessons for Training in Psychiatry of Old Age

Christos Plakiotis

Abstract Clinical simulation encompasses a broad range of methods and techniques that allow clinical skills to be rehearsed and practiced away from the clinic before being applied to real patients. As such, preparation of doctors and other healthcare professionals for safe clinical practice is one of its main aims. The objective of this paper was to review the evidence regarding the use of clinical simulation training in geriatric medicine education and consider how the findings may be translated to education in the closely related field of psychiatry of old age. Original papers and descriptive case studies of clinical simulation training programs for medical professionals were considered for inclusion. Papers were grouped according to the participants' level of training: (1) undergraduate medical education; (2) postgraduate medical education; and (3) multiple levels of medical learners. A diverse range of effective simulation modalities for teaching geriatric medicine was identified across all levels of learning. The evidence suggests that there is much fertile ground for trainees in geriatric medicine and psychiatry of old age to participate in joint simulation training programs, thereby maximising their reach while minimising associated resource requirements and financials costs. Given the prominent position of psychiatry of old age at the interface between psychiatry and medicine, old age psychiatrists potentially have much to offer in advancing the field of clinical simulation while simultaneously improving patient care.

Keywords Geriatric medicine • Psychiatry • Old age • Medical education • Clinical simulation

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1 Introduction

1.1 Clinical Simulation in Healthcare Education

One of the main aims of clinical simulation is to prepare doctors and other healthcare professionals for safe clinical practice. It includes a broad array of methods and techniques applicable to both novice and expert learners and to cognitive, affective or psychomotor learnings tasks [1]. Simulations are 'approximations to reality that require trainees to react to problems or conditions as they would under genuine circumstances' [2]. Whether involving clinical vignettes, standardised patients (SPs), mannequins, computerised case management scenarios, virtual reality or a combination of these techniques, clinical simulation has great potential as a training and assessment tool in medical education [3].

While clinical simulation cannot substitute for clinical experience, it is a potent educational technique when learning outcomes are clearly specified. It is a relatively safe, learner-centred approach that should be implemented as part of a broader curriculum rather than in isolation. It allows the acquisition of procedural competence through 'deliberate' task repetition but debriefing and feedback are also necessary for translation of learning into practice [1].

The level of fidelity (realism or authenticity) should be suited to the type of task and learning stage and occurs on a continuum from completely artificial to a real-life situation. At the low end of this continuum is the stem of a clinical vignette that requires the learner to reach a clinical decision. At the high end of the spectrum, use of standardised patients provides a more convincing setting for teaching and assessing clinical skills and competencies [3].

Useful at the undergraduate, graduate and continuing medical education levels, clinical simulation has been shown to lead to improvements in procedural performance in laparoscopic surgery and advanced cardiac life support as well as in medical knowledge (basic science and clinical), the level of comfort with which procedures are performed, teamwork and communication, and retest performance of simulated scenarios. More studies are needed, however, to see if patient outcomes are improved [4].

1.2 Clinical Simulation in Psychiatry

The nature of psychiatry as a largely non-interventional specialty may influence the types of simulation that are most appropriate for delivering educational content to learners. From the author's personal experience as both a trainee and instructor in a large metropolitan tertiary care centre in Victoria, Australia, simulation in psychiatry appears to be most frequently used in relation to preparing trainees for Objective Structured Clinical Examinations (OSCEs) at both undergraduate and postgraduate levels; namely, as part of a general OSCE for fourth-year medical

students at Monash University and as part of the Royal Australian and New Zealand College of Psychiatrists' OSCE for psychiatry trainees. When clinical simulation is used as a teaching modality, it is most frequently used in staging 'mock OSCEs' to prepare candidates for the real examinations. The skills assessed in such examinations are largely related to history taking, physical examination, case formulation and management and are generally non-procedural in the conventional medical sense. As such, availability of sophisticated simulation laboratories with equipment such as mannequins is generally not required. Simulated patients or carers are frequently used in such examinations. Due to the associated costs, these tend to be volunteers in practice examinations (e.g. other learners, candidates or supervisors) and professional actors in real examinations.

Electroconvulsive therapy and, increasingly, transcranial magnetic stimulation are the key procedures performed in psychiatry and are notable exceptions to the above rules. While training in transcranial magnetic stimulation currently tends to be limited to practising specialists, all trainees are expected to undergo training in electroconvulsive therapy. At the author's clinical service, this takes the form of a 3-day teaching program and refresher courses (for psychiatry trainees and nurse and psychiatrist ECT practitioners). The former includes familiarisation in the use of an ECT machine facilitated by a training mannequin followed by practical observation and participation in actual treatments. The other key simulation experience in which all mental health staff are expected to participate annually is basic life support training in a dedicated simulation setting.

1.3 Psychiatry of Old Age and Geriatric Medicine

Psychiatry of old age is a psychiatric sub-specialty that partially overlaps with other areas of medicine and geriatric medicine in particular. In undertaking the comprehensive psychiatric assessment of older adults, it is essential that medical problems that may predispose to, precipitate or perpetuate mental illness are identified and appropriately managed, either directly or through referral to geriatricians or other medical specialists. The 'Curricular Milestones for Graduating Geriatric Fellows'—identified by the American Geriatrics Society and Association of Directors of Geriatric Academic Programs and covering the general and systems-based care of older adults and geriatric syndromes—also resonate as topics of interest to practicing old age psychiatrists [5].

Also common to both specialties is the short supply of their practitioners in an increasingly aging world. A 2012 workforce report by The Institute of Medicine in the United States identified that it will never be possible to train enough specialists in geriatric medicine and psychiatry of old age to care for the rapidly growing numbers of older adults with mental illness and substance use disorders [6]. More than half the training positions in these two specialties go unfilled each year in the United States. Furthermore, the number of old age psychiatrists in that country is predicted to fall from less than 1800 in 2013 to only 1650 by 2030—less than 1 per 6000 older

adults requiring their services [7]. Recruitment and retention to these professions is therefore paramount. In a systematic review examining why geriatric medicine is an unpopular career choice among medical students, the importance of exposure to the rewarding aspects of this profession—as a counter to deterrents such as geriatric patient complexity—was identified as an important goal of pre-clinical and clinical medical education in this field [8]. The need for more resources in teaching old age psychiatry to medical students has also been identified [9]. To facilitate these goals, it is essential that educators in geriatric medicine and psychiatry of old age make full use of modern pedagogical techniques, including clinical simulation, in training doctors at all levels of learning.

2 Objectives

The objective of this paper was to review the literature regarding the use of clinical simulation training in geriatric medicine education and consider the applicability of the findings to education in the closely related field of psychiatry of old age. Relevant papers were identified by entering the search term combination 'geriatric AND simulation' into the PubMed and ERIC databases. Original papers and descriptive case studies of clinical simulation training programs were considered for inclusion and additional relevant studies identified from reference lists. Due to the many papers identified in the related fields of aged care (gerontologic) nursing education and interprofessional healthcare education (i.e. involving learners from more than one profession), it was decided to focus this review on papers examining geriatric clinical simulation for doctors from all specialties and at all levels of learning (from medical students, though to junior and senior residents and specialist physicians). Because of the diverse range of simulation modalities described, papers were grouped according to the participants' level of training: (1) undergraduate medical education; (2) postgraduate medical education; and (3) multiple levels of medical learners.

3 Clinical Simulation Training in Undergraduate Geriatric Medicine Education

The 'Geriatrix' board game of Hoffman et al. [10] was one of the earliest reported uses of clinical simulation in teaching geriatric medicine to medical students. It was conducted as part of a mandatory 2-week geriatrics clerkship for third-year students at the Upstate Medical Centre College of Medicine and involved six to eight students and three instructors at each session. Students and their instructors were able to assume the roles of doctor, staff member, family member and older adult during the game. In immediate post-game evaluations, students indicated that

the game inspired their enthusiasm, resulted in attitude change regarding aging and imparted essential information about geriatric patient care.

In another early initiative, McVey et al. [11] developed an 'Aging Game' modified from the earlier 'Into Aging' simulation game by Hoffman and Reif [12]. The game was designed to encourage medical students at Duke University to consider their own aging process and attitudes towards aging in general, while sensitising them to difficulties confronting older adults, through simulated sensory deficits and functional dependency arising from physical, psychosocial and financial decline. Course evaluations completed by 81 medical students suggested the program was successful in achieving this aim. This Aging Game approach has proven very popular in undergraduate education ever since, as will be seen below.

Lorraine et al. [13] described a geriatric medicine training program in which fourth-year medical students at Eastern Virginia Medical School performed a range of activities of daily living, such as dressing, eating, toileting, taking pills, shopping and paying bills, after being 'impaired' by means of clothes, bindings and other appliances to simulate rheumatoid arthritis, Parkinson's disease, stroke or diabetes. Students' attitudes towards older adults were significantly improved following program participation.

Robinson and Rosher [14] described a 'Half-Full Aging Simulation Experience' at the Southern Illinois University School of Medicine, in which 49 third-year medical students experienced simulated functional deficits associated with normal aging and learnt how these could be overcome through modest environmental changes. Evaluation of students' attitudes before and after the experience demonstrated an improvement in their ability to view older adults as having potential and being able to change and pursue goals, thereby challenging the 'half-empty' perspective on aging they might otherwise hold [15].

Pacala et al. [16] developed an Aging Game simulation workshop and evaluated its effectiveness as a geriatric educational tool over 10 years as part of the University of Minnesota's medical school curriculum [17]. In this workshop, age-related physical, sensory, and cognitive deficits were experienced in simulation by medical students. Considerable personnel requirements notwithstanding, the workshop successfully drew attention to key issues in geriatric medicine and aged care. Positive student feedback was received in relation to the learning modality, attitudinal change and educational merit.

Varkey et al. [18] evaluated a 3-h aging game conducted among 84 first-year medical students at Mayo Medical School and involving experiential learning about medication use, functional impairment and living in residential care. The course was positively received, with 93% of participants indicating they would take the course even if not compulsory. The percentage of participants who felt the game significantly or moderately improved their knowledge and skills in aged care were 61.5% and 37.3% respectively.

Koh et al. [19] examined whether medical students' knowledge and attitudes towards geriatric medicine could be improved via a holistic education program incorporating early exposure, aging simulation and small group teaching. Glasses were worn to simulate cataracts and adhesive tape applied to fingertips to simulate

peripheral neuropathy. Medication boxes were filled with coloured mints to simulate medications with different dosing regimens and water was drawn up in microsyringes to simulate insulin. Applied learning using ambulatory aids and appliances was also facilitated. Two hundred and sixty one students at the Yong Loo Lin School of Medicine at the National University of Singapore were included in the intervention cohort and compared to 254 control students who undertook the old curriculum. Both knowledge and attitudes among the intervention group improved upon completion of the module.

Rull et al. [20] described an 'Aging Couple Across the Curriculum' program in which a couple aged from 60 to 90 over the duration of a 4-year medical course at the Southern Illinois University School of Medicine. Both aging with multiple illnesses and healthy aging were represented in the man and woman respectively. Students acquired a wholistic training experience in complex aged care issues via standardised patient experiences, small-group encounters, and paper-based learning modules. Participants rated sessions as good or excellent and found interactions with the aging couple particularly valuable.

Tunuguntla et al. [21] randomised 156 first-year medical students at the University of Miami Miller School of Medicine to undertake an interactive online module in home safety assessment—an important topic in geriatric medicine education—with either animated or static graphics. The authors found no advantage in employing the more expensive animated graphics in terms of students' cognitive burden scale scores, competency assessment test scores, and time spent on task.

Sutin et al. [22] reported on the use of a standardised patient to teach 42 third-year medical students at the New York University School of Medicine about assessing older adults with recurrent falls and other geriatric syndromes. Played by one of nine actresses, the patient aged from 75 years (with falls) to 80 years (with memory problems) to 82 years (with an acute confusional state) over the course of a 3-h simulation. In an end-of-year clinical skills examination that included a 79-year-old man with falls, students who had participated in the prior simulation performed significantly better at this particular station and were three times as likely to undertake gait examination.

van Zuilen et al. [23] reported on a competency-based program—including a small-group simulated patient interview—for teaching second-year medical students to identify the risks of polypharmacy and provide recommendations to improve medication safety in a non-threatening setting. On average, 16.1 out of 18 concerns were identified and 15.4 out of 18 recommendations were made following the simulated patient interview and participant satisfaction was high. At the competency test some months later, 176 students were given a case scenario and asked to recognize seven possible risks and provide seven recommendations. The standard (score of 8 out of 14) was attained by 97.2% of students at their first attempt.

As part of a 'Chronic Illness, Disability and Rehabilitation' module at Newcastle Medical School, Fisher and Walker [24] implemented a simulation session in which four geriatric medicine clinical scenarios (delirium, falls, elder abuse and breaking bad news) were taught to 74 third-year medical students using mannequins,

professional role-players and simulated clinical documentation. Student performance on a knowledge test was significantly better after the clinical simulation training and was maintained when reassessed 1 month later. Participants' views of the simulation session and its effects on their attitudes towards geriatric medicine were unequivocally favourable.

Medical students at the University of Louisville School of Medicine were given the opportunity to have 19 encounters with a simulated patient depicted by the same role-player as part of a Longitudinal Standardized Patient Project over their two pre-clinical years [25]. Each of the nine simulated patients (including two aged over 65 years) had a distinctive medical history and illness course aligned with history-taking and communication skills learning objectives. Familiarity with patients' personal and medical histories allowed students to concentrate on communication tasks and documentation of continuity of care learning experiences. An unexpected advantage of this project was the teaching relationship that developed between role-players and students over time.

4 Clinical Simulation Training in Postgraduate Geriatric Medicine Education

In an interesting randomized trial undertaken at the University of Indiana School of Medicine, Westmoreland et al. [26] used clinical simulation to evaluate first-year residents' ability to clinically apply geriatric medicine training delivered by web-based and paper-based learning methodologies. First-year geriatric medicine residents on a 1-month ambulatory care placement underwent block randomisation to web- or paper-based teaching regarding four topics: dementia, depression, urinary incontinence and falls. Pre- and post-testing was undertaken in both groups to ascertain the effectiveness of theoretical knowledge acquisition.

Residents were advised during the learning process that they would also encounter unannounced standardised patients and activated standardised patients in a real world outpatient setting over a 1-year period to evaluate their ability to apply the knowledge learnt. Standardised patients were actors trained to depict particular clinical scenarios. They were employed in a non-traditional manner by inserting them directly into an outpatient clinic to assess residents' performance in an encounter the latter perceived to be real. The aim was for residents to be unaware they were seeing an 'unannounced standardised patient'. In addition, some of these patients were 'activated standardised patients' who had been trained to ask residents pre-set questions about their healthcare during the clinical episode [26].

Knowledge test scores were significantly better following web-based rather than paper-based learning. Scores from standardised patient and activated standardised patient interactions were not significantly different apart from a better chart abstraction score (rating of residents' paper medical record charts) for dementia following web-based learning. As the study progressed, residents became increasingly able

to identify unannounced patients and their use was met with dissatisfaction by both study participants (who felt they detracted from real patient care) as well as their supervisors (who were not alerted to their presence), resulting in study termination 1 month early. The authors concluded that while web-based teaching may be useful in improving residents' knowledge of geriatric medicine theory, more rigorous educational endeavours may be needed to improve their practical clinical performance [26].

Andrade et al. [27] evaluated an avatar-mediated, 3-dimensional (3-D) OSCE station (of 15 minutes' duration) as a more expedient and flexible alternative to traditional home visits for examining eight geriatric medicine fellows' competency in home safety assessments. Fellows had to identify home safety hazards in a 3-D virtual environment—which they navigated using their avatar, a graphical representation of themselves—and provide written management recommendations which were independently marked by two examiners against an inventory-based checklist. The fellows identified less than half of the fifty hazards (mean score of 43%) located in seven simulated living areas. Six of eight participants rated the simulation as 'excellent' despite four of five women (but none of three men) reporting navigation difficulties. The authors suggested that avatar-mediated, 3-D virtual worlds may facilitate examination of challenging or impractical skills beyond home safety assessment, such as disaster management, team-based skills and cultural competencies.

Webb and Duthie [28] described the development of a geriatrics training curriculum for second postgraduate year surgical trainees. Inadequate educational attention to aged care topics and discomfort in caring for older adults was revealed by a needs assessment survey of the seven trainees in the program. Instruction was undertaken by general surgeons and geriatricians over 10 h in 1 year and was based on adult learning methods, including patient simulation using Objective Structured Video Examination (OSVE). Evaluation of the initial, case-based session of the curriculum, concerning critical care and end-of-life issues in older adults, showed a 57–86% improvement in participants' pre- to post-test scores respectively and content and teaching quality were highly rated. The authors considered their approach to be promising in improving the care of older surgical patients through enhancing surgeons' knowledge of their medical and social needs.

A more recent study by Duane et al. [29] demonstrated the limitations of traditional educational approaches in teaching geriatric medicine to 49 surgical interns (51%), residents and critical care fellows, as assessed by a patient simulation examination. Residents received educational materials in polypharmacy, delirium and end-of-life care for review in their own time, including three suggested readings and four websites. Pre- and post-test scores (1 month later) for knowledge acquisition were consistently better for polypharmacy. The poorest post-test scores were for end-of-life issues. Irrespective of pre- and post-test performance, there was no correlation with better patient care as assessed by objective (physician) and subjective (patient satisfaction) grading on a simulation examination involving an older adult patient and his or her carer. The authors concluded that alternative models of geriatric medicine education for surgeons are required.

Biese et al. [30] evaluated the impact of a 1-year geriatric curriculum on 29 emergency medicine residents' knowledge, attitudes and decision-making in treating older patients. The program included six lectures (medication management, care transitions, iatrogenic injuries, confusional states, trauma and abdominal pain) and seven high-fidelity simulations (pressure ulcer infection, drugs of abuse, salicylate toxicity, medication-induced gastrointestinal bleeding, mesenteric ischemia, myocardial infarction and aortic aneurysm). Pre- to post-test knowledge scores improved from 58.5% to 68.0% respectively. Residents' attitudes and the percentage of older adults receiving chemical sedation and urinary catheterisation did not change. However, there was a significant reduction (from 8 of 49 to 1 of 47) in inappropriate urinary catheterisation. Improvements in knowledge and decision-making were suggested to reduce the risk of adverse outcomes among older emergency department attendees.

5 Clinical Simulation Training in Geriatric Medicine for Multiple Levels of Medical Learners

Williams et al. [31] examined the utility of a Geriatrics Standardized Patient Instructor (GSPI) in assessing and teaching geriatrics skills to medical learners from different specialties and training levels. Teaching revolved around the functional assessment of a patient prior to hospital discharge. Seventeen standardised patients were trained and assisted geriatrician assessors in rating learners on functional assessment and communication skills. The GSPI was implemented as a formative assessment among 138 house officers from nine specialties. House officers positively received the entire experience and obtained mean scores of 78 and 86 (out of 100) for functional assessment and communication skills respectively. The GSPI was also implemented as part of an intensive, multifaceted educational initiative among 171 first-year medical students. The mean score for both functional assessment and communication skills was 93 and the experience was again positively received. The GSPI was subsequently effectively utilised among second- and third-year medical students and house officers from 12 different disciplines and included in OSCE-based training programs for new third-year medical students and house officers [31].

Orton and Mulhausen [32] described the development of the University of Iowa's GeriaSims e-learning virtual patient programs and their effectiveness as geriatric medical education tools for medical students, primary care residents and physicians. Nine modules were developed covering delirium, dementia, functional assessment, polypharmacy, falls, urinary incontinence, failure to thrive or undernutrition, ischemic stroke and palliative care. The modules simulated one or a series of clinical encounters, in either longitudinal format (same patient) or thematic format (common theme but different patients). The effectiveness of the GeriaSims modules in meeting learning objectives was verified in survey results of multiple levels of learners. The self-paced flexibility of the online learning environment was important in the uptake of these modules.

In a related paper, Ruiz and Leipzig [33] outlined the benefits of the GeriaSims Falls Module in particular, which covered important aspects of falls assessment, management and prevention through four realistic, interactive and evidence-based patient vignettes. Although the module was targeted at second- to fourth-year medical students, residents, fellows, and physicians, some of the content was identified as probably being too difficult for most second-year students. The learners' main goal was to make two decisions based on data available in the 'patient chart.' The authors concluded that development of the GeriaSims Falls Module was worthwhile despite the expense and time required.

Shield et al. [34] outlined a program for integrating geriatric medicine training into the entire curriculum at the Warren Alpert Medical School of Brown University, from medical student, clerkship and residency training to practising physician levels. One learning activity involved the use of interactive media cases of older virtual patients. In particular, geriatricians assisted in developing a lively DVD of a longitudinal, multi-year case of an older woman who falls with resulting serious consequences and comorbidities. Epidemiological context, screening tests and management were included as were accounts of falls from several older patients. No further details about this program or its perceived utility were provided.

Tan et al. [35] described the experiences of Harvard Medical School and the Miller School of Medicine at the University of Miami in developing and implementing geriatric virtual patients into their curriculum. Virtual patient simulation proved to be a valuable asset in standardising geriatric medicine training at Harvard Medical School, which occurred longitudinally across the educational curriculum. Using a pre-existing Harvard Medical School virtual patient template, seven geriatrics modules were developed: delirium, dementia, hypertension and falls, pain management, skin and chronic wound care, osteoporosis and ethics. The modules were offered to internal medicine interns and residents at affiliated internal medicine residency programs. Several modules were also offered to clinicians globally as continuing medical education.

At the University of Miami, medical students, internal medicine residents and geriatric medicine fellows studied mandatory curricula incorporating virtual patient simulations. To improve trainees' skills in the management of urinary incontinence and the overactive bladder, six virtual patient simulations with graphics and video media were developed. Participants reported enhanced self-confidence in assessing and managing urinary incontinence and satisfaction with the program [35].

Andrade et al. [36] evaluated the practicality, utility and acceptability of virtual geriatric home safety assessments, including whether spatial ability, cognitive load (increased in participants with low spatial ability), presence (a participant's feeling of actually being in the simulated environment) and self-efficacy influenced the efficiency with which hazards were identified. Participants included 30 medical trainees (13 men and 17 women): ten senior medical students, ten internal medicine interns and ten geriatric medicine fellows. The home safety simulation was simple to use and improved trainees' self-efficacy. Performance was better among men than women and significantly correlated to spatial ability and presence, prompting the authors to urge educators to consider these factors in implementing virtual reality training in this field.

6 Discussion

6.1 The Scope for Clinical Simulation Training in Psychiatry of Old Age

Review of the literature regarding the utilisation of clinical simulation training by our geriatric medicine colleagues suggests that there may be merit in the wider adoption of this approach as a teaching modality in psychiatry of old age. There is considerable theoretical and practical overlap between these two fields, as evidenced, for example, by the inclusion of a web-based training module on depression for geriatric medicine residents in the study by Westmoreland et al. [26]. The link extends much further, however. Delirium and dementia are core topics for both disciplines. The geriatric medical syndromes covered in the papers reviewed herein, such as polypharmacy, falls, incontinence, pain management and palliative care, frequently arise in old age psychiatry care settings and awareness of their importance by old age psychiatrists is imperative both for the safe practice of their own discipline and for appropriate referral to, and shared management with, geriatric physicians. Similarly, geriatricians frequently encounter the entire spectrum of mental illness in patients they see and identification of the interplay between psychiatric and medical illness in older adults and appropriate liaison with psychiatric services is essential for effective geriatric medicine practice.

Consequently, there is much fertile ground for advanced trainees in both disciplines to undergo joint simulation training experiences. Psychiatry trainees may benefit from structured simulation training experiences in key medical syndromes affecting older adults, akin to those offered to geriatric medicine trainees and described in detail in this review. In turn, geriatric medicine trainees may benefit from the greater inclusion of psychiatric content in their simulation training curriculum so they are better prepared to identify key psychiatric conditions, such as mood and anxiety disorders and psychosis, and how these may impact on the manifestation and management of medical illnesses.

In considering the types of simulation activities described in this review, it seems likely that some types of tasks may be more suitable or acceptable than others depending on trainees' level of experience. Experiential activities designed to simulate the aging process [11–19] may be more suitable for medical students rather than advanced trainees in psychiatry of old age who are already immersed in clinical settings in which unhealthy aging is unfortunately common. Conversely, the use of online simulations akin to those of the GeriaSims training modules [32, 33] or 3-D, avatar-based, virtual home safety assessments [27] may represent more useful templates for teaching clinical skills to advanced learners. The use of the same simulated patient over a 2-year training experience, as described by Kodner and Bohnert [25], is an interesting concept that is worth exploring further in both a medical student context as described but also in postgraduate specialist training. While advanced trainees in geriatric medicine and psychiatry of old age may be more likely than medical students to have prolonged continuity of care experiences

with actual patients, this cannot always be guaranteed, and simulated patients may be a useful vehicle for teaching core competencies. Ideally, at least some of the simulated patient cases should have psychiatric and medical comorbidity and be jointly offered to trainees of both disciplines.

The paper by Westmoreland et al. [26] also strikes a note of caution in relation to the limitations of clinical simulation as an assessment tool in medical education more broadly. Clinical simulation is indeed frequently and overtly used to assess clinical performance in the OSCE format. What the paper of Westmoreland et al. [26] draws attention to is the lack of acceptability to trainees, and potentially supervisors also, of unpredictably interspersing simulation-based assessment tasks in real world clinical practice. In this study, prior warning about the presence of unannounced simulated patients was inadequate in reducing participant and supervisor dissatisfaction related to their appearances. The authors' findings suggest that transparency is paramount in designing clinical simulation activities for both training and assessment purposes, so that learners feel safe and not inadvertently misled by the process.

6.2 Recommendations for Future Practice and Clinical Research

According to Ker and Bradley [1], a greater emphasis on accountability will see doctors having to rehearse and practice their skills away from the clinic before exercising these with real patients. For this reason, the future of clinical simulation appears to be well-established at this point in time. From the perspective of psychiatry of old age it seems reasonable to address this increased focus on accountability by exploring ways of better utilising clinical simulation at all levels of fidelity in teaching this discipline at the undergraduate, postgraduate and continuing medical education levels. Given the potential resources and financial costs associated with implementing large-scale clinical simulation activities, teaming up with geriatricians on the one hand and general adult and consultation-liaison psychiatrists on the other may represent an efficient means of maximising the reach of any programs that are developed. Extending these programs to involve nursing and allied health clinicians may achieve further benefits in economy of scale and provide for a true interprofessional learning experience that bolsters team management of vulnerable older adults with mental illness.

At the same time, it is important to critically evaluate the effectiveness of such endeavours as a basis for their further refinement. A research agenda for simulation-based healthcare education, developed following an Utstein Style Meeting in Copenhagen, provides a useful template for undertaking future research in this field, providing a range of research questions clustered under the themes of instructional design, outcomes measurement and translational research [37]. Given the prominent position of psychiatry of old age at the interface between psychiatry and medicine,

old age psychiatrists potentially have much to offer in advancing this important area of medical education while simultaneously improving patient care.

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References

1. Ker, J., and P. Bradley. 2014. Simulation in Medical Education. In *Understanding Medical Education: Evidence, Theory and Practice*, ed. T. Swanwick, 2nd ed., 175–192. Chichester, West Sussex: John Wiley & Sons, Ltd.
2. Tekian, A., C.H. McGuire, and W.C. McGaghie. 1999. *Innovative Simulations for Assessing Professional Competence: From Paper-and-Pencil to Virtual Reality*. Chicago, Illinois: University of Illinois at Chicago, Department of Medical Education.
3. Munshi, F., H. Lababidi, and S. Alyousef. 2015. Low- Versus High-Fidelity Simulations in Teaching and Assessing Clinical Skills. *Journal of Taibah University Medical Sciences* 10: 12–15. doi:10.1016/j.jtumed.2015.01.008.
4. Okuda, Y., E.O. Bryson, S. DeMaria, et al. 2009. The Utility of Simulation in Medical Education: What is the Evidence? *MSJ The Mount Sinai Journal of Medicine* 76: 330–343.
5. Parks, S.M., G.M. Harper, H. Fernandez, et al. 2014. American Geriatrics Society/Association of Directors of Geriatric Academic Programs Curricular Milestones for Graduating Geriatric Fellows. *Journal of the American Geriatrics Society* 62: 930–935. doi:10.1111/jgs.12821.
6. Institute of Medicine (U.S.), J. Eden, K. Maslow, et al. 2012. *The Mental Health and Substance Use Workforce for Older Adults: In Whose Hands?* Washington, DC: National Academies Press.
7. Bartels, S.J., and J.A. Naslund. 2013. The Underside of the Silver Tsunami—Older Adults and Mental Health Care. *The New England Journal of Medicine* 368: 493–496.
8. Meiboom, A.A., H. de Vries, C.M.P.M. Hertogh, and F. Scheele. 2015. Why Medical Students Do Not Choose a Career in Geriatrics: A Systematic Review. *BMC Medical Education*. doi:10.1186/s12909-015-0384-4.
9. Gregory, C.A., and T. Dening. 1995. Teaching Old Age Psychiatry to Medical Students in England. *International Journal of Geriatric Psychiatry* 10: 883–886.
10. Hoffman, S.B., F.R. Brand, P.G. Beatty, and L.A. Hamill. 1985. Geriatrix: A Role-Playing Game. *The Gerontologist* 25: 568–572.
11. McVey, L.J., D.E. Davis, and H.J. Cohen. 1989. The 'Aging Game': An Approach to Education in Geriatrics. *JAMA* 262: 1507–1509.
12. Hoffman, T.L., and S.D. Reif. 1978. *Into Aging: A Simulation Game*. Thorofare, New Jersey: Charles B Slack Inc.
13. Lorraine, V., S. Allen, A. Lockett, and C.M. Rutledge. 1998. Sensitizing Students to Functional Limitations in the Elderly: An Aging Simulation. *Family Medicine* 30: 15–18.
14. Robinson, S.B., and R.B. Rosher. 2001. Effect of the "Half-Full Aging Simulation Experience" on Medical Students' Attitudes. *Gerontology & Geriatrics Education* 21: 3–12. doi:10.1300/J021v21n03_02.
15. Damron-Rodriguez, J., B.J. Kramer, and D. Gallagher-Thompson. 1998. Effect of Geriatric Clinical Rotations on Health Professions Trainees' Attitudes About Older Adults. *Gerontology & Geriatrics Education* 19: 67–79. doi:10.1300/J021v19n02_07.
16. Pacala, J.T., C. Boult, C. Bland, and J. O'Brien. 1995. Aging Game Improves Medical Students' Attitudes Toward Caring for Elders. *Gerontology & Geriatrics Education* 15: 45–57. doi:10.1300/J021v15n04_05.

17. Pacala, J.T., C. Boult, and K. Hepburn. 2006. Ten Years' Experience Conducting the Aging Game Workshop: Was It Worth It? *Journal of the American Geriatrics Society* 54: 144–149. doi:10.1111/j.1532-5415.2005.00531.x.
18. Varkey, P., D.S. Chutka, and T.G. Lesnick. 2006. The Aging Game: Improving Medical Students' Attitudes Toward Caring for the Elderly. *Journal of the American Medical Directors Association* 7: 224–229. doi:10.1016/j.jamda.2005.07.009.
19. Koh, G.C., R.A. Merchant, W.S. Lim, and Z. Amin. 2012. The Knowledge-Attitude Dissociation in Geriatric Education: Can It Be Overcome? *Annals Academy of Medicine Singapore* 41: 383.
20. Rull, G., R.B. Rosher, N. McCann-Stone, and S.B. Robinson. 2006. A Simulated Couple Aging Across the Four Years of Medical School. *Teaching and Learning in Medicine* 18: 261–266. doi:10.1207/s15328015tlm1803_12.
21. Tunuguntla, R., O. Rodriguez, J.G. Ruiz, et al. 2008. Computer-Based Animations and Static Graphics as Medical Student Aids in Learning Home Safety Assessment: A Randomized Controlled Trial. *Medical Teacher* 30: 815–817. doi:10.1080/01421590802263508.
22. Sutin, D., L. Rolita, N. Yeboah, et al. 2011. A Novel Longitudinal Geriatric Medical Student Experience: Using Teaching Objective Structured Clinical Examinations. *Journal of the American Geriatrics Society* 59: 1739–1744. doi:10.1111/j.1532-5415.2011.03538.x.
23. van Zuilen, M.H., R.M. Kaiser, and M.J. Mintzer. 2012. A Competency-Based Medical Student Curriculum: Taking the Medication History in Older Adults. *Journal of the American Geriatrics Society* 60: 781–785. doi:10.1111/j.1532-5415.2011.03871.x.
24. Fisher, J.M., and R.W. Walker. 2014. A New Age Approach to an Age Old Problem: Using Simulation to Teach Geriatric Medicine to Medical Students. *Age and Ageing* 43: 424–428. doi:10.1093/ageing/aft200.
25. Kodner, C., and C. Bohnert. 2015. The Longitudinal Standardized Patient Project: Innovation from Necessity. *Academic Medicine* 90: 317–320. doi:10.1097/ACM.0000000000000565.
26. Westmoreland, G.R., S.R. Counsell, W. Tu, et al. 2010. Web-Based Training in Geriatrics for Medical Residents: A Randomized Controlled Trial Using Standardized Patients to Assess Outcomes. *Journal of the American Geriatrics Society* 58: 1163–1169. doi:10.1111/j.1532-5415.2010.02869.x.
27. Andrade, A.D., P. Cifuentes, M.C. Oliveira, et al. 2011. Avatar-Mediated Home Safety Assessments: Piloting a Virtual Objective Structured Clinical Examination Station. *Journal of Graduate Medical Education* 3: 541–545. doi:10.4300/JGME-D-11-00236.1.
28. Webb, T.P., and E. Duthie. 2008. Geriatrics for Surgeons: Infusing Life into an Aging Subject. *Journal of Surgical Education* 65: 91–94. doi:10.1016/j.jsurg.2007.12.004.
29. Duane, T.M., L. Fan, A. Bohannon, et al. 2011. Geriatric Education for Surgical Residents: Identifying a Major Need. *The American Surgeon* 77: 826–831.
30. Biese, K.J., E. Roberts, M. LaMantia, et al. 2011. Effect of a Geriatric Curriculum on Emergency Medicine Resident Attitudes, Knowledge, and Decision-Making. *Academic Emergency Medicine* 18: S92–S96. doi:10.1111/j.1553-2712.2011.01170.x.
31. Williams, B.C., K.E. Hall, M.A. Supiano, et al. 2006. Development of a Standardized Patient Instructor to Teach Functional Assessment and Communication Skills to Medical Students and House Officers. *Journal of the American Geriatrics Society* 54: 1447–1452. doi:10.1111/j.1532-5415.2006.00857.x.
32. Orton, E., and P. Mulhausen. 2008. E-Learning Virtual Patients for Geriatric Education. *Gerontology & Geriatrics Education* 28: 73–88. doi:10.1300/J021v28n03_06.
33. Ruiz, J.G., and R.M. Leipzig. 2008. GeriaSims: Falls Module. *Journal of the American Geriatrics Society* 56: 130–131. doi:10.1111/j.1532-5415.2007.01480.x.
34. Shield, R.R., T.F. Wetle, and R.W. Besdine. 2008. Preparing for the Silver Tsunami: The Integration of Geriatrics into the New Medical Curriculum at the Warren Alpert Medical School of Brown University. *Medicine and Health, Rhode Island* 91: 247–249.
35. Tan, Z.S., P.L. Mulhausen, S.R. Smith, and J.G. Ruiz. 2010. Virtual Patients in Geriatric Education. *Gerontology & Geriatrics Education* 31: 163–173. doi:10.1080/02701961003795813.

36. Andrade, A.D., P. Cifuentes, M.J. Mintzer, et al. 2012. Simulating Geriatric Home Safety Assessments in a Three-Dimensional Virtual World. *Gerontology & Geriatrics Education* 33: 233–252. doi:10.1080/02701960.2011.611553.
37. Issenberg, S.B., C. Ringsted, D. Østergaard, and P. Dieckmann. 2011. Setting a Research Agenda for Simulation-Based Healthcare Education: A Synthesis of the Outcome from an Utstein Style Meeting. *Simulation in Healthcare: The Journal of the Society for Simulation in Healthcare* 6: 155–167. doi:10.1097/SIH.0b013e3182207c24.

Integrating Omic Technologies in Alzheimer's Disease

Antigoni Avramouli and Panayiotis M. Vlamos

Abstract Scientific advances in biomedical disciplines have allowed us to identify the underlying causes of many diseases with increased comprehension—leading the way towards precision medicine. In this context, unique disease and medical traits pave the way for the development of adapted disease management, drugs and therapies tailored to each patient. Bearing in mind that reductionism, an approach that has dominated biomedical research for many years and has resulted in the identification of definite cellular phenotypes and human diseases which are linked with specific integral molecules, we strongly believe that Alzheimer's Disease, one of the most common neurodegenerative diseases, could not be applied to the model of one disease—one assay—one drug. Regarding the discrete complexities in the molecular pathogenesis combined with the limited knowledge of inherited and sporadic forms of Alzheimer's disease, the great heterogeneity in the clinical development, as well as the plethora of validated biomarkers that have been proposed for early diagnosis or prognosis of the disease, we presume that a radically different way of thinking is in demand for comprehensive explanations of the molecular pathogenesis of the disease. In this article we highlight the most recent advances made in the omics field of systems biology towards a more complete understanding of Alzheimer's disease mechanisms, emphasizing to the paramount emergence of the development of various high-throughput strategies applied to the omics sciences.

Keywords Precision medicine • Omics • Alzheimer's disease • Systems biology • Biomarkers

1 Introduction

Complex diseases are caused by a combination of genetic, biological, and environmental factors. The determination of disease etiology necessitates the alignment of clinical phenotypes with underlying biomolecular mechanisms. Consequently,

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researchers have traditionally tried to identify distinct clinical phenotypes at first and then to compare biomolecular factors that might explain differentiations in disease manifestation.

Since 1906, when Dr Alois Alzheimer [1], first described the presence of dense deposits outside the neurons and bands of tangles within the brain cells in the autopsied brain of a woman, we still have a slight knowledge about the underlying mechanisms that cause Alzheimer's Disease (AD). AD is described as a multifactorial disease and is subjected to exhaustive investigation. Most relevant discoveries on mechanisms and pathways underlying AD have come from comprehensive studies on genetic factors and their contribution to the disease. Analysis at this level demands not only the study of gene sequences, searching for single nucleotide polymorphisms or mutations, but also genomic alterations and consequently protein dysfunction or differences in concentration levels. All these facts may lead to the clinical manifestation of the disease, as they affect the regulation of pathways and networks underlying susceptibility to AD.

Omic data have gained a lot of attention in the last decade, in particular in those areas of biomedicine where we face clear unmet medical needs. Created as a new model for complex problem solving, system biology approaches seem to open promising perspectives particularly for a better understanding of complex diseases such as Alzheimer's disease and other dementias. In this review, we shall provide a brief overview of high-throughput technologies used in the omics field of research and we shall describe briefly recent advances on AD investigation.

2 High-Throughput Technologies in Systems Biology

Phenotype is the result of complex system interconnections of a large number of components whose interactions accounts for a variety of biological functions. A fundamental aspect that drove the development of systems biology is the advances in various high-throughput biotechnologies. Omic technologies primarily aim to the detection of genes (genomics), mRNA (transcriptomics), proteins (proteomics) and metabolites (metabolomics) precisely in a specific biological sample. These technologies not only allow the quantification of single molecules (genes, microRNAs, protein levels, and metabolites) of a biological system, but also provide the generation of massive interactomes that describe the complex interconnections of these molecules, and even decipher the function of the system.

The term genomics provides sequence information on the genetic mapping and DNA sequencing of sets of genes or the complete genomes of selected organisms. Whole-genome sequencing efforts of the previous century have produced many fully sequenced genomes, following the completion of the Human Genome Project [2, 3]. Sanger sequencing and next-generation sequencing (NGS) techniques are used in order to analyze the complete DNA sequence of an organism's genome [4, 5]. Sanger sequencing reads one base-pair at a time, while NGS utilizes numerous sequencing reactions at the same run, allowing immediate sequencing and

examination of millions of base-pairs. Nonetheless, our lack of understanding of the functional consequences of most genetic alterations encountered in many diseases, greatly limits our capacity to incorporate Whole Genome Sequencing widely into the clinic [6].

Transcriptomics use gene expression microarray technology to study gene expression by quantifying mRNA transcripts on thousands of genes at the same time [7]. In this method DNA molecules are attached to each slide and act as probes to detect the set of messenger RNA (mRNA) transcripts expressed by a group of genes. The data from microarray analysis are stored in computer databases and can be used further to generate gene expression profiles, that show simultaneous variations in the expression of many genes in response to a specific condition or treatment. Furthermore, an other technique, NGS-based RNA sequencing (RNA-seq) can be used to estimate gene expression levels at a higher resolution and sample throughput [8]. It can also reveal alternative gene spliced transcripts. This data can be integrated furthermore, in many database repositories that store massive microarray- and sequence-based gene expression datasets and can be reused as a basis for new biological studies [9].

In many cases, however, it is protein altered functionalities that are enabled in many pathological conditions. This third downstream "omics" field is known as proteomics [10]. Proteomic studies elucidate the protein complement of cells, including identification, quantification, localization, and modification. The most comprehensive and versatile tool in large-scale proteomics is mass spectrometry (MS), that uses mass analysis for protein characterization. Mass spectrometry (MS) can be used to determine the concentration of thousands of proteins in a single experiment [11, 12]. Whereas DNA microarray technology is based on a highly sensitive and specific hybridization reaction between nucleic acid fragments, inherent limitations of biological MS [13] require several different approaches to protein analysis. Implementation of these strategies (e.g., sample preparation, front-end separation, ionization, data acquisition, and data analysis) differs depending on the sample complexity and the goals of the analysis [14].

Finally, global metabolomic profiling by nuclear magnetic resonance (NMR) and liquid chromatography (LC) or gas chromatography (GC) coupled with MS is used to measure the concentration and composition of both targeted and untargeted metabolites [15]. In particular, mass cytometry facilitates high-dimensional quantitative analysis of the effects of molecules at single-cell resolution [16]. Such single-cell genomic analyses greatly enhance diagnostic and experimental analyses.

3 Application of Omic Technologies in Alzheimer's Disease

Many genetic loci have been identified to be associated with susceptibility to AD and have been reviewed in the recent article by Tosto and Reitz [17]. Most common mutations have been mapped in APP (49 pathogenic mutations), in PSEN1 (216 mutations) and PSEN2 (16 mutations) genes (http://www.molgen.ua.ac.be/

ADMutations/) [18–20]. Also Apolipoprotein e4 allele (APOEe4) increases Late Onset Alzheimer's Disease (LOAD) risk [21, 22]. Moreover, this allele is implicated in lower memory function and earlier onset of AD [23–27]. The molecular mechanisms relating APOE and AD risk increase are not fully elucidated yet, whereas many research teams have found significant differences among ethnic groups [28, 29]. Over the past decade, the development of high-throughput technologies assisted in our better comprehension of diseases at the molecular level. This fact revolutionized biomedical science and has notably advanced the understanding of the molecular grounds of AD. In addition to the genes mentioned above, over 20 common functional variants exerting large effects on AD risk have been identified by recent NGS studies [17]. The conducted studies indicate significant locus heterogeneity; in addition, they clearly indicate that not only common variants with small effect sizes, but also many other low-frequency or rare coding variants with moderate to large effect sizes contribute to AD risk [17].

Despite these recent advances in AD genomics, a major part of the genetic contribution to AD remains still unexplained. Furthermore, the direct impact of the identified loci on clinical diagnosis and prognosis is very limited at the moment. Scientists remain far of understanding if the observed effect sizes for all these variants have clinical implications, whereas the genetic loci that have been recognized have demonstrated limited statistical power to predict disease risk.

Transcriptomic studies have identified a large amount of genes and putative pathways associated with AD pathogenesis. These studies compare mRNA transcript expression levels, as mentioned above, among AD patients and healthy controls. In 2008, Liang et al. [30] published an influential study describing gene expression profiles from postmortem investigation of six anatomically and functionally distinct brain regions in AD patients. Notably, the authors have disclosed significant regional differential expression in AD brains compared with healthy control brains, including expression variations of genes previously implicated in AD pathogenesis and remarkably concerning the formation of plaques and tangles. Tan et al. [31] also described consistent patterns of alteration in the gene expression profile in the neocortex of AD patients compared to healthy subjects. The exploration of the transcriptome in those patients revealed synaptic dysfunction, disrupted neurotransmission, and generation of neuroinflammation.

Many studies have noticed a distinctive perturbation that characteristic of AD when compared to blood transcriptomes from other neurological diseases, as well as among AD patients and MCI subjects [32]. In light of this, there have been many blood tests have been developed and discriminate AD patients from non-demented control subjects. They also simplify the assessment of gene dysregulation in different stages of MCI and AD. These tests include a blood RNA test with a sensitivity of 100% and specificity of 96% in AD diagnosis [33], a blood gene expression evaluation with high accuracy of 87% [34] and AclarusDx™, a blood-based transcriptomic test [35].

Proteins are supposed to have a high potential as dynamic biomarkers in the prognosis, diagnosis, and progress monitoring of a given disease [36]. Plethora of studies have been employed to study proteome alterations in AD during the last

decade (reviewed in [37, 38]). The use of high-throughput systems aided in the examination of different types of clinical biofluids, especially cerebrospinal fluid (CSF) and blood (i.e., plasma/serum). Proteomic analyses of AD patients have recorded over 1000 proteins that are differentially expressed. Nevertheless, the interpretation of the results is difficult, and a direct association between specific proteomic reports and AD pathogenesis remains unclear [38]. Most of these studies conclude that the diagnostic accuracy is increased by the measurement of currently recognized CSF markers of AD such as Aβ42, total tau (t-tau), phosphorylated tau (p-tau), and the recently added neurofilament light protein (NFL) [38, 39]. These biomarkers, at least on a group level, robustly separate AD patients from controls, or serve to differentiate patients according to their disease status. Recently an important initiative launched by the Human Proteome Organization (HUPO), attempts to elucidate the CNS proteome in both normal ageing and neurodegenerative diseases [40].

Metabolic alterations have been recognized for many pathological conditions, including AD [41]. Metabolomic profiling is an approach that suits clinical applications as it can examine quite easily biofluids or peripheral tissues [42]. Numerous metabolites levels are dysregulated in AD. However, efforts to combine data obtained from these studies have been limited since metabolomic platforms and biofluids employed were unrelated and the studies diverged in the range of the detected metabolites. Notably, a recent study analysis of MCI individuals and AD patients, revealed that the altered pathways among these subjects have been mostly associated with energy metabolism and mitochondrial activity as well as biosynthesis, trafficking or metabolism of amino acids, lipids, neurotransmitters and hormones [42]. The evidence that APOE, a lipid chaperone protein, is the most valuable biomarker for sporadic late onset of AD, points to the importance of lipid and metabolic dynamics as a field that needs to be explored in AD research. Furthermore, projects like HUSERMET [43, 44] and PredictAD (available at http://www.predictad.eu/), are currently available and aim to the disclosure of serum-derived metabolic markers in AD. Intriguingly, metabolomic profiling offers the opportunity to associate the molecular pathway variations with the physiology and structure of cells and tissues.

4 Conclusion

Although systems biology is in its infancy and faces many challenges, it will undoubtedly transform the medical and healthcare practice. With the development of high throughput technologies, the insights of networks in cells and tissues were combined with multivariate analyses, resulting in an integrative approach that starts with the patient. Such methods when combined with omic data information could advance AD research and development. This may not only suggest new insights by creating a system-based understanding of this heterogeneous disease, but also it could offer new opportunities into disease diagnosis and prediction yielding clinical benefit in the improvement of the clinical management of the disease.

References

1. Alzheimer, A. 1898. Neuere Arbeiten über die Dementia senilis und die auf atheromatöser Gefässerkrankung basierenden Hirnkrankheiten. *Monatsschrift für Psychiatrie und Neurologie* 3: 101–115.
2. Lander, E.S., L.M. Linton, B. Birren, C. Nusbaum, M.C. Zody, et al. 2001. Initial Sequencing and Analysis of the Human Genome. *Nature* 409: 860–921.
3. Venter, J.C., M.D. Adams, E.W. Myers, P.W. Li, R.J. Mural, et al. 2001. The Sequence of the Human Genome. *Science* 291: 1304–1351.
4. Mardis, E.R. 2008. Next-Generation DNA Sequencing Methods. *Annual Review of Genomics and Human Genetics* 9: 387–402.
5. Sanger, F., S. Nicklen, and A.R. Coulson. 1977. DNA Sequencing with Chain-Terminating Inhibitors. *Proceedings of the National Academy of Sciences* 74: 5463–5467.
6. Chrystoja, C.C., and E.P. Diamandis. 2014. Whole Genome Sequencing as a Diagnostic Test: Challenges and Opportunities. *Clinical Chemistry* 60 (5): 724–733.
7. Taub, F.E., J.M. DeLeo, and E.B. Thompson. 1983. Sequential Comparative Hybridizations Analyzed by Computerized Image Processing Can Identify and Quantitate Regulated RNAs. *DNA* 2 (4): 309–327.
8. Wang, Z., M. Gerstein, and M. Snyder. 2009. RNA-Seq: A Revolutionary Tool for Transcriptomics. *Nature Reviews Genetics* 10: 57–63.
9. Rung, J., and A. Brazma. 2013. Reuse of Public Genome-Wide Gene Expression Data. *Nature Reviews Genetics* 14: 89–99.
10. Yarmush, M.L., and A. Jayaraman. 2002. Advances in Proteomic Technologies. *Annual Review of Biomedical Engineering* 4: 349–373.
11. Ong, S.E., L.J. Foster, and M. Mann. 2003. Mass Spectrometric-Based Approaches in Quantitative Proteomics. *Methods* 29: 124–130.
12. Wiese, S., K.A. Reidegeld, H.E. Meyer, and B. Warscheid. 2007. Protein Labeling by iTRAQ: A New Tool for Quantitative Mass Spectrometry in Proteome Research.*Proteomics* 7: 340–350.
13. Ghaemmaghami, S., W.K. Huh, K. Bower, R.W. Howson, A. Belle, et al. 2003. Global Analysis of Protein Expression in Yeast. *Nature* 425: 737–741.
14. Ruse, C.I., and J.R. Yates III. 2007. Mass Spectrometry in Systems Biology. In *Systems Biology*, ed. I. Rigoutsos and G. Stephanopoulos, 3–42. Oxford: Oxford University Press.
15. Shah, S.H., W.E. Kraus, and C.B. Newgard. 2012. Metabolomic Profiling for the Identification of Novel Biomarkers and Mechanisms Related to Common Cardiovascular Diseases: Form and Function. *Circulation* 126: 1110–1120.
16. Bendall, S.C., E.F. Simonds, P. Qiu, A.D. Amir el, P.O. Krutzik, et al. 2011. Single-Cell Mass Cytometry of Differential Immune and Drug Responses Across a Human Hematopoietic Continuum. *Science* 332: 687–696.
17. Tosto, G., and C. Reitz. 2016. Genomics of Alzheimer's Disease: Value of High Throughput Genomic Technologies to Dissect Its Etiology. *Molecular and Cellular Probes* 30 (6): 397–403.
18. Goate, B.A., M.C. Chartier-Harlin, M. Mullan, et al. 1991. Segregation of a Missense Mutation in the Amyloid Precursor Protein Gene with Familial Alzheimer's Disease. *Nature* 349: 704–706.
19. Levy-Lahad, E., W. Wasco, P. Poorkaj, et al. 1995. Candidate Gene for the Chromosome 1 Familial Alzheimer's Disease Locus. *Science* 269: 973–977.
20. Sherrington, R., E.I. Rogaev, Y. Liang, et al. 1995. Cloning of a Gene Bearing Missense Mutations in Early-Onset Familial Alzheimer's Disease. *Nature* 375: 754–760.
21. Ashford, J.W., and J.A. Mortimer. 2002. Non-familial Alzheimer's Disease is Mainly Due to Genetic Factors. *Journal of Alzheimer's Disease* 4 (3): 169–177.
22. Slooter, A.J., M. Cruts, S. Kalmijn, et al. 1998. Risk Estimates of Dementia by Apolipoprotein E Genotypes from a Population-Based Incidence Study: The Rotterdam Study. *Archives of Neurology* 55 (7): 964–968.

23. Breitner, J.C., B.W. Wyse, J.C. Anthony, et al. 1999. APOE-Epsilon4 Count Predicts Age When Prevalence of AD Increases, Then Declines: The Cache County Study. *Neurology* 53 (2): 321–331.
24. Corder, E.H., A.M. Saunders, W.J. Strittmatter, et al. 1993. Gene Dose of Apolipoprotein E Type 4 Allele and the Risk of Alzheimer's Disease in Late Onset Families. *Science* 261: 921–923.
25. Gomez-Isla, T., H.L. West, G.W. Rebeck, et al. 1996. Clinical and Pathological Correlates of Apolipoprotein E Epsilon 4 in Alzheimer's Disease. *Annals of Neurology* 39 (1): 62–70.
26. Holmes, C., R. Levy, D.M. McLoughlin, J.F. Powell, and S. Lovestone. 1996. Apolipoprotein E: Non-cognitive Symptoms and Cognitive Decline in Late Onset Alzheimer's Disease. *Journal of Neurology, Neurosurgery, and Psychiatry* 61: 580–583.
27. Kurz, A., K. Altland, N. Lautenschlager, et al. 1996. Apolipoprotein E Type 4 Allele and Alzheimer's Disease: Effect on Age at Onset and Relative Risk in Different Age Groups. *Journal of Neurology* 243: 452–456.
28. Romas, S.N., V. Santana, J. Williamson, et al. 2002. Familial Alzheimer Disease Among Caribbean Hispanics: A Reexamination of Its Association with APOE. *Archives of Neurology* 59: 87–91.
29. Tang, M.X., Y. Stern, K. Marder, et al. 1998. The APOE-Epsilon4 Allele and the Risk of Alzheimer Disease Among African Americans, Whites, and Hispanics. *JAMA* 279: 751–755.
30. Liang, W.S., T. Dunckley, T.G. Beach, et al. 2008. Altered Neuronal Gene Expression in Brain Regions Differentially Affected by Alzheimer's Disease: A Reference Data Set. *Physiological Genomics* 33: 240–256.
31. Tan, M.G., W.T. Chua, M.M. Esiri, et al. 2010. Genome Wide Profiling of Altered Gene Expression in the Neocortex of Alzheimer's Disease. *Journal of Neuroscience Research* 88: 1157–1169.
32. Han, G., J. Wang, F. Zeng, et al. 2013. Characteristic Transformation of Blood Transcriptome in Alzheimer's Disease. *Journal of Alzheimer's Disease* 35: 373–386.
33. Fehlbaum-Beurdeley, P., A.C. Jarrige-Le Prado, D. Pallares, et al. 2010. Toward an Alzheimer's Disease Diagnosis Via High-Resolution Blood Gene Expression. *Alzheimer's & Dementia* 6: 25–38.
34. Booij, B.B., T. Lindahl, P. Wetterberg, et al. 2011. A Gene Expression Pattern in Blood for the Early Detection of Alzheimer's Disease. *Journal of Alzheimer's Disease* 23: 109–119.
35. Fehlbaum-Beurdeley, P., O. Sol, L. Désiré, et al. 2012. Validation of AclarusDx™, a Bloodbased Transcriptomic Signature for the Diagnosis of Alzheimer's Disease. *Journal of Alzheimer's Disease* 32: 169–181.
36. Thambisetty, M., and S. Lovestone. 2010. Blood-based biomarkers of Alzheimer's disease: challenging but feasible. *Biomarkers in Medicine* 4: 65–79.
37. Cacabelos, R. 2016. Proteomic Biomarkers in Alzheimer's Disease. *Diagnostic Pathology Open Access* 1: 1.
38. Lista, S., Z.S. Khachaturian, D. Rujescu, F. Garaci, B. Dubois, and H. Hampel. 2016. Application of Systems Theory in Longitudinal Studies on the Origin and Progression of Alzheimer's Disease. *Methods in Molecular Biology* 1303: 49–67.
39. Olsson, B., R. Lautner, U. Andreasson, A. Öhrfelt, et al. 2016. CSF and Blood Biomarkers for the Diagnosis of Alzheimer's Disease: A Systematic Review and Meta-Analysis. *Lancet Neurology* 8: 1474–4422.
40. Hamacher, M., and H.E. Meyer. 2005. HUPO Brain Proteome Project: Aims and Needs in Proteomics. *Expert Review of Proteomics* 2: 1–3.
41. Han, X., D. MHoltzman, and D.W. McKeel Jr. 2002. Substantial Sulfatide Deficiency and Ceramide Elevation in Very Early Alzheimer's Disease: Potential Role in Disease Pathogenesis. *Journal of Neurochemistry* 82: 809–818.
42. Trushina, E., T. Dutta, X.M. Persson, et al. 2013. Identification of Altered Metabolic Pathways in Plasma and CSF in Mild Cognitive Impairment and Alzheimer's Disease Using Metabolomics. *PLoS One* 8: e63644.

43. Cottingham, K. 2008. HUSERMET Researchers Look to the Metabolome for Answers. *Journal of Proteome Research* 7: 4213.
44. Dunn, W.B., D. Broadhurst, P. Begley, et al. 2011. Procedures for Large-Scale Metabolic Profiling of Serum and Plasma Using Gas Chromatography and Liquid Chromatography Coupled to Mass Spectrometry. *Nature Protocols* 6: 1060–1083.

Anxiety and Depression in Staff of Mental Units: The Role of Burnout

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Abstract One of the most investigated areas is the study of job stress and anxiety and its effects on the professionals' mental health status. The purpose of this study was to research the levels of anxiety and depression in staff that works in mental units and if burnout is related with these mental health parameters. The sample consisted of 217 mental health care professionals from mental health care units of all over Greece. The Greek version of the Symptoms Rating Scale for Depression and Anxiety (SRSDA) questionnaire was used to evaluate the levels of anxiety and depression and the Greek version of Maslach's Burnout Inventory (MBI) were used. Descriptive statistics were initially generated for sample characteristics. General linear models with MBI dimensions as independent variables and the anxiety and depression subscales of SRSDA as dependent variables were used to determine the relation between burnout and mental health parameters. Statistics were processed with SPSS v. 19.0. Statistical significance was set at $p = 0.05$. The average age of the sample was 39.00 ± 8.19 years. Regarding gender the percentage of men was 24.88% ($N = 54$) and of women 75.11% ($N = 163$). The means for the subscales of SRSDA were 4.91 ± 4.87 for Anxiety, 6.21 ± 5.92 for Depression Beck-21 and 2.83 ± 3.41 for Depression Beck-13. The results of general linear models are

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shown that Emotional Exhaustion and Depersonalization are statistically correlated with Anxiety and Depression Subscales of SRSDA. Burnout plays an important role in anxiety and depression levels of the staff that works in mental health units all over Greece.

Keywords Anxiety • Depression • Burnout • Mental health staff

1 Introduction

One of the most investigated areas in terms of empirical and applied research, is the study of job stress and anxiety and its effects on the professionals' mental health [1–5].

Many studies correlate stress in the workplace with factors, such as doubt, conflict and overload [6–9]. Another approach, which draws on its reasoning of the Cherniss model [10], includes other sources of stress and anxiety, such as personal factors and factors related to the working environment [11–14]. This approach is largely motivated by the perception that stress and anxiety are caused by the working environment and this fact triggers a chain reaction that eventually ends to pathological situations, physical and mental effects [15–17].

Anxiety and welfare are reported in literature as mental health indicators [18–21]. Stress in mental health research likely constitutes a problematic variable, since the corresponding theoretical position that exists, seems not to have been clearly formulated. Typically, stress is seen as a mental health indicator [22–24].

Regarding depression, its connection to the working environment is relatively recent. Depression is defined as a set of negative feelings, suicidal thoughts, emotional fatigue and social resignation [25, 26]. The depression related to job justifiably is examined in the context of job burnout [27]. The few studies on depression in work context were guided by the theories on stress and burnout [28, 29]. Although it is difficult to draw firm conclusions from the relatively limited evidence of researchers, it seems that the examination of depression in the job context enriches the understanding of the relationship between job and mental health [30–32]. Some researchers have developed the concept of "professional depression" as a more comprehensive concept, which includes common elements of depression and burnout [33].

Research on burnout appears more structured and largely guided by the work of Maslach and Jackson [34]. Burnout was seen as a syndrome, mainly caused by chronic and prolonged occupational stress, manifested in emotional exhaustion, depersonalization and reduced feeling for personal achievements, especially among humanitarian and of social contribution professions.

While recognizing the similarities between the two concepts of depression and burnout, Leiter and Durup have highlighted the fundamental difference between them [26]. Specifically, they argued that while burnout is a social construct, depression is clinical in nature that reflects personal thoughts and human feelings. Therefore, burnout can be considered as a matter related to the work environment,

while depression is more comprehensive as a concept and it has not any specific professional connectivity to the business environment [26]. In this context, Leiter and Durup have presented empirical evidence demonstrating the validity of the distinction between burnout and depression [26].

Regarding to anxiety, the burnout syndrome may be distinguished from anxiety, since the first has long-term nature, while the latter is usually considered as something more transient [35].

Within this rationale that tries to distinguish anxiety and depression from burnout, the question that comes out, refers to the way that anxiety, depression and burnout can be identified in a mental health model. Another question refers to what should be the appropriate levels of their analysis. The attempt to find out a clinical concept in a fundamental social-psychological framework seems to be quite difficult. Furthermore, the nature of the relationship between these three parameters has not yet been theoretically elucidated. Some questions as if anxiety and depression can lead to burnout or vice versa, or whether they both have some common and some unique causes, have been the focus of several studies.

2 Purpose

The purpose of this study was to investigate the levels of anxiety and depression in staff of mental units and if burnout is related with these mental health parameters.

The research questions were:

1. Which are the anxiety and depression levels of the personnel that works in mental health units?
2. What is the level of burnout among mental health care staff?
3. Is there a correlation between burnout, anxiety and depression? Which dimensions of burnout are correlated the most?

3 Material and Methods

3.1 Sample and Sampling

The sample in this study consisted of 217 mental health care professionals who were randomly selected from mental health care units of all over Greece. Local ethical committees approved the study protocol. Doctors, nurses, psychologists and other mental healthcare specialists participated in the study. The response rate was 72.33% (217 out of 300 questionnaires).

3.2 Demographic Data

For the demographic data of the sample questions about gender, age, marital status, number of children, educational status, profession, years in profession and years in department were used.

3.3 Anxiety and Depression Levels Measuring

The Greek version of the Symptoms Rating Scale for Depression and Anxiety (SRSDA) questionnaire was used to evaluate the levels of anxiety and depression among the staff of mental units [36]. SRSDA includes 42 items and contains six subscales; the 21-item Beck Depression Subscale, the 13-item Beck Depression Subscale, the Melancholia Subscale, the Asthenia Subscale, the Anxiety Subscale and the Mania Subscale.

As concerning the anxiety and depression subscales of the SRSDA the composition is as follows:

(1) The 14-item Anxiety Subscale includes the items 3, 4, 5, 12, 15, 17, 21, 24, 25, 27, 33, 39, 40 and 42. These are scored as: a = 0, b = 1, c = 2, d = 3.
(2) The 21-item Beck Depression Subscale (BDI-21) includes the items 1, 8, 11, 13, 14, 17, 18, 19, 20, 21, 22, 23, 25, 26, 27, 28, 29, 31, 32, 34 and 41. These are scored as: a = 0, b = 1, c = 2, d = 3.
(3) The 13-item Beck Depression Subscale (BDI-13) includes the items 1, 8, 11, 13, 14, 19, 20, 22, 28, 29, 32, 34 and 41. These are scored as: a = 0, b = 1, c = 2, d = 3.

The optimal cut-off points established for the Greek population in these subscales are: Anxiety:10/11, BDI-21:14/15 and BDI-13:7/8.

3.4 Burnout Measuring

The Greek version of Maslach's Burnout Inventory (MBI) was used for measuring burnout levels [37]. MBI is a seven point Likert scale of 22 items (0: never happens to me, 6: it happens to me every day). The 22 questions are consisted in three dimensions of burnout; emotional exhaustion, personal accomplishment and depersonalization. Questions 5, 10, 11, 15, 22 refer to depersonalization, 4, 7 9, 12, 17, 18, 19, 21 refer to personal accomplishment and the rest refer to depersonalization. To discriminate between the levels of each dimension of burnout, scores that provided by the Greek version of MBI were used, referring to burnout dimensions in health care professionals in Greece, classifying burnout in low, moderate and high category [37].

3.5 Statistics

Descriptive statistics were initially generated for sample characteristics. Normality was checked by the Kolmogorov-Smirnov test. The results of the SRSDA subscales and of the MBI dimension were mixed. Some subscales exhibited marginally normal distributions (emotional exhaustion, personal accomplishment and anxiety) while others were clearly not normally distributed (depersonalization, Beck-21 and Beck-13). As there are articles in the literature arguing that parametric tests can successfully be applied to non-normally distributed data [38, 39], data were processed with parametric tests. Chronbach's α for the SRSDA and MBI subscales was ranged between 0.81 and 0.90. The cut-off points of the Greek version of SRSDA were used to determine the anxiety and depression status of the sample. The classification of the Greek version of MBI was used in order to find out if the levels of burnout are low, moderate or high. General linear models with MBI dimensions as independent variables and Anxiety and Depression Subscales of SRSDA as dependent variables were used to determine the role of burnout in anxiety and depression levels of the staff. Statistics were processed with SPSS v.19.0 for Windows. P values <0.05 were defined as reflecting the acceptable level of statistical significance.

4 Results

4.1 Demographic Characteristics

Table 1 shows the demographic and job features of the sample. The average age of the staff that works in mental health units is 39.00 ± 8.19 years. Regarding gender the percentage of men is 24.88% (N = 54) and of women 75.11% (N = 163). As for marital status, 60.20% declared married (mean score = 0.94, 0 = single, 1 = married, 2 = divorced) and 38.90% of the married declared that they do not have children. The 47.50% of the sample members has graduated from higher education institutes (universities and technological educational institutes) and as for the profession 63.13% are nurses, 14.28% doctors and 22.59% other specialties. The mean score of years in profession for the sample is 9.31 ± 7.50 and for years in department is 4.07 ± 4.27.

4.2 Anxiety and Depression Levels

The results for the Anxiety and Depression Subscales of SRSDA are presenting in Tables 2 and 3. Table 2 is showing the results for each question of the Anxiety and Depression Subscales and Table 3 the results for the sum of Anxiety and Depression

Table 1 Demographic characteristics of the sample

Demographic features	Mean	SD
Age	39.00	8.19
Marital status	0.94	0.88
Number of children	1.20	1.11
Degree	0.69	0.62
Graduate year	1994	7.75
Years in profession	9.31	7.50
Years in department	4.07	4.27

Gender	N	%
Men	54	24.88
Women	163	75.11

Profession	N	%
Nurses	137	63.13
Doctors	31	14.28
Others	49	22.59

Subscales. The means for the subscales of SRSDA is 4.91 $\pm$ 4.87 for Anxiety, 6.21 $\pm$ 5.92 for Depression Beck-21 and 2.83 $\pm$ 3.41 for Depression Beck-13. The cut off points of the Greek version of SRSDA are the F scale in Table 3. According to the mean scores of the subscales and the F scale, most of the mental health care workers do not appear anxiety or depression disorder. The percentages of the sample members with a possible psychiatric disorder (scores over cut off points) are 12.20% for Anxiety, 7.50% for Depression Beck-13 and 9.90% for Depression Beck-21.

4.3 Burnout Levels

Burnout levels are shown in Table 4 and are compared with the mean scores for each dimension that have been provided by the Greek version of MBI (28). Overall the burnout level of the staff that works in mental health units is moderate. The mean score for emotional exhaustion is 18.46 (low $\leq$ 20.00), for personal accomplishment 37.28 (moderate = 36.00–41.00) and for depersonalization 6.73 (moderate = 6.00–10.00). Regarding the percentages of the sample, the 13.36% (N = 29) is presenting high levels of emotional exhaustion, the 29.95% (N = 65) high levels of personal accomplishment and finally 26.26% (N = 57) is presenting high levels of depersonalization.

Table 2 Descriptive statistical results for each question of the depression and anxiety subscales of SRSDA

Beck-21 Questions for depression			Beck-13 Questions for depression			14 Questions for anxiety		
Question	Mean	±SD	Question	Mean	±SD	Question	Mean	±SD
1	0.17	0.51	1	0.17	0.51	3	0.35	0.93
8	0.25	0.54	8	0.25	0.54	4	0.34	0.65
11	0.14	0.45	11	0.14	0.45	5	0.27	0.49
13	0.08	0.33	13	0.08	0.33	12	0.22	0.61
14	0.22	0.48	14	0.22	0.48	15	0.13	0.35
17	0.42	0.85	19	0.39	0.58	17	0.42	0.85
18	0.41	0.87	20	0.23	0.51	21	0.62	0.86
19	0.39	0.58	22	0.11	0.40	24	0.33	0.66
20	0.23	0.51	28	0.08	0.29	25	0.35	0.66
21	0.62	0.86	29	0.23	0.48	27	0.45	0.80
22	0.11	0.40	32	0.50	0.60	33	0.07	0.28
23	0.27	0.72	34	0.44	0.80	39	0.84	0.80
25	0.35	0.66	41	0.03	0.23	40	0.10	0.35
26	0.22	0.53				42	0.44	0.77
27	0.45	0.80						
28	0.08	0.29						
29	0.23	0.48						
31	0.69	0.69						
32	0.50	0.60						
34	0.44	0.80						
41	0.03	0.23						
Mean	0.30	0.58	Mean	0.22	0.48	Mean	0.35	0.65

Table 3 Descriptive statistical results for the anxiety and depression Su scales of SRSDA

SRSDA subscales	F scale	Mean score	SD	Percentage (%) over F scale
Anxiety	10	4.91	4.87	12.20
Depression Beck-21	14	6.21	5.92	7.50
Depression Beck-13	7	2.83	3.41	9.90

4.4 The Role of Burnout in Anxiety and Depression Levels

In order to search if burnout plays a significant role in anxiety and depression levels of mental health staff general linear regression models were conducted, in which MBI dimensions were the independent variables and Anxiety and Depression Subscales the dependent variables. Results are presenting in Table 5. Emotional Exhaustion and Depersonalization are statistically correlated with Anxiety and Depression Subscales of SRSDA, while Personal Accomplishment is not correlated with any subscale.

Table 4 Levels of the MBI dimensions

Burnout dimension	Low values		Moderate values		High values	
Emotional exhaustion	Mean score ≤ 20.00		Mean score = 21.00–30.00		Mean score ≥ 31.00	
Mean score = 18.46	N	%	N	%	N	%
	136	62.67	52	23.96	29	13.36
Personal accomplishment	Mean score ≥ 42.00		Mean score = 36.00–41.00		Mean score ≤ 35.00	
Mean score = 37.28	N	%	N	%	N	%
	66	30.41	86	39.63	65	29.95
Depersonalization	Mean score ≤ 05.00		Mean score = 06.00–10.00		Mean score ≥ 11.00	
Mean score = 6.73	N	%	N	%	N	%
	115	52.99	45	20.73	57	26.26

Table 5 General linear regression with burnout dimensions as independent variables and anxiety and depression subscales as dependent variables

Independent	Dependent		
	Anxiety	Depression, Beck-21	Depression, Beck-13
Constant			
Coefficient β	0.05	0.07	−0.01
t	1.22	2.10	−0.28
p value	0.23	0.04	0.78
Emotional exhaustion			
Coefficient β	0.09	0.05	0.06
t	3.90	2.96	3.74
p value	**0.00**	**0.00**	**0.00**
Personal accomplishment			
Coefficient β	–	–	–
t	–	–	–
p value	**NS**	**NS**	**NS**
Depersonalization			
Coefficient β	0.09	0.09	0.07
t	4.26	4.91	4.46
p value	**0.00**	**0.00**	**0.00**

The positive scores of coefficient β for Emotional Exhaustion show that, higher levels of Emotional Exhaustion implicate higher levels of Anxiety ($\beta = 0.09$, $p = 0.00$), Depression Beck-21 ($\beta = 0.05$, $p = 0.00$) and Depression Beck-13 ($\beta = 0.06$, $p = 0.00$). Also, the positive scores of coefficient β for Depersonalization show that higher levels of Depersonalization implicate higher levels of Anxiety ($\beta = 0.09$, $p = 0.00$), Depression Beck-21 ($\beta = 0.09$, $p = 0.00$) and Depression Beck-13 ($\beta = 0.07$, $p = 0.00$).

5 Discussion

In recent years, various mental disorders such as depression, anxiety and mania are in focus of research regarding burnout. Particular emphasis is given to depression, which seems to disturb a significant proportion of the working population worldwide. Several researchers suggested that the concepts of depression and burnout are similar [32, 33, 40], while several evidence showed that the MBI results are correlated with employees satisfaction and depression [41]. However, there are several research findings that significantly distinguish the concepts of depression and burnout [42, 43].

This study was focused on the research of the anxiety's and depression's level of mental health's employees, as well as their correlation with burnout. The SRSDA questionnaire was used; in which apart of questions about the work depression, there were also included questions that aimed to examine levels of anxiety [36]. Analysis of the data showed that while the majority of the sample members has no anxiety or depressive disorder, there is a significant proportion, around 10%, which indicates psychiatric symptoms. The MBI was used to study the burnout syndrome. The levels of the personnel's burnout are moderate, except of the emotional exhaustion, which is low, but it is also close to the average limit.

While examining the existence of possible correlations between burnout, anxiety and depressive disorder, it was found that two dimensions of burnout, emotional exhaustion and depersonalization, show statistically significant positive correlation with anxiety and depression. These results are consistent with the findings of other studies, in which depression, anxiety and vulnerability to disease are significantly related to emotional exhaustion and depersonalization [19, 41]. But they are partly in contrast with the study of Iakovides et al., in which it was shown that nurses suffering from burnout syndrome had no signs of depression [44], fact which could be explained by todays' working conditions in Greek hospitals and the current socioeconomic status of the country.

In literature there are many studies focusing on the efforts of finding suitable strategies of dealing with the mental effects of work as well as with the professional burnout. The addressing modes can be applied to individual and administrative level [45]. Every health professional should promptly be able to recognize the symptoms or some indications in order to directly ask the help of experts. These indications may be in the form of organic symptoms or dysfunctions in human's mental status [46].

In mental health facilities, health professionals need to regularly reassess and update their goals and expectations set by them and by the working environment. When, for example, they expect that the care provided should always be effective and that their interventions should influence patients' lives, and the patients in turn will consistently recognize their tender, they will receive their advice and guidance without any doubt, then the employees are particularly vulnerable to frustrations as these expectations are too high and unrealistic [47].

The development of self-defense and personal dealing or handling of difficult situations is a qualification for the mental health professional, who is daily experiencing problems and adverse conditions in the workplace. Self-control and regulation of emotions help in efficient and without trouble resolving the issues and defusing the anxiogenic situations [48].

The quest of support from friends, colleagues, but also by specific mental health professionals, can contribute to the prevention and treatment of mental disorders and burnout [49]. It is difficult, however, for the mental health professionals to seek for assistance for themselves by an expert, as they have mainly learned to support and help patients and not their own selves.

Dealing with personal interests and activities, helps each employee to escape from the everyday routine, to ease tensions, to rest, to express himself creatively and renew his strength [50].

Finally, good rest, good nutrition and exercise also contribute to the employees' protection from the effects of chronic stress and the prevention of burnout. Furthermore, an application for leave by the employee, when he/she feels very tired and mentally overworked, is particularly useful and prevents overwork to permanently exhaust the body [50].

6 Conclusion

Burnout plays an important role in anxiety and depression levels of the staff that works in mental health units all over Greece. Emotional exhaustion and depersonalization are the two burnout dimensions that are statistically correlated with the employees' anxiety and depression. Further studies about factors that are affecting anxiety and depression levels of mental health care professionals in Greece should be conducted.

Finally, another point that merits further study and has occupied several researchers is the direction of the relationship between depression and other health disorders with burnout. There is a belief that in the future these issues will be at the sight of many empirical studies [5, 6, 40, 51].

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References

1. Abd El-aal, N.H., and N.I. Haasan. 2014. Relationship Between Staff Nurses' Satisfaction with Quality of Work and Their Levels of Depression, Anxiety and Stress in Critical Care Units. *Journal of American Science* 10 (1s): 91–101. http://www.jofamericanscience.org.
2. Bianchi, R., C. Boffy, C. Hingray, D. Truchot, and E. Laurent. 2013. Comparative Symptomatology of Burnout and Depression. *Journal of Health Psychology* 18 (6): 782–787.
3. Gökçen, C., S. Zengin, M.M. Oktay, G. Alpak, B. Al, and C. Yıldırım. 2013. Burnout, Job Satisfaction and Depression in the Healthcare Personnel Who Work in the Emergency Department. *Anadolu Psikiyatri Derg* 14 (2): 122–128.
4. Marchand, A., P. Durand, R.P. Juster, and S.J. Lupien. 2014. Workers' Psychological Distress, Depression, and Burnout Symptoms: Associations with Diurnal Cortisol Profiles. *Scandinavian Journal of Work, Environment & Health* 40 (3): 305.
5. Papathanasiou, I.V. 2015. Work-Related Mental Consequences: Implications of Burnout on Mental Health Status Among Health Care Providers. *Acta Informatica Medica* 23 (1): 22–28. doi:10.5455/aim.2015.23.22-28.
6. Fradelos, E.C., K. Tsaras, O. Velenzta, E. Vasiliadou, N. Aouant, P. Palla, D. Mitsi, K. Anastopoulou, and I.V. Papathanasiou. 2016. Relation Between Burnout, Mental Health and Aggression of Nursing Personnel. *Journal of Disease and Global Health* 7 (3): 156–163.
7. Kelloway, E.K., and J. Barling. 1991. Job Characteristics, Role Stress, and Mental Health. *Journal of Occupational Psychology* 64: 291–304.
8. Papathanasiou, I., D. Damigos, and V. Mavreas. 2011. Burnout in Greek Medical and Mental Health Care Workers. *Global Journal of Health Science* 3 (2): 206–210.
9. Zohar, D. 1995. The Justice Perspective of Job Stress. *Journal of Organizational Behavior* 16: 487–495.
10. Cherniss, C. 1980. *Professional Burnout in Human Service Organizations*. New York: Praeger.
11. Burke, R.J., and E.R. Greenglass. 1995. A Longitudinal Study of Psychological Burnout in Teachers. *Human Relations* 48 (2): 187–202.
12. ———. 1990. Type A Behavior and Nonwork Activities. *Personality and Individual Differences* 18: 322–328.
13. Carod-Artal, F.J., and C. Vazquez-Cabrera. 2013. Burnout Syndrome is an International Setting. In *Burnout for Experts*, 15–35. New York: Springer US.
14. Chen, X., X. Tan, and L. Li. 2013. Health Problem and Occupational Stress Among Chinese Doctors. *Chinese Medicine* 4: 1.
15. Fradelos, E.C., G. Tzitzikos, V. Giannouli, P. Argyrou, C. Vassilopoulou, and P. Theofilou. 2014. Assessment of Burn-Out and Quality of Life in Nursing Professionals: The Contribution of Perceived Social Support. *Health Psychology Research* 2 (1): 984. doi:10.4081/hpr.2014.984.
16. Ganster, D.C., and J. Schaubroeck. 1991. Work Stress and Employee Health. *Journal of Management* 17 (2): 235–271.
17. Papathanasiou, I.V., K. Tsaras, A. Neroliatsiou, and A. Roupa. 2015. Stress: Concepts, Theoretical Models and Nursing Interventions. *American Journal of Nursing Science* 4 (2-1): 45–50. doi:10.11648/j.ajns.s.2015040201.19.
18. Greenglass, E.R. 1993. The Contribution of Social Support to Coping Strategies. *In Special Issue on Women and Management, Guest Editors: Esther R. Greenglass and Judi Marshall, Applied Psychology: An International Review* 42 (4): 323–340.
19. Papathanasiou, I.V., D. Damigos, and V. Mavreas. 2011. Higher Levels of Psychiatric Symptomatology Reported by Health Professionals Working in Medical Settings in Greece. *Annals of General Psychiatry* 10: 28.
20. Roskies, E., C. Louis-Guerin, and C. Fournier. 1993. Coping with Job Insecurity: How Does Personality Make a Difference? *Journal of Organizational Behavior* 14: 617–630.

21. Rossouw, L., S. Seedat, R.A. Emsley, S. Suliman, and D. Hagemeister. 2013. The Prevalence of Burnout and Depression in Medical Doctors Working in the Cape Town Metropolitan Municipality Community Healthcare Clinics and District Hospitals of the Provincial Government of the Western Cape: A Cross-Sectional Study. *South African Family Practice* 55 (6): 567–573.
22. Adebowale, T.O., and A.O. Adelufosi. 2013. Stress and Minor Psychiatric Morbidity Among Nigerian Executives: Some Socio-Demographic and Biological Correlates. *Annals of Medical and Health Sciences Research* 3 (3): 412–416.
23. Derogatis, L.R., R.S. Lipman, K. Rickels, et al. 1997. The Hopkins Symptom Checklist (HSCL): A Self-Report Symptom Inventory. *Behavioral Science* 19: 1–15.
24. Machado, T., V. Sathyanarayanan, P. Bhola, and K. Kamath. 2013. Psychological Vulnerability, Burnout, and Coping Among Employees of a Business Process Outsourcing Organization. *Industrial Psychiatry Journal* 22 (1): 26.
25. Bakker, A.B., and P.L. Costa. 2014. Chronic Job Burnout and Daily Functioning: A Theoretical Analysis. *Burnout Research* 1 (3): 112–119.
26. Leiter, M.P., and J. Durum. 1994. The Discriminant Validity of Burnout and Depression: A Confirmatory Factor Analytic Study. *Anxiety, Stress and Coping* 7: 357–373.
27. Gito, M., H. Ihra, and H. Ogata. 2013. The Relationship of Resilience, Hardiness, Depression and Burnout Among Japanese Psychiatric Hospital Nurses. *Journal of Nursing Education and Practice* 3 (11): 12–18.
28. Arsenault, A., S.L. Dolan, and M.R. Van Ameringen. 1991. Stress and Mental Strain in Hospital Work: Exploring the Relationship Beyond Personality. *Journal of Organizational Behavior* 12: 483–493.
29. Bluen, S.D., J. Barling, and W. Burns. 1990. Predicting Sales Performance, Job Satisfaction, and Depression by Using the Achievement Strivings and Impatience-Irritability Dimensions of Type-A Behavior. *Journal of Applied Psychology* 75 (2): 212–216.
30. McGrath, A., and N.R. Boore. 2003. Occupational Stress in Nursing. *International Journal of Nursing Studies* 40: 555–565.
31. Soares, J.F., and B. Jablonska. 2004. Psychosocial Experiences Among Primary Care Patients with and without Musculoskeletal Pain. *European Journal of Pain* 3: 79–89.
32. Tanaka, J.S., and G.J. Huba. 1984. Confirmatory Hierarchical Factor Analysis of Psychological Distress Measures. *Journal of Personality and Social Psychology* 46: 621–635.
33. Firth, H., P. McKeown, J. McIntee, et al. 1987. Professional Depression, "Burnout" and Personality in Longstay Nursing. *International Journal of Nursing Studies* 24: 227–237.
34. Maslach, C., and S.E. Jackson. 1981. The Measurement of Experienced Burnout. *Journal of Occupational Behavior* 2: 99–113.
35. Reilly, N.P. 1994. Exploring a Paradox: Commitment as a Moderator of the Stressor-Burnout Relationship. *Journal of Applied Psychology* 24: 297–314.
36. Fountoulakis, K.N., A. Iacovides, S. Kleanthous, S. Samolis, K. Gougoulias, G. Kaprinis, and P. Bech. 2003. The Greek Translation of the Symptoms Rating Scale for Depression and Anxiety: Preliminary Results of the Validation Study. *BMC Psychiatry* 10: 21.
37. Anagnostopoulos, F., and D. Papadatou. 1992. Reliability and Validity of the Maslach Burnout Inventory to a Population of Nurses. *Journal of Psychological Issues* 5: 183–202.
38. Vickers, A.J. 2005. Parametric Versus Non-parametric Statistics in the Analysis of Randomized Trials with Non-normally Distributed Data. *BMC Medical Research Methodology* 5: 35.
39. Zimmerman, D.W. 2000. Statistical Significance Levels of Nonparametric Tests Biased by Heterogeneous Variances of Treatment Groups. *Journal of General Psychology* 127: 354–364.
40. Maslach, C., W. Schaufeli, and M.P. Leiter. 2001. Job Burnout. *Annual Review of Psychology* 52: 397–422.
41. Jeanneau, M., and K. Armelius. 2000. Self-Image and Burnout in Psychiatric Staff. *Journal of Psychiatric and Mental Health Nursing* 7: 399–406.
42. Bakker, A.B., and W.B. Schaufeli. 2000. Burnout Contagion Processes Among Teachers. *Journal of Applied Social Psychology* 30: 2289–2308.
43. Glass, D.C., and J.D. McKnight. 1996. Perceived Control, Depressive Symptomatology, and Professional Burnout: A Review of the Evidence. *Psychology and Health* 11: 23–48.

44. Iacovides, A., K.N. Fountoulakis, C. Moysidou, et al. 1999. Burnout in Nursing Staff: Is There a Relationship Between Depression and Burnout? *Journal of Psychiatry in Medicine* 29: 421–433.
45. Lazarus, R.S., and S. Folkman. 1984. *Stress, Appraisal and Coping*. New York: Springer.
46. Beck, J., M. Gerber, S. Brand, U. Puhse, and E. Holsboer-Trachsler. 2013. Executive Function Performance is Reduced During Occupational Burnout but can Recover to the Level of Healthy Controls. *Journal of Psychiatric Research* 47 (1): 1824–1830.
47. Papadatou, D., F. Anagnostopoulos, and D. Monos. 1994. Factors Contributing to the Development of Burnout in Oncology Nursing. *British Journal of Medical Psychology* 67: 187–199.
48. Papathanasiou, I.V., E. Fradelos, Ch. Kleisiaris, K. Tsaras, M. Kalota, and L. Kourkouta. 2014. Motivation, Leadership, Empowerment and Confidence: Their Relation with Nurses' Burnout. *Materia Socio Medica* 26 (6): 405–410. doi:10.5455/MSM.2014.26.405-410.
49. Fradelos, E., S. Mpelegrinos, C. Mparo, C. Vassilopoulou, P. Argyrou, S. Zyga, M. Tsironi, and P. Theofilou. 2014. Burnout Syndrome Impacts on Quality of Life in Nursing Professionals: The Contribution of Perceived Social Support. *Progress in Health Sciences* 4 (1): 102–109.
50. Papathanasiou, I.V., Ch. Kleisiaris, K. Tsaras, E. Fradelos, and L. Kourkouta. 2015. General Satisfaction Among Healthcare Workers: Differences Between Employees in Medical and Mental Health Sector. *Materia Socio Medica* 27 (4): 225–228. doi:10.5455/msm.2015.27.225-228.
51. Glass, D.C., J.D. McKnight, and H. Valdimarsdottir. 1993. Depression, Burnout and Perceptions of Control in Hospital Nurses. *Journal of Consulting and Clinical Psychology* 61 (1): 147–155.

Scheduling and Modeling a Cognitive Assessment Guide for Screening AD by Primary Care Physicians

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Abstract New research data on Alzheimer's Disease define it as a clinicobiological entity, which has a long preclinical and presymptomatic phase. Emphasis has been given in early detection and diagnosis, which will allow professionals, caregivers and patients themselves to plan and adjust better to the response of the disease. Primary care physicians, who most often are the first to witness and perceive cognitive impairment, may play a central role in the diagnostic procedure, but frequently they are reluctant to be engaged to the screening procedure. The aim of this study is to model a practical guideline for mental assessment and screening, which will be part of a whole step-to-step medical instruction policy for primary care physicians. After a careful review of the literature, we propose a two-visits approach. This approach combines the measures to be administered in each visit, with a detailed list of close-ended questions on the factors concerning Alzheimer's screening. The tests are automatically available to the physician through hyperlink connection and the scores are immediately calculated. Clinical trials will follow to test the validity of the proposed guidance.

Keywords Alzheimer's disease • Primary care • Primary care physicians • Diagnosis • Dementia • Guidelines • Cognitive assessment

1 Introduction

Alzheimer's Disease (AD), a progressive neurodegenerative condition, is the most common cause of cognitive impairment among elder people [1]. Many studies point out the emerging numbers of AD patients and foresee that in 2020 48.1 million people will be affected by dementia and this score will reach up to 90.3 million patients in 2040 [2]. As noted, after 65 years of age, the risk of developing dementia

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is approximately 17–20% and 70% of patients with dementia have AD, 17% have vascular dementia and 13% have a combination of dementia with Parkinson related dementia, Lewy-bodies, alcoholic dementia or frontal lobe dementia [3].

Even though dementia is a growing problem in the ageing population, it still may often go unrecognized or misdiagnosed in its earlier stages [4]. Previous studies show an unrecognized cognitive impairment number between 27 and 81% among affected patients in primary care settings [5]. Although primary care physicians (PCP) are the first experts that patients respond to, they have identified a number of obstacles in their attempt to diagnose and treat dementia, such as lack of time, negative perception to the significance of early diagnosis, difficulties related to behavior and general problems attributed to dementia as well as poor connections with community social service agencies [1, 6, 7].

Despite the fact that there is no disease modification therapy yet available, screening of dementia could help patients, family members and physicians to plan a care strategy, discuss of medical therapy, search for comorbidities and provide support through the demanding phases of the disease. As mentioned by the Alzheimer's Association, unrecognized dementia, may lead to unnecessary workups iatrogenic illness, costly and inappropriate utilization of hospital and emergency room care as well as pure outcome [8].

A long way went through, since 1984, when the National Institute of Neurological and Communicative Disorders and Stroke in collaboration with the Alzheimer's Disease and Related Disorders Association, developed the first diagnostic criteria for Alzheimer dementia (NINCDS-ADRA) [9]. In 2007 the International Working Group (IWG) for New Research Criteria for the Diagnosis of Alzheimer's Disease established the use of biomarkers in the diagnosis of AD [10]. This fact changed the conceptual of AD from a clinicopathological disease which could be accurately diagnosed only after death, to a clinicobiological entity, which could be recognized in vivo, providing guidelines through the diagnostic procedure. In 2014 the IWG updated their clinical entity of prodromal AD and introduced improved biomarkers for AD. They also defined the criteria for atypical and non-AD dementia, recognizing that the occurrence of specific and sensitive biomarkers in neurological conditions other than AD are just as important [10].

Family physicians are the most accessible professionals that people with dementia usually present themselves to, although an estimated 39% of suspected patients present to specialists, such as neurologists, psychiatrists, geriatricians [11]. So the PCP is regularly the first physician to observe the problem and may be the only one involved in making the diagnosis. This fact points out the key role of the PCP in recognizing early signs and symptoms of the disease, ordering appropriate tests, establishing a diagnosis, treating the patient and supporting the family [12].

However, the diagnosis of AD remains a challenging process. For this purpose, several tools are available and especially for early screening of dementia. These tools may not be specifically diagnostic for AD, but they can provide valuable evidence for cognitive impairment and guide a further evaluation [12]. In order to achieve an earlier diagnosis of dementia, Alzheimer's Association and National Chronic Care Consortium proposed the use of questionnaires and specific tools, applied either to an informer or directly to the potential patient, by primary health

care providers [8]. These tools will be used besides the triggers based on the "10 Warning Signs." Many studies recommend that a combination of tests is better for the detection of dementia, than applying them separately [13]. In this study it was also highlighted that the combination of Mini Mental State Evaluation test (MMSE) and the Preffer's Functional Activities Questionnaire (FAQ) had an excellent performance in dementia detection.

2 Previous Guidelines

Several educational programs have focused on enhancing the diagnostic ability of PCPs in the case of dementia [14]. A 2010 position paper on the PCP and AD, proposed a strategy, according to which the PCP could identify, proceed to the diagnosis of "probable Alzheimer" and then a specialist would confirm the presence of the disease. The suggested strategy includes anamnestic interview with the patient and the family/caregiver as well, search for episodic memory impairment with specific measures and clinical evaluation, during which the PCP examines the weight record, blood pressure, hearing and vision, neurological features, gait and balance disturbances and could ask for additional laboratory testing and brain imaging [15].

Over the last years an effort is attempted through user-friendly practical guidelines, so as to improve the diagnostic procedure by general physicians. As many other major national organizations, the US Department of Health and Human Services has developed a National Plan to Address Alzheimer's Disease concluding in recommendations for practical guidelines in primary care [1]. The Care Management Advisory Group of the Chronic Care Networks for Alzheimer's Disease, concluded to a guideline regarding dementia assessment in primary care settings (brochure, tools). It is based in a three- step assessment approach, which includes interviews of the caregivers and the patients, cognitive, behavioral and mood tests, as well as biomarkers and other laboratory tests (glucose, liver function, B12 and folate levels, genetic tests etc.) and brain images (Table 1).

This table also depicts Galvin's and Sadowsky's practical guidelines algorithm [14], a stepwise approach is proposed to diagnose and assess AD in primary care, as well as the algorithm that was conducted by Alzhrimer's Association [16]. The last one includes both structured assessment and less structured evaluations based on the patients and the informer and it follows steps according to the outcome of each assessment. Cordell et al. selected three tests as the most suitable for assessment in primary care: Memory Impairment Screen (MIS), General Practitioner Assessment of Cognition (GPCOG) and Mini-Cog. Among the advantages of these tests are the administration time, which is about 5′ each, their validity in community and primary care settings, the fact that they are easy to administer by non-specialists, are relatively free from language, education, culture bias and are free from cost.

McCarten [17] in his study describes more thoroughly the clinical evaluation of dementia symptoms, analyzing each phase. The phase of the Interview includes

Table 1 Algorithms for the assessment of cognition in Alzheimer's disease

Care management advisory group of the chronic care networks for Alzheimer's disease algorithm
Level 1 examination
(a) Interview with the client and the family
(b) Examination (physical exam, neurologic exam, functional status, mental status, depression assessment)
(c) Laboratory tests
(d) Therapeutic "diagnostic" tests
Level 2 examination
(a) Advanced laboratory tests
(b) Brain imaging
Level 3 examination
(a) Consultation by neurologists or psychiatry
(b) Neuropsychological evaluation
(c) Advanced laboratory tests
(d) Lumbar puncture
Galvin's and Sadowsky's practical guidelines algorithm
(1) Prediagnostic tests
(2) Cognitive assessment with screening tests (Mini mental state evaluation, montreal cognitive assessment, Mini-Cog)
(3) Evaluation of the level of independence and the degree of disability
(4) Determination of the presence and the degree of behavioral symptoms
(5) Identification of the caregiver and assessment of the adequacy of the support systems
Alzheimer's association medicare annual wellness visit algorithm for assessment of cognition
A. Review HRA, clinician observation, self-reported concerns, responses to queries
B. Conduct brief structured assessment
• Patient assessment: Mini-Cog or GPCOG or MIS
• Informant assessment of patient: Short IQCODE, AD8 or GPCOG
Brief assessment(s) triggers concerns:
Patient: Mini-Cog $\leq$3 or GPCOG <8 or MIS $\leq$ 4 or
Informant: Short IQCODE $\geq$3.38 or AD8 $\geq$ 2 or
GPCOG informant score $\leq$ 3 with patient score < 8
C. Refer OR conduct full dementia evaluation

several domains: overview, setting the ground rules, interviewing patient and family, onset and course, major causes of brain disease, nature of symptoms, investigating behavioral symptoms, personality change, delusions and hallucinations, motor disturbances, sleep past medical history, medications, family history, social history. The Examination phase includes: neurologic examination, localizing cognitive functions, mental-status examination, language, recent memory, visuospatial skills, generative naming, executive function, sequencing, set-shifting. Third phase Additional Testing, includes: brain imaging, laboratory tests, neuropsychological tests, functional assessment.

3 Cognitive Measures

Plethora of tools has been available concerning detection of dementia and the follow-up of the disease. Even though there is no gold standard for an accurate and final diagnosis, it is acknowledged that a large body of surveys conducted has used specific tools, both for the patient and the caregiver/informer besides. Some of the most frequent used measures are mentioned in Table 2.

Previous studies aiming on reviewing measures for screening dementia in primary care settings concluded among many tools in GPCOG (General Practitioner Assessment of Cognition), Mini-Cog, MIS, along with MMSE [35, 36]. Others suggest that the use of GPGOG, Mini-Cog, MIS in primary care screening is preferable than MMSE, due to bias of agelanguage, education and social class), however GPCOG has limited amount of evidence. [37]. A review of the best tools for dementia detection, with criteria of negative predictive value, at least equal to that of MMSE and ith an administration time less than 5′, concluded in four out of 16 instruments: MMSE, GPCOG, 7-Minute Screen and the Clock Drawing Test [29]. In a review position paper MMSE, GPCOG and the Clock Drawing Test seem to be practical, validated tools and the most useful to detect dementia in primary care [15].

4 Methodology

The search of the literature was based on Pubmed search engine using the keywords: Alzheimer's disease AND Primary Care OR Primary Care Physicians, Diagnosis, Dementia, Guidelines, Cognitive Assessment. Only English-language articles were included and references within articles were subsequently searched.

The aim was to roughly review a great amount of already existing guidelines for the diagnosis of AD and to proceed in recreation and modeling of a practical guideline for mental assessment and screening, which will be a part of a whole step-to-step medical instruction tool for PCPs.

The tests were selected according to the rationale of the time spending for the assessment, availability, low cost, frequency of use in the majority of studies conducted, the free of charge character accompanied with online accessibility and finally their availability in Greek version. Their ability to test directly the patient and not through the informer was also a parameter for selection. Although it is important for the physician to gather information about the patient from a good informer, the emphasis here is given directly to the patient and his/her experience. The choice of getting information from the informer is given by assessing the General Practitioner Assessment of Cognition (GPCOG-Informant Interview). After reviewing the related literature, we concluded in the tests shown in Table 3.

All of the above tests are used in the vast majority of studies and have been positively evaluated for their validity and consistency. Although MMSE is the one

Table 2 Tests for detecting dementia

Test	Description	References
Mini mental state examination	The most used test for detecting dementia worldwide. It includes items associated with recent memory, calculation, language and visuospatial area, scoring ranges from 0 (worst) to 30 (best) and it can be completed in 15 min. It measures orientation, calculation and attention, immediate memory, recall, various aspects of language and visuospatial-skills. It provides cut off scores for mild, moderate and severe dementia. Its limitations are due to age, language and educational barriers.	[18, 19]
Clock Drawing Test (CDT)	It is administered in most of the screening processes and it measures spatial and executive function. The client has to draw a clock face reading a specific time. Although its clinical use is valuable, the lack of a universal administrative and scoring method results in ambiguities in interpretation	[20–22]
Mini-Cog	It has been validated in population- based studies and in older community-dwelling adults, heterogeneous with respect to language, culture and education) and is completed in 10 min. It includes a clock drawing test and a verbal memory task.	[16, 23, 24]
Memory Impairment Screen (MIS)	It includes a verbal memory task with a specific encoding procedure. There are available cut-off scores for populations with varying base rates. However, executive function and visuospatial-skills are not tested.	[25, 26]
Behavioral Pathology in Alzheimer's Disease rating scale (BEHAVE-AD)	It is an instrument for screening and evaluating behavioral disturbances in AD patients. It includes 25 items, which measure the behavioral disturbances in 7 major categories. The categories are paranoid, delusional ideation, hallucinations, activity disturbance, aggressiveness, affective disturbances, diurnal rhythm disturbances, phobias and anxieties. Each symptom is scored on a 4-point scale of severity. It addresses also to the caregiver's disturbance	[27–29]
Geriatric Depression Scale (GDS)	A simple, 15-item, yes/no questionnaire that can be helpful in identifying possible depression among elderly people. GDS is mostly appropriate for screening in early stages of dementia, when problems with memory are less confounding	[30, 31]
General Practioner Assessment of Cognition (GPCOG)	It evaluates time orientation, word recall, recall of recent event and includes a clock drawing test. This tool has the option of an additional 6-questions set addressing the informer examining changes that have been noticed lately. The combined score of the two scales is found to have higher sensitivity and specificity than each section separately	[32]
Montreal Cognitive Assessment (MoCa) test	It is designed to test for mild cognitive impairment and tests several domains. It takes approximately 10 min to administer. The following cognitive domains are tested: Attention, memory, orientation, language, visuoconstruction abilities and executive functions	[33]
Animal naming-short verbal term	It is the most commonly used category fluency measure for assessing of cognitive impairment. In 60″ a person is called to name as many animals as he/she can think. It is useful as a brief initial screen for dementia and milder degrees of cognitive impairment	[34]

Table 3 Tests selected

Mini Mental State Evaluation (MMSE)	Quick and easy to administer (20′) Covers six areas (a) orientation, (b) registration, (c) attention & calculation, (d) recall, (e) language, (f) ability to copy a figure Can track the overall progression of cognitive decline
General Practitioner Assessment of Cognition (GPCOG-Patient examination and informant interview)	Quick and easy to administer (less than 4′ the patient section and 2′ informant section) Studied in a primary care setting The combined score and 2-stage method had higher sensitivity and specificity than patient than patient and informant sections separately
Mini-Cog	Quick and easy to administer (2′–4′) Studied in a general population sample High specificity and sensitivity Education and language/race biases found absent in U.S. samples Combines an un-cued 3-item recall test with a Clock Drawing Test, that serves as a recall distractor
Montreal Cognitive Assessment (MoCa)	Quick and easy to administer (10′–15′) Studied in a memory clinic population High sensitivity
Memory Impairment Screening test (MIS)	Quick and easy to administer (4′) It's a 4-item test that uses controlled learning to maintain attention, includes specific semantic processing and optimize encoding specificity, so as to improve the screening outcome No education and language biases
Geriatric Depression Scale (GDS) short form	Quick and easy to administer (5′–7′) It includes 15 items (instead of 30 items included in the full form) It is most commonly used by physically ill and demented patients with short attention span, who feel easily fatigue
Behavioral Pathology in Alzheimer's Disease (BEHAVE-AD-FW)	Quick and easy to administer (20′) Includes 25 items which are grouped in 7 major categories It has frequency-weighted severity score
Short Verbal Test (Animal naming)	Quick and easy to administer (60′′) Free of education/language/age biases Examines semantic memory and semantic memory organization

[a]The Clock Drawing Test (CDT) is included both in Mini-Cog and in GPCOG

used widely in most of the studies, a research review concluded that MIS and mini-Cog are both psychometrically and clinically more appropriate and robust for use in primary care [37]. GDS and Behavioral pathology rating scale were included, as it is important for the PCP to be able to proceed to differential diagnosis, with the

use of suitable tools. Most often same symptoms make their appearance to multiple diseases or coexist with others. This is the case especially with depression, which is associated with cognitive-functional impairment [31] and that is the reason why GDS and Behavioral pathology rating scale are necessary tools as well.

Then an approach of two visits was designed in details, so as for the PCP not to be pressed to conclude hastily to a diagnosis, before having time to evaluate the patient's general condition and also for the patient not to feel overloaded by the evaluation process. This approach is also supported by the work of Simmons et al. [3].

4.1 First Visit

First Visit includes an interview with the patient, with an aim to collect all the necessary demographic data and medical history, both patient's and his/her family. Time is given also to psychosocial history, which provides valuable information about patient's living conditions, support system, financial state, occupation, activities, physical activity, diet, weight loss, sleeping pattern and current important life events. Medical history and mental state examination are basic tools for supporting the diagnosis. Medical history provides necessary information for patient's daily functioning and degree of impairment, whereas mental status examination provides the evidence for cognitive impairment [38]. Each of the above aspects is documented by literature overview [39]. Then in order to adjust gradually the patient according to the dementia screening process, the 10 Signs According to the Alzheimer's Association are applied and evaluated by means of frequency and intensity (Table 4).

First Visit proceeds on with cognitive assessment, by means of variable and standardized cognitive tests. A set of specific cognitive tools is proposed, taking into high consideration of their duration. This is a very important aspect of the selected tests, because, especially in the First Visit plenty amount of time is spent by health professionals in order to gather all the necessary information about the patient's medical and psychosocial history and to record carefully all the symptoms of the disease.

Table 4 Ten signs according to the Alzheimer's association

1. Memory loss that disrupts daily life
2. Challenges in planning or solving problems
3. Difficulty completing familiar tasks
4. Confusion with time or place
5. Trouble understanding visual images and spatial relationships
6. New problems with words in speaking or writing
7. Missing thing and losing the ability to retrace steps
8. Decreased or poor judgment
9. Withdrawal from work or social activity
10. Changes in mood or personality

The set of the selected cognitive tools is as follows:

- GPCOG
- MIS
- Mini-Cog
- the short verbal test
- GDS-short term

First Visit is concluded with the unity of clinical evaluation, where clinical characteristics about the patient are estimated, such as general behavior, external appearance, flow of thought, eye contact etc.

If the outcome of the cognitive tests clearly indicates dementia, the PCP prescribes basic medical test and moves on with a full cognitive evaluation in the Second Visit.

4.2 Second Visit

Second visit proceeds with a complete cognitive evaluation, assessing the following tests: MMSE, MoCa and Behavioral Pathology in AD Rating Scale. A more thorough examination of the symptoms is taking place, so as to elicit the critical features of the problem, regarding the domains of aphasia (speech disorder), apraxia (decrease concerning the ability for moving coordination despite the unharmed motor functioning), agnosia (disability of recognizing or identifying objects, despite the unharmed sensor functioning) and disability in execution functioning (planning, organization). Each of the above domains is evaluated by a number of tasks which the patient is called to perform. A list of paradigms is available for the physician which includes specific paradigms.

ex. of evaluating Aphasia

- After placing the objects, ask: show me the pencil, the clock, the notebook
- Name this object (ex. Chair, pen, paper)
- Name the days of the week
- Fill the missing word (day and..., black and ...)
- What is the use of the lighter? Which is the color of strawberries?
- Describe in steps what do you do after you get off the bed in the morning
- Name in 1′ as many words as possible begin with the letter "f, or s"

Again if indication for dementia occurs then a prescription for extended blood/urine tests and tomography tests (EEG, fMRI, PET) is needed. The sequence of the steps follows gradation from basic to extended and specialized medical examination.

Apart from the tests' assessment and the medical history, the rest of the guidance is designed in a form of close-ended and directed questions, which is answered by a YES or NO statement, so as to be easy and quick for the PCP to apply and perceive

an immediate first idea of the patient's condition. It is also convenient to the patient, as it is neither fatigue nor time costly.

When the completion of the two visits is over, the PCP has available the screening outcome of the patient. In addition the tests are provided with hyperlink, making the grade procedure easy and quick to obtain.

5 Conclusion

With the entrance of biomarkers in the diagnostic procedure of AD, the full spectrum of the disease from the asymptomatic to the severe stages of the disease is covered [10], making the need for early screening of dementia much more essential. With an emphasis given on early detection and health maintenance, cognitive assessment is becoming a basic component of the PCP wellness visits [1]. It is estimated that when physicians are provided with data and suggestions about the cognitive status of older adults, they can manage better the treatment strategy aiming at remediating cognitive decline [4]. As mentioned before, early detection and medical record documentation of dementia may improve medical care; help patient and family to plan for the future and general improve quality of life [16]. For this reason, studies are throwing light into PCP's understanding the sequence of cognitive decline in AD's patients, which could help setting expectations on disease progression [40].

It is acknowledged that the implementation of a comprehensive cognitive assessment especially among older community-dwellers with comorbidities of chronic diseases and disability is a difficult task [41]. Findings show that general physicians are concerned about their ability to detect symptoms and make a diagnosis and even in treatment management, when it comes to AD, raising the necessity for educational training in the above matters [42]. However important early interventions are in dementia, literature shows that PCP are reluctant taking action and proceed to cognitive evaluation when dementia is suspected for a number of reasons [4]. In a recent survey in Italian general practitioners, results uncovered a 53% of the sample not using screening tests or protocols to diagnose and manage dementia and that 55% of the participants considered the training on the recognition on AD symptoms and signs inadequate [2].

The search of early dementia requires, besides clinical evaluation and cognitive assessment, a series of tests, such as imaging (including MRI, FDG-PET, amyloid PET), and cerebrospinal fluid (CSF) examination assaying Aβ 1-42, T-τ, P-τ [39]. In Alzheimer's Disease, cerebrospinal fluid (CSF) shows reduced levels of amyloid β 1-42 (Aβ42) and increased levels of phosphorylated tau (P-tau) and total tau (T-tau). This pattern is proposed for use in the research concerning the diagnostic criteria [43]. It is now well known that Alzheimer's pathophysiological process begins decade or decades before the presence of symptoms and the clinical diagnosis and that research in CSF, neuroimaging and others biomarkers can provide the ability for early detection of the disease [44]. Cognitive assessment could play an important role enhancing the diagnostic procedure.

The likelihood of early diagnosis and its disclosure is a matter of strong controversy. Possible negative aspects of early dementia screening, such as initiating anxiety or/and depression, stigma [39], should be taken into serious consideration. Early disclosure of dementia is accompanied with risks both for the patient and the family and friends, including preoccupation with the diagnosis, restriction of activities, higher vigilance from the family, distress and increased anxiety [45, 46].

A variety of new tools for cognitive assessment which take under consideration the issues of validity, sensitivity and time saving are being under construction [47]. These tools will be tested in future studies and then they may be carefully incorporated in new guidelines. We are in need for longitudinal studies which will combine biomarkers and sensitive cognitive measures that will detect early cognitive decline.

Testing and evaluation of the proposed cognitive assessment guidance is needed, through pilot studies and clinical trials with specific methodology plan. It is essential to mention that no diagnostic conclusion can be made only by the cognitive assessment, without thorough medical examination. Furthermore, the need for retest and reevaluation should be mentioned. Through cognitive assessment, the reexamination of the patient is easy to be applied without the burdens of cost or invasive methods.

References

1. Geldmacher, D.S., and D.R. Kerwin. 2013. Practical Diagnosis and Management of Dementia Due to Alzheimer's Disease in the Primary Care Setting: An Evidence-Based Approach. *The Primary Care Companion for CNS Disorders* 15 (4): 1–18.
2. Veneziani, F., B. Aldo, B. Aldo, B.P. Imbimbo, A. Leo, M. Copetti, A. Fontana, S. Arcuti, B. Aldo, and G. Logroscino. 2016. Examination of Level of Knowledge in Italian General Practitioners Attending an Education Session on Diagnosis and Management of the Early Stage of Alzheimer's Disease: Pass or Fail. *International Psychogeriatrics* 28 (7): 1111–1124.
3. Simmons, B.B., B. Hartmann, and D. Dejoseph. 2011. Evaluation of Suspected Dementia. *American Family Physician* 84 (8): 895–902.
4. Fowler, N.R., L. Morrow, L. Chiappetta, B. Snitz, K. Huber, E. Rodriguez, and J. Saxton. 2015. Cognitive Testing in Older Primary Care Patients: A Cluster-Randomized Trial. *Alzheimer's & Dementia (Amsterdam, Netherlands)* 1 (3): 349–357.
5. Boustani, M., C.M. Callahan, F.W. Unverztag, M.G. Austrom, A.J. Perkins, B.A. Fultz, S.L. Hui, and H.C. Hendry. 2005. Implementing a Screening and Diagnosis Program for Dementia in Primary Care. *Journal of General Internal Medicine* 20: 272–277.
6. Apesoa-Varano, E.C., J.C. Barker, and L. Hinton. 2011. Curing and Caring: The Work of Primary Care Physicians with Dementia Patients. *Qualitative Health Research* 21 (11): 1469–1483.
7. Hinton, L., C.E. Franz, G. Reddy, Y. Flores, R.L. Kravitz, and J.C. Barker. 2007. Practice Constraints, Behavioral Problems, and Dementia Care: Primary Care Physicians' Perspectives. *Journal of General Internal Medicine* 22 (11): 1487–1492.
8. National Chronic Care Consortium and the Alzheimer's Association. 2003. Tools for Early Identification, Assessment, and Treatment for People with Alzheimer's Disease and Dementia, pp. 1–40.

9. McKhann, G., D. Drachman, M. Folstein, and R. Katzman. 1984. Views & Reviews Clinical Diagnosis of Alzheimer's Disease. *Neurology* 34 (7): 939.
10. Dubois, B., H.H. Feldman, C. Jacova, H. Hampel, J.L. Molinuevo, K. Blennow, and P. Scheltens. 2014. Position Paper Advancing Research Diagnostic Criteria for Alzheimer's Disease: The IWG-2 Criteria. *Lasnset Neurology* 13: 614–629.
11. National Institutes of Health and Clinical Excellence. 2006. Dementia: Supporting People with Dementia and Their Careers in Health and Social Care. Available at: https://www.nice.org.uk/guidance/cg42?unlid=539472848201646613.
12. Galvin, J.E., T.M. Meuser, and J.C. Morris. 2012. Improving Physician Awareness of Alzheimer Disease and Enhancing Recruitment: The Clinician Partners Program. *Alzheimer Disease and Associated Disorders* 26 (1): 61–67.
13. Cruz-Orduña, I., J.M. Bellón, P. Torrero, E. Aparicio, A. Sanz, N. Mula, and J. Olazarán. 2012. Detecting MCI and Dementia in Primary Care: Efficiency of the MMS, the FAQ and the IQCODE. *Family Practice* 29 (4): 401–406.
14. Galvin, J.E., C.H. Sadowsky, and NINCDS-ADRDA. 2012. Practical Guidelines for the Recognition and Diagnosis of Dementia. *Journal of the American Board of Family Medicine* 25 (3): 367–382.
15. Villars, H., S. Oustric, S. Andrieu, J.P. Baeyens, R. Bernabei, H. Brodaty, and B. Vellas. 2010. The Primary Care Physician and Alzheimer's Disease: An International Position Paper. *The Journal of Nutrition, Health & Aging* 14 (2): 110–120.
16. Cordell, C.B., S. Borson, M. Boustani, J. Chodosh, D. Reuben, J. Verghese, and L.B. Fried. 2013. Alzheimer's Association Recommendations for Operationalizing the Detection of Cognitive Impairment During the Medicare Annual Wellness Visit in a Primary Care Setting. *Alzheimer's & Dementia* 9 (2): 141–150.
17. McCarten, J.R. 2013. Clinical Evaluation of Early Cognitive Symptoms. *Clinics in Geriatric Medicine* 29 (4): 791–807.
18. Ritchie, C.W., G.M. Terrera, and T.J. Quinn. 2015. Dementia Trials and Dementia Tribulations: Methodological and Analytical Challenges in Dementia Research. *Alzheimer's Research & Therapy* 7: 31.
19. Leoutsakos, J.S., S.N. Forrester, C.D. Corcoran, M.C. Norton, P.V. Rabins, M.I. Steinberg, J.T. Tschanz, and C.G. Lyketsos. 2015. Latent Classes of Course in Alzheimer's Disease and Predictors: The Cache County Dementia Progression Study. *International Journal of Geriatric Psychiatry* 30 (8): 824–832.
20. Yoo, D.H., and J.S. Lee. 2016. Clinical Usefulness of the Clock Drawing Test Applying Rasch Analysis in Predicting of Cognitive Impairment. *Journal of Physical Therapy Science* 28: 2140–2143.
21. Kim, M.-J., C.-W. Han, K.-Y. Min, C.-Y. Cho, C.-W. Lee, Y. Ogawa, and M. Kohzuki. 2016. Physical Exercise with Multicomponent Cognitive Intervention for Older Adults with Alzheimer's Disease: A 6-Month Randomized Controlled Trial. *Dementia and Geriatric Cognitive Disorders Extra* 6 (2): 222–232.
22. de Paula, J.J., L. Bertola, R.T. Ávila, L. Moreira, G. Coutinho, E.N. de Moraes, and L.F. Malloy-Diniz. 2013. Clinical Applicability and Cutoff Values for an Unstructured Neuropsychological Assessment Protocol for Older Adults with Low Formal Education. *PLoS One* 8 (9): e73167.
23. Borson, S., J.M. Scanlan, P. Chen, and M. Ganguli. 2003. The Mini-Cog as a Screen for Dementia: Validation in a Population-Based Sample. *Journal of the American Geriatrics Society* 51 (10): 1451–1454.
24. Borson, S.O.O., J. Scanlan, M. Brush, P. Vitaliano, and A. Dokmak. 1999. The Mini-Cog a Cognitive Vital Signs Measure for Dementia Screening in Multi Lingual Elderly. *International Journal of Geriatric Psychiatric* 15: 1021–1027.
25. Alsaffar, H., L. Wilson, D.P. Kamdar, F. Sultanov, D. Enepekides, and K.M. Higgins. 2016. Informed Consent: Do Information Pamphlets Improve Post-Operative Risk-Recall in Patients Undergoing Total Thyroidectomy: Prospective Randomized Control Study. *Journal of Otolaryngology Head & Neck Surgery* 45 (1): 1–6.

26. Barral, A.G., J. Cacho, and E. Navarro. 2016. Practical Application of Brief Cognitive Tests. *Neurología* 31 (3): 183–194.
27. Wu, Y., W. Xu, X. Liu, Q. Xu, L. Tang, and S. Wu. 2015. Adjunctive Treatment with High Frequency Repetitive Transcranial Magnetic Stimulation for the Behavioral and Psychological Symptoms of Patients with Alzheimer's Disease: A Randomized, Double-Blind, Sham-Controlled Study. *Shanghai Archives of Psychiatry* 27 (5): 280–288.
28. Sampson, E.L., N. White, K. Lord, B. Leurent, V. Vickerstaff, and S. Scott. 2015. Pain, Agitation, and Behavioural Problems in People with Dementia Admitted to General Hospital Wards: A Longitudinal Cohort Study. *Journal of the International Association for the Study of Pain* 156 (4): 675–683.
29. Boada Rovira, M., L. Tárraga, G. Modinos, S. Diego, and B. Reisberg. 2006. Behavioral Pathology in Alzheimer s Disease Rating Scale (BEHAVE-AD): Validación Española. *Behavioral Pathology in Alzheimer S Disease Rating Scale (BEHAVE-AD): Spanish Validation* 21 (1): 19–25.
30. Radloff, L.S., and L. Teri. 1986. Use of the Center for Epidemiological Studies-Depression Scale with Older Adults. *Clinical Gerontologist* 5 (1–2): 119–136.
31. de Paula, J.J., M.A. Bicalho, R.T. Ávila, M.T.G. Cintra, B.S. Diniz, M.A. Romano-Silva, and L.F. Malloy-Diniz. 2015. A Reanalysis of Cognitive-Functional Performance in Older Adults: Investigating the Interaction Between Normal Aging, Mild Cognitive Impairment, Mild Alzheimer's Disease Dementia, and Depression. *Frontiers in Psychology* 6 (January): 2061.
32. Aprahamian, I., B.S. Diniz, R. Izbicki, M. Radanovic, P.V. Nunes, and O.V. Forlenza. 2011. Optimizing the CAMCOG Test in the Screening for Mild Cognitive Impairment and Incipient Dementia: Saving Time with Relevant Domains. *International Journal of Geriatric Psychiatry* 26 (4): 403–408.
33. Vogel, S.J., S.J. Banks, J.L. Cummings, and J.B. Miller. 2015. Concordance of the Montreal Cognitive Assessment with Standard Neuropsychological Measures. *Alzheimer's & Dementia Diagnosis, Assessment & Disease Monitoring* 1 (3): 289–294.
34. Sager, M.A., B.P. Hermann, A. La Rue, and J.L. Woodard. 2006. Screening for Dementia in Community-Based Memory Clinics. *WMJ: Official Publication of the State Medical Society of Wisconsin* 105 (7): 25–29.
35. Brodaty, H., D. Pond, N.M. Kemp, G. Luscombe, L. Harding, K. Berman, and F.A. Huppert. 2002. The GPCOG: A New Screening Test for Dementia Designed for General Practice. *Journal of the American Geriatrics Society* 50 (3): 530–534.
36. Lorentz, W.J., J.M. Scanlan, and Ss Borson. 2002. Brief Screening Tests for Dementia. *Canadian Journal of Psychiatry* 47: 723–733.
37. Milne, A., A. Culverwell, R. Guss, J. Tuppen, and R. Whelton. 2008. Screening for Dementia in Primary Care: A Review of the Use, Efficacy and Quality of Measures. *International Psychogeriatrics* 20 (5): 911–926.
38. Knopman, D.S., and R.C. Petersen. 2014. Mild Cognitive Impairment and Mild Dementia: A Clinical Perspective. *Mayo Clinic Proceedings* 89 (10): 1452–1459.
39. Panegyres, P.K., R. Berry, and J. Burchell. 2016. Early Dementia Screening. *Diagnostics (Basel, Switzerland)* 6 (1): 6.
40. Henneges, C., C. Reed, Y.F. Chen, G. Dell'Agnello, and J. Lebrec. 2016. Describing the Sequence of Cognitive Decline in Alzheimer's Disease Patients: Results from an Observational Study. *Journal of Alzheimer's Disease* 52 (3): 1065–1080.
41. Beauchet, O., C.P. Launay, C. Merjagnan, A. Kabeshova, and C. Annweiler. 2014. Quantified Self and Comprehensive Geriatric Assessment: Older Adults are Able to Evaluate Their Own Health and Functional Status. *PLoS One* 9 (6): e100636.
42. Somme, D., A. Gautier, S. Pin, and A. Corvol. 2013. General Practitioner's Clinical Practices, Difficulties and Educational Needs to Manage Alzheimer's Disease in France: Analysis of National Telephone-Inquiry Data. *BMC Family Practice* 14: 81.
43. Mattsson, N., U. Andreasson, S. Persson, M.C. Carrillo, S. Collins, S. Chalbot, and S.A. Käser. 2013. CSF Biomarker Variability in the Alzheimer's Association Quality Control Program. *Alzheimer's & Dementia* 9 (3): 251–261.

44. Sperling, R.A. 2012. Toward Defining the Preclinical Stages of Alzheimer's Disease: Recommendations from the National Institute on Aging-Alzheimer's Association Workgroups on Diagnostic Guidelines for Alzheimer's Disease. *Alzheimer's & Dementia* 7 (3): 280–292.
45. Bamford, C., S. Lamont, M. Eccles, L. Robinson, C. May, and J. Bond. 2004. Disclosing a Diagnosis of Dementia: A Systematic Review. *International Journal of Geriatric Psychiatry* 19 (2): 151–169.
46. Wilcock, J., and R.O. Carroll. 2009. Concordance with Clinical Practice Guidelines for Dementia in General Practice. *Ageing and Mental Health* 13 (2): 155–161.
47. Belmin, J., S. Pariel-Madjlessi, P. Surun, C. Bentot, D. Feteanu, Lefebvre des Noettes, and J.L. Golmard. 2007. The Cognitive Disorders Examination (Codex) is a Reliable 3-Minute Test for Detection of Dementia in the Elderly (Validation Study on 323 Subjects). *Presse Médicale* 36 (9 I): 1183–1190.

Sarcopenia and Its Impact on Quality of Life

Maria Tsekoura, Alexandros Kastrinis, Marianna Katsoulaki, Evdokia Billis, and John Gliatis

Abstract Sarcopenia is recognized as a major health problem among older adults. This syndrome is associated with serious health consequences in terms of frailty, disability, morbidity and mortality. The aim of this study is to review sarcopenia and its impact on quality of life (QoL). MEDLINE database was searched from January to March 2016 using predefined search terms "sarcopenia", quality of life". Based on extensive literature search, 32 articles were identified while only 6 studies met the inclusion criteria and were associated with sarcopenia and QoL. Quality of life level was measured using generic self-reported tools; the Medical Outcomes Survey Short-form General Health Survey (SF-36) in 4 studies and EuroQol-5D instrument (EQ-5D) in 2 studies. Subjects with sarcopenia demonstrated a significantly high proportion of problems relating to several dimensions of QoL. More studies based on Sarcopenia and QoL are needed. Although the impact of sarcopenia on QoL was assessed in all studies with QoL generic instruments, it would be more insightful to utilise a disease-specific quality of life questionnaire, such as the SarQoL for sarcopenic subjects.

Keywords Sarcopenia • Quality of life • Disability • Consequences

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1 Introduction

The name sarcopenia is derived from Greek sarx (flesh) and penia (loss), literally meaning poverty of flesh [1]. The term was first introduced in 1989 by Irwin Rosenberg to define the loss of muscle mass that occurs with advancing age [2, 3] and since then its definition has seen a number of modifications [4]. The European Working Group on Sarcopenia in Older People (EWCSOP) suggested diagnosing sarcopenia when at least two of three criteria apply: (1) low muscle mass, (2) low muscle strength, and/or (3) low physical performance [5]. The EWCSOP proposed a diagnosis for sarcopenia using the criteria of low muscle mass (estimated by the ratio of appendicular lean mass over height squared, $\leq$7.23 kg/ht^2 for men and $\leq$5.67 kg/ht^2 for women) and low muscle function (either low muscle strength and/or low physical performance). Low muscle strength (measured by grip strength <30 kg for men and <20 kg for women) or low physical performance (measured by gait speed <0.8 m/s) [6].

This syndrome leads to a decline in muscle strength and power [3]. It is associated with physical disability, mobility limitations, poor quality of life, mortality [5, 7, 8]. However, the association between sarcopenia and altered quality of life (QoL) has not been thoroughly explored. The aim of this study is to review sarcopenia and its impact on quality of life.

2 Methods

2.1 Search Strategy and Selection Criteria

Pubmed was searched from January 2016 to March 2016 using the term "sarcopenia", "muscle mass", "quality of life", "disability". The reference lists of systematic review articles and meta analyses were scanned for any additional references missed from the Pub Med search. The search was limited to publications in English. Inclusion criteria consisted of studies investigated the association between variables related to QoL and sarcopenia.

3 Results

The current search identified a total of 32 studies related to sarcopenia and related factors, such as bone density, muscle strength, nutritional status etc. Only 6 studies investigated the association between sarcopenia and QoL (Table 1). Outcome measures which were used were SF-36, EuroQoL (EQ-5D).

Sayer et al. [13] investigated the relationship between loss of muscle strength and Health related quality of life (HRQoL) in 2987 men and women aged 59–73 years

Table 1 Studies about Sarcopenia and quality of life

Author	Type of study	Participants (N)	Outcome measures	Results
Beaudart et al. [9]	Longitudinal study	534 (73 with sarcopenia)	SF 36, EQ-5D, EQ-VAS	Decrease of QoL in physical function (PF) using the SF 36 ($p = 0.001$)
Patel et al. [10]	Cross sectional study	103 (with sarcopenia)	SF 36	Men and women with sarcopenia reported poorer (i.e. lower) SF-36 General health ($p < 0.001$) and PF scores ($p < 0.002$)
Go et al. [11]	Cross sectional study	1397 (219 with Sarcopenia)	EuroQol-5 dimension (EQ-5D)	Subjects with sarcopenia showed a significantly higher proportion of problems in all items of the EQ-5D descriptive system of HRQoL (mobility self-care, usual activity anxiety & depression: $p = 0.001$, pain & discomfort: $p = 0.054$) than those without sarcopenia
Silva Neto et al. [12]	Cross sectional study	56 (11 with sarcopenic obesity)	SF-36	Mean values were lower in the affected women. (Statistically significant differences were not observed between the studied variables) handgrip strength correlated positively and significantly with all of the SF-36 dimensions except Vitality ($p = 0.08$) and mental health ($p = 0.25$)
Sayer et al. [13]	Cross sectional study	2987	SF 36	Lower grip strength was associated with increased prevalence of having poor scores for all of the SF-36 domains ($p < 0.001$ in men and women)
Kull et al. [14]	Population based cohort	304	SF 36	Sarco-osteoporotic individuals show markedly lower scores in the role: physical (women: $p = 0.001$, men: $p = 0.001$) and role emotional (women: $p = 0.002$, men: $p = 0.001$) subscales of the SF-36 questionnaire

of age. Their objective was to investigate the relationship between grip strength, as a marker of sarcopenia, and Short Form-36 (SF-36) score, as a marker of HRQoL, in a community-dwelling elderly population. They showed that lower grip strength was associated with reduced HRQoL in older men and women. This study suggested that the resulting association reflects the link between sarcopenia and generalized frailty.

Kull et al. [14] found a reduced QoL in two domains (i.e. physical function and vitality) of the SF-36 questionnaire in sarcopenic subjects. A population sample of 304 patients aged 25–70 years old was analyzed with a Lunar DPX-IQ dual energy X-ray absorptiometry machine and their HRQoL was assessed with the Short-Form-36 (SF-36) questionnaire. The authors aimed to define sarco-osteopenia (SOP) in healthy subjects using both muscle functional parameters and assessment of the impact on HRQoL. Combining osteopenia and sarcopenia could identify a frailer, higher-fracture-risk population. These individuals also show markedly lower scores in the role-physical ($p = 0.01$), vitality ($p = 0.03$), and role-emotional ($p = 0.02$) subscales of the SF-36 questionnaire.

Patel et al. [10] reported reduced QoL in sarcopenic patients living in UK. The domains were physical function and general health. Men and women with sarcopenia had poor self-reported general health and functional domains scores.

The SarchoPhage project is a longitudinal study in 534 elderly subjects assessing health and functional consequences on Sarcopenia. Seventy three (73) subjects were diagnosed sarcopenic (prevalence 13.7%). Sarcopenic subjects were older, had a lower Body Mass Index, lower calf, wrist and arm circumference, presented more cognitive impairments, comorbidities and worse physical quality of life (SF 36) for the domain of physical functioning, were more frail (Fried), had higher risk of falls (Timed up and Go) and presented tiredness for the activities of daily living (Mobility-test) [9].

Go et al. [11] presented significantly more problems of mobility, self-care, usual activity and anxiety than non-sarcopenic subjects. This study evaluated the association between sarcopenia and bone density HRQoL in Korean men, suggesting that sarcopenia may have a greater influence on dimensions of physical functioning rather than social functioning or mental health. Sarcopenic patients due to loss of muscle mass and decrease in muscle strength through sarcopenia may present with discomfort during movement and usual activity.

Silva Neto et al. [12] investigated the association between variables related to QoL and sarcopenia, sarcopenic obesity and muscle mass in 56 elderly women. Of the 56 participants, 13 (23.21%) were classified as sarcopenic, while 43 (76.78%) were not. QoL was measured using the SF-36.

4 Discussion

Sarcopenia is associated with multiple adverse outcomes such as comorbidities, poor physical performance, physical disability, depression, hospitalisation, functional decline, falls and mortality [10, 15–17]. It does not come as a surprise that

the likelihood of having worse quality of life is higher in elderly subjects diagnosed with Sarcopenia.

The association between sarcopenia and altered QoL has been supported by 6 studies. Studies using SF-36 and EQ-5D instruments indicates decrease in domains of QoL especially physical function (Sarcph study). More studies based on Sarcopenia and QoL seem necessary to assess the real impact of Sarcopenia on QoL [9].

The impact on sarcopenia on quality of life is assessed in all studies by generic tools. Quality of life assessments via questionnaires are obviously important and necessary for healthcare staff to understand the needs, of elderly people and people with sarcopenia. QoL measures prioritise problems, facilitate communication and monitor changes or response to treatment. Using the appropriate QoL measure in clinical practice ensures that treatment plans and evaluations focus on the patient rather than the disease [18]. It would be useful to have at our disposal a sarcopenia specific QoL questionnaire to assess the prospective quality of life of sarcopenic subjects. For that reason Beaudart and colleagues in 2015 report the development of the first disease-specific, self-administrated sarcopenia-related QoL questionnaire, the SarQoL questionnaire. This instrument includes 22 questions and seven domains of dysfunction: Physical and Mental Health, Locomotion, Body composition, Functionality, Activities of daily living, Leisure activities and Fear [9].

Gender differences cannot be discussed based on these particular studies. For example Go et al. [11] studied only men and Silva Neto et al. [12] studied only women. Patel in their study indicated that men and women with sarcopenia were on average shorter, weighed less, had lower waist, hip and mid-thigh circumferences, and also recorded slower Timed up and Go test and chair rise test, than their counterparts without sarcopenia.

The consequences of sarcopenia on QoL, disability and mortality are important and it is recommended that physicians and therapists should consider screening for sarcopenia in different settings [6]. The next step is to use the SarQoL questionnaire and to develop new studies for sarcopenic individuals.

5 Conclusions

The results of this literature review showed that sarcopenia is associated with healthy outcomes and an obvious decline in QoL. This indicates the importance of preventative and interventional management strategies for managing sarcopenic individuals. However, additional research is necessary to further determine the impact of sarcopenia on quality of life.

References

1. Von Haeling, S., J.E. Morley, and S.D. Anker. 2010. An Overview of Sarcopenia: Facts and Numbers on Prevalence and Clinical Impact. *Journal of Cachexia, Sarcopenia and Muscle* 1: 129–133.
2. Rosenberg, I.H. 1997. Sarcopenia: Origins and Clinical Relevance. *The Journal of Nutrition* 127: 990S–991S.
3. Morley, J.E. 2008. Sarcopenia: Diagnosis and Treatment. *The Journal of Nutrition, Health & Aging* 12: 452–456.
4. Rizzoli, R., J.Y. Reginster, J.F. Arnal, I. Bautmans, C. Beuadart, H. Bischoff-Ferrari, et al. 2013. Quality of Life in Sarcopenia and Frailty. *Calcified Tissue International* 93: 101–120.
5. Cruz-Jentoft, A.J., J.P. Baeyens, J.M. Baur, Y. Boirie, T. Cederholm, F. Landi, et al. 2010. Sarcopenia: European Consensus on Definition and Diagnosis: Report of the European Working Group on Sarcopenia in Older People. *Age and Ageing* 39: 412–423.
6. Cruz-Jentoft, A.J., F. Landi, S.M. Schneider, C. Zuniga, H. Arai, Y. Boirie, and L.K. Chen. 2014. Prevalence of and Interventions for Sarcopenia in Ageing Adults: A Systematic Review. Report of the International Sarcopenia Initiative (EWGSOP and IWGS). *Age and Ageing* 43: 748–759.
7. Bijma, A.Y., C.G.M. Meskers, N. Van Den Eshof, R.G. Westendrop, S. Sipila, L. Strenroth, et al. 2014. Diagnostic Criteria for Sarcopenia and Physical Performance. *AGE* 36: 275–285.
8. Pongchaoiyakul, C., P. Limpawattana, P. Kotruchin, and R. Rajatanavin. 2013. Prevalence of Sarcopenia and Associated Factors Among Thai Population. *Journal of Bone and Mineral Metabolism* 31: 346–350.
9. Beaudart, C., J.Y. Reginster, J. Petermanns, S. Gillain, A. Quabron, M. Locquet, J. Slomian, F. Buckinx, and O. Bryuere. 2015. Quality of Life and Physical Components Linked to Sarcopenia: The SarchPhAge Study. *Experimental Gerontology* 69: 103–110.
10. Patel, H.P., H.E. Syddall, K. Jameson, S. Robinson, H. Denison, H.C. Roberts, et al. 2013. Prevalence of Sarcopenia in Community-Dwelling Older People in the UK Using the European Working Group on Sarcopenia in Older People (EWGSOP) Definition: Findings from the Hertfordshire Cohort Study (HCS). *Age and Ageing* 42 (3): 378–384.
11. Go, S.W., Y.H. Cha, J.A. Lee, et al. 2013. Association Between Sarcopenia, Bone Density, and Health-Related Quality of Life in Korean Men. *Korean Journal of Family Medicine* 34: 281–288.
12. Silva Neto, L.S., M.G. Karnikowiski, A.B. Tavares, et al. 2012. Association Between Sarcopenia, Sarcopenic Obesity, Muscle Strength and Quality of Life Variables in Elderly Women. *Revista Brasileira de Fisioterapia* 16: 360–367.
13. Sayer, A.A., H.E. Syddall, H.J. Martin, E.M. Dennison, H.C. Roberts, and C. Cooper. 2006. Is Grip Strength Associated with Health-Related Quality of Life? Findings from the Hertfordshire Cohort Study. *Age and Ageing* 35 (4): 409–415.
14. Kull, M., R. Kallikorm, and M. Lember. 2012. Impact of a New Sarco-Osteopenia Definition on Health-Related Quality of Life in a Population-Based Cohort in Northern Europe. *Journal of Clinical Densitometry* 15 (1): 32–38.
15. Visser, M., D.J.H. Deeg, and P. Lips. 2004. Low Vitamin D and High Parathyroid Hormone Levels as Determinants of Loss of Muscle Strength and Muscle Mass (Sarcopenia): The Longitudinal Aging Study Amsterdam. *The Journal of Clinical Endocrinology & Metabolism* 88 (12): 5766–5772.
16. Sayer, A.A., S.M. Robinson, H.P. Patel, T. Shavlakadze, C. Cooper, and M.D. Grounds. 2013. New Horizons in the Pathogenesis, Diagnosis and Management of Sarcopenia. *Age and Ageing* 42: 145–150.
17. Beaudart, C., R. Rizzoli, O. Bruyere, J.Y. Regintsre, and E. Biver. 2014. Sarcopenia: Burden and Challenges for Public Health. *Archives of Public Health* 72: 45.
18. Higginson, I.J., and A.J. Carr. 2001. Measuring Quality of Life: Using Quality of Life Measures in the Clinical Setting. *BMJ* 322 (7297): 1297–1300.

Molecular Chaperones in Neurodegenerative Diseases: A Short Review

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Abstract Stress and misfolded proteins result to dysfunction in the cell, often leading to neurodegenerative diseases and aging. Misfolded proteins form toxic aggregates that threaten cell's stability and normal functions. In order to restore its homeostasis, the cell activates the UPR system. Leading role in the restoration play the molecular chaperones which target the misfolded proteins with the purpose of either helping them to unfold and refold to their natural state or lead them degradation. This paper aims to present some of the most known molecular chaperones and their relation with diseases associated to protein misfolding and neurodegeneration, as well as the role of chaperones in proteostasis.

Keywords Molecular chaperones • Neurodegenerative diseases • Alzheimer disease • Huntington's disease • Misfolded proteins • UPR • Protein folding

1 Introduction

The concept of proteostasis maintenance involves the biological pathways which are critical for post-mitotic cells, such as neurons. Disruption of the mechanisms of proteostasis is increasingly referred in many studies, to be involved in neurodegenerative diseases. Dysregulation of some of the key mechanisms which have been evolved to maintain cellular homeostasis, such as protein folding, as well as the presence of molecular chaperones and degradation mechanisms, are implicated as pathological hallmark of these diseases.

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Protein misfolding and formation of toxic aggregates within affected neurons are thought to be involved in the pathogenesis many age-related diseases, such as Alzheimer's disease. Whilst neurodegenerative diseases differ in the proteins which appear to misfold, one common feature is the disruption of the protein quality control system.

Chaperone machinery is involved in the assembly of the unique three-dimensional structure of proteins. Chaperones play a ubiquitous role in the cytoplasmic meshwork: they are highly abundant, they form a complex with all the elements of the cytoskeleton and they also attach to a plethora of other proteins [1]. Molecular chaperones serve to prevent protein misfolding, participate in the loose folding procedure, assist in folding and refolding of damaged proteins and target severely damaged proteins to degradation and sequester them to larger aggregates. Therefore, chaperones are key components to this protein quality control system [2].

The native folding of proteins can be disrupted by stress, molecular crowding and mutations [3]. Driven by ATP, chaperones gradually convert toxic misfolded protein substrates, into non-toxic, natively refolded. Stress and protein damages can lead to cellular dysfunction, causing degenerative diseases and aging [4]. These conditions activate cellular stress-response pathways, including the UPR, which counteract stress and help restore homeostasis to protein folding in the ER [5].

Chaperone complex disruption results in instability and incorrect subcellular localization of the signaling protein [6]. Besides the major role of chaperones, which is to target all misfolded proteins with hydrophobic surfaces, there are also specialized chaperones that stabilize several signal transduction networks important to cancer cells [7].

2 Why Proteins Misfold?

Misfolding may originate from improper interactions between regions of the folding polypeptide chain. Another factor is the crowded nature of the intracellular environment that may prevent folding from proceeding at a biologically relevant time scale [8]. Incompletely folded proteins, often tend to inappropriate interactions with other molecules [9] and aggregate in the cell due to high local concentration of nascent chains [10]. There may be several kinds of aggregates, including disordered or 'amorphous' aggregates, but amyloid fibrils are the most characteristic [11]. Deposition of abnormal protein aggregation characterizes most neurodegenerative disorders, not just AD and Parkinson's, but also motor neuron diseases, as well as diseases of peripheral tissue like familial amyloid polyneuropathy, Charcot-Marie-Tooth [12–15].

3 How Chaperones Prevent Aggregation

The cellular strategy to counteract the aggregation of non-native proteins is the chaperone machinery. The heat shock proteins (HSPs) are the main chaperone classes which are referred to prevent the accumulation of misfolded conformers (e.g. HSP60 and HSP70) [16]. The reversibility of protein aggregation has been first observed to be mediated by heat-shock protein Hspl04 [17]. Chaperone binding blocks intermolecular aggregation of non-native polypeptides and may also prevent, or reverse intramolecular misfolding [18, 19]. Those proteins that do not succeed to fold correctly are selected for degradation by the protein control system. This is achieved through the proteasome and the chaperone mediated autophagy [20]. Increasing evidence show that perturbation of these functions plays a key role in the pathogenesis of severe human disorders [21, 22].

Moreover, the ability of maintaining cellular proteostasis declines with aging, which results in the accumulation of misfolded proteins, deposition of aggregates, cellular toxicity and eventually cell death [23–25]. The age-related accumulation of oxidized proteins has been proposed to be due to either, or both, increased protein oxidative damage and decreased oxidized protein degradation and repair [26]. This deterioration of proteostasis is a characteristic risk factor of many human pathologies and represents a hallmark which is considered to contribute to the aging process [27].

The role of chaperone function and the simultaneous protein oxidation, misfolding and aggregation in aged organisms, suggest that preservation of protein homeostasis and long-range protein organization have a major role in neurodegeneration and aging [1, 28, 29].

4 ER Stress and the Unfolded Protein Response

Although differential mechanisms have been described to be involved in different neurodegenerative conditions, endoplasmic reticulum (ER) stress is increasingly implicated as a key factor relevant to pathogenesis of AD and other neurodegenerative diseases [30, 31]. The accumulation of misfolded or deficiently modified proteins within the ER, disturbs ER homeostasis, giving rise to ER stress, which results in activation of the unfolded protein response (UPR) [32, 33]. The UPR involves [31, 34]:

- up-regulation of protein chaperones to promote protein folding,
- translational attenuation to reduce the load of proteins within the ER to prevent further accumulation of misfolded proteins,
- up-regulation of ER-associated protein degradation (ERAD) and
- autophagy to promote degradation of misfolded proteins

Therefore, ER stress triggering of UPR plays a pivotal role in maintaining cell proteostasis [35, 36]. In circumstances of chronic or prolonged ER stress, however, the UPR seems to trigger apoptotic signalling cascades [37, 38]. The ER response

includes changes in specific proteins, which might cause translational attenuation, induction of ER chaperones and degradation of misfolded proteins [39]. In case of prolonged or aggravated ER stress, apoptotic signalling is stimulated, which leads to cell death [40]. The dual functions of ER stress appears to switch from pro-survival to pro-apoptosis [41–43].

5 Chaperones and Diseases

Molecular chaperones have a ubiquitous role in the protein quality control system. Misfolded proteins are usually refolded with the assistance of molecular chaperons and/or degraded by the ubiquitin-proteasome system. In many studied the protective effects of overexpressing chaperones in models of misfolding disease is promoted. The role of different chaperones, their function and their association in different neurological diseases is summarized in the table in Appendix section.

5.1 Alzheimer's Disease

Amyloid fibrils, Aβ aggregation, are a well-characterized aggregation state associated with Alzheimer's disease [44]. Several studies have investigated the role of heat shock proteins in AD [45]. Some studies suggest that the induction of small heat-shock proteins, Hsp70, protects neurons from protein aggregation and toxicity [46, 47]. In addition to HSP70 the complex of Hsp90 can also inhibit Aβ formation and slow the rate of aggregation [48]. Other studies showed that ubiquitin (a heat-shock protein, which labels damaged proteins and directs them for proteolytic degradation) is affected by Alzheimer's disease, in neurons and in surrounding astrocytes [49]. The role that HSPs may act therapeutically as neuroprotective agents, by modulating innate immune activation is reviewed in [50].

5.2 Parkinson's Disease

Parkinson' s disease is the second most common movement disorder, characterized by motor impairments—bradykinesia, rigidity, and resting tremor, caused by a progressive degeneration of dopaminergic neurons in the substantia nigra [51]. An increasing number of evidence shows that endoplasmic reticulum stress and the unfolded protein response are also key elements of Parkinson' s disease etiology [52]. These results suggest a proposed therapeutic strategy to ensure appropriate protein folding and to avoid ER stress. This requires an efficient chemical or molecular chaperone network to promote the appropriate folding of proteins [53]. A number of chaperone-based therapies are under development, which aim to prevent the formation of potentially toxic—synuclein oligomers and aggregates [54].

5.3 Amyotrophic Lateral Sclerosis

Amyotrophic Lateral Sclerosis also known as ALS is a neurodegenerative disease that affects the upper and lower motor neurons. Molecular analyses have shown that the disease is primarily caused due to a mutation either to SOD1 (Cu/Zn superoxide dismutase) or to FUS (fused in sarcoma) [55]. Mutant SOD1 is related to the Hsp70/Hsp90 network and its degradation seems to be regulated by CHIP, another co-chaperone [56]. Ubiquitinated SOD1 forms aggregates, that are associated with Hsc70 which might be able to protect Sod1 from degradation while Hsp70 favors it [57]. Therapeutic strategies that inhibit Hsp90 and increase Hsp70 activity are investigated in some cellular and animal ALS models [58].

5.4 Huntington's Disease

Amyloid-like inclusions have been associated also with Huntington's disease (HD), which is caused by expanded polyglutamine repeats in the Huntingtin protein [59]. HD is an autosomal, dominant and inherited neurodegenerative disease that is focused on the region of basal ganglia causing mental, emotional and motor problems to the patients. In Huntington's, the responsible gene (IT15) for the production of protein huntingtin is mutated causing expanded repetition of the trinucleotide CAG, which in turn makes the produced polyglutamine strand toxic for the brain. As a result, neurons which contain the mutant protein begin to atrophy [60, 61]. The normal function of huntingtin is to maintain brain cells in a good condition and to help in intracellular procedures. The mutation at the beginning of the huntingtin gene causes the destabilization of the protein leading to problems; it interferes with the typical function of [62]. Mutant huntingtin (m-htt) affects many cytoplasmic proteins which are related to apoptosis, transcription, mitochondrial function and other vital for the cell procedures. M-htt joins together with other proteins forming protein aggregates. Protein aggregation is the phenomenon when misfolded proteins clump together inside or outside the cell. Those forms are toxic for the cell and reduce the Ubiquitin Proteasome System function (UPS) [63, 64].

5.5 Charcot-Marie-Tooth Disease

Autosomal Dominant Demyelinating Neuropathies CMT1 (Charcot-Marie-Tooth Disease type1) is associated with an autosomal dominant duplication on a chromosome that includes the peripheral myelin protein 22 gene (PMP22) [65]. When overexpressed in cultured cells, PMP22 has been observed to form protein

aggregates [66]. In other studies were found that misfolded protein SIMPLE forms abnormal cytosolic aggregates. These findings suggest that demyelinating CMT may be a protein-misfolding disease of Schwann cells [67].

5.6 Creutzfeldt-Jakob Disease and Other Prion Encephalopathies

Prion proteins from mammalian species, are prone to amyloid-like prion diseases, like Creutzfeld-Jakob disease [68]. In many studies it is referred that chaperones try to block the contact surfaces of prion molecules [1]. Many chaperones, such as Hsp60, Hsp70, or its co-chaperone, Hsp40 were found to fight against prion aggregation [69, 70]. The "chaperone overload" hypothesis emphasises the need for efficient ways to enhance chaperone-capacity in ageing subjects and calls for the identification and future "repair" of silent mutations [1].

5.7 Conclusions

Researches suggest that protein aggregation is part of the cellular response to an imbalanced protein homeostasis. Intense research interest to unravel the pathophysiological significance of these protein aggresome has unveiled the important role of chaperones in proteostasis, by promoting the correct folding of proteins into their native conformations. Therefore, novel therapeutic strategies should aim at the role of chaperones to prevent aberrant protein misfolding and promoting maintenance of cellular homeostasis.

Appendix

Chaperone	Location	Role	Characteristics	Related disease
UPR	Lumen of ER	Halting protein translation Degrading misfolded proteins Activating signaling pathways that increase the production of molecular chaperones apoptosis		Creutzfeldt-Jakob disease, Alzheimer's disease, Parkinson's disease, Huntington's disease Prion diseases
First chaperone	Nucleus	Assembly of nucleosomes form histones to DNA		
Steric chaperone		Convey folding information into some other proteins		
Calnexin/Calreticulin	Lumen of ER	Calnexin forms part of the quality control monitor that recognize and target abnormally folded proteins for rapid degradation Protein folding Functions as a chaperone for the folding of MHC class I α-chain in the membrane of the ER Calreticulin binds to misfolded proteins and prevents them from being exported from the endoplasmic reticulum to the Golgi apparatus Calreticulin, an abundant ER chaperone was shown to participate in the quality control of the amyloid precursor protein	Lectin chaperones Glycan processing	HD

(continued)

(continued)

Chaperone	Location	Role	Characteristics	Related disease
Crystallin				AD
Hsp47/ERp29	ER	Non classical molecular chaperones	Hsp47 Procollagen chaperone	
PDI/PPI/ERp57	ER	Folding chaperones		
Transfer chaperones (Sec61 membrane protein)	Mitochondria & ER of eukaryotes	Transport across membranes		
GroEl/GroEs, Dnak/DnaJ/GrpC		Foldases	Dnak is an Hsp70 protein. One of Hsp40 chaperones is DnaJ (75-residue protein), which interacts with DnaK (a Hsp70 chaperone) and assists in capturing substrate proteins	
DnaJ/Hsp53		Holdases	DnaJ is one of Hsp40	
GRP78/BiP,GRP94	ER	General chaperones		AD,PD
GRP170				
Erp57/BiP	ER	Quality control	Recognize misfolded proteins and help their retention in the ER allowing only correctly folded proteins to the cytosol	
Hsp60/Hsp100/Hsp90		Hsp100/Hsp90 Protein disaggregation and refolding	Heat shock proteins,ATP/ADP	HD, prion diseases
Hsp70/Hsp40		Are involved in blocking aggregation of misfolded proteins by binding to their hydrophobic segments	Hsp70 consists of ATP-binding N-terminal domain and peptide binding C-terminal domain Hsp70 works in tandem with Hsp40 co-chaperone	AD, PD, HD, prion diseases

			Proteins in a cell may experience partial unfolding due to variety of factors, such as temperature increase, pH change etc. Some proteins may also fail to reach their native states after synthesis. As result such proteins adopt aggregation-prone states. To prevent this Hsp70 binds to such proteins and act as a general “safe keeper” for misfolded proteins	
Cdc48p (valosin containing protein (VCP/p97))	ER	Ubiquitin binding protein	Transport substances from ER to cytoplasm	
PDIA3 (Protein disulfide isomeric A3)	ER	Interacts with lectin chaperones Modulate folding of newly synthesized glycoproteins		
X-box binding protein (XbP1)	Part of UPR	Correlates with the expression level of expressed proteins in order to adapt the folding capacity of the ER to the respective requirements		AD, Crohn’s disease

References

1. Söti, C., and P. Csermely. 2002. Chaperones and aging: role in neurodegeneration and in other civilizational diseases. *Neurochemistry International* 41(6):383–389.
2. Tiroli-Cepeda, A.O., and C.H.I. Ramos. 2011. An overview of the role of molecular chaperones in protein homeostasis. *Protein and Peptide Letters* 18(2):101–109.
3. Sharma, S.K., P. Christen, and P. Goloubinoff. 2009. Disaggregating chaperones: an unfolding story. *Current Protein and Peptide Science* 10(5):432–446.
4. Fulda, S., A.M. Gorman, O. Hori, and A. Samali. 2010. Cellular stress responses: cell survival and cell death. *International Journal of Cell Biology* 2010:214074.
5. Pincus, D., A. Aranda-Díaz, I.A. Zuleta, P. Walter, and H. El-Samad. 2014. Delayed Ras/PKA signaling augments the unfolded protein response. *Proceedings of the National Academy of Sciences* 111(41):14,800–14,805.
6. Pratt, W.B., and D.O. Toft. 2003. Regulation of signaling protein function and trafficking by the Hsp90/Hsp70-based chaperone machinery. *Experimental Biology and Medicine* 228(2):111–133.
7. Hong, D.S., U. Banerji, B. Tavana, G.C. George, J. Aaron, and R. Kurzrock. 2013. Targeting the molecular chaperone heat shock protein 90 (Hsp90): lessons learned and future directions. *Cancer Treatment Reviews* 39(4):375–387.
8. Moreno-Gonzalez, I., and C. Soto. 2011. Misfolded protein aggregates: mechanisms, structures and potential for disease transmission. In *Seminars in Cell and Developmental Biology*, vol. 22, 482–487. New York: Elsevier.
9. Dobson, C.M. 2003. Protein folding and misfolding. *Nature* 426(6968):884–890.
10. Jha, S. 2009. *Molecular Biology of Protein Folding*, vol. 84. Amsterdam: Academic.
11. Ross, C.A., and M.A. Poirier. 2004. Protein aggregation and neurodegenerative disease. *Nature Reviews Neuroscience* 5:S10–S17.
12. Berger, P., A. Niemann, and U. Suter. 2006. Schwann cells and the pathogenesis of inherited motor and sensory neuropathies (Charcot–Marie–Tooth disease). *Glia* 54(4):243–257.
13. Koo, E.H., P.T. Lansbury, and J.W. Kelly. 1999. Amyloid diseases: abnormal protein aggregation in neurodegeneration. *Proceedings of the National Academy of Sciences* 96(18):9989–9990.
14. Planté-Bordeneuve, V., and G. Said. 2011. Familial amyloid polyneuropathy. *The Lancet Neurology* 10(12):1086–1097.
15. Wood, J., T. Beaujeux, and P. Shaw. 2003. Protein aggregation in motor neurone disorders. *Neuropathology and Applied Neurobiology* 29(6):529–545.
16. Tyedmers, J., A. Mogk, and B. Bukau. 2010. Cellular strategies for controlling protein aggregation. *Nature Reviews Molecular Cell Biology* 11(11):777–788.
17. Parsell, D.A., A.S. Kowal, M.A. Singer, and S. Lindquist. 1994. Protein disaggregation mediated by heat-shock protein Hspl04. *Nature* 372(6505):475–478.
18. Bukau, B., and A.L. Horwich. 1998. The Hsp70 and Hsp60 chaperone machines. *Cell* 92(3):351–366.
19. Hartl, F.U., and M. Hayer-Hartl. 2002. Molecular chaperones in the cytosol: from nascent chain to folded protein. *Science* 295(5561):1852–1858.
20. Arias, E., and A.M. Cuervo. 2011. Chaperone-mediated autophagy in protein quality control. *Current Opinion in Cell Biology* 23(2):184–189.
21. Koga, H., and A.M. Cuervo. 2011. Chaperone-mediated autophagy dysfunction in the pathogenesis of neurodegeneration. *Neurobiology of Disease* 43(1):29–37.
22. Orenstein, S.J., and A.M. Cuervo. 2010. Chaperone-mediated autophagy: molecular mechanisms and physiological relevance. *Seminars in Cell and Developmental Biology* 21(7):719–726.
23. David, D.C., N. Ollikainen, J.C. Trinidad, M.P. Cary, A.L. Burlingame, and C. Kenyon. 2010. Widespread protein aggregation as an inherent part of aging in C. elegans. *PLoS Biol* 8(8):e1000,450.

24. Morley, J.F., H.R. Brignull, J.J. Weyers, and R.I. Morimoto. 2002. The threshold for polyglutamine-expansion protein aggregation and cellular toxicity is dynamic and influenced by aging in Caenorhabditis elegans. *Proceedings of the National Academy of Sciences* 99(16):10,417–10,422.
25. Olzscha, H., S.M. Schermann, A.C. Woerner, S. Pinkert, M.H. Hecht, G.G. Tartaglia, M. Vendruscolo, M. Hayer-Hartl, F.U. Hartl, and R.M. Vabulas. 2011. Amyloid-like aggregates sequester numerous metastable proteins with essential cellular functions. *Cell* 144(1):67–78.
26. Friguet, B. 2006. Oxidized protein degradation and repair in ageing and oxidative stress. *FEBS Letters* 580(12):2910–2916.
27. López-Otín, C., M.A. Blasco, L. Partridge, M. Serrano, and G. Kroemer. 2013. The hallmarks of aging. *Cell* 153(6):1194–1217.
28. Leak, R.K. 2014. Heat shock proteins in neurodegenerative disorders and aging. *Journal of Cell Communication and Signaling* 8(4):293–310.
29. Verbeke, P., J. Fonager, B.F. Clark, and S.I. Rattan. 2001. Heat shock response and ageing: mechanisms and applications. *Cell Biology International* 25(9):845–857.
30. Pereira, C.M. 2013. Crosstalk between endoplasmic reticulum stress and protein misfolding in neurodegenerative diseases. *ISRN Cell Biology* 2013:1–22.
31. Perri, E.R., C.J. Thomas, S. Parakh, D.M. Spencer, and J.D. Atkin. 2015. The unfolded protein response and the role of protein disulfide isomerase in neurodegeneration. *Frontiers in Cell and Developmental Biology* 3:80.
32. Salminen, A., A. Kauppinen, T. Suuronen, K. Kaarniranta, and J. Ojala. 2009. ER stress in alzheimer's disease: a novel neuronal trigger for inflammation and alzheimer's pathology. *Journal of Neuroinflammation* 6(1):1.
33. Schröder, M., and R.J. Kaufman. 2005. The mammalian unfolded protein response. *Annual Review of Biochemistry* 74:739–789.
34. Rao, R.V., and D.E. Bredesen. 2004. Misfolded proteins, endoplasmic reticulum stress and neurodegeneration. *Current Opinion in Cell Biology* 16(6):653–662.
35. Manié, S.N., J. Lebeau, and E. Chevet. 2014. Cellular mechanisms of endoplasmic reticulum stress signaling in health and disease. 3. Orchestrating the unfolded protein response in oncogenesis: an update. *American Journal of Physiology-Cell Physiology* 307(10):C901–C907.
36. Tabas, I., and D. Ron. 2011. Integrating the mechanisms of apoptosis induced by endoplasmic reticulum stress. *Nature Cell Biology* 13(3):184–190.
37. Logue, S.E., P. Cleary, S. Saveljeva, and A. Samali. 2013. New directions in ER stress-induced cell death. *Apoptosis* 18(5):537–546.
38. Rutkowski, D.T., and R.J. Kaufman. 2007. That which does not kill me makes me stronger: adapting to chronic er stress. *Trends in Biochemical Sciences* 32(10):469–476.
39. Lindholm, D., H. Wootz, and L. Korhonen. 2006. ER stress and neurodegenerative diseases. *Cell Death and Differentiation* 13(3):385–392.
40. Sano, R., and J.C. Reed. 2013. ER stress-induced cell death mechanisms. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research* 1833(12):3460–3470.
41. Kadowaki, H., H. Nishitoh, and H. Ichijo. 2004. Survival and apoptosis signals in er stress: the role of protein kinases. *Journal of Chemical Neuroanatomy* 28(1):93–100.
42. Kim, I., W. Xu, and J.C. Reed. 2008. Cell death and endoplasmic reticulum stress: disease relevance and therapeutic opportunities. *Nature Reviews Drug Discovery* 7(12):1013–1030.
43. Nishitoh, H. 2012. Chop is a multifunctional transcription factor in the er stress response. *Journal of Biochemistry* 151(3):217–219. doi:10.1093/jb/mvr143, http://jb.oxfordjournals.org/content/151/3/217.abstract.
44. Frokjaer, S., and D.E. Otzen. 2005. Protein drug stability: a formulation challenge. *Nature Reviews Drug Discovery* 4(4):298–306.
45. Asea, A.A., and I.R. Brown. 2008. *Heat Shock Proteins and the Brain: Implications for Neurodegenerative Diseases and Neuroprotection*, vol. 3. London: Springer Science and Business Media.

46. Franklin, T., A. Krueger-Naug, D. Clarke, A.P. Arrigo, and R. Currie. 2005. The role of heat shock proteins Hsp70 and Hsp27 in cellular protection of the central nervous system. *International Journal of Hyperthermia* 21(5):379–392.
47. Turturici, G., G. Sconzo, and F. Geraci. 2011. Hsp70 and its molecular role in nervous system diseases. *Biochemistry Research International* 2011:1–18.
48. Ou, J.R., M.S. Tan, A.M. Xie, J.T. Yu, and L. Tan. 2014. Heat shock protein 90 in alzheimer's disease. *BioMed Research International* 2014:796869.
49. Lajtha, A., G. Gibson, and G. Dienel. 2007. *Handbook of Neurochemistry and Molecular Neurobiology: Neuroactive Proteins and Peptides*, Ed. Krieglstein, J., 123142. New York: Springer.
50. Amor, S., M. Bugiani, and J. van Noort. 2016. Heat shock proteins: old and novel roles in neurodegenerative diseases in the central nervous system. *CNS and Neurological Disorders Drug Targets*.
51. Alexander, G.E. 2004. Biology of parkinson's disease: pathogenesis and pathophysiology of a multisystem neurodegenerative disorder. *Dialogues in Clinical Neuroscience* 6:259–280.
52. Ryu, E.J., H.P. Harding, J.M. Angelastro, O.V. Vitolo, D. Ron, and L.A. Greene. 2002. Endoplasmic reticulum stress and the unfolded protein response in cellular models of parkinson's disease. *The Journal of Neuroscience* 22(24):10,690–10,698.
53. Varma, D., and D. Sen. 2015. Role of the unfolded protein response in the pathogenesis of parkinson's disease. *Acta Neurobiologiae Experimentalis* 75:1–26.
54. Ebrahimi-Fakhari, D., L.J. Saidi, and L. Wahlster. 2013. Molecular chaperones and protein folding as therapeutic targets in parkinson's disease and other synucleinopathies. *Acta Neuropathologica Communications* 1(1):1.
55. Redler, R.L., and N.V. Dokholyan. 2012. The complex molecular biology of amyotrophic lateral sclerosis (ALS). *Progress in Molecular Biology and Translational Science* 107:215.
56. Mayer, M., and B. Bukau. 2005. Hsp70 chaperones: cellular functions and molecular mechanism. *Cellular and Molecular Life Sciences* 62(6):670–684.
57. Kubota, H. 2009. Quality control against misfolded proteins in the cytosol: a network for cell survival. *Journal of Biochemistry* 146(5):609–616.
58. Carman, A., S. Kishinevsky, W.L. John Koren III, and G. Chiosis. 2013. Chaperone-dependent neurodegeneration: a molecular perspective on therapeutic intervention. *Journal of Alzheimer's Disease and Parkinsonism* 2013 (Suppl 10).
59. Melkani, G.C., A.S. Trujillo, R. Ramos, R. Bodmer, S.I. Bernstein, and K. Ocorr. 2013. Huntington's disease induced cardiac amyloidosis is reversed by modulating protein folding and oxidative stress pathways in the drosophila heart. *PLoS Genet* 9(12):e1004,024.
60. Plerou, A., and P. Vlamos. 2015. Evaluation of mathematical cognitive functions with the use of eeg brain imaging. In *Experimental Multimedia Systems for Interactivity and Strategic Innovation*, 284–306. Hershey, P.A.: IGI Global.
61. Plerou, A., C. Bobori, and P. Vlamos. 2015. Molecular basis of huntington's disease and brain imaging evidence. In *2015 IEEE International Symposium on Signal Processing and Information Technology (ISSPIT)*, 387–391. Piscataway, NJ: IEEE.
62. Schulte, J., and J.T. Littleton. 2011. The biological function of the huntingtin protein and its relevance to huntingtons disease pathology. *Current trends in neurology* 5:65.
63. Scherzinger, E., R. Lurz, M. Turmaine, L. Mangiarini, B. Hollenbach, R. Hasenbank, G.P. Bates, S.W. Davies, H. Lehrach, and E.E. Wanker. 1997. Huntingtin-encoded polyglutamine expansions form amyloid-like protein aggregates in vitro and in vivo. *Cell* 90(3):549–558.
64. Stefani, M., and C.M. Dobson. 2003. Protein aggregation and aggregate toxicity: new insights into protein folding, misfolding diseases and biological evolution. *Journal of Molecular Medicine* 81(11):678–699.
65. Lupski, J.R., R.M. de Oca-Luna, S. Slaugenhaupt, L. Pentao, V. Guzzetta, B.J. Trask, O. Saucedo-Cardenas, D.F. Barker, J.M. Killian, and C.A. Garcia, et al. 1991. DNA duplication associated with Charcot–Marie–Tooth disease type 1A. *Cell* 66(2):219–232.

66. Juárez, P., and F. Palau. 2012. Neural and molecular features on Charcot–Marie–Tooth disease plasticity and therapy. *Neural Plasticity* 2012:171636.
67. Lee, S.M., L.S. Chin, and L. Li. 2012. Protein misfolding and clearance in demyelinating peripheral neuropathies: therapeutic implications. *Communicative and Integrative Biology* 5(1):107–110.
68. Zhao, J.H., H.L. Liu, H.Y. Lin, C.H. Huang, H.W. Fang, S.S. Chen, Y. Ho, W.B. Tsai, and W.Y. Chen. 2007. Chemical chaperone and inhibitor discovery: potential treatments for protein conformational diseases. *Perspectives in Medicinal Chemistry* 1:39.
69. DebBurman, S.K., G.J. Raymond, B. Caughey, and S. Lindquist. 1997. Chaperone-supervised conversion of prion protein to its protease-resistant form. *Proceedings of the National Academy of Sciences* 94(25):13,938–13,943.
70. Newnam, G.P., R.D. Wegrzyn, S.L. Lindquist, and Y.O. Chernoff. 1999 Antagonistic interactions between yeast chaperones Hsp104 and Hsp70 in prion curing. *Molecular and Cellular Biology* 19(2):1325–1333.

Interactome of Obesity: Obesidome

Genetic Obesity, Stress Induced Obesity, Pathogenic Obesity Interaction

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Abstract Obesity is a chronic disease of increasing prevalence reaching epidemic proportions. Genetic defects as well as epigenetic effects contribute to the obesity phenotype. Investigating gene (e.g. *MC4R* defects)-environment (behavior, infectious agents, stress) interactions is a relative new field of great research interest. In this study, we have made an effort to create an interactome (henceforth referred to as "obesidome"), where extrinsic stressors response, intrinsic predisposition, immunity response to inflammation and autonomous nervous system implications are integrated. These pathways are presented in one interactome network for the first time. In our study, obesity-related genes/gene products were found to form a complex interactions network.

Keywords Interactome • Obesidome • Genetic obesity • Autonomic nervous system • Inflammation • Stress induced obesity • Pathogenic obesity

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1 Introduction

Obesity is an epidemic in the western societies linked to behavioral, genetic, metabolic, psychological disorders, predicting chronic diseases as cardiovascular diseases, polycystic ovary syndrome, cardiometabolic syndrome, hepatocellular carcinoma etc. It is a major problem for Public Health, as its constantly increasing prevalence influences the mortality rates as well. Visceral fat accumulation is age dependent. The dominant pathogenic mechanism is that of oxidative stress and research on antioxidant genes is on going along the scientific community [1]. On the other hand, a percentage of bariatric patients gain weight soon after been operated. The failure is suspected to have genetic cause, and is under investigation.

Network-based approaches are utilized in the biomedical research in order to investigate the complex associations between the biological molecules (e.g., genes, proteins, hormones etc) that are implicated in various disorders/diseases [2]. In this study, we constructed a network diagram displaying interactions among gene/gene products, either known or predicted, that are associated with obesity.

2 Methods

Obesity-related genes or gene products were extracted from the biomedical literature [3, 4]. The interactions among them were investigated through STRING v10 [5], a database of known and predicted, physical and indirect associations among genes/proteins. In this study, a high confidence interaction score of 0.7–0.97 was selected. The intermediate nodes were also predicted, showing no more than 20 interactors.

3 Results

Collectively, 54 gene/gene products (Table 1) were identified which are implicated in different mechanisms/pathways (discussed below) that appear to form a highly interconnected network (Fig. 1).

3.1 Genetically Induced Severe Obesity

Proopiomelanocortin (POMC) appears as a "hub" in the network (Fig. 1). POMC protein is cleaved into several peptides. Of those, the α-melanocyte-stimulating hormone (α-MSH) or γ-melanocortin, which is activated by leptin (LEP) (Fig. 1), was demonstrated to transduce the anorexigenic effect of LEP by binding to the melanocortin-4 receptor (MC4R) [6]. LEP itself binds selectively to the leptin receptor (LEPR) which is implicated in fat metabolism. LEPR mediates signal

Table 1 The symbols and names of the genes used to generate the network in Fig. 1

Gene symbol	Gene description
ACE	Angiotensin I converting enzyme (peptidyl-dipeptidase A) 1
ACE2	Angiotensin I converting enzyme (peptidyl-dipeptidase A) 2
ADRB2	Adrenoceptor beta 3
ADRB3	Adrenoceptor beta 3
AGRP	Agouti related protein homolog
AGT	Angiotensinogen
AVP	Arginine vasopressin
BCHE	Butyrylcholinesterase
CARTPT	Cocaine and amphetamine regulated transcript
CCK	Cholecystokinin
CHRNA7	Cholinergic receptor, nicotinic, alpha 7
COMT	Catechol-O-methyltransferase
CREB1	cAMP responsive element binding protein 1
CRH	Corticotropin releasing hormone
FOS	FBJ murine osteosarcoma viral oncogene homolog
FTO	Fat mass and obesity associated
GCG	Glucagon
GHRL	Ghrelin
GHSR	Growth hormone secretagogue receptor
GIPR	Gastric inhibitory polypeptide receptor
HNMT	Histamine N-methyltransferase
HTR2A	5-hydroxytryptamine (serotonin) receptor 2A
ICAM1	Intercellular adhesion molecule 1
IFNG	Interferon, gamma
IGF1	Insulin-like growth factor 1 (somatomedin C)
IL6	Interleukin 6 (interferon, beta 2)
IL6R	Interleukin 6 receptor
INS	Insulin
INSIG2	Insulin induced gene 2
JAK2	Janus kinase 2
LEP	Leptin
LEPR	Leptin receptor
MAOA	Monoamine oxidase A
MC1R	Melanocortin 1 receptor
MC2R	Melanocortin 2 receptor
MC3R	Melanocortin 3 receptor
MC4R	Melanocortin 4 receptor
MC5R	Melanocortin 5 receptor
NMU	Neuromedin U
NPS	Neuropeptide S

(continued)

Table 1 (contiinued)

Gene symbol	Gene description
NPY	Neuropeptide Y
NR4A1	Nuclear receptor subfamily 4, group A, member 1
PCK1	Phosphoenolpyruvate carboxykinase 1
PCSK1	Proprotein convertase subtilisin/kexin type 1
PDYN	Prodynorphin
PLIN	Perilipin 1
POMC	Proopiomelanocortin
PPARG	Peroxisome proliferator-activated receptor gamma
REN	Renin
STAT3	Signal transducer and activator of transcription 3
TNF	Tumor necrosis factor
UCP1	Uncoupling protein 1
UCP2	Uncoupling protein 2
UCP3	Uncoupling protein 3

The genes used as initial input to STRING are shown in bold

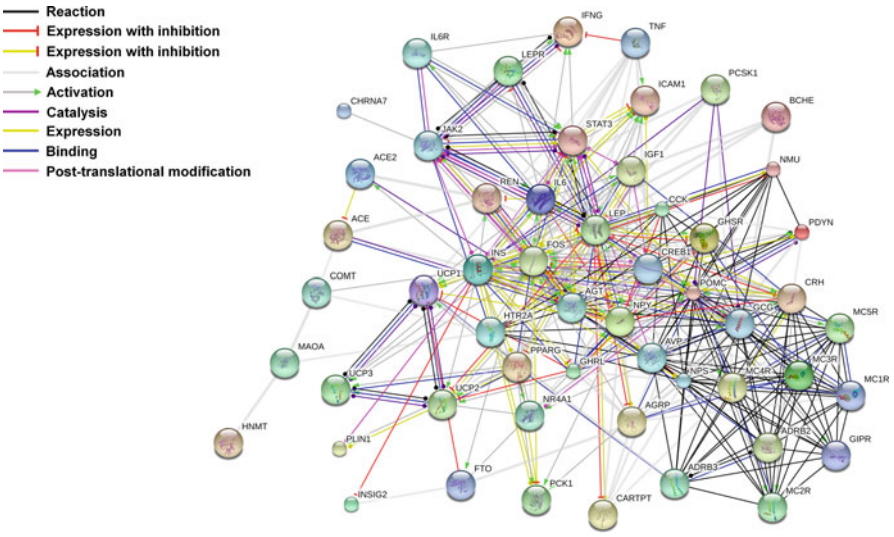

Fig. 1 Network showing the interactions among the gene/gene products listed in Table 1. The *connecting lines* indicate the predicted mode of action

transduction through JAK2/STAT3 (Fig. 1). Furthermore, MC4R is functionally linked to several other melanocortin receptors (MC1R, MC2R, MC3R and MC5R), as well as obesity-associated factors (Fig. 1).

Single nucleotide polymorphisms (SNP) in the genes *PPARG (peroxisome proliferator-activated receptor gamma)*, *ADRB2-3 (adrenoceptor beta 2-3)*, *UCP1-3 (uncoupling protein 1-3)* and *PLIN (perilipin 1)* are suggested to contribute to

susceptibility to obesity [7]. These genes/gene products appear to be connected in the network (Fig. 1).

Interactions between dietary fatty acids and the genes *LEP*, *TNF (tumor necrosis factor)* and *PPARG (peroxisome proliferator-activated receptor gamma)*, the latter of which regulates fatty acid metabolism, are suggested by Nieters and colleagues [8]. PPARG is suggested to suppress *LEP* gene expression (Fig. 1).

Insulin (INS), which regulates glucose levels in blood, appears also as a central hub in the network (Fig. 1). Herbert and coworkers reported a strong correlation between a genetic variant near *INSIG2 (insulin induced gene 2)*, which is inhibited by INS (Fig. 1), and childhood and adulthood obesity risk [9]. INS also regulates PLIN1, PPARG and activates UCP1/2/3. Of note, PCK1 (*phosphoenolpyruvate carboxykinase 1*), another important obesity-relevant factor is suggested to be activated by PPARG, CREB and GCG and inhibited by FOS and INS.

Functional polymorphisms in *PCSK1* (*proprotein convertase subtilisin/kexin type 1*) are associated with obesity risk [10]. PCSK1 hydrolyzes proglucagon (GCG) to glucagon-like peptide-1 (GLP-1), processes proINS to INS and catalyzes the cleavage of POMC into corticotropin (Fig. 1).

Moreover, a functional polymorphism in the *FTO (fat mass and obesity associated)* gene is significantly associated with obesity risk in a Danish population [11]. FTO deficiency is suggested to induce *UCP1* expression (Fig. 1).

3.2 Stress Induced Obesity

Several genes related to stress-induced obesity are connected to each other and to genes/gene products of other pathways. The GHSR (growth hormone secretagogue receptor) is a receptor for ghrelin (GHRL), expressed at high levels in the hypothalamus and the pituitary gland, both of which are involved in the physiological response to stress [3]. GHSR regulates body weight and energy homeostasis [12]. GHRL stimulates appetite, induces adiposity and affects gastric acid secretion [13]. GHRL is suggested to decrease *UCP1* and *UCP2* expression and stimulate POMC expression. Also, GHRL is suggested to interact with GLP-1. Moreover, INS is predicted to affect *GHRL* expression (Fig. 1). The gastrointestinal hormone CCK (cholecystokinin), which is synthesized and released in the duodenum, the first segment of the small intestine [14], is shown to inhibit NPY (neuropeptide Y) (Fig. 1), a neuropeptide known to be implicated in the control of feeding behavior [15]; *CCK* gene expression appears to be induced by LEP (Fig. 1). The satiety factor CART (cocaine and amphetamine regulated transcript) is suggested to be regulated by LEP (Fig. 1).

3.3 Inflammation-Autonomics Nervous System Implications

Inflammation-associated factors were found to contribute to obesity. For instance, ACE2 (angiotensin I converting enzyme 2) [16] inhibits ACE (angiotensin I convert-

ing enzyme 1) expression. ACE and ACE2 are connected to AGT (angiotensinogen); ACE and ACE2 hydrolyze Ang I (angiotensin I) to Ang II (angiotensin II). Moreover, the antidiuretic hormone AVP (arginine vasopressin) is functionally linked to ACE and ACE2 through AGT (Fig. 1). TNF is suggested to decrease the expression of the fellow inflammation-related gene *IFNG* and may indirectly interact to JAK2. The inflammatory factors IL6 (interleukin 6) and its receptor IL6R (interleukin 6 receptor) exert their effect through JAK2 which interacts with leptin and insulin. Both latter hormones play key role on obesity onset and progress.

Factors of the autonomic nervous system are also suggested to be implicated in obesity. For instance, the hormone and neurotransmitter CRH (corticotropin releasing hormone), which is secreted by the hypothalamus in response to stress, appears to be associated functionally with several components of the network that are involved in different pathways such as AVP, POMC, MC1/2/3/4/5R, ADRB2-3, IL6. Of note, the cholinesterase BCHE (butyrylcholinesterase) is suggested to be linked to several components of the interaction network (Fig. 1). Furthermore, CHRNA7 (cholinergic receptor, nicotinic, alpha 7) is shown to activate JAK2 which interacts with LEP, IL6R and the neurotransmitter HTR2A (serotonin receptor 2A) (Fig. 1). In addition, HNMT (histamine N-methyltransferase) is functionally linked to HTR2A through MAOA (monoamine oxidase A) and COMT (catechol-O-methyltransferase). HTR2A also interacts with CCK and GHSR (Fig. 1).

4 Discussion

4.1 Genetically Induced Severe Obesity

The genetic contribution to obesity has been shown in family targeted population studies [17, 18]. Although 127 different candidate genes have been associated with obesity-related phenotypes [4], only few of them are chosen from areas containing pure linkage signals. In this study, we included only genes presenting pure linkage signals.

The MC4-mediated signaling pathway plays a pivotal role in regulating independently the energy equilibrium (homeostasis) and appetite, thus, body weight gain. Genetic defects along this pathway result in early onset of severe (also called pathogenic) obesity (often defined by BMI >40 kg/m^2) [19].

Proopiomelanocortin (POMC) deficiency obesity is a very rare genetic disorder with severe, early-onset obesity and profound hyperphagia as characteristic clinical symptoms. It may be originated from hormonal deficiencies (i.e. hypoadrenalism) or PCSK1 enzyme need for POMC protein product. Both of them result to the lack of α-MSH that activates MC4R [19].

Lack of signaling in the LEPR leads to MC4 pathway non functionality. Our network illustrates in detail all these interactions. Additionally, our network shows a prognosis that PPARG, ADRB2-3, UCP1-3 and PLIN contribute to susceptibility to obesity [7].

4.2 Stress Induced Obesity

The hypothalamic CRH–AVP and brainstem norepinephrine centers of the stress system innervate and stimulate each other. Chronic stress may lead to chronic diseases with obesity one of them [20]. The pathogenesis of chronic-stress related obesity includes effects on HPA released hormones, visceral fat accumulation as a result of chronic hypercortisolism, reactive insulin hyper secretion, low growth hormone secretion and hypogonadism [21–25]. The interactions retrieved in our network confirm the current literature. In addition, we predicted that GHRL decreases *UCP1* and *UCP2* expression (Fig. 1). Furthermore, cholecystokinin (CCK) seems to inhibit NPY (neuropeptide Y) (Fig. 1), a neuropeptide known to be implicated in the control of feeding behavior [15]; *CCK* gene expression appears to be induced by LEP (Fig. 1). Moreover, the network predicted that the satiety factor CART (cocaine and amphetamine regulated transcript) is regulated by LEP (Fig. 1). Though CART is questioned as drug target (due to its triggering extracellular signal-regulated kinases release inside the cell), the predicted interactions are of high confidence level, therefore could be investigated for drug targeting.

4.3 Inflammation-Autonomic Nervous System Implications

Hypothalamic dysfunction resulting to autonomic nervous system dysfunction is significantly associated to obesity [26]. The implication of inflammation factors to autonomic nervous system response and the pathogenesis of obesity is quite new, and been reported in animal models. It has been recently shown that in the gut, the cross talk of the stimulated vagus nerve with immune cells increases the cholinergic tone [27]. An intricate communication network exists between the nervous and immune systems; this interplay could advocate in the regulation of the immune response. TGF-β (and thymic stromal lymphopoietin) produced by the enterocytes and/or immune cells, contribute to the maintenance of immune homeostasis [28]. The interactions between the inflammatory and/or autonomic nervous system biomarkers and their encoding genes becomes more clear in this network. JAK2 serves as a key hub for leptin and insulin activity, thus, providing the foundation to further investigation.

Furthermore, BCHE is suggested to be linked to GCG, LEP, INS, IGF1 of the interaction network.

5 Conclusion

Interactions networks may lead to better insight of the protein/genes interactions and pathogenesis of obesity. Gut hormones (with leptin a hallmark node) implicate with the autonomic nervous system, stress system, homeostasis system, genetic factors.

Other nodes appear also as "hubs" in the network, connected to several nodes that represent molecules of the obesidome, JAK2, including CRH (in the hypothalamus), POMC (in the pituitary gland) and INS (in the liver).

References

1. Ruperez, A.I., A. Gil, and C.M. Aguilera. 2014. Genetics of Oxidative Stress in Obesity. *International Journal of Molecular Sciences* 15: 3118–3144.
2. Kontou, P.I., A. Pavlopoulou, N.L. Dimou, G.A. Pavlopoulos, and P.G. Bagos. 2016. Network Analysis of Genes and Their Association with Diseases. *Gene* 590: 68–78.
3. Patterson, Z.R., and A. Abizaid. 2013. Stress Induced Obesity: Lessons from Rodent Models of Stress. *Frontiers in Neuroscience* 7: 130.
4. Qi, L., and Y.A. Cho. 2008. Gene-Environment Interaction and Obesity. *Nutrition Reviews* 66: 684–694.
5. Szklarczyk, D., A. Franceschini, S. Wyder, K. Forslund, D. Heller, J. Huerta-Cepas, M. Simonovic, A. Roth, A. Santos, K.P. Tsafou, M. Kuhn, P. Bork, L.J. Jensen, and C. Von Mering. 2015. String V10: Protein-Protein Interaction Networks, Integrated Over the Tree of Life. *Nucleic Acids Research* 43: D447–D452.
6. Farooqi, I.S., and S. O'Rahilly. 2008. Mutations in Ligands and Receptors of the Leptin-Melanocortin Pathway that Lead to Obesity. *Nature Clinical Practice. Endocrinology & Metabolism* 4: 569–577.
7. Lohmueller, K.E., C.L. Pearce, M. Pike, E.S. Lander, and J.N. Hirschhorn. 2003. Meta-Analysis of Genetic Association Studies Supports a Contribution of Common Variants to Susceptibility to Common Disease. *Nature Genetics* 33: 177–182.
8. Nieters, A., N. Becker, and J. Linseisen. 2002. Polymorphisms in Candidate Obesity Genes and Their Interaction with Dietary Intake of N-6 Polyunsaturated Fatty Acids Affect Obesity Risk in a Sub-Sample of the Epic-Heidelberg Cohort. *European Journal of Nutrition* 41: 210–221.
9. Herbert, A., N.P. Gerry, M.B. Mcqueen, I.M. Heid, A. Pfeufer, T. Illig, H.E. Wichmann, T. Meitinger, D. Hunter, F.B. Hu, G. Colditz, A. Hinney, J. Hebebrand, K. Koberwitz, X. Zhu, R. Cooper, K. Ardlie, H. Lyon, J.N. Hirschhorn, N.M. Laird, M.E. Lenburg, C. Lange, and M.F. Christman. 2006. A Common Genetic Variant is Associated with Adult and Childhood Obesity. *Science* 312: 279–283.
10. Benzinou, M., J.W. Creemers, H. Choquet, S. Lobbens, C. Dina, E. Durand, A. Guerardel, P. Boutin, B. Jouret, B. Heude, B. Balkau, J. Tichet, M. Marre, N. Potoczna, F. Horber, C. Le Stunff, S. Czernichow, A. Sandbaek, T. Lauritzen, K. Borch-Johnsen, G. Andersen, W. Kiess, A. Korner, P. Kovacs, P. Jacobson, L.M. Carlsson, A.J. Walley, T. Jorgensen, T. Hansen, O. Pedersen, D. Meyre, and P. Froguel. 2008. Common Nonsynonymous Variants in PCSK1 Confer Risk of Obesity. *Nature Genetics* 40: 943–945.
11. Andreasen, C.H., K.L. Stender-Petersen, M.S. Mogensen, S.S. Torekov, L. Wegner, G. Andersen, A.L. Nielsen, A. Albrechtsen, K. Borch-Johnsen, S.S. Rasmussen, J.O. Clausen, A. Sandbaek, T. Lauritzen, L. Hansen, T. Jorgensen, O. Pedersen, and T. Hansen. 2008. Low Physical Activity Accentuates the Effect of the FTO rs9939609 Polymorphism on Body Fat Accumulation. *Diabetes* 57: 95–101.
12. Pazos, Y., F.F. Casanueva, and J.P. Camina. 2008. Basic Aspects of Ghrelin Action. *Vitamins and Hormones* 77: 89–119.
13. Peeters, T.L. 2005. Ghrelin: A New Player in the Control of Gastrointestinal Functions. *Gut* 54: 1638–1649.
14. Chaudhri, O., C. Small, and S. Bloom. 2006. Gastrointestinal Hormones Regulating Appetite. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 361: 1187–1209.

15. Zimanyi, I.A., Z. Fathi, and G.S. Poindexter. 1998. Central Control of Feeding Behavior by Neuropeptide Y. *Current Pharmaceutical Design* 4: 349–366.
16. Dandona, P., S. Dhindsa, H. Ghanim, and A. Chaudhuri. 2007. Angiotensin II and Inflammation: The Effect of Angiotensin-Converting Enzyme Inhibition and Angiotensin II Receptor Blockade. *Journal of Human Hypertension* 21: 20–27.
17. Maes, H.H., M.C. Neale, and L.J. Eaves. 1997. Genetic and Environmental Factors in Relative Body Weight and Human Adiposity. *Behavior Genetics* 27: 325–351.
18. Stunkard, A.J., J.R. Harris, N.L. Pedersen, and G.E. Mcclearn. 1990. The Body-Mass Index of Twins Who have been Reared Apart. *The New England Journal of Medicine* 322: 1483–1487.
19. Kuhnen, P., K. Clement, S. Wiegand, O. Blankenstein, K. Gottesdiener, L.L. Martini, K. Mai, U. Blume-Peytavi, A. Gruters, and H. Krude. 2016. Proopiomelanocortin Deficiency Treated with a Melanocortin-4 Receptor Agonist. *The New England Journal of Medicine* 375: 240–246.
20. Chrousos, G.P. 2009. Stress and Disorders of the Stress System. *Nature Reviews Endocrinology* 5: 374–381.
21. ———. 2000. The Role of Stress and the Hypothalamic-Pituitary-Adrenal Axis in the Pathogenesis of the Metabolic Syndrome: Neuro-Endocrine and Target Tissue-Related Causes. *International Journal of Obesity and Related Metabolic Disorders* 24 (Suppl. 2): S50–S55.
22. De Bellis, M.D., G.P. Chrousos, L.D. Dorn, L. Burke, K. Helmers, M.A. Kling, P.K. Trickett, and F.W. Putnam. 1994. Hypothalamic-Pituitary-Adrenal Axis Dysregulation in Sexually Abused Girls. *The Journal of Clinical Endocrinology and Metabolism* 78: 249–255.
23. Grundy, D., E.D. Al-Chaer, Q. Aziz, S.M. Collins, M. Ke, Y. Tache, and J.D. Wood. 2006. Fundamentals of Neurogastroenterology: Basic Science. *Gastroenterology* 130: 1391–1411.
24. Pervanidou, P., G. Kolaitis, S. Charitaki, A. Margeli, S. Ferentinos, C. Bakoula, C. Lazaropoulou, I. Papassotiriou, J. Tsiantis, and G.P. Chrousos. 2007. Elevated Morning Serum Interleukin (Il)-6 or Evening Salivary Cortisol Concentrations Predict Posttraumatic Stress Disorder in Children and Adolescents Six Months after a Motor Vehicle Accident. *Psychoneuroendocrinology* 32: 991–999.
25. Vgontzas, A.N., M. Zoumakis, E.O. Bixler, H.M. Lin, P. Prolo, A. Vela-Bueno, A. Kales, and G.P. Chrousos. 2003. Impaired Nighttime Sleep in Healthy Old Versus Young Adults is Associated with Elevated Plasma Interleukin-6 and Cortisol Levels: Physiologic and Therapeutic Implications. *The Journal of Clinical Endocrinology and Metabolism* 88: 2087–2095.
26. Snitker, S., I. Macdonald, E. Ravussin, and A. Astrup. 2000. The Sympathetic Nervous System and Obesity: Role in Aetiology and Treatment. *Obesity Reviews* 1: 5–15.
27. Matteoli, G., and G.E. Boeckxstaens. 2013. The Vagal Innervation of the Gut and Immune Homeostasis. *Gut* 62: 1214–1222.
28. Pabst, O., and A.M. Mowat. 2012. Oral Tolerance to Food Protein. *Mucosal Immunology* 5: 232–239.

Fatigue in Arthritis: A Multidimensional Phenomenon with Impact on Quality of Life

Fatigue and Quality of Life in Arthritis

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Abstract An important factor which influences the quality of life of patients with arthritis is the fatigue they experience. The purpose of this study was to assess the relationship between fatigue and quality of life among patients with osteoarthritis and rheumatoid arthritis. Between January 2015 and March 2015, 179 patients with osteoarthritis and rheumatoid arthritis completed the Fatigue Assessment Scale and the Missoula-VITAS Quality of Life Index-15 (MVQoLI-15). The study was conducted in Rehabilitation Centers located in the area of Peloponnese, Greece. Data related to sociodemographic characteristics and their individual medical histories were recorded. Statistical analysis was performed using the IBM SPSS Statistics version 19. The analysis did not reveal statistically significant correlation between fatigue and quality of life neither in the total sample nor among patients

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with osteoarthritis ($r = -0.159$; $p = 0.126$) or rheumatoid arthritis. However, there was a statistically significant relationship between some aspects of fatigue and dimensions of quality of life. Osteoarthritis patients had statistically significant lower MVQoLI-15 score than rheumatoid arthritis patients (13.73 ± 1.811 vs 14.61 ± 1.734) and lower FAS score than rheumatoid patients (26.14 ± 3.668 vs 29.94 ± 3.377) (p-value < 0.001). The finding that different aspects of fatigue may affect dimensions of quality of life may help health care professionals by proposing the early treatment of fatigue in order to gain benefits for quality of life.

Keywords Fatigue • Multidimensional • Osteoarthritis • Quality of life • Rheumatoid arthritis

1 Introduction

Fatigue is a complex and multifactorial phenomenon including physiological and psychological parameters [1]. Fatigue is often characterized as a multidimensional continuous condition which accompanies chronic illnesses and has a significant impact on the patients' overall quality of life [2]. Fatigue has been recognized as a serious symptom that can significantly reduce the functionality of an individual suffering from chronic diseases such as cancer [3], multiple sclerosis [4], end stage renal disease [2], systemic lupus erythematosus [5], rheumatoid arthritis (RA) [6], and osteoarthritis (OA) [7, 8]. The cause of fatigue in chronic diseases is difficult to be proved, even if it is the main or a characteristic symptom of a disease. Also, sleep disorders, stress, pain, anxiety about the outcome of the disease, depression and patient's personality contribute to the subjective experience of fatigue in chronic diseases [9, 10].

Clinically significant fatigue has been reported by over 40% of patients with RA or OA. Although fatigue is a common feature in both diseases, these disorders are characterized by different etiological mechanisms, which may result in different types of fatigue between these two diseases. Rheumatoid arthritis is an inflammatory disease that causes pain, edema (swelling), stiffness and loss of function of the joints [11]. Fatigue among RA patients has been associated with pain, poor sleep, depression and functional disability, while in OA, pain in single joints is accompanied by symptoms of fatigue [12].

Fatigue, as the main and most frequent of the symptoms in arthritis, is strongly associated with patient's quality of life [13]. According to Rupp et al. [14] RA patients view fatigue as the most important factor which affect their quality of life. In addition, Swedish RA patients regard that decreasing of fatigue should be the basic goal of the treatment [15]. Fatigue, related to RA and OA, effect negatively the daily activities and the quality of sleep of these patients [16, 17] while Doeglas et al. [18] found that fatigue affects not only the physical dimensions of the quality of life but, also, influences the mental well-being, social aspects and interpersonal relationships.

2 Materials and Methods

The aim of the study was to assess the levels of fatigue among patients with osteoarthritis and rheumatoid arthritis and its effect on the quality of life. This study adopts an analytic correlational study design. Patients undergoing physiotherapy in Rehabilitation Centers located in the area of Peloponnese, Greece, participated in this study. The inclusion criteria were: (a) age > 18 years, (b) diagnosis of osteoarthritis or rheumatoid arthritis, (c) ability to write and read fluently the Greek language. The exclusion criteria were: history of mental illness and serious eye problems. Ultimately, 179 patients met the criteria. The study was carried out during the period January 2015–March 2015.

2.1 Data Collection

Firstly, patients were given the Greek version of the Missoula-VITAS Quality of Life Index-15 (MVQoLI-15) [19]. MVQoLI-15 is a measurement tool for collecting information on patients' QoL during an advanced disease. The questionnaire consists of five aspects: symptoms, functionality, interpersonal relationships, wellness and spirituality. Three types of information are collected in each area: (a) Assessment (b) Satisfaction (c) Importance. Responses use a five-point Likert scale, so that the lowest score indicating the least desirable condition and vice versa. The score of the MVQoLI-15 is as follows: Assessment: -2 to $+2$, Satisfaction: -4 to $+4$, Importance: 1 to 5. (Assessment + Satisfaction) $\times$ Importance = QoL in each dimension. The Assessment and Satisfaction scores can range from -6 to $+6$ and indicate whether the patient assesses his/her situation negatively or positively. When multiplied by the Importance factor, the overall dimension score is magnified by how important that domain is. The final score in each dimension reflects the overall impact of that domain on quality of life (QoL). The internal validity of the Greek version of the questionnaire was satisfactory with Cronbach's alpha equal to 0.74 [19].

In addition, patients were given the Greek version of the "Fatigue Assessment Scale (FAS)" [20]. Specifically, FAS is a tool which collects information related to the perceived fatigue. The original form of FAS was constructed by Michielsen et al. [21] and has, also, been used in Dutch patients with sarcoidosis [22]. FAS consist of 10 questions. Responses use a five-point Likert scale as follows: 1 = never, 2 = sometimes, 3 = regularly, 4 = often, 5 = always. Therefore, the score ranges between 10 and 50. Patients are categorized as "non-fatigued" if the FAS score is <22, "fatigued" if the FAS score is $\geq$ to 22 and "extremely fatigued" if the FAS score is $\geq$ to 35. Psychometric properties of the Greek version have been tested and the internal consistency of the scale was found to be equal to 0.761 [20].

Finally, data related to sociodemographic characteristics, disease duration, medication and sleep activities were recorded.

2.2 Data Analysis

Quantitative variables are described as mean (± standard deviation) and qualitative variables as absolute frequencies and relative frequencies. Where appropriate, parametric (t test and ANOVA) and non-parametric (Mann-Whitney and Kruskal-Wallis) tests were applied. The association between quantitative variables was assessed using Spearman's r. The significance level was set up at 5%.

2.3 Ethical Considerations

The study was approved by the Administration of the Private Rehabilitation Center (27/6/2014) and the Hellenic Data Protection Authority (Approval Number 1356, 24/9/2014). Patients, who met the criteria, after being informed about the aim of the study, gave their written consent and completed the above mentioned questionnaires. Participants' anonymity was protected and safety of the material was maintained.

3 Results

3.1 Participants' Characteristics

In the current study, 179 Greek patients undergoing physiotherapy participated. Of these, 86.6% were female. The mean age was 61.35 (±9.682) years old (Table 1).

3.2 Content Validity of the FAS

Applying factor analysis with the Varimax method of axes rotation, five statistically significant factors were emerged explaining 67.9% of the total dispersion. Factor 1 "General tiredness" explains 17.91% of the total variation, factor 2 "Lack of interest" interprets 13.76%, factor 3 "Excessive fatigue—feelings of worthlessness" explains the 12.51% of total variability, factor 4 "Difficulty in starting activities" interprets the 11.95% of the total variability and factor 5 "Thought disorders—weakness objective and critical thinking" the 11.76% of the total variability in the data. The responses of patients, the 5 factors, the variables and the percentage of variance are presented in Table 2.

Six fatigue indices were used: total FAS score (calculated as the sum of the 10 items after reversing questions 4 and 10), and the five factors. The mean FAS score was 27.93 (±4.601). A percentage of 5.6% (n = 10) from the participants were "non-fatigued", 91.6% (n = 163) were "fatigued" while 2.8% (n = 5) were "extremely fatigued".

Table 1 Participants' characteristics

Demographic data		Frequency	Percentage (%)
Gender	Female	155	86.6
	Male	24	13.4
Residence	Urban area	140	78.2
	Suburban	39	21.8
Educational level	Primary school	43	24.0
	High school	114	63.7
	University	22	12.3
Marital status	Unmarried	1	0.6
	Married	159	88.8
	Divorced	7	3.9
	Widowed	12	6.7
Occupation	Private employee	31	17.3
	State employee	11	6.1
	Freelancing	16	8.9
	Housework	9	5.0
	Farmer	8	4.5
	Retired	104	58.1
Type of arthritis	Osteoarthritis	95	53.1
	Rheumatoid arthritis	84	46.9
Disease duration	<1 year	16	8.9
	1–5 years	36	20.1
	>5 years	127	70.9
Do you take medications?	No	56	31.3
	Yes	123	68.7
How many hours do you sleep at night?	<4	27	15.1
	4–6	32	17.9
	6–8	120	67.0
Do you take medication before sleep?	No	90	50.3
	Rarely	10	5.6
	Sometimes	79	44.1

	Mean	SD
Age (years)	61.35	9.682
Number of children	1.68	0.927

SD Standard Deviation

3.3 MVQoLI-15

The dimension "Functionality" seems to have the highest mean (5.67 ± 9.300) while "Interpersonal Relationships" and "Symptoms" follows with mean 5.64 ± 5.390 and 1.90 ± 8.856 respectively. "Wellness" and "Spirituality" took negative mean (−14, 17 ± 10.402 and −7.60 ± 10.434 respectively). Also,

Table 2 The fatigue assessment scale and the loadings of the variables on the factors

	Never	Sometimes	Regularly	Often	Always	Loading	Eigen value	Range (%)
I am bothered by fatigue	0 (0.0)	34 (19.0)	64 (35.8)	44 (24.6)	37 (20.7)	0.827	Factor 1 1.791	17.91
I get tired very quickly	1 (0.6)	38 (21.2)	57 (31.8)	70 (39.1)	13 (7.3)	0.848		
I have problems thinking clearly	43 (24.0)	93 (52.0)	25 (14.0)	13 (7.3)	5 (2.8)	0.713	Factor 2 1.376	13.76
I feel no desire to do anything	13 (7.3)	103 (57.5)	42 (23.5)	18 (10.1)	3 (1.7)	0.754		
I don't do much during the day	2 (1.1)	46 (25.7)	80 (44.7)	45 (25.1)	6 (3.4)	−0.521	Factor 3 1.251	12.51
Physically, I feel exhausted	3 (1.7)	84 (47.2)	44 (24.7)	35 (19.7)	12 (6.7)	0.513		
Mentally, I feel exhausted	44 (24.6)	112 (62.6)	10 (5.6)	10 (5.6)	3 (1.7)	0.801		
I have problems getting started	28 (15.6)	55 (30.7)	45 (25.1)	29 (16.2)	22 (12.3)	0.883	Factor 4 1.195	11.95
I have enough energy for everyday life	30 (16.8)	96 (53.6)	32 (17.9)	17 (9.5)	4 (2.2)	0.847	Factor 5 1.176	11.76
When I am doing something, I can concentrate quite well	3 (1.7)	22 (12.3)	47 (26.3)	63 (35.2)	44 (24.6)	0.646		

Percentage (%)

regarding the question "How would you rate the overall quality of your life?", 52.5% of the patients answered "moderate", 30.2% answered "poor", 12.3% "good", 3.9% "very poor" while 1.1% "very good". The mean score of MVQoLI-15 was 14.14 (±1.824).

3.4 Correlations

The analysis did not reveal statistically significant correlation between fatigue and quality of life neither in the total sample nor among patients with OA ($r = -0.159$; $p = 0.126$) or RA ($r = 0.114$; $p = 0.301$). However, there was a statistically significant relationship between "General tiredness" and "Spirituality" ($r = 0.198$; $p = 0.008$), between "Excessive fatigue—feelings of worthlessness" and "Functionality" ($r = 0.178$; $p = 0.017$) and between "Difficulty in starting activities" and "Spirituality" ($r = 0.225$; $p = 0.002$). In all cases, the correlation is low.

The analysis revealed a statistically significant correlation between the effect of the disease on the FAS score ($t(176) = -7.166$; $p < 0.001$), with OA patients having lower FAS score than RA patients (26.14 ± 3.668 vs 29.94 ± 3.377) (p-value <0.001).

Table 3 shows the effect of the disease on the five dimensions of MVQoLI-15 score. The disease seems to significantly affect the dimension "Spirituality" ($t(177) = -4.532$; $p < 0.001$). OA patients had significantly lower "Spirituality" score than RA patients (−10.76 ± 9.756 vs −4.04 ± 10.069) as well as lower total MVQoLI-15 score ($t(177) = -3.315$; $p = 0.001$). OA patients had statistically significant lower MVQoLI-15 score than RA patients (13.73 ± 1.811 vs 14.61 ± 1.734).

3.5 Association of FAS with Demographic Characteristics

FAS was affected significantly by place of birth (Mann-Whitney $U = 1847.0$; $p = 0.004$) and marital status (Kruskal-Wallis $X^2(2) = 6.931$; $p = 0.031$). Higher FAS values were women born in an urban area and married. The FAS correlated significantly with age (Spearman's $r = -0.245$; $p = 0.001$).

"General tiredness" was affected significantly only by place of birth (Mann-Whitney $U = 1968.0$; $p = 0.014$) while "Lack of interest" was not affected significantly by any demographic feature. "Excessive fatigue—feelings of worthlessness" was influenced significantly by place of birth (Mann-Whitney $U = 1983.0$; $p = 0.016$) and marital status (Kruskal-Wallis $X^2(2) = 7.714$; $p = 0.021$). The same applies for the "Difficulty in starting activities". Finally, "Thought disorders—weakness objective and critical thinking" was significantly affected only by education (Kruskal-Wallis $X^2(2) = 7.791$; $p = 0.020$). Patients living

Table 3 Association of MVQoLI-15 score with the disease

		N	Mean	SD	p-value
Symptoms	OA	95	1.99	8.017	0.887
	RA	84	1.80	9.767	
Functionality	OA	95	4.72	10.803	0.147
	RA	84	6.74	7.149	
Interpersonal relationships	OA	95	6.00	5.794	0.339
	RA	84	5.23	4.895	
Wellness	OA	95	−14.65	11.017	0.509
	RA	84	−13.62	9.695	
Spirituality	OA	95	−10.76	9.756	<0.001
	RA	84	−4.04	10.069	
MVQoLI-15 Score	OA	95	13.73	1.811	0.001
	RA	84	14.61	1.734	

SD Standard Deviation
OA Osteoarthritis
RA Rheumatoid Arthritis

at urban regions preferred "General tiredness", "Excessive fatigue—feelings of worthlessness" and "Difficulty in starting activities". Married patients preferred "Excessive fatigue—feelings of worthlessness" while divorced patients preferred "difficulty in starting activities". Secondary educated people preferred "Thought disorders—weakness objective and critical thinking".

3.6 Association of FAS with the Characteristics of Disease and Medication

FAS was affected significantly by the disease duration (Kruskal-Wallis $X^2(2) = 8.042$; $p = 0.018$), by taking medication ($t(176) = -5.238$; $p < 0.001$) and by taking medication before bedtime (Kruskal-Wallis $X^2(2) = 6.724$; $p = 0.035$). Higher FAS values had patients who suffered many years (>4–6 years), patients who take medication and those taking medication before bedtime.

"General tiredness" of FAS was significantly affected by the disease duration (Kruskal-Wallis $X^2(2) = 8.333$; $p = 0.016$), by taking medication ($t(176) = -3.326$; $p = 0.001$) and sleep hours (Kruskal-Wallis $X^2(2) = 8.735$; $p = 0.013$). Higher values of "General tiredness" had patients who suffered many years, patients who take medication and patients who sleep less. "Lack of interest" and "Excessive fatigue—feelings of worthlessness" were not affected significantly by any feature of the disease and drugs. "Difficulty in starting activities" was influenced significantly by taking medication ($t(176) = -4.249$; $p < 0.001$), sleeping hours (Kruskal-Wallis $X^2(2) = 8.603$; $p = 0.014$) and taking medication before sleep (Kruskal-Wallis $X^2(2) = 19.099$; $p < 0.001$). Higher values of "Difficulty in starting activities" had

patients who take medication, patients who sleep 4–6 h, and patients who rarely take medication before bedtime. Finally, "Thought disorders—weakness objective and critical thinking" was only influenced by the disease duration (Kruskal-Wallis $X^2(2) = 6.381$; $p = 0.041$), with patients who learned about their disease before 1–5 years having higher values.

3.7 Association of QoL with the Demographic Characteristics

MVQoLI-15 score was significantly affected by gender (Mann-Whitney $U = 1279.0$; $p = 0.014$) and place of birth (Mann-Whitney $U = 2093.5$; $p = 0.026$). Women and people born in an urban area had higher MVQoLI-15 values. MVQoLI-15 was uncorrelated both with age (Spearman's $r = -0.072$; $p = 0.336$) and the number of children (Spearman's $r = 0.009$; $p = 0.903$).

3.8 Association of QoL with the Characteristics of Disease and Medication

MVQoLI-15 Score was significantly affected by the sleeping hours (Kruskal-Wallis $X^2(2) = 7.487$; $p = 0.024$) and taking medication before bedtime (Kruskal-Wallis $X^2(2) = 7.655$; $p = 0.022$). Patients who slept 4–6 h and those taking medication before bedtime had higher MVQoLI-15 score.

4 Discussion

This study was conducted in Peloponnese region, Greece, aiming to explore the association between fatigue and quality of life among 179 patients with osteoarthritis and rheumatoid arthritis. Michielsen et al. [23] and Smith et al. [24] assessed fatigue using FAS among Croatian patients with sarcoidosis and chronic heart failure patients respectively. The Greek version of the Fatigue Assessment Scale has been used to assess the levels of fatigue among hemodialysis patients [2]. In the current study, factor analysis revealed five factors while in the Greek study of Zyga et al. [2] two factors were emerged (mental and physical fatigue). According to our findings, RA patients experience higher levels of fatigue and higher levels of QoL than OA patients. Dimensions of quality of life such as "Functionality" and "Spirituality" are affected by fatigue.

The total score of fatigue in our sample was 27.93 (±4.601) and the majority (91.6%) of the sample were classified as "fatigued". RA patients had higher FAS score (29.94 ± 3.377) than OA patients (26.14 ± 3.66) and higher MVQoLI-15

score 14.61 ± 1.734) than OA patients (13.73 ± 1.811). Michielsen et al. [23] using FAS among sarcoidosis patients found a mean score of FAS 24.3 ± 8.1 which is lower than the total score of our study. Zyga et al. [2] using the same scale among hemodialysis patients found, also, a lower mean score of FAS (24.99 ± 8.093).

As far as the fatigue among OA patients is concerned, a series of studies related to fatigue and OA describe fatigue as exhaustive and often responsible for the limitation of activity. Gignac et al. [7] reported that adults with moderate OA who participate in focus groups describe their fatigue as debilitating and sometimes responsible for curbing their activities. The study of Power et al. [8] showed a significant amount of stress in OA patients with a negative impact on their quality of life and a great negative effect of the symptoms of the disease on the levels of perceived fatigue.

Fatigue in RA is experienced as a daily symptom with various duration and intensity which affect daily activities and behaviors [17]. In general, if we would try making a mention about the etiology of fatigue in RA we would say that little is known. According to Mancuso et al. [25], fatigue among RA patients is associated with pain, physical dysfunction and psychosocial factors. Wolfe and Michaud [26] reported that 40% of patients with RA have significant fatigue as well as that fatigue was associated with pain as the most powerful prognostic indicator. Sleep disorders and depression was also very important prognostic factors as it was three times more common in people with significant fatigue. Also, other studies linked RA with increased fatigue levels considering, also, other factors such as depression, anemia, weight and pain [27].

As far as the effect of fatigue on QoL is concerned, our study did not reveal a strong association between fatigue and QoL. An explanation for this is that our sample did not experience high levels of fatigue. Thus, it is possible that the majority of the sample have benefits from treatment which may reduce the amount of fatigue that is experienced. However, we found an association between "General tiredness" of FAS and "Spirituality", between "Excessive fatigue—feelings of worthlessness" and "Functionality" and between "Difficulty in starting activities" and "Spirituality". Studies exploring the relationship between fatigue and QoL among patients with RA have shown contradictory results. Wolfe [28] did not found a significant relationship between fatigue scores, physical function and vitality. In contrast, vitality which is a subscale of SF-36 has been found significantly correlated with fatigue [27]. Rupp et al. [14] highlight that different aspects of fatigue in RA are associated with different aspects of QoL of RA patients. Similar results are referred by Van Tubergen et al. [29] in study among patients with ankylosing spondylitis.

"Spirituality", as a dimension of QoL of patients, is an important aspect which may influence fatigue. Many OA and RA patients, who participate in religious activities such as prayer, are influenced by beliefs. According to the perceptions of patients, faith and participation in religious activities can be helpful in reducing levels of perceived fatigue, pain, and improving quality of life. Baetz and Bowen [30] exploring the role of spirituality in chronic conditions and fatigue found that spirituality is associated with better psychological well-being.

In our study, a major risk factor associated with fatigue was the duration of sleep. Particularly, subjects with less than 4–6 h of sleep had higher values of "General tiredness" of FAS. Insufficient sleep may be a predictor of fatigue and its effect has been demonstrated previously in arthritis. Especially, a correlation between fatigue and poor quality of sleep has been noted previously in RA [31]. The presence of sleep disturbance in combination with other variables (pain, depressive symptoms and psychosocial factors) might be the responsible mechanism for fatigue in RA and OA patients [32].

The present study showed a correlation between fatigue and disease duration. Butbul Aviel et al. [33] have also found a significant relationship between fatigue and disease duration. In contrast, no significant association was noted between fatigue and disease duration in RA patients [34].

FAS found to be unidimensional when completed by Dutch patients [22] and Croatian patients with sarcoidosis [23]. It seems that this does not apply in Greek population with arthritis. Although the study of Zyga et al. [2] in Greek renal patients proposed a two-dimensional interpretation of FAS (physical and mental—which is in agreement with other studies), this study suggest a multidimensional interpretation of FAS. This is probably due to the different cultural background among people.

Fatigue is a common symptom in patients with OA and RA. We associated the levels of fatigue with quality of life among patients with OA and RA but these interrelations may not be too strong. Our findings suggest that different aspects of fatigue may affect dimensions of QoL. Therefore, we conclude that fatigue is a multidimensional and multifactory symptom and should be investigated systematically by clinicians.

5 Conclusions

After researching the international literature, we propose the early treatment of fatigue in primary care as it can prevent chronicity while the detection of fatigue in patients with osteoarthritis and rheumatoid arthritis can reduce the number of patients resorting to a numerous of unacceptable and harmful alternative approaches. There is a great need for the development of improved fatigue evaluation methods. The complexity of fatigue's pathogenic mechanism makes the effective intervention by health care professionals difficult. Thus, we suggest investigating the role of other factors contributing to fatigue in patients with osteoarthritis and rheumatoid arthritis. An assessment of all these factors can identify potential targets to which we can intervene therapeutically. Nurses and physicians should encourage patients to express their perceptions and feelings, to adhere to their treatment regimen and apply techniques for a qualitative sleep. Health staff should, also, recognize the factors of fatigue such as sleepless and disease duration. Thus, psychological support and the involvement of patient in clinical decision making can decrease the severity of fatigue [35]. Ultimately,

continuing research is necessary to assess the effectiveness and impact of these interventions in order to improve the quality of life of patients with osteoarthritis and rheumatoid arthritis.

As already mentioned, the study was conducted in Rehabilitation Centers in Peloponnese Region, Greece. As a result, we cannot generalize the results for all RA and OA patients. In addition, the questionnaires were given during the physiotherapy session. Therefore, the presence of physiotherapists, physicians and other patients may have influenced the objectivity of the results. However, the fact that the results are in agreement with a part of the published studies limits the subjectivity of the responses.

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References

1. Jorgensen, R. 2008. Chronic Fatigue: An Evolutionary Concept Analysis. *Journal of Advanced Nursing* 63: 199–207.
2. Zyga, S., V. Alikari, A. Sachlas, et al. 2015. Assessment of Fatigue in End Stage Renal Disease Patients Undergoing Hemodialysis: Prevalence and Associated Factors. *Medieval Archaeology* 69: 376–380.
3. Frank, M. 2014. Cancer Related Fatigue Syndrome in Neoplastic Diseases. *Przegląd Menopauzalny* 13: 352–355.
4. Tabrizi, F.M., and M. Radfar. 2015. Fatigue, Sleep Quality, and Disability in Relation to Quality of Life in Multiple Sclerosis. *International Journal of MS Care* 17: 268–274.
5. Yuen, H.K., and M.A. Cunningham. 2014. Optimal Management of Fatigue in Patients with Systemic Lupus Erythematosus: A Systematic Review. *Therapeutics and Clinical Risk Management* 10: 775–786.
6. Gong, G., and J. Mao. 2016. Health-Related Quality of Life Among Chinese Patients with Rheumatoid Arthritis: The Predictive Roles of Fatigue, Functional Disability, Self-Efficacy, and Social Support. *Nursing Research* 65: 55–67.
7. Gignac, M.A., A.M. Davis, G. Hawker, et al. 2006. "What Do You Expect? You're Just Getting Older": A Comparison of Perceived Osteoarthritis-Related and Aging-Related Health Experiences in Middle- and Older-Age Adults. *Arthritis and Rheumatism* 55: 905–912.
8. Power, J.D., E.M. Badley, M.R. French, et al. 2008. Fatigue in Osteoarthritis: A Qualitative Study. *BMC Musculoskeletal Disorders* 9: 63.
9. DeJean, D., M. Giacomini, M. Vanstone, et al. 2013. Patient Experiences of Depression and Anxiety with Chronic Disease: A Systematic Review and Qualitative Meta-Synthesis. *Ontario Health Technology Assessment Series* 13: 1–33.
10. McBeth, J., B. Tomenson, C.A. Chew-Graham, et al. 2015. Common and Unique Associated Factors for Medically Unexplained Chronic Widespread Pain and Chronic Fatigue. *Journal of Psychosomatic Research* 79: 484–491.
11. Nikolaus, S., C. Bode, E. Taal, et al. 2013. Fatigue and Factors Related to Fatigue in Rheumatoid Arthritis: A Systematic Review. *Arthritis Care and Research* 65: 1128–1146.
12. Stebbings, S., P. Herbison, T.C. Doyle, et al. 2013. A Comparison of Fatigue Correlates in Rheumatoid Arthritis and Osteoarthritis: Disparity in Associations with Disability, Anxiety and Sleep Disturbance. *Rheumatology (Oxford)* 49: 361–367.

13. Stone, A.A., J.E. Broderick, L.S. Porter, et al. 1997. The Experience of Rheumatoid Arthritis Pain and Fatigue: Examining Momentary Reports and Correlates Over One Week. *Arthritis Care & Research* 10: 185–193.
14. Rupp, I., H.C. Boshuizen, C.E. Jacobi, et al. 2004. *Arthritis and Rheumatism* 51: 578–585.
15. Ahlmén, M., U. Nordenskiöld, B. Archenholtz, et al. 2005. Rheumatology Outcomes: The Patient's Perspective. A Multicentre Focus Group Interview Study of Swedish Rheumatoid Arthritis Patients. *Rheumatology (Oxford)* 44: 105–110.
16. Hewlett, S., Z. Cockshott, M. Byron, et al. 2005. Patients' Perceptions of Fatigue in Rheumatoid Arthritis: Overwhelming, Uncontrollable, Ignored. *Arthritis and Rheumatism* 53: 697–702.
17. Repping-Wuts, H., R. Uitterhoeve, P. van Riel, et al. 2008. Fatigue as Experienced by Patients with Rheumatoid Arthritis (RA): A Qualitative Study. *International Journal of Nursing Studies* 45: 995–1002.
18. Doeglas, D. 2000. *Functional Ability, Social Support and Quality of Life: A Longitudinal Study in Patients with Early Rheumatoid Arthritis [Dissertation]*. Groningen: University of Groningen.
19. Theofilou, P., A. Aroni, M. Ralli, et al. 2013. Measuring Health Related Quality of Life in Hemodialysis Patients. Psychometric Properties of the Missoula-VITAS Quality of Life Index (MVQOLI-15) in Greece. *Health Psychology Research* 1: e17.
20. Alikari, V., E. Fradelos, A. Sachlas, et al. 2016. Reliability and Validity of the Greek Version of "The Fatigue Assessment Scale". *Archives of Hellenic Medicine* 33: 231–238.
21. Michielsen, H.J., J. De Vries, and G.L. Van Heck. 2003. Psychometric Qualities of a Brief Self-Rated Fatigue Measure: The Fatigue Assessment Scale. *Journal of Psychosomatic Research* 54: 345–352.
22. De Vries, J., H. Michielsen, G.L. Van Heck, et al. 2004. Measuring Fatigue in Sarcoidosis: The Fatigue Assessment Scale (FAS). *British Journal of Health Psychology* 9: 279–291.
23. Michielsen, H.J., J. De Vries, M. Drent, et al. 2005. Psychometric Qualities of the Fatigue Assessment Scale in Croatian Sarcoidosis Patients. *Sarcoidosis, Vasculitis, and Diffuse Lung Diseases* 22: 133–138.
24. Smith, O.R., H.J. Michielsen, and A.J. Pelle. 2007. Symptoms of Fatigue in Chronic Heart Failure Patients: Clinical and Psychological Predictors. *European Journal of Heart Failure* 9: 922–927.
25. Mancuso, C.A., M. Rincon, W. Sayles, et al. 2006. Psychosocial Variables and Fatigue: A Longitudinal Study Comparing Individuals with Rheumatoid Arthritis and Healthy Controls. *The Journal of Rheumatology* 33: 1496–1502.
26. Wolfe, F., and K. Michaud. 2004. Fatigue, Rheumatoid Arthritis, and Anti-Tumor Necrosis Factor Therapy: An Investigation in 24,831 Patients. *The Journal of Rheumatology* 31: 2115–2120.
27. Pollard, L.C., E.H. Choy, J. Gonzalez, et al. 2006. Fatigue in Rheumatoid Arthritis Reflects Pain, Not Disease Activity. *Rheumatology (Oxford)* 45: 885–889.
28. Wolfe, F. 2004. Fatigue Assessments in Rheumatoid Arthritis: Comparative Performance of Visual Analog Scales and Longer Fatigue Questionnaires in 7760 Patients. *The Journal of Rheumatology* 31: 1896–1902.
29. van Tubergen, A., J. Coenen, R. Landewé, et al. 2002. Assessment of Fatigue in Patients with Ankylosing Spondylitis: A Psychometric Analysis. *Arthritis and Rheumatism* 47: 8–16.
30. Baetz, M., and R. Bowen. 2008. Chronic Pain and Fatigue: Associations with Religion and Spirituality. *Pain Research & Management* 13: 383–388.
31. Wolfe, F., K. Michaud, T. Li, et al. 2006. Sleep Disturbance in Patients with Rheumatoid Arthritis: Evaluation by Medical Outcomes Study and Visual Analog Sleep Scales. *The Journal of Rheumatology* 33: 1942–1951.
32. Repping-Wuts, H., P. van Riel, and T. van Achterberg. 2009. Fatigue in Patients with Rheumatoid Arthritis: What is Known and What is Needed. *Rheumatology (Oxford)* 48: 207–209.

33. Butbul Aviel, Y., R. Stremler, S.M. Benseler, et al. 2011. Sleep and Fatigue and the Relationship to Pain, Disease Activity and Quality of Life in Juvenile Idiopathic Arthritis and Juvenile Dermatomyositis. *Rheumatology (Oxford)* 50: 2051–2060.
34. Turan, Y., Z. Kocaağa, H. Koçyiğit, et al. 2010. Correlation of Fatigue with Clinical Parameters and Quality of Life in Rheumatoid Arthritis. *Turkish Journal of Rheumatology* 25: 63–67.
35. Zyga, S. 2012. Maintaining the Identity and Self-Worth in Patients with Severe Illness or Injury. *International Journal of Caring Sciences* 5 (1): 1–2.

Association of Nutrients with Biomarkers of Alzheimer's Disease

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Abstract Prospective cohort studies, cross-sectional surveys, autopsy studies and intervention clinical trials that investigated the association between nutrients and Alzheimer's disease (AD) have been reviewed. To estimate the relationship between specific nutrient intake and the risk of AD, Cochrane Library, PubMed, EMBASE, and the Fisher Center for Alzheimer's Research Foundation were searched for this purpose. Most published observational studies found an inverse relationship between vitamins, n-3 fatty acids and AD. The majority of intervention studies support the beneficial effect of combined vitamins and n-3 fatty acids providing them in the early stages of the disease. Only vitamin E and Zn supplementation failed to show any significant difference on the study population. On the other hand, high dietary intake of saturated fat and brain metal accumulation were positively associated with the incidence of AD.

Keywords Neurodegenerative disease • Alzheimer's disease • Cognitive impairment • Nutrients • Vitamins • Fatty acids • Minerals • Metals • Polyphenols

1 Introduction

A considerable amount of research indicates that nutrients or their absence may influence the brain's neurochemistry and thus they are involved in the pathogenesis of neurodegenerative disorders. As indicated in the majority of epidemiological studies, specific dietary intake or nutrient deficiencies appear to be related to the onset of the Alzheimer's disease (AD).

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2 Objective

The purpose of this report is to review critically the results of epidemiological studies and clinical trials investigating the association between nutrient intake and the incidence of Alzheimer's disease as well as to assess the scientific validity and the generalizability of these results.

3 Methods

The literature was searched by using the services of Cochrane Library, PubMed/MEDLINE, EMBASE, Fisher Center for Alzheimer's Research Foundation. The following key words in the above databases were used to search for published articles: nutrients, metals, antioxidants, phenolic compounds, fatty acids, vitamin intake or vitamin supplementation and Alzheimer's disease or mild cognition impairment (MCI) or dementia or cognitive impairment. A study was included in this review if (1) it was written in English, (2) it assessed the relationship between nutrients and MCI or/and AD or dementia, (3) study population varied from postmenopausal age (60 years) to elderly age (85 years), (4) the outcomes measured by widely accepted neuropsychological tests or neuroimaging tools. There were no restrictions regarding race, gender, ethnicity or publication date. Cohort, cross-sectional studies, histochemical studies and intervention clinical trials were included as they met the above criteria.

4 Beneficial Effects of Nutrients on Cognitive Function

4.1 Ascorbic Acid

Ascorbic acid (AA) is a strong reducing agent and upon oxidation, dehydroascorbic acid is formed. Although it is clearly concentrated in the human brain, the mechanisms by which AA affect neurodegenerative diseases are under investigation.

Only three of the eight well-designed cohort studies that included 33,252 subjects, have shown benefits from vitamin C intake [1]. There are only seven combined studies (with $n = 1951$) that showed a significant inverse association of vitamin C in plasma with cognitive impairment and AD. Finally, only three of four small studies that examined directly biological levels of vitamin C at CSF, revealed positive relationship between vitamin C and cognitive function.

Bowman et al. [2] conducted a biomarker study in order to investigate the effects of brain levels of ascorbic acid (AA) on the neurodegeneration in AD. Thirty-two diagnosed subjects with mild to moderate AD (10 females, mean age 71 ± 7 years) were examined at baseline and 12 months. Cerebrospinal fluid (CSF) and peripheral

blood were collected at baseline for AA and albumin content. According to the results, CSF and plasma AA failed to alter cognitive reduction independently. However, CSF: plasma AA ratio seems that may predict the rate of AD progression over 1 year, after controlling for age, gender, education, APOE-4 carrier status, and baseline cognitive function. The association between this ratio and the cognitive decline in AD was modified by blood brain barrier integrity. Obviously, BBB dysfunction fails to maintain high CSF: plasma AA radio, as AA diffuse out of central nervous system, following its concentration gradient.

4.2 Vitamin E

Tocopherol is a major antioxidant protecting cell membranes from free radical damage. Since neurodegenerative diseases are associated with oxidative stress, the null hypothesis is that AD may be prevented or cured by the dietary intake of vitamin E. From a systematic search of relevant publication databases in The Cochrane Library, ALOIS, MEDLINE, EMBASE, PsycINFO, only three randomized placebo-controlled trials met the inclusion criteria [3]. According to the results, no significant difference observed in the progression of MCI to AD between the two groups either at a dose of 800 IU/d or at 2000 IU/d (hazard ratio 1.02; 95% CI 0.74 to 1.41; $P = 0.91$).

4.3 Vitamin C in Combination with Vitamin E and α-Lipoic Acid

Vitamin C could replenish oxidized vitamin E and in turns vitamin E at the membrane–cytoplasmic interface can restore oxidized vitamin C. In this way, antioxidant vitamins are shown to block initiation and break free radical chain reactions. A cross-sectional and prospective study was conducted by Zandi et al. [4] to determine if taking vitamins reduces the risk of Alzheimer disease. The initial study population consisted of 5092 elderly residents (65 years or older) of Cache County. First priority was to examine the genotype at the gene for apolipoprotein E (APOE) via buccal DNA collection. 97% of the population was diagnosed for dementia following multistage assessment protocol and screening (e.g. Mini-Mental State Examination, Dementia Questionnaire, structured neurological examination, neuropsychological tests). At this initial assessment 200 prevalent cases of AD were identified. During next follow-up assessment, between 1998 and 2000, 104 elderly subjects among 3227 survivors, had been diagnosed with AD. In unadjusted analyses, intake of vitamin E, vitamin C and multivitamin all showed an inverse relationship with AD prevalence. However, after adjusting the findings for age, sex, years of education, number of APOE alleles, the inverse association remained

significant only for vitamin E(>400 IU) and multivitamin users. Using vitamin C and vitamin E in combination, was associated with reduced prevalence and incidence of AD. Other case control study comparing the vitamin status and other metabolites between mild dementia/MCI/AD subjects and healthy controls is in progress (Multiple Nutritional Deficiencies Causing Dementia of the Alzheimer Type, NCT01479885). In a double-blind, placebo-controlled clinical trial [5], 75 subjects, 60–85 years, with mild to moderate Alzheimer disease were randomly assigned to receive a daily dose of 800 IU of vitamin E plus 500 mg of vitamin C plus 900 mg of α-lipoic acid or 400 mg of coenzyme Q (x3/d) or placebo. Using the MMSE and ADCS-ADL scores, there were no differences between the three groups in CSF αβ42, tau, p-tau$_{181}$ but a 19% reduction in CSF F_2-isoprostane levels in the E/C/ALA group was observed.

4.4 Folic Acid, B_6 and B_{12}

There is a wealth of evidence from epidemiological studies that demonstrate a relationship between vitamin-B complex and cognition. The Oxford researchers [6] allocated 156 elderly subjects with mild cognitive impairment (according to 2004 Petersen criteria), to receive B vitamins (folic acid 0.8 mg, vitamin B12 0.5 mg, vitamin B6 20 mg) or placebo, over the course of 2 years. Their purpose was to examine whether high-dose B-vitamin treatment can slow the atrophy of specific brain regions (gray matter tissue). These brain regions play a key role in Alzheimer's disease (AD) process and they are associated with cognitive decline.

A significant body of epidemiological studies has constantly revealed the role of plasma homocysteine as a risk factor for dementia and Alzheimer's disease [7–9]. Participants were also divided into two groups according to baseline measurements of homocysteine levels: those with high levels of homocysteine (above 11.06 μmol/L) and those with average levels or below (equal to or lower than 11.06 μmol/L).

The analysis of the data showed that the two groups although were similar in gray matter volume at baseline, those receiving B vitamins showed a significant reduction of atrophy in posterior brain regions, including bilateral hippocampus and parahippocampal gyrus, retrosplenial precuneus, lingual and fusiform gyrus, as well as in the cerebellum, compared to the placebo group. Interestingly, it was noted that the average brain atrophy measured by loss of gray matter, over a 2-year period, was 3.7% in the placebo group, compared to 0.5% in the B-vitamin group.

The beneficial effects of B vitamins appeared in subjects with high blood homocysteine levels (>11 μmol/L). Over 2 years, those with high homocysteine levels reduced brain atrophy from 5.2% to 0.6% in the B-vitamin group ($p < 0.05$). B-vitamin supplementation also lowered homocysteine levels by an average of 29%.

4.5 N-3 Fatty Acids

It has been speculated that supplementation with DHA, in particular, could alter membrane fluidity and neural membrane function and could also modulate gene expression at the transcription level [10].

In the light of the research that demonstrates the preventive role of n-3 fatty acids in the AD pathology, investigators such as Pottala et al. [11] proposed that higher levels of red blood cell EPA and DHA were associated with hippocampal brain volumes. This cohort study aimed to examine if there was any association between the n-3 fatty acid levels in red blood cell and the brain volume measured with MRI, 8 years after blood was drawn.

Based on its methodology, the study population consisted of 1111 post-menopausal women from the Women's Health Initiative Memory Study and was all free from dementia. Red blood cell EPA and DHA levels as well as MRI brain volumes were measured. The findings were adjusted for hormone therapy, time since randomization, demographics, intracranial volume and cardiovascular disease risk factor. The results were controlled for racial differences. Volumetric measurements were taken at different regions (4 lobes, limbic, basal ganglia, corpus callosum, hippocampus) by summing up the number of voxels.

In fully adjusted models it was found that a 2SD higher omega-3 index was strongly associated with 2.1 cm^3 larger brain volume, measured 8 years later, showing significant p value equal to 0.048. Nearly significant association found between DHA and total brain volume ($p = 0.063$) whereas EPA was not significantly related to total brain volumes ($p = 0.11$).

The available findings from RCTs, using omega-3 fatty acid supplementation ranging from 3 to 12 months, in patients diagnosed with MCI ended up with a positive effect on cognitive performance following supplementation. In agreement with the above, Volkert et al. [12], also reported that n-3 fatty acids might be effective in early stages of neurodegenerative disease but failed to improve cognition in people with dementia.

4.6 Multinutrient Supplementation

Researchers [13] conducted a randomized double-blind, placebo-controlled trial in order to test the hypothesis that daily supplementation of combined nutrients improves cognition in older women. The authors followed a stratified randomization according to nonfrail, prefail and frail subjects. Non acute ill, postmenopausal women (60–84 years) were randomized to receive for 6 months, placebo or 4 capsules per day of Efalex Active 50+ (1 g DHA and 160 mg EPA per day in addition to Ginkgo biloba, phosphatidylserine, α-tocopherol, folic acid, and vitamin B_{12}).

According to the results, the administration of encapsuled DHA plus other nutrients exerts positive effects on verbal memory and significantly improved the PAL memory test, the psychomotor reaction speed and finally the mobility (HWspeed). The findings suggest that it may be that the synergistic combination of nutrients found in the multinutrient supplement has a superior effect than individual nutrients in older persons.

4.7 Medical Food (Souvenaid)

Souvenaid (Nutricia N.V., Zoetermeer, the Netherlands) is a specially designed medical food that contains several combined nutrients and precursors (phospholipids, uridine monophosphate, EPA, DHA, choline, vitamin C, vitamin E, selenium, vitamin B_{12}, vitamin B_6, folic acid-Fortasyn® Connect). Souvenaid is speculated to support synapse formation and function in patients with AD.

Shah et al. [14], following a 24-week, double-blinded clinical trial, examined whether Souvenaid could reduce cognitive decline in treated subjects with mild to moderate AD, at 48 clinical centres. Treated persons (n = 527) with AD medications (52% women, mean age 76.7 years) were randomized to receive either placebo (isocaloric) or an oral intake of souvenaid (125 ml, 125 kcal), in addition to unchanged dosages of standard medications. According to their personal taste preferences, participants chose one of the two available flavors (strawberry or vanilla), once per day. This S-Connect clinical trial failed to show benefits from the oral intake intervention. Other randomized controlled trials to obtain more information on the mode of action and long-term efficacy of Souvenaid currently are ongoing.

4.8 Vitamin D

Animal studies have shown that vitamin D receptors (VDR) are expressed in many brain regions including the hippocampus, amygdala, hypothalamus, thalamus, cortex, cerebellum, *accumbens nuclei* and throughout the olfactory system. Combined studies reveal that $1,25(OH)_2D$ is present in the cerebrospinal fluid, it is synthesized in neurons and microglia and seems to exert a paracrine/autocrine action in nervous system. Vitamin D regulates the nerve growth factor (NGF), influences the plasticity of neurons and inhibits the synthesis of Inducible Nitric Oxide Synthase and via these mechanisms it acts as a neuroprotective compound [15]. A number of studies reveal an association between vitamin D deficiency and dementia [16]. A large multi-centre randomized clinical trial (DO-HEALTH) has already recruited a total of 2152 community-dwelling men and women aged 70 years in order to assess the combined benefits of 2000 IU of vitamin D/day, 1 g of omega-3 fatty acids/day, and a simple home exercise program (http://do-health.eu/wordpress/). This promising study will be ongoing till 2017.

4.9 Zinc

In a randomized double-blind, placebo controlled intervention trial 387 healthy adults (196 males; 191 females) aged 55–87 years were daily treated with 0 mg Zn (placebo), 15 mg Zn, 30 mg Zn, for 6 months, in order to investigate the effects of zinc supplementation on their cognitive functioning [17]. Visual memory, working memory, attention, and reaction time were measured at baseline and after 3 and 6 months. Zn supplementation at doses of 15 and 30 mg/d was associated with improved scores in tests of spatial working memory, at 3 months only. However, 15 mg/d of zinc supplementation had a detrimental effect on the attention test. The study showed significant interactions only for two out of the eight cognitive tests.

4.10 Polyphenols

Polyphenols that are the largest class of phytochemicals found in nature are the responsible molecules for the bitter and astringent properties associated with certain foods. Over 4000 polyphenols have been identified and mainly found in vegetables, fruits, grains, roots, flowers, seeds, herbs and spices. They are especially abundant in red wine and green tea, as well as in plants such as grapes, apples, onions and wild berries. The resveratrol present in grapes and in red wine, the curcumin in turmeric and the catechols in green tea are all extremely powerful molecules against cancer and against neuronal damage. Undoubtedly, dietary polyphenolic compounds have a protective role against diseases involving oxidative stress, such as cancers, cardiovascular and neurodegenerative diseases (Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS), and stroke). Recent studies reveal that they exhibit neuroprotection through a numerous different mechanisms such as free radicals scavengers, inhibitors of inflammatory response and modulators of intracellular signaling pathways and gene expression [18]. Intervention clinical trials that supplemented AD or MCI subjects with either EGCG or resveratrol or curcumin at several doses from 6 to 18 months, are in progress.

5 Detrimental Effects of Dietary Factors on Cognition

5.1 Iron (Fe)

Resent research highlights the role of metal ions in the development of AD. Available data from bibliography, suggest that excess iron could be a concern for older people at risk for AD, Parkinson's, dementia and other neurodegenerative diseases. Iron is referred as a metal with high reactivity and increased oxidation from this metal can damage brain cells.

Smith et al. [19], conducted an in vacuom research, in order to determine whether redox-active iron was elevated in MCI and preclinical AD as compared to cognitively-intact age-matched control patients. The question is whether iron deposition at the preclinical stage of AD may be useful as a diagnostic tool or as an index of AD progression.

The results showed that the pre-clinical AD/MCI cases appear to have more iron accumulated in both cortex and cerebellum, compared with cognitively normal patients. In a representative MCI case, many of the iron-positive structures are associated with glial cells. Moreover, the redox-active iron in the cerebellum tends to increase as cognition was progressively impaired. The authors concluded that an imbalance in iron homeostasis occurs not only in the affected cortical regions but also in the cerebellum, and it precedes the neurodegenerative processes. Interestingly, other studies [20–22] confirmed a significantly higher redox active iron in the CSF in the MCI subjects, and proposed that, increased iron make tau to accumulate.

5.2 Copper (Cu)

Excess of this metal can be toxic and thus excess copper intake may contribute to cognitive problems. A meta-analysis [23, 24] suggested that levels of copper not bound to ceruloplasmin are increased in AD subjects compared to healthy controls. In conclusion copper dysfunction appears to play a role in AD pathology.

5.3 Saturated and Trans Fatty Acids

Increased dietary intake of saturated fat may induce cognitive decline since it was positively associated with AD risk in the majority of published studies. Bernard et al. (2013) undertook a systematic review to identify the association between fat intake and incidence of AD, dementia, MCI or cognitive decline. Dietary intake of saturated fat was positively associated with the incidence of AD (3/4 studies), with total dementia (1/2 studies), with MCI (1/4 studies) and with cognitive decline (2/4 studies). Inconclusive results arise from studies that estimated the relationship between trans fat intake and risk of AD.

6 Discussion

Vitamin C Methodological rigour of studies is likely to restrict the validity of the findings. Considering the possible role of ascorbic acid, the plasma AA levels are considered as short term result, therefore could not predict long term intake of vitamin C, that possible related to neurological effects.

Vitamin E There is need to conduct clinical trials assessing the possible preventive role of all types of vitamin E (α-, β-, γ-tocopherol).

A Combination of Vitamin E & C & α-Lipoic Acid The cross sectional study examined the synergistic effect of vitamin C and vitamin E, used a large sample size and appropriately took into account risk factors for dementia including education, occupational history, medical history, tobacco and alcohol use, and medication or vitamin intake. However, the AD incidence data derived from a relative small period of time. Another concern is that user and not user vitamin group were not matched for possible confounding variables. For example, those who use vitamins may have a tendency to follow a healthier lifestyle. Considering the clinical trial conducted by Galasco et al. [5], the decision for combined supplementation at that doses is based on other clinical trials and a meta-analysis indicating an increase in all cause mortality when given more than 800 IU/d of vitamin E. However, one of the major strengths of the study is that participants' care-givers, and investigators were blind to treatment allocation.

Folic Acid, B_6 and B_{12} Considering the study design, the use of structural neuroimaging tool contrary to neuropsychological tool, allow to assess any effect of supplementation and thus increases the power of the study. Moreover, all statistical tests (FEW-correction) were performed in this study a priori and had no impact on the treatment group. However, using the whole-brain voxelwise approach instead of a region-of-interest approach increases the probability of experimental type I error. A further challenge in mild or severe hyperhomocysteinemia would be to assess B_{12}, B_6 and folic acid deficiencies based on metabolic indicators and initiate supplementation at appropriate doses.

N-3 Fatty Acids The findings of cohort study may hide a series of methodological limitations, since the sample population was only women recruited from Women's Health Initiative. The lack of sample representativeness involves in selection bias and minimizes the strength of the study. Secondly, as a cross sectional study, cannot permit a possible causal relationship between omega-3 index and total brain volume. Furthermore, the study method did not include as variable any alteration or initiation of fish oil supplement after the screening, causing detection bias.

Results obtained from a number of controlled studies investigating the efficacy of DHA/EPA supplementation have been so far conflicting and inconclusive [25]. The validity of most findings from intervention studies with omega-3 supplementation in MCI and AD subjects, may be restricted from small sample size, relatively low dose of omega-3 fatty acids, short intervention period since most studies report 6 months duration of supplementation and bias from carer's awareness of the treatment regimen.

Further well-designed clinical trials, for a longer duration (12–24 months) and intervention at therapeutic doses are needed to confirm any efficacy of n-3PUFA supplementation in any stage of the AD and particularly in early to moderate AD.

Efalex 50+ The small sample size (n = 12 for placebo group; n = 15 for supplementation group) and the short term intervention (6 months) do not allow to conclude anything about the effectiveness of this supplementation.

Souvenaid The available evidence, so far, is insufficient to draw any positive or negative conclusions. This clinical study, used an adequate sample size, however the pharmacological treatments of the participants and the overall drop-out rate (14.4%) during the trial could bias the results.

Vitamin D Cross-sectional studies cannot establish a causal relationship between vitamin D deficiency and cognitive decline. Different results from systematic reviews and meta-analyses may be partially explained by different cut-off points for vitamin D status and different potential confounders that authors took into account (age, sex, race, level of education, diabetes, hypertension, kidney disease, depression, physical activity, seasonality in vitamin D status).

Zinc Although, the present study is based on accurate cognitive tests and measurements and took into account different characteristics of study population (education level, age, gender), two major methodological limitations should be underlined. First, the low dose of zinc supplementation (15 and 30 mg/d) seems to be insufficient to show any measurable effect, since the tolerable upper intake level (UL) is 40 mg/d for both males and females and for both age groups 19–70 years and >70 years [26]. Second, the outcomes of the study cannot generalize to older adults with significant cognitive impairment since this population was excluded from the study.

Saturated or Trans Fat Current review has several methodological flaws that we should note. The small number of observational studies and the absence of intervention clinical trials compromise the findings. The heterogeneity in the results arises from differences in the participant characteristics (age, ethnicity, APOE $\varepsilon 4$ status), duration of observation and diet at baseline. Participants in the Rotterdam Study reported an unusual high vitamin E in parallel with saturated fat intake which may influence the results. Moreover, most observational studies assessed dietary intake using food frequency questionnaires that introduce recall bias and measurement errors.

7 Conclusions and Future Directions for Research

Evidence so far, suggest that antioxidant compounds such as vitamin E and C, as well as B-vitamins, omega-3 fatty acids, polyphenols, and vitamin D might be useful first in a combined formula and second in very early stages of the disease e.g. in MCI stage. On the other hand, excess metal accumulation of iron and copper and high dietary intake of saturated and trans fat associate with risk for dementia. Notably, numerous methodological issues and inconsistencies arise from this review. Both observational and clinical studies should be interpreted with caution taking into account any possible limitation of study design such as sample

size, presence of confoundings, selection bias, lack of causality, diagnostic tools and testing procedures. In order to develop neuroprotective strategies through nutrient intake, well designed clinical studies are needed of adequate nutrient doses and with appropriate intervention period.

References

1. Bowman, G.L. 2012. Ascorbic Acid, Cognitive Function, and Alzheimer's Disease: A Current Review and Future Direction. *BioFactors* 38: 114–122.
2. Bowman, G.L., H. Dodge, B. Frei, C. Calabrese, B.S. Oken, J.A. Kaye, and J.F. Quinn. 2009. Ascorbic Acid and Rates of Cognitive Decline in Alzheimer's Disease. *Journal of Alzheimer's Disease* 16 (1): 93–98.
3. Farina, Nicolas, Mokhtar Gad El Kareem Nasr Isaac, Annalie R. Clark, Jennifer Rusted, and Naji Tabet. 2012. Vitamin E for Alzheimer's Dementia and Mild Cognitive Impairment. *Cochrane Database of Systematic Reviews* 11: CD002854.
4. Zandi, P.P., J.C. Anthony, A.S. Khachaturian, S.V. Stone, D. Gustafson, J.T. Tschanz, M.C. Norton, K.A. Welsh-Bohmer, J.C. Breitner, and Cache County Study Group. 2004. Reduced Risk of Alzheimer's Disease in Users of Antioxidant Vitamin Supplements: The Cache County Study. *Archives of Neurology* 61: 82–88.
5. Galasco, R., E. Peskind, Clark C. MD, J. Quinn, J. Ringman, Jicha G. MD, C. Cotman, B. Cottrell, T. Montine, R. Thomas, P. Aisen, and Antioxidants for Alzheimer Disease. 2012. A Randomized Clinical Trial with Cerebrospinal Fluid Biomarker Measures. *Archives of Neurology* 69 (7): 836–841.
6. Gwenaëlle, Douaud, Helga Refsum, Celeste A. de Jager, Robin Jacoby, Thomas E. Nichols, Stephen M. Smith, and A. David Smith. 2013. Preventing Alzheimer's Disease-Related Gray Matter Atrophy by B-Vitamin Treatment. *Proceedings of the National Academy of Sciences* 110: 9523–9528.
7. Morris, M.S. 2012. The Role of B Vitamins in Preventing and Treating Cognitive Impairment and Decline. *Advances in Nutrition* 3: 801–812.
8. Smith Mark, A., Xiongwei Zhu, Massimo Tabaton, Gang Liu, Daniel W. McKeel Jr., Mark L. Cohen, Xinglong Wang, Sandra L. Siedlak, Takaaki Hayashi, Masao Nakamura, Akihiko Nunomura, and George Perry. 2010. Increased Iron and Free Radical Generation in Preclinical Alzheimer Disease and Mild Cognitive Impairment. *Journal of Alzheimer's Disease* 19 (1): 363–372.
9. Vogel, T., N. Dali-Youcef, G. Kaltenbach, and E. Andrès. 2009. Homocysteine, Vitamin B12, Folate and Cognitive Functions: A Systematic and Critical Review of the Literature. *International Journal of Clinical Practice* 63: 1061–1067.
10. Horrocks, L.A., and A.A. Farooqui. 2004. Docosahexaenoic Acid in the Diet: Its Importance in Maintenance and Restoration of Neural Membrane Function. *Prostaglandins, Leukotrienes and Essential Fatty Acids* 70 (4): 361–372.
11. Pottala, J., K. Yaffe, J. Robinson, M. Espeland, R. Wallace, and S.W. Harris. 2014. Higher RBC EPA+DHA Corresponds with Larger Total Brain and Hippocampal Volumes. *Neurology* 82 (5): 435–442.
12. Volkert, D., M. Chourdakis, G. Faxen-Irving, T. Frühwald, F. Landi, M.H. Suominen, M. Vandewoude, R. Wirth, and S.M. Schneider. 2015. ESPEN Guidelines on Nutrition in Dementia. *Clinical Nutrition* 34 (6): 1052–1073.
13. Strike, A.C., Carlisle Alison, Gibson E. Leigh, and C. Dyall Simon. 2015. A High Omega-3 Fatty Acid Multinutrient Supplement Benefits Cognition and Mobility in Older Women: A Randomized, Double-Blind, Placebo-Controlled Pilot Study. *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences*: 1–9.

14. Shah, R.C., P.J. Kamphuis, S. Leurgans, S.H. Swinkels, C.H. Sadowsky, A. Bongers, S.A. Rappaport, F. Joseph, J.F. Quinn, R. Wieggers, P. Scheltens, and D. Bennet. 2013. The S-Connect Study: Results from a Randomized, Controlled Trial of Souvenaid in Mild-to-Moderate Alzheimer's Disease. *Alzheimer's Research & Therapy* 5: 59.
15. Fernandes de Abreu, D.A., D. Eyles, and F. Féron. 2009. Vitamin D, a Neuro-Immunomodulator: Implications for Neurodegenerative and Autoimmune Diseases. *Psychoneuroendocrinology* 34 (Suppl. 1): S265–S277.
16. Annweiler, C., Y. Rolland, A.M. Schott, H. Blain, B. Vellas, F.R. Herrmann, and O. Beauchet. 2012. Higher Vitamin D Dietary Intake is Associated with Lower Risk of Alzheimer's Disease: A 7-Year Follow-Up. *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences* 67 (11): 1205–1211.
17. Maylor, E.A., E.E.A. Simpson, D.L. Secker, N. Meunier, M. Andriollo-Sanchez, A. Polito, B. Stewart-Knox, C. McConville, J.M. O'Connor, and C. Coudray. 2006. Effects of Zinc Supplementation on Cognitive Function in Healthy Middle-Aged and Older Adults: The ZENITH Study. *British Journal of Nutrition* 96 (4): 752–760.
18. Choi, D.-Y., Y.-J. Lee, J.T. Hong, and H.-J. Lee. 2012. Antioxidant Properties of Natural Polyphenols and Their Therapeutic Potentials for Alzheimer's Disease. *Brain Research Bulletin* 87: 144–153.
19. Smith, A.D., S.M. Smith, C.A. de Jager, P. Whitbread, C. Johnston, G. Agacinski, A. Oulhaj, K.M. Bradley, R. Jacoby, and H. Refsum. 2010. Homocysteine-Lowering by B Vitamins Slows the Rate of Accelerated Brain Atrophy in Mild Cognitive Impairment: A Randomized Controlled Trial. *PLoS One* 5: e12244.
20. Lavados, M., M. Guillón, M.C. Mujica, L.E. Rojo, P. Fuentes, and R.B. Maccioni. 2008. Mild Cognitive Impairment and Alzheimer Patients Display Different Levels of Redox-Active CSF Iron. *Journal of Alzheimer's Disease* 13 (2): 225–232.
21. Tofaris, G.K., T. Revesz, T.S. Jacques, S. Papacostas, and J. Chataway. 2007. Adult-Onset Neurodegeneration with Brain Iron Accumulation and Cortical Alpha-Synuclein and tau Pathology: A Distinct Clinicopathological Entity. *Archives of Neurology* 64: 280–282.
22. Yamamoto, A., et al. 2002. Iron (III) Induces Aggregation of Hyperphosphorylated tau and Its Reduction to Iron (II) Reverses the Aggregation: Implications in the Formation of Neurofibrillary Tangles of Alzheimer's Disease. *Journal of Neurochemistry* 82: 1137–1147.
23. Squitti, R., et al. 2014. Meta-Analysis of Serum Non-ceruloplasmin Copper in Alzheimer's Disease. *Journal of Alzheimer's Disease* 38 (4): 809–822.
24. Squitti, R., M. Siotto, and R. Polimanti. 2014. Low-Copper Diet as Preventive Strategy to Decrease the Risk for Copper Phenotype in Alzheimer's Disease. *Neurobiology of Aging* 35 (Suppl. 2): S40–S50.
25. Thomas, J., C.J. Thomas, J. Radcliffe, and C. Itsiopoulos. 2015. Omega-3 Fatty Acids in Early Prevention of Inflammatory Neurodegenerative Disease: A Focus on Alzheimer's Disease. *BioMed Research International*. Volume, Article ID 172801, 13 pp.
26. Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine. 2001. *(US) Panel on Micronutrients*. Washington: National Academies Press.
27. Annweiler, C., B. Fantino, D. Le Gall, A.M. Schott, G. Berrut, and O. Beauchet. 2011. Severe Vitamin D Deficiency is Associated with Advanced-Stage Dementia in Geriatric Inpatients. *Journal of the American Geriatrics Society* 59 (1): 169–171.
28. Annweiler, C., D.J. Llewellyn, and O. Beauchet. 2013. Low Serum Vitamin D Concentrations in Alzheimer's Disease: A Systematic Review and Meta-Analysis. *Journal of Alzheimer's Disease* 33 (3): 659–674.
29. Barnard Neal, D., E. Bunner Anne, and Agarwal Ulka. 2014. Saturated and Trans Fats and Dementia: A Systematic Review. *Neurobiology of Aging* 35 (2): S65–S73.

Searching for Correlations Between the Development of Neurodegenerative Hallmarks: Targeting Huntingtin as a Contributing Factor

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Abstract This paper aims to study four general hallmarks of neurodegeneration and the correlations between them, with emphasis on the huntingtin (htt) interactions contributing to their prevention or promotion in its wild-type and mutated forms. Most of the neurodegenerative diseases share same or similar cell dysfunctions and huntingtin seems to associate in an polyglutamine-length dependent manner with components of the mechanisms that can go impaired. Therefore, the protein is proposed as contributing factor to the development of selective neurodegeneration.

Keywords Huntingtin • PolyQ tract • Neurodegeneration • Transcription • Protein aggregation • Degradation pathways • Mitochondria

1 Introduction

Neurodegenerative diseases are characterized by progressive dysfunction of the nervous system and although they don't seem to appear under same causes, they all lead to selective neurodegeneration with very few features to distinguish the one from the other, especially when it comes to early diagnosis. There are multiple changes in the natural functions of a cell, connected with neurodegeneration and considered as its hallmarks. In this paper, four common neurodegenerative hallmarks that seem to trigger one another are discussed: problems in gene expression processes that lead to misfolding proteins, protein aggregations that result from the binding of the misfolding proteins, impairments in the degradating systems of the cell and impairments of the mitochondria, the main power supply of the cell. Neurodegenerative diseases like Alzheimer's, Parkinson's and Huntington's disease share all these complications. This similarity raises the question of whether all the cases start with same modifiers, which depending on their functioning impair other components that later specify the disease. In the case of HD, the

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only major neurodegenerative disease for which the genetic mutation is known and widely accepted, huntingtin protein and its mutations are connected with the cascade of the disease [1]. But apart from HD, huntingtin's wild-type interactions seem mostly neuroprotective and are essential for the cell's well regulated functions [2]. Many of its interactions and functions are lost, impaired or enhanced in an polyglutamine length-dependent manner, meaning that they are determined mostly of the polyQ tract that the protein contains. A polyQ tract containing more than ∼40 repeats is capable of causing HD, but is that the length and starting point from where the protein loses or alters its interactions? If its not and its interactions are altered one by one much earlier depending on the length of its polyQ tarct, can htt be connected with more than one neurodegenerative diseases acting like a contributing factor to their cascade?

2 HD and Huntingtin Protein

Huntington disease is a progressive inherited brain disorder, that has a broad impact on a person's functional abilities. Talking, thinking and moving abilities gradually worsen over time, as a result of the progressive neurodegeneration that takes place in the individual's brain. HD is caused by a mutation in a single gene called Huntington gene (HTT), inherited in an autosomal dominant pattern [1]. Its cytogenetic location is 4p16.3. There are multiple different alleles of the gene that can be categorized to the ones that will result in Huntington's disease and to the ones that won't. A C-A-G codon sequence that is repeated a few times near the beginning of the gene determines whether an allele will result in HD through the length of the polyQ tract that it will encode in the product of the gene, the huntingtin protein. The more CAG codons the gene has, the larger the polyQ tract of the protein that it produces is. The bigger the polyQ tract is, the more unstable the htt protein that is produced is, altering its proper interactions that seem to be polyglutamine-dependent and causing problems to the mechanisms that it normally seems to protect. Generally, the polyQ tract's lengths that can cause HD are from 36 codons and above while the normal lengths range up to 26 repeats long. From 27 to 35 repeats, the individuals carrying the tract are called "intermediate alleles" with no great risk of developing the disease but with a risk of passing it to their progeny. The CAG repeat length seems to be highly correlated to age of onset too, as well as in the progression and severity of the disease [3, 4]. The age of onset has divided the disease into 2 forms, the adult one and the juvenile one. As juvenile HD are considered cases with age of onset before 20 years of age. The years of survival that a patient has after the onset of the disease, range from 10 to 17. Although Huntingtin (htt) is mostly considered as the protein that causes HD, its natural role in the human body seem to be very important too as researchers have tried in early studies to solve the enigma behind its functions, connecting their loss with neurodegeneration [2, 5]. It's exact role in an organism is still under investigation but generally it is mostly thought as a molecular scaffold, allowing different proteins to come together and

interact [6]. It appears to be quite an old protein in evolution made up of 3144 amino acids, that has no sequence homology with other proteins. As it seems to be essential for normal development before birth [7], some of its functions also include transcriptional regulation, endocytosis, mitochondrial functioning, regulating cell death and other. It also contains a functional nuclear export signal (NES): a sequence of 17 amino acids at the beginning of the chain that allows the protein to leave the nucleus and regulates other functions of htt. Removing it causes htt to accumulate in the nucleus and enhances the aggregate's formation [8]. It can also shuttle in and out of the nucleus through it's many binding partners and their interactions. Htt interacts directly with at least 19 other proteins and undirectly with over 100 ones. It interacts with proteins responsible for transcription, transport and cell signaling, endocytosis, programmed cell death (PCD) and other, making it's proper subcellular localization and it's proper functioning essential for cell's proper regulation [9]. Many of these interactions are altered when the protein contains a large polyQ tract. Htt's posttranslational modifications (PTMs) seem to be very important too as they regulate it's proper localization, levels of toxicity, inclusion formation, aggregation and degradation. As htt normaly goes under processes of phosphorylation, SUMOylation, ubiquitination, acetylation, proteolytic cleavage and palmitoylation, most of these PTMs are altered in a polyglutamine-length dependent manner [10].

3 Hallmarks of Neurodegeneration

Neurodegenerative diseases are connected with impairments in the cell's proper functions. There are many impairments with the major ones seen in the development of almost every neurodegenerative disease and considered as neurodegenerative "hallmarks". Some of them are causative and other resultative. Some are thought as protective against neuronal death and other as contributing to it. At most cases, these categorizations are difficult as the results of studies trying to characterize each hallmark remain contradictory. Here are some neurodegenerative hallmarks, the correlations between them, some of the neurodegenerative diseases with which they are connected and the role of wild-type and mutated huntingtin in their prevention or promotion.

3.1 Gene Expression Impairments

Gene expression is the process by which a gene codes for a product. Most commonly this product is a protein. It is a combination of two main stages, transcription and translation. In the transcription process, a DNA segment is copied into RNA with the help of transcription factors (proteins) that bind to DNA controlling the rate of transcription. They can promote (activator) or prevent (repressor) the expression

of a gene. REST/NRSF is one of the most important transcriptional repressors as it regulates the expression of neural genes in both neural and non-neural cells by binding to their RE1/NRSE domains. It is thought to repress more than 2000 genes in non-neural tissues and participate as an activator in neural differentiation processes through its interaction with RCOR1 protein, regulating neurogenesis. Its nuclear levels are found increased with aging in non-AD humans, showing also a neuroprotective role as it can repress genes that promote cell death, induce the expression of stress response genes and protect from oxidative stress and amyloid-b protein toxicity [11]. In individuals with dementias such as AD, frontotemporal dementia and dementia with Lewy bodies, REST is found lost from the nucleus [12]. These evidences show that REST acts not only as silent repressor but also as an active protector and its translocation or dysfunction affects dramatically the cell well being. Wild type htt is observed to have a part in the regulation of transcription in two main ways: Firstly, it is observed in vitro to bind directly to DNA, regulating transcription in the most direct way possible [13], and secondly, it participates in transcription processes by interacting with many transcription factors like REST/NRSF [14], sp1 [15], p53, CREB binding proteins [16] and others. Huntingtin interacts with the REST repressor element by binding to it and keeping it in the cytoplasm, regulating its availability. It prevents it from entering the nucleus and repressing the expression of important for the cell genes, such as the one that expresses BDNF, a neurotrophin that helps and supports the survival and growth of neurons and is important for long-term memory. In the presence of a big polyQ tract size, these htt functions are altered as it is observed that gene expression is modulated differentially in a polyglutamine-dependent manner [14], as it is and the htt's direct binding to DNA [13]. For example, BDNF's transcription is downregulated in the presence of mhtt [17]. So at first level, when wild type htt is lost, the proper controls over the transcription factors with which it interacts are lost too. At second level, when mhtt is present, the regulation of the binding activities of the transcriptional factors that controls are not just lost but altered. The transcription stage is followed by the translation stage, where the protein exist as an unfolded polypeptide. To reach it's final three-dimensional structure and be biological functional, it must fold properly. At the time of folding, a protein follows a path that leads to its native state. Proteins with unstable or complicated conformation some times have difficulties in finding their native state and instead misfold in useless or even toxic forms. The information needed for a protein to fold properly is hidden in its amino acids sequence and the amino acid sequence is highly correlated with the first step of gene expression, as every codon of the DNA codes for a single amino acid. The diseases connected with protein misfolding are called proteopathies and many of them are neurodegenerative ones, making protein misfolding a major hallmark of neurodegeneration. For example, Parkinson's disease is a major neurodegenerative disease linked with the misfolding of α-Synuclein. As for Alzheimer's, many years of research have connected the development of the disease with the misfolding of amyloid beta (Aβ) and tau proteins. Huntington's disease is connected with the misfolding of huntingtin protein. As said above, huntingtin is thought as mutated when it contains an enlarged

polyQ tract. At that form, htt not only looses it's normal functions that are thought as neuroprotective, but also functions in a toxic way to the cells, like aggregating in the nucleus and interrupting the folding processes of other proteins.

3.2 Protein Aggregation

Problems in the translation stage of gene expression can lead to misfolded proteins. Misfolded proteins can form protein aggregations. If aggregations are contributing to neurodegeneration or are a defenensive mechanism of the cell against it is still under investigation, but it is a fact that they are a hallmark of neurodegeneration. Many diseases are connected with the formation of protein aggregates [18]. For example, Lewy bodies are protein aggregations composed mainly of the protein alpha-synuclein associated with other proteins such as tau, ubiquitin, neurofilament protein and others, and are present in patients of Parkinson's disease, dementia with Lewy bodies (DLB), Pick's disease and some tauopathies. As for Alzheimer's, amyloid plaques made mostly of beta-amyloid and neurofibrillary tangles with tau protein as a main component are the major hallmarks of the disease. At the case of HD, huntingtin misfolds and forms aggregations as its polyQ fragments that the UPS fails to dygest, group into tangled rigid bodies held together by hydrogen bonds. When found in the nucleus, those mhtt aggregates are thought to damage the cell membrane and entrap other proteins and key cell regulatory factors by its hydrophobic bonds, preventing important processes like the one of gene expression. When found in the axons and dendrites, they are thought to impair the axonal trafficking of the cell [19]. The first 17 amino acids of mhtt's chain consist the most phosphorilated region of the protein called T3 region and are considered as the most responsible for the intranuclear accumulation and forming of the aggregates, as T3 phosphorilation is associated with enchanced mhtt aggregation [10]. Generally, phosphorylation of htt is polyglutamine-length dependent and mhtt is less phosphorilated than wild-type htt at all known sites. Additionally, mhtt exhibits less palmitoylation also in an polyglutamine-length dependent manner while reduced palmitoylation often leads to the formation of aggregates [10]. More recent studies have shown that oligomers, molecules of intermediate relative molecular masses that consist a few monomer units, may be the most toxic kind of molecular complexes when talking about aggregations. In a very interesting study were the effects of the polyQ length on htt's aggregations were examined, in vitro and in vivo results showed clear polyglutamine-length dependent forming of htt oligomers, with oligomers detected even in htt fragments consisting 20 polyQ repeats [20]. Studies that have examined the correlations between the different disease's aggregation forming have shown connections between the proteins involved in each disease's cascade. For Alzheimer's, the huntingtin-associated protein 1(HAP1) that binds to htt in a polyglutamine-dependent manner, seems to regulate APP subcellular trafficking (APP is the amyloid precursor protein from which the Aβ peptide results) [21]. Also, interestingly, HD is the most recently described tauopathy as

in HD brains tau levels are increased and mutations of the HTT lead to pathogenic tau alterations [22]. Furthermore, htt and α-synuclein are two main proteins that misfold, form aggregates and are the main causes of two of the most major neurodegenerative diseases, HD and PD. The lots that they have in common made the research field study their possible connections with in vivo studies founding the two proteins coaggregating in mouse models of HD and suspecting that α-syn acts as an additional mediator of polyQ's toxicity [23].

3.3 Impaired Degradation Pathways

Many neurodegenerative diseases are linked with high levels of proteins which later form aggregations and inclusion bodies damaging the cell. But the levels of these proteins depend not only on their synthesis rates but on their degradation rates too, implicating an impairment in the degradation pathways of the cell at the development of neurodegeneration [24]. There are two main cell processes, by which the protein degradation is regulated in eukaryotic cells: the ubiquitin-proteasome pathway (UPP) and the autophagy-lysosomal pathway. Both of them are studied for changes on their natural functions in the development of diseases. Ubiquitin is a small regulatory protein that binds to substrate proteins. The type of binding and the number of ubiquitin molecules involved in it (monoubiquitination–polyubiquitination) may signal for their degradation by proteasome, alter their activity or cellular location, or regulate their protein interactions. The UPS system is responsible for the degradation of almost 80% of the intracellular proteins so its impairment is the first that comes in mind when talking about extracellular deposits of mutated proteins. The process of ubiquitination is carried out in three main steps: activation by an ubiquitin-activating enzyme (E1), conjucation by an ubiquitin-carrier protein (E2) and ligation by ubiquitin-protein ligases. Ubiquitin is coded from 4 genes (UBB, UBC, UBA52, RPS27A) and the brain has a fixed amount of it, which means that it has to be recycled to continue doing its job properly by a reversible process to ubiquitination. This recycling is done by a family of proteases called the deubiquitinating enzymes (DUBs), that can remove ubiquitin from its targets substrates. At the case of Alzheimer's, evidences show that Aβ's accumulations impairs the proteasome system. But also, mutations or dysfunctions of the UPS system components induce the Aβ accumulation, thus generating an endless loop that leads to neurodegeneration [25]. For example, the inhibition of the UPS downregulates the expression of Parkin (E3 ligase), which's high levels are connected with decreased levels of Aβ-42 accumulation and higher proteasome activity. A mutant form of ubiquitin (UBB+1) from the gene UBB is found in the brains of AD patients, other tauopathies and polyglutamine diseases like HD, and was reported to impair the proteasome activity in vitro. Also, the overexpression of UCHL1(a DUB enzyme) is connected with decreased levels of Aβ. The most interesting of that is that both Parkin and UCHL1 are connected with the cascade of Parkinson's Disease, while UBB+1 is regulated

from a protein-partner of htt. Parkin is a protein encoded by the PARK2 gene, which's mutations are linked with the development of a familial form of PD, the autosomal recessive juvenile Parkinson's disease. The UCHL1 is also known as PARK5 gene and its polymorphisms and mutations may reduce or increase the risk of developing PD. Generally for Parkinson's Disease, early studies showed alterations in the proteasomal functions in it's sporadic forms [26]. In HD, there are studies that investigate the impairment of the UPS system, it's capability of interacting with huntingtin and its capability of degradating it when it contains a large polyQ tract, but the results are contradictory. An ubiquitin-conjugating enzyme E2 K called also Hip2, a protein coded from the UBE2K gene and known for binding to the N-terminus of huntingtin, is found upregulated in patients of AD and is the suspicious of having a role in the neurotoxicity of UBB+1 by promoting its polyubiquitination that leads to proteasome inactivity [27]. The second important degradation pathway is the lysosome-autophagy pathway which is responsible mostly for the degradation of long-lived proteins. Autophagy is connected with neurodegeneration development as autophagic vacuoles are observed to accumulate in the brains of AD, HD, PD and other neurodegenerative diseases, but it is not clear if at these cases autophagy is just induced or its flux is impaired [28]. During autophagy, the material to be degraded called cargo is isolated from the rest of the cell sequestered in an double-membraned vesicle (autophagosome) that later fuses with a lysosome. There are two kinases connected with the regulation of autophagy, mTOR and AMPK. mTOR regulates autophagy by suppressing it when activated and promoting it when not activated and is thought to have a part in the development of neurodegeneration. First, it is suggested as contributing factor to the development of AD [29]. Generally, there are evidences of hyperactivity of mTOR in AD brains, something totally reasonable as Aβ is found in vitro to indirectly activate it. Interestingly, this effect seems to be dose-dependent as physiological levels of Aβ seem to increase mTOR signaling but significantly higher levels of Aβ decrease it. Also, there is a link between mTOR and tau protein too as mTOR is suspected to regulate tau concentrations by hyperactivating and increasing tau translation and phosphorylation. In Parkinson's disease the studies showed that inhibition of some of mTOR's functions by rapamycin protected in vivo and in vitro models of PD against neuron death [30]. As for HD, a study of the earlies 2000 showed an interaction between mhtt and mTOR in an polyglutamine-dependent manner when mTOR was found inactivated and sequestered in polyglutamine aggregates. Its inactivation induced autophagy and reduced the toxicity of polyglutamine expansions but the chronically impaired mTOR activity may ultimately lead to the brain atrophy seen in HD, as some of its functions are also connected with important processes of cell-growth [31]. A later study confirmed an impairment of the autophagy process in HD, when a difficulty of the autophagosomes to recognize and sequester their cargo-targets where observed [32]. Wild-type huntingtin was also connected with the selective-autophagy process by being observed to genetically interact with autophagy pathway components (p62, ULK1) in Drosophila [33]. ULK1 is a kinase that acts downstream for the mTOR complex and regulates negatively AMPK activity. The last study also showed that

htt-ULK1 and mTOR-ULK1 complexes are mutually exclusive suggesting that htt promotes activation of selective autophagy even in the presence of active mTOR by directly competing away ULK1 from the ULK-mTORC1 complex. P62 on the other hand is a receptor that mediate cargo recognition and htt is found to facilitate its efficiency. If the study where in HD autophagosomes are found failing to recognize and sequester their cargo is combined with the fact that wild-htt enhances this recognition, the loss of the wild-type htt or/and the mutation of the protein may contribute to the impairment of selective autophagy. Interestingly, recent evidences show connections and correlations between the two systems too as proteins normally degraded by the one pathway can be degraded by the other too under certain conditions. In vitro studies showed also a connection between the two systems as when the UPS system was overwhelmed or inhibited, the ubiquitinated misfolded proteins were transported to "aggresomes", cytoplasmic juxtanuclear structures that are thought to be mediated by autophagy, protecting the cell from misfolded-protein stress in times of UPS impairments. This connection is further verificated from other in vitro studies, where the impairment of the UPS system induced autophagy [34]. But what happens when the UPS pathway is impaired and the autophagy-pathway is unable to efficiently replace it?

3.4 Mitochondrial Dysfunction

Mitochondria are the main generator of cell's energy supply with the production of ATP. They also have other processes to maintain as mitochondrial biogenesis, fission–fusion, transport and degradation (mitophagy). As mitochondria are the organelles that are responsible for the production of the energy currency of the cell (ATP) and the regulation of the cellular metabolism, their dysfunctioning is thought as a main cause when talking about ATP-dependent systems's impairments (like the one of UPS), and many studies have marked them as treatment targets and tried to use their dysfunctioning as a starting point for the development of efficient disease's diagnosis tools [35, 36]. Mitochondria are showed to regulate calcium's (Ca^{2+}) homeostasis and having a role in pathways leading to cell death. AD is connected with altered Ca^{2+} homeostasis after Aβ was found to perturb neuronal Ca^{2+} homeostasis that lead to intracellular Ca^{2+} overload, increasing the vulnerability of neurons to excitotoxicity and contributing to the increased oxidative stress seen in AD [37]. Furthermore, studies have also connected elevated Ca^{2+} to neurofibrillary pathology. In PD, a heterogeneous mixture of small oligomers of α-synuclein can also lead to Ca^{2+} dysregulation [38], while furthermore the genes that with their mutations are connected with Parkinson's disease are connected with mitochondria too [39]. An important example is the Parkin protein encoded from the PARK2 gene, that is found to have a protective but not fully understood function in mitochondria. In HD, an early study tried to show a Ca^{2+} homeostasis disruption in an polyglutamine-dependent manner as an early effect of the disease [40]. Many studies after that had contradictory results upon the issue, but that isn't

the only way that htt seems to participate in processes connected with mitochondria. First, mhtt is showed in vitro and in vivo to inhibit protein import into mitochondria through its interactions [41]. At mitochondrial-biogenesis level, the transcriptional co-activator PGC-1α that seem to regulate the process is found downregulated in HD brains, with evidences showing that mhtt inhibits PGC-1α transcription modulating its expression [42]. For mitochondrial fission and fusion processes, Drp1 is thought as the main mediator. Studies showed that mhtt may have a direct interaction with Drp1, as evidences of in vitro studies suggested that their interaction increased Drp1 activity and may promote mitochondrial fragmentation in HD [43]. The affinity of mhtt to Drp1 is higher than that of wild-type htt. As for the transport, htt seem to form a scaffolding complex alongside with its binding partner HAP1, that enhances autophagosomal transport. The polyQ expansion in mhtt may on the other hand impair it, as its affinity for HAP1 is greater and disrupts the htt-HAP1 interaction [44]. This has an impact on autophagy and mitophagy too, as an impairment in autophagosome transport equals to downregulated and impaired autophagy. There is a study suggesting that mhtt compromises mitochondrial ATP production too, showing that htt is involved also in the regulation of the main task of mitochondria: cell's energy supply [45]. Furthermore, there are evidences that mHtt induces PTP opening in isolated mitochondria [46]. MPT (mitochondrial permeability transition pore) is a protein pore formed in the inner mitochondrial membrane under certain pathological conditions and its induction can lead to mitochondrial swelling and cell death. Among other pathological conditions that can cause MPT are also conditions where there is exposition in toxic amounts of Ca^{2+}. Mhtt seem to significantly decrease the necessary amount of it for triggering the MPT pore opening [46]. When there are MPT pores opened in a mitochondrion, ATP production reduces and calcium leaves the mitochondrion stressing other mitochondria nearby. Reactive oxygen species (ROS) and free radicals such as cytochrome c are produced and released triggering pathological processes that result from the failure of the antioxidant and antiexcitatory mechanisms of the cell, such as oxidative stress and excitotoxicity that are observed in many neurodegenerative diseases. Cytochrome c can also activate pro-apoptotic factors and trigger apoptosis. Interestingly, when it was observed that mhtt induces MPT opening, it was also observed that its N-terminus induced cytochrome-c release too in an MPT-dependent manner [46].

4 Discussion

Based on the above findings, the impairment of the one mechanism triggers the dysfunctioning of the other. Four of the most common hallmarks of neurodegeneration were discussed with emphasis on the interactions between huntingtin and the components of the mechanism that goes impaired in each case. Htt seems to participate in the regulation of almost every cell mechanism directly, or indirectly through interacting with their components. Most of these interactions are changed in an polyglytamine-dependent manner. The lost of the wild-type htt function is

one of the ways that htt can contribute to the development of neurodegeneration. But htt in most cases is not just lost but mutated and many of these functions are changed and altered depending on the length of the polyQ tract that the protein contains, leading to the second way in which htt may contribute to the development of neurodegeneration. As huntingtin is mostly thought as the protein that cause the development of HD, it is studied for its mutations under HD conditions. The patients of the disease determinded which length of the polyQ tract of htt keeps the protein to its wild-type form and under which it's capable of causing HD. Generally, someone threatened by HD must carry around ∼40 repeats long polyQ tract, but there have been also reported cases of patients of HD or HD-like diseases with very less than these repeats [47, 48]. If the protein looses and alters its interactions depending on the polyQ tract that it contains and with ∼40 repeats and above it's capable of causing a major neurodegenerative disease on its own, does it mean that these ∼40 repeats are the treshold beyond which all the protein's interactions are beginning to change? Or there are other tresholds of smaller tract sizes upon which some of htt's interactions alter, but they are not enough for causing a disease. And if this hypothesis is true and different polyQ tract sizes mean different alterations, can polyQ tract sizes that cause changes not able to cause a neurodegeneration by their own, act like a contributing factor to the development of other neurodegenerative diseases? Here it is proposed that there are tresholds upon the length of the polyQ tract of htt, minimum lengths above which the protein starts to alter each of its interactions and contribute to the development of neurodegeneration. This contribution may refer to the age of onset of each neurodegenerative disease, the progression of it and/or the severity of its symptoms. Altered interactions of htt isoforms with shorter polyQ tracts than the ones causing HD in patients with different neurodegenerative diseases can configurate htt as contributing factor to selective neurodegeneration.

References

1. MacDonald, M.E., C.M. Ambrose, M.P. Duyao, R.H. Myers, C. Lin, L. Srinidhi, and P.S. Harper. 1993. A Novel Gene Containing a Trinucleotide Repeat That is Expanded and Unstable on Huntington's Disease Chromosomes. *Cell* 72 (6): 971–983. doi:10.1016/0092-8674(93)90585-E.
2. Borrell-Pagès, M., D. Zala, S. Humbert, and F. Saudou. 2006. Huntington's Disease: From Huntingtin Function and Dysfunction to Therapeutic Strategies. *Cellular and Molecular Life Sciences* 63 (22): 2642–2660. doi:10.1007/s00018-006-6242-0.
3. Brandt, J., J. Brandt, F.W. Bylsma, R. Gross, O.C. Stine, N. Ranen, and C.A. Ross. 2006. The Association of CAG Repeat Length with Clinical Progression in Huntington Disease. *Neurology* 66: 1016–1020. doi:10.1212/01.wnl.0000204230.16619.d9.
4. Brinkman, R.R., M.M. Mezei, J. Theilmann, E. Almqvist, and M.R. Hayden. 1997. The Liklihood of Being Affected with Huntington Disease by a Particular Age for a Specific CAG Size. *American Journal of Human Genetics* 60 (5): 1202–1210.
5. Schulte, J., and J.T. Littleton. 2011. The Biological Function of the Huntingtin Protein and Its Relevance to Huntington's Disease Pathology. *Current Trends in Neurology* 5: 65–78.

6. Caviston, J.P., and E.L.F. Holzbaur. 2009. Huntingtin as an Essential Integrator of Intracellular Vesicular Trafficking. *Trends in Cell Biology* 19: 147–155. doi:10.1016/j.tcb.2009.01.005.
7. Nasir, J., S. Floresco, and J. O'Kusky. 1995. Targeted Disruption of the Huntington's Disease Gene Results in Embryonic Lethality and Behavioral and Morphological Changes in Heterozygotes. *Cell* 81: 811–823. doi:10.1016/0092-8674(95)90542-1.
8. Zheng, Z., A. Li, B.B. Holmes, J.C. Marasa, and M.I. Diamond. 2013. An N-terminal Nuclear Export Signal Regulates Trafficking and Aggregation of Huntingtin (Htt) Protein Exon 1. *Journal of Biological Chemistry 288* (9): 6063–6071. doi:10.1074/jbc.M112.413575.
9. Kaltenbach, L.S., E. Romero, R.R. Becklin, R. Chettier, R. Bell, A. Phansalkar, and R.E. Hughes. 2007. Huntingtin Interacting Proteins are Genetic Modifiers of Neurodegeneration. *PLoS Genetics* 3 (5): 689–708. doi:10.1371/journal.pgen.0030082.
10. Ehrnhoefer, D.E. 2011. Small Changes, Big Impact: Posttranslational Modifications and Function of Huntingtin in Huntington Disease. *The Neuroscientist* 17: 475–492. doi:10.1177/1073858410390378.
11. Zhao, Y., M. Zhu, Y. Yu, L. Qiu, Y. Zhang, and L. He. 2016. Brain REST/NRSF is not Only a Silent Repressor but Also an Active Protector. *Molecular Neurobiology* 54: 541–550. doi:10.1007/s12035-015-9658-4.
12. Lu, T., T. Lu, L. Aron, J. Zullo, Y. Pan, H. Kim, et al. 2014. REST and Stress Resistance in Ageing and Alzheimer's Disease. *Nature* 507 (7493): 448–454. doi:10.1038/nature13163.
13. Benn, C.L., T. Sun, G. Sadri-Vakili, et al. 2008. Huntingtin Modulates Transcription, Occupies Gene Promoters In Vivo and Binds Directly to DNA in a Polyglutamine-Dependent Manner. *Journal of Neuroscience* 28: 10720–10733. doi:10.1523/JNEUROSCI.2126-08.2008.
14. Conforti, P., C. Zuccato, N. Belyaev, P. Conforti, L. Ooi, M. Tartari, et al. 2007. Widespread Disruption of Repressor Element-1 Silencing Transcription Factor/Neuron-Restrictive Silencer Factor Occupancy at Its Target Genes in Huntington's Disease. *Journal of Neuroscience* 27: 6972–6983. doi:10.1523/JNEUROSCI.4278-06.2007.
15. Li, S., A.L. Cheng, H. Zhou, S. Lam, M. Rao, H. Li, and X. Li. 2002. Interaction of Huntington Disease Protein With Transcriptional Activator Sp1. *Molecular and Cellular Biology* 22 (5): 1277–1287. doi:10.1128/mcb.22.5.1277-1287.2002.
16. Natl, P., J.S. Steffan, E.E. Wanker, G. Bates, J.S. Steffan, A. Kazantsev, et al. 2000. The Huntington's Disease Protein Interacts with p53 and CREB-Binding Protein and Represses Transcription. *Proceedings of the National Academy of Sciences* 97: 6763–6768. doi:10.1073/pnas.100110097.
17. Zuccato, C., A. Ciammola, D. Rigamonti, B.R. Leavitt, D. Goffredo, L. Conti, and E. Cattaneo. 2001. Loss of Huntingtin-Mediated BDNF Gene Transcription in Huntington's Disease. *Science* 293: 493–498. doi:10.1126/science.1059581.
18. Ross, C.A., and M.A. Poirier. 2004. Protein Aggregation and Neurodegenerative Disease. *Nature Medicine* 10: S10–S17. doi:10.1038/nm1066.
19. Lee, W.M., M. Yoshihara, and J.T. Littleton. 2004. Cytoplasmic Aggregates Trap Polyglutamine-Containing Proteins and Block Axonal Transport in a Drosophila Model of Huntington's Disease. *Proceedings of the National Academy of Sciences* 101: 3224–3229. doi:10.1073/pnas.0400243101.
20. Legleiter, J., and L.M. Thompson. 2010. Mutant Huntingtin Fragments Form Oligomers in a Polyglutamine Length-Dependent Manner In Vitro and In Vivo. *Journal of Biological Chemistry* 285: 14777–14790. doi:10.1074/jbc.M109.093708.
21. Wang, T., and X. Zhou. 2012. Huntingtin Associated Protein 1 Regulates Trafficking of the Amyloid Precursor Protein and Modulates Amyloid Beta Levels in Neurons. *Journal of Neurochemistry* 122: 1010–1022. doi:10.1111/j.1471-4159.2012.07845.x.
22. Fernandez-Nogales, M., J.R. Cabrera, M. Santos-Galindo, J.J. Hoozemans, I. Ferrer, A.J. Rozemuller, et al. 2014. Huntington's Disease is a Four-Repeat Tauopathy with Tau Nuclear Rods. *Nature Medicine* 20 (8): 881–885. doi:10.1038/nm.3617.
23. Chanez-cardenas, M. E., E.Vazquez-contreras, and E. Ch. 2012. The Aggregation of Huntingtin and α-Synuclein. doi:10.1155/2012/606172.

24. Rubinsztein, D.C. 2006. The Roles of Intracellular Protein-Degradation Pathways in Neurodegeneration. *Nature* 443: 780–786. doi:10.1038/nature05291.
25. Tramutola, A., F. Di Domenico, E. Barone, M. Perluigi, and D.A. Butterfield. 2016. It Is All About (U) Biquitin: Role of Altered Ubiquitin-Proteasome System and UCHL1 in Alzheimer Disease. *Oxidative Medicine and Cellular Longevity* 2016: 1–12.
26. Isacson, O., K.S.P. Mcnaught, R. Belizaire, O. Isacson, P. Jenner, and C.W. Olanow. 2003. Altered Proteasomal Function in Sporadic Parkinson's Disease. *Experimental Neurology* 179: 38–46. doi:10.1006/exnr.2002.8050.
27. Ko, S., G.B. Kang, S.M. Song, J. Lee, D.Y. Shin, J. Yun, et al. 2010. Structural Basis of E2 – 25K/UBB+1 Interaction Leading to Proteasome Inhibition and Neurotoxicity. *Journal of Biological Chemistry* 285: 36070–36080. doi:10.1074/jbc.M110.145219.
28. Mccray, B.A., and J.P. Taylor. 2008. The Role of Autophagy in Age-Related Neurodegeneration. *Neurosignals* 16: 75–84. doi:10.1159/000109761.
29. Oddo, S. 2014. The Role of mTOR Signaling in Alzheimer Disease. *Frontiers in Bioscience* S4: 941–952.
30. Malagelada, C. 2010. Rapamycin Protects Against Neuron Death in In Vitro and In Vivo Models of Parkinson's Disease NIH Public Access. *Journal of Neuroscience* 30: 1166–1175. doi:10.1523/JNEUROSCI.3944-09.2010.
31. Ravikumar, B., C. Vacher, Z. Berger, D. Je, O. Lg, F. Scaravilli, et al. 2004. Inhibition of mTOR Induces Autophagy and Reduces Toxicity of Polyglutamine Expansions in Fly and Mouse Models of Huntington Disease. *Nature Genetics* 36: 585–595. doi:10.1038/ng1362.
32. Martinez-Vicente, M., et al. 2010. Cargo Recognition Failure is Responsible for Inefficient Autophagy in Huntington's Disease. *Nature Neuroscience* 13: 567–576. doi:10.1038/nn.2528.
33. Rui, Y., Z. Xu, B. Patel, Z. Chen, D. Chen, A. Tito, and G. David. 2015. Huntingtin Functions as a Scaffold for Selective Macroautophagy. *Nature Cell Biology* 17: 262–275. doi:10.1038/ncb3101.
34. Acta, B.B., P.K. Todd, and J.P. Taylor. 2008. Autophagy and the Ubiquitin-Proteasome System: Collaborators in Neuroprotection. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease* 1782: 691–699. doi:10.1016/j.bbadis.2008.10.002.
35. Alexiou, A., J. Rekkas, and P. Vlamos. 2011. Modeling the Mitochondrial Dysfunction in Neurogenerative Diseases Due to High H^+ Concentration. *Bioinformation* 6 (5): 5–7.
36. Alexiou, A. and Vlamos, P. 2012. Evidence for Early Identification of Alzheimer's Disease. arXiv:1209.4223v2 [q-bio.NC].
37. Neuroscience, C., Q.C. Rutgers, and P.T. Rutgers. 2016. Alterations in Mitochondrial Quality Control in Alzheimer's Disease. *Frontiers in Cellular Neuroscience* 10. doi:10.3389/fncel.2016.00024.
38. Danzer, K., K.M. Danzer, D. Haasen, A.R. Karow, S. Moussaud, M. Habeck, et al. 2007. Different Species of α-Synuclein Oligomers Induce Calcium Influx and Seeding. *Journal of Neuroscience* 27: 9220–9232. doi:10.1523/JNEUROSCI.2617-07.2007.
39. Von Stockum, S., et al. 2015. Mitochondrial Dynamics and Mitophagy in Parkinson's Disease: A Fly Point of View. *Neurobiology of Disease* 90: 58–67. doi:10.1016/j.nbd.2015.11.002.
40. Panov, A.V., J.R. Burke, J.T. Greenamyre, B.R. Leavitt, C.A. Gutekunst, W.J. Strittmatter, and M.R. Hayden. 2002. Early Mitochondrial Calcium Defects in Huntington's Disease Are a Direct Effect of Polyglutamines. *Nature Neuroscience* 5: 731–736. doi:10.1038/nn884.
41. Yano, H., S.V. Baranov, O.V. Baranova, J. Kim, Y. Pan, S. Yablonska, et al. 2014. Inhibition of Mitochondrial Protein Import by Mutant Huntingtin. *Nature Neuroscience* 17 (6): 822–831. doi:10.1038/nn.3721.
42. Cui, L., H. Jeong, F. Borovecki, C.N. Parkhurst, N. Tanese, and D. Krainc. 2006. Transcriptional Repression of PGC-1 a by Mutant Huntingtin Leads to Mitochondrial Dysfunction and Neurodegeneration. *Cell* 127: 59–69. doi:10.1016/j.cell.2006.09.015.
43. Guedes-Dias, P., et al. 2015. Mitochondrial Dynamics and Quality Control in Huntington's Disease. *Neurobiology of Disease* 90: 51–57. doi:10.1016/j.nbd.2015.09.008.

44. Wong, Y.C., and E.L.F. Holzbaur. 2014. The Regulation of Autophagosome Dynamics by Huntingtin and HAP1 is Disrupted by Expression of Mutant Huntingtin, Leading to Defective Cargo Degradation. *Journal of Neuroscience* 34 (4): 1293–1305. doi:10.1523/JNEUROSCI.1870-13.2014.
45. Milakovic, T., and G.V.W. Johnson. 2005. Mitochondrial Respiration and ATP Production are Significantly Impaired in Striatal Cells Expressing Mutant Huntingtin. *Journal of Biological Chemistry* 280 (35): 30773–30782. doi:10.1074/jbc.M504749200.
46. Choo, Y.S., G.V.W. Johnson, M. Macdonald, P.J. Detloff, and M. Lesort. 2004. Mutant Huntingtin Directly Increases Susceptibility of Mitochondria to the Calcium-Induced Permeability Transition and Cytochrome c Release. *Human Molecular Genetics* 13 (14): 1407–1420. doi:10.1093/hmg/ddh162.
47. Powell, S.Z., J. Jankovic, C. Kenney, S. Powell, and J. Jankovic. 2007. Autopsy-Proven Huntington's Disease with 29 CAG Repeats Autopsy-Proven Huntington Disease with 29 Trinucleotide Repeats. *Movement Disorders* 22: 127–130. doi:10.1002/mds.21195.
48. Academisch, B., and M. Centrum. 2010. Late-Onset Huntington Disease with Intermediate CAG Repeats: True or False. *Journal of Neurology, Neurosurgery, and Psychiatry* 81: 228–230. doi:10.1136/jnnp.2008.170902.

The Effects of Anodal Transcranial Direct Current Stimulation on Working Memory

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Abstract The aim of this paper was to review the effects of anodal transcranial direct current stimulation (tdcs) on working memory in healthy population. Ten studies were identified involving 319 subjects. Working memory performance was measured using cognitive tasks such as the *n*-back task, digit span forward or digit span backwards test, Sternberg WM task, the Pacet Auditory Serial Addition Task (PASAT) and the Pacet Auditory Serial Subtraction Task (PASST), verbal and visuospatial tasks and the Operation Span (OSpan) task. All studies showed that anodal tdcs co-administered with cognitive tasks can significantly enhance working memory performance by inducing cortical excitability. Further research should be made towards older population as aging is accompanied with a decline in cognitive abilities and patients with memory deficits to demonstrate whether tdcs can be used as an interventional mean in clinical context as well.

Keywords Anodal transcranial direct current stimulation • Dorsolateral prefrontal cortex (DLPFC) • Working memory

1 Introduction

Normal ageing is accompanied by a decline in cognitive and motor abilities which result in the decrease of dexterity skills, speed and accuracy [1, 2]. Working memory (WM) is a cognitive system that is able to store, process and manipulate information for transient use [3]. According to Neuroimaging studies, during working memory tasks different areas of the prefrontal cortex are activating in young and older adults indicating that each group performs the tasks differently [1]. Older adults use compensatory mechanisms in order to perform the same as the younger adults in a motor task however they retain their ability to learn through practice [1, 2]. Recent

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studies have shown that non-invasive brain techniques such as the transcranial direct current stimulation (tdcs) can increase cortical excitability leading to the enhancement of working memory and motor learning in healthy subjects [4–6]. In most of the studies the dorsolateral prefrontal cortex (DLPFC), which includes Brodmann Areas 46 and 9, is stimulated because it has been shown to be highly involved in WM processing [7, 8].

2 Search Strategy and Selection Criteria

Pubmed, Science Direct, Springer and Sage databases were searched from June 2016 to August 2016 using the terms "working memory", "anodal transcranial direct current stimulation", "dorsolateral prefrontal cortex", "healthy population". The reference lists of systematic review articles and meta-analyses were scanned for any additional references missed from the above databases' search. The studies selected were examining only healthy population and were conducted the last decade. Only English literature was included for the current review.

3 Studies' Findings

Andrews et al. [7] investigated the relationship between cognitive activity and anodal tdcs on the left DLPFC (areas 9, 46) to enhance working memory in 10 participants aged 20–51 years old. All participants took part in the following three conditions at intervals of 1 week to prevent any carry-over effects from tdcs: Active or sham tdcs applied for 10 min during an *n*-back task or active tdcs applied for 10 min at rest. Before and after each condition a digit span forward & digit span backwards test was administered verbally by the experimenter. Their objective was to explore whether tdcs applied to the left DLPFC during the *n*-back task would improve performance on a digit span forward or digit span backwards test, to a greater extent than either tdcs or cognitive activity alone. Their results showed that active tdcs co-administered with the *n*-back task enhanced the performance on digit span forward, compared with the two other conditions. However, no significant result was found regarding the digit span backwards test. This study suggested that there may be potential benefit from the use of adjunctive cognitive activity to enhance the effects of tdcs.

Fregni et al. [8] examined the effect of anodal tdcs (active or sham) on working memory by stimulating the left DLPFC during a 3-back memory task in 15 subjects aged 19–22 years old. Seven participants also undertook a session of anodal tdcs on M1 and cathodal tdcs on the left DLPFC. Their study concluded that tdcs co-administered with a 3-back working memory task had significant results ($p = 0.0042$) comparing to sham stimulation, tdcs on primary motor cortex (M1) or cathodal tdcs.

Giglia et al. [9] compared anodal tdcs stimulation on right and left DLPFC to investigate any different effects on cognitive performance in ten right-handed participants. All participants undertook a sham condition as control. They concluded that only anodal tdcs on the right DLPFC ($p < 0.01$) was able to enhance performance on the memory guided visuospatial task compared to the other two conditions.

Hoy et al. [10] found that 1 mA of anodal tdcs produced the most significant effects ($p = 0.038$) compared to higher current of 2 mA or sham tdcs on the left DLPFC. Eighteen subjects were examined in all three conditions over a period of 3 weeks.

Mulquiney et al. [11] investigated whether transcranial random noise stimulation (tRNS) on left DLPFC can significantly enhance WM performance compared to anodal tdcs or sham stimulation. Ten subjects were examined in three conditions (tRNS or sham tdcs whilst performing the Sternberg WM task or anodal tdcs) at intervals of minimum 1 week. All participants performed the *n*-back task before and after each intervention to assess speed and accuracy. Results showed that only anodal tdcs significantly improved the speed of performance on 2-back memory task.

Pope et al. [12], following previous studies that showed improvement in cognitive performance due to anodal tdcs stimulation, aimed to determine whether anodal tdcs on the left DLPFC could similarly enhance WM performance when the cognitive task required is on higher demand. Sixty-three participants were separated in three equal groups receiving 20 min of anodal, cathodal or sham tdcs. Accuracy, latency and variability of correct verbal responses were assessed using the Pacet Auditory Serial Addition Task (PASAT) and the Pacet Auditory Serial Subtraction Task (PASST) before and after each intervention. Significant effects were found only on PASST after the anodal tdcs concluding that anodal tdcs can selectively improve difficult cognitive performance.

Stephens and Berryhill [13] examined cognitive performance in 90 older adults paired with 15 min of 1, 2 mA anodal tdcs or sham tdcs on the right prefrontal cortex (PFC). Their results found that 2 mA anodal tdcs induced significantly greater long-lasting results after 1 month without stimulation.

Zaehle et al. [14] investigated the effect of tdcs on WM performance and neural activity using a letter 2-back task after sham, anodal and cathodal stimulation on the left DLPFC. Their study showed that tdcs can change WM performance by modulating the underlying neural oscillation.

Jones et al. [15] tested 72 participants in 10 sessions of sham or anodal tdcs along with verbal and visuospatial tasks and the Operation Span (OSpan) task. All participants undertook a follow up testing after 1 month. Results showed that all subjects improved after WM tasks however only the participants who received anodal tdcs maintain significant effects after 1 month follow up.

Ohn et al. [16] investigated the effects of 1 mA anodal or sham tdcs on 15 young healthy participants. Their results showed that 1 mA of tdcs enhanced WM performance and the effects lasted for 30 min after the end of stimulation (Table 1).

Table 1 Studies of tdcs stimulation and working memory performance

Author	Type of study	Participants (N)	Anode electrode position	Duration	Amplitude/Electrode	Results
Andrews et al. [7]	Cross-over study	10 (mean age 28.1 ± 8.72)	F3 left DLPFC	10 min	1 mA: 35 cm^2	Active tdcs co-administered with the n-back task enhanced the performance on digit span forward, compared with the two other conditions
Fregni et al. [8]	Randomized controlled trial	15 (mean age 20.2)	F3 left DLPFC	10 min	1 mA: 35 cm^2	Tdcs on DLPFC co-administered with the 3-back task showed significant results comparing to sham and tdcs on Ml or cathodal tdcs
Giglia et al. [9]	Quasi experimental study	10 (mean age 27 ± 2.3)	F3 left DLPFC or F4 right DLPFC	10 min	1 mA: 35 cm^2	Only anodal tdcs on the right DLPFC was able to enhance performance on the memory guided visuospatial task compared to the other two conditions
Hoy et al. [10]	Quasi experimental study	18 (mean age 24.71 ± 6.97)	F3 left DLPFC	20 min	1 or 2 mA: 35 cm^2	l mA of anodal tdcs produced the most significant effects compared to the other conditions
Mulquiney et al. [11]	Cross-over study	10 (mean age 29.5)	F3 left DLPFC	10 min	1 mA: 35 cm^2	Only anodal tdcs significantly improved the speed of performance on 2-back memory task
Pope et al. [12]	Randomized controlled trial	63 (mean age 22 ± 2.3)	F3 left DLPFC	20 min	2 mA: 25 cm^2	Significant effects were found only on PASST after the anodal tdcs concluding that anodal tdcs can selectively improve difficult cognitive performance
Stephens and Berryhill [13]	Randomized controlled trial	90 (mean age 69)	F4 right DLPFC	15 min	1 mA or 2 mA: 35 cm^2	2 mA anodal tdcs induced significantly greater long-lasting results after 1 month without stimulation
Zaehle et al. [14]	Randomized controlled trial	16 (mean age 25 ± 2)	F3 left DLPFC	15 min	1 mA: 35 cm^2	Tdcs can change WM performance by modulating the underlying neural oscillation
Jones et al. [15]	Randomized controlled trial	72 (mean age 64.38 ± 5.08)	F4 right DLPFC	10 min	1.5 mA: 35 cm^2	All subjects improved after WM tasks however only the participants who received anodal tdcs maintain significant effects after 1 month follow up
Ohn et al. [16]	Cross-over study	15 (mean age 27.7 ± 6.97)	F3 left DLPFC	30 min	1 mA: 25 cm^2	1 mA of anodal tdcs produced the most significant effects compared to sham tdcs and the effects lasted for 30 min after the end of stimulation

4 Discussion

Working memory is associated with complex cognitive tasks such as learning and reasoning that tend to decline while ageing [1, 17]. The aim of this review was to investigate whether tdcs stimulation whilst administered with a cognitive task can induce cortical excitability in the DLPFC and enhance WM performance in healthy subjects.

All studies showed that anodal tdcs co-administered with cognitive tasks can significantly enhance working memory performance. Six studies [7, 8, 10, 11, 13, 14] used the *n*-back task which has been found to activate the DLPFC [14]. The rest of the studies [9, 12, 15] used other verbal and visuospatial tasks such as the PASAT, the PASST and the OSpan task. Their results demonstrated that only anodal tdcs, compared to the other conditions tested, produced significant results.

The findings suggested that anodal tdcs combined with a cognitive task can modulate working memory performance implicating that tdcs can be used as a therapeutic mean in clinical context. Two studies [18, 19] have investigated the effect of tdcs stimulation on patients. Ulam et al. [19] demonstrated significantly positive effects of tdcs on WM of patients with Traumatic Brain Injury (TBI). Boggio et al. [18] examined tdcs stimulation on patients with Parkinson's disease (PD) and their results highlighted the important effects of 2 mA anodal tdcs on WM performance as indexed by task accuracy. Boggio and colleagues concluded that tdcs can induce positive effects on WM of PD patients but it depends on the intensity and site of stimulation.

There is also evidence of some non significant effects of tdcs on WM in three studies [3, 11, 12]. Berryhill and Jones tested 25 older subjects (mean age 63.7) in WM performance according to their educational background. The less educated group presented no benefit from tdcs stimulation on cognitive tasks contrary to the educated group that was uniformly affected. Berryhill and Jones hypothesized that possibly the educated group employed different strategy on WM tasks by recruiting better structures of PFC. Their results are supported from previous studies [20, 21] that demonstrated that expert participants showed greater activation of the PFC and performed better on cognitive tasks compared to novice participants. Mulquiney et al. [11] and Pope et al. [12] concluded that tdcs does provide evidence of cortical excitability but only in some aspects of DLPFC and can selectively enhance cognitive performance in difficult cognitive tasks.

Eight studies [7–12, 14, 16] examined only young healthy participants. Six of these studies set the tdcs current at 1 mA except from Pope and colleagues (2 mA) and Hoy and colleagues (1 and 2 mA). Hoy and colleagues, found the most significant effects after 1 mA of tdcs which contradicts their hypothesis that higher current would have greater improvement on WM performance. Stephens and Berryhill [13] and Jones et al. [15] included older participants and the current was set at 2 and 1.5 mA respectively showing that possibly older adults need higher intensity for long lasting effects. Although the results of the studies reviewed were promising, further research should be made towards older population as aging is accompanied

with a decline in cognitive abilities. Future studies, may also compare young and old participants on their performance in a WM task, to demonstrate whether tdcs will boost the performance of older participants compared to the young group.

5 Conclusion

There are several evidence that tdcs can induce cortical excitability leading to better cognitive performance. This indicates the importance for further research to demonstrate whether tdcs can be used as an interventional mean in patients with memory deficits. Future studies should focus on including larger sample size and exploring possible differences between young and old participants, to demonstrate whether it is tdcs that boosts cognitive performance or practice of a cognitive task itself. Also, more studies are needed to investigate the variations of current density, the duration of stimulation and the number of sessions for long-lasting effects.

References

1. Glisky, E.L. 2007. Changes in Cognitive Function in Human Aging. In *Brain Aging: Models, Methods, and Mechanisms*, ed. D.R. Riddle. Boca Raton, FL: CRC Press.
2. Newell, K.M., D.E. Vaillancourt, and J.J. Sosnoff. 2006. Aging, Complexity and Motor Performance. In *Handbook of the Psychology of Aging*, ed. J.E. Birren and K.W. Schaie, 6th ed. New York: Elsevier Academic Press.
3. Berryhill, M.E., and K.T. Jones. 2012. tDCS Selectively Improves Working Memory in Older Adults with More Education. *Neuroscience Letters* 521: 148–151.
4. Hummel, F.C., K. Heise, P. Celnik, A. Floel, C. Gerloff, and L.G. Cohen. 2010. Facilitating Skilled Right Hand Motor Function in Older Subjects by Anodal Polarization Over the Left Primary Motor Cortex. *Neurobiology of Aging* 31: 2160–2168.
5. Oliviero, A., P. Profice, P.A. Tonali, F. Pilato, E. Saturno, M. Dileone, et al. 2006. Effects of Aging on Motor Cortex Excitability. *Neuroscience Research* 55 (1): 74–77.
6. Wu, T., Y. Zang, L. Wang, X. Long, M. Hallett, Y. Chen, et al. 2007. Age Influence on Functional Connectivity of the Motor Network in the Resting State. *Neuroscience Letters* 422: 164–168.
7. Andrews, S.C., K.E. Hoy, P.G. Enticott, Z.J. Daskalakis, and P.B. Fitzgerald. 2011. Improving Working Memory: The Effect of Combining Cognitive Activity and Anodal Transcranial Direct Current Stimulation to the Left Dorsolateral Prefrontal Cortex. *Brain Stimulation* 4: 84–89.
8. Fregni, F., P.S. Boggio, M. Nitsche, F. Bermpohl, A. Antal, E. Feredoes, M.A. Marcolin, S.P. Rigonatti, M.T.A. Silva, W. Paulus, and A. Pascual-Leone. 2005. Anodal Transcranial Direct Current Stimulation of the Prefrontal Cortex Enhances Working Memory. *Experimental Brain Research* 166: 23–30.
9. Giglia, G., F. Brighina, S. Rizzo, et al. 2014. Anodal Transcranial Direct Current Stimulation of the Right Dorsolateral Prefrontal Cortex Enhances Memory-Guided Responses in a Visuospatial Working Memory Task. *Functional Neurology* 29 (3): 189–193.
10. Hoy, K.E., M.R. Emonson, S.L. Arnold, R.H. Thomson, Z.J. Daskalakis, and P.B. Fitzgerald. 2013. Testing the Limits: Investigating the Effect of tDCS Dose on Working Memory Enhancement in Healthy Controls. *Neuropsychologia* 51: 1777–1784.

11. Mulquiney, P.G., K.E. Hoy, Z.J. Daskalakis, and P.B. Fitzgerald. 2011. Improving Working Memory: Exploring the Effect of Transcranial Random Noise Stimulation and Transcranial Direct Current Stimulation on the Dorsolateral Prefrontal Cortex. *Clinical Neurophysiology* 122: 2384–2389.
12. Pope, P.A., J.W. Brenton, and R.C. Miall. 2015. Task-Specific Facilitation of Cognition by Anodal Transcranial Direct Current Stimulation of the Prefrontal Cortex. *Cerebral Cortex* 25: 4551–4558.
13. Stephens, J.A., and M.E. Berryhill. 2016. Older Adults Improve on Everyday Tasks After Working Memory Training and Neurostimulation. *Brain Stimulation* 9: 553–559.
14. Zaehle, T., P. Sandmann, J.D. Thorne, et al. 2011. Transcranial Direct Current Stimulation of the Prefrontal Cortex Modulates Working Memory Performance: Combined Behavioural and Electrophysiological Evidence. *BMC Neuroscience* 12 (2): 1–2.
15. Jones, K.T., J.A. Stephens, M. Alam, M. Bikson, and M.E. Berryhill. 2015. Longitudinal Neurostimulation in Older Adults Improves Working Memory. *PLoS ONE* 10 (4): 1–18.
16. Ohn, S.H., C.I. Park, W.K. Yoo, et al. 2008. Time-Dependent Effect of Transcranial Direct Current Stimulation on the Enhancement of Working Memory. *NeuroReport* 19: 43–47.
17. Baddeley, A. 1992. Working Memory. *Science* 255: 556.
18. Boggio, P.S., R. Ferrucci, S.P. Rigonatti, et al. 2006. Effects of Transcranial Direct Current Stimulation on Working Memory in Patients with Parkinson's Disease. *Journal of the Neurological Sciences* 249: 31–38.
19. Ulam, F., C. Shelton, L. Richards, et al. 2015. Cumulative Effects of Transcranial Direct Current Stimulation on EEG Oscillations and Attention/Working Memory During Subacute Neurorehabilitation of Traumatic Brain Injury. *Clinical Neurophysiology* 126: 486–496.
20. Grabner, R.H., A.C. Neubauer, and E. Stern. 2006. Superior Performance and Neural Efficiency: The Impact of Intelligence and Expertise. *Brain Research Bulletin* 4: 422–439.
21. Solso, R.L. 2001. Brain Activities in a Skilled Versus a Novice Artist: An fMRI Study. *Leonardo* 34 (1): 31–34.

Bioimpedance Measurements in Adolescents with Polycystic Ovary Syndrome: A Pilot Study

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Abstract Limited data are available on the body composition of adolescent women with polycystic ovary syndrome (PCOS). The aim of this study was to examine differences in body composition indices of metabolism, homeostasis and inflammation, between Greek adolescent females suffering from PCOS and age- and body mass index (BMI)-matched non-PCOS controls. Thirteen PCOS patients and nine non-PCOS controls, aged 13–24 years participated in this cross-sectional study. Study participants underwent assessment by a novel dual frequency bioimpedance device (BIA-ACC). The following body composition indices were measured in each adolescent: extra cellular water (ECW) as inflammation marker, total body water (TBW) as homeostasis marker, extracellular mass to body cell mass ratio (ECM/BCM), fat mass (FM), fat-free mass (FFM) and intracellular water (ICW) as markers of body mass composition and metabolism. Non-linear analysis showed no statistically significant differences in the body composition characteristics between PCOS patients and controls. Further studies with larger sample sizes are needed to confirm whether adolescents with PCOS actually have similar body composition profile with their non-PCOS peers.

Keywords Polycystic ovary syndrome • Adolescents • Bioimpedance • Body composition • Inflammation • Homeostasis

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1 Introduction

Polycystic ovary syndrome (PCOS) is a heterogeneous endocrine disorder, affecting up to 15% of reproductive age women [1, 2]. It is a multifactorial disorder that may manifest early in adolescence. Genetic as well as environmental factors are implicated in its pathogenesis [3]. Obesity and excess abdominal adipose tissue exacerbate metabolic and endocrine aberrations that are central in the pathogenesis of PCOS. Although, it is considered a primarily gynecological problem, it is, in fact, a multisystem disorder with, not only reproductive, but also severe metabolic implications; women with PCOS are at increased risk for developing infertility, insulin resistance, metabolic syndrome and type II diabetes mellitus [1, 4].

Differences in hormonal and metabolic profiles are observed in patients with PCOS according to their body mass index (BMI) [5]. However, BMI is an inadequate means of body composition assessment, as it measures the degree of excess body weight, but does not differentiate fat mass and fat free mass (muscle mass or bone mass). Considering the implications of PCOS, studying body composition would give a deeper insight of the physiology of the disease, as it seems to contribute to the reproductive and metabolic dysfunction of the syndrome.

Body composition can be defined by the added proportions of muscle, fat and bone masses and water. Bioelectrical impedance analysis (BIA) is a safe, quick, inexpensive, reproducible and non-invasive method of body composition assessment [6]. The technique has been compared with and validated against traditional measures of body composition analysis in various patient populations [7–15]. BIA measures the varying bioelectric resistance and reactance of different body tissues by recording a voltage drop of an applied low-voltage alternating current through the body. Lean tissue and fluids containing electrolytes conduct the current and cell membranes serve as capacitors and account for capacitive resistance. Fat and bone are poor conductors [16]. Resistance and reactance are used with height, weight, age and gender in a number of multiple regression relationships to predict body composition compartments such as fat-free mass, lean body mass, extracellular mass, body cell mass [16], mineral distribution between different body compartments and the percentage of body fat [17].

Limited data are available on body composition assessed by bioelectrical impedance in adolescent women with PCOS. In the current study, we aimed to evaluate the differences in body composition between Greek adolescent females suffering from PCOS and non-PCOS controls.

2 Methods

2.1 Population

This was a cross-sectional study of Greek adolescents suffering from PCOS and age- and BMI-matched non-PCOS controls. Adolescent females, aged 13–24 years, with PCOS as well as controls who presented for annual check up, were recruited from outpatients attending the Center for Adolescent Medicine and UNESCO Chair on Adolescent Health Care of the First Department of Pediatrics, at the "Aghia Sophia" Children's Hospital, in Athens, Greece, over a period of 1 year, from January 2014 to December 2014.

Participants underwent a detailed physical, biochemical, hormonal and sonographic assessment. A history of physical exercise (hours per week) for each adolescent was also recorded. Polycystic ovary syndrome was diagnosed according to the Rotterdam definition, in the presence of at least two of the following three criteria (a) chronic anovulation, (b) clinical and/or biochemical hyperandrogenism, and (c) polycystic ovaries on ultrasound, after exclusion of related disorders. At least 2 years should have elapsed from menarche in order to participate in the study. Exclusion criteria included pregnancy, other genetic or endocrine disorder, psychiatric illness and the presence of metallic dental braces or metallic orthopaedic implants.

The study was approved by the institutional ethics committee. Adolescent girls and their parents were informed about the nature of the study and written informed consent was obtained prior to participation.

2.2 Bioimpedance Measurements

Each study participant was placed in supine position on a flat, electrically non-conductive surface, away from any metallic element. Two electrodes were placed on the dorsal surface of the right hand and foot at the third metacarpophalangeal and metatarsophalangeal joints respectively; one electrode on the dorsal surface of the right wrist, medially between the distal prominences of the radius and the ulna; and one electrode on the right ankle, between the medial and lateral malleoli.

Body composition analysis of study participants was performed by a novel dual frequency bioimpedance BIA-ACC device (BIOTEKNA, Inc., Venice, Italy) at the Clinical Research Center of the Biomedical Research Foundation of the Academy of Athens. This device applies alternating currents, using a bi-frequency (50 and 1.5 kHz) method, to measure body composition based on a multi-compartment model. A three compartment model (body mass = water + fat mass + residual) [18] of the body composition type II formula for research purposes [19, 20] was

used to assess body composition in each study participant. This model was preferred because it is suitable for adolescents [21, 22], and is robust to inter-individual variability in fat-free mass [22, 23].

The following body composition characteristics were assessed: extra cellular water (ECW) as inflammation marker, total body water (TBW) as homeostasis biomarker, extra cellular mass to body cell mass ratio (ECM/BCM), fat mass (FM), fat-free mass (FFM) and intra cellular water (ICW) as markers of body mass composition and metabolism. All measurements were predicted by the equations suggested by Bray and Ramirez and validated by Tsigos and Wan studies [6, 24–26].

3 Statistical Analysis

Albeit Shapiro Wilks showed normality of all distributions, however as the size of both groups was small, an assumption was made that the distributions were unknown. Thus, differences between the two groups were assessed with the Mann-Whitney non-parametric tests and the exact U value of the Mann-Whitney test as well as the resulted exact p-value were calculated. All p-values were two-sided and a value of $p < 0.05$ was considered statistically significant. Statistical analysis was performed with SPSS17 software. Results are presented as mean value ± standard deviation (SD).

4 Results

Thirteen PCOS patients (age range 13–24 years, BMI range 19.5–32.1 kg/m^2) and nine non-PCOS controls (age range 13–22 years, BMI range 21.5–35.7 kg/m^2), participated in the study. All participants except one reported physical exercise performance of less than 9 h per week. Table 1 presents the anthropometric and body composition characteristics of the PCOS and non-PCOS groups. Participants' bioimpedance measurements for both groups are also shown in Fig. 1. There were no statistically significant differences in ECW, ICW, TBW, FM, FFM and ECM/BCM between PCOS and non-PCOS adolescents (Table 1).

5 Discussion

Our data indicated that PCOS girls had similar body composition profile for adiposity, free-fat mass, total, intra- and extra-cellular body water, ECM/BCM ratio, with non-PCOS controls.

Table 1 Participants' anthropometric and body composition characteristics

Variable	PCOS n = 13	Non-PCOS n = 9	U[a]	p value
Age (years)	16.6 ± 2.99	16.9 ± 2.71	55	0.813
BMI (kg/m^2)	25.7 ± 3.9	25.4 ± 4.71	42.3	0.265
ECW (lt)	13.95 ± 1.30	16.48 ± 6.87	29	0.775
ICW (lt)	18.92 ± 6.46	19.95 ± 6.03	26	0.913
TBW (lt)	32.66 ± 7.56	34.86 ± 8.17	38	0.966
FM (kg)	34.73 ± 47.78	33.64 ± 16.34	22.5	0.301
FFM (kg)	47.08 ± 8.79	51.26 ± 7.76	33	0.639
ECM/BCM	1.148 ± 0.42	1.101 ± 0.24	36	0.831

PCOS polycystic ovary syndrome, *BMI* body mass index, *ECW* extra cellular water, *ICW* intra cellular water, *TBW* total body water, *FM* fat mass, *FFM* fat-free mass, *ECM/BCM* extra cellular mass to body cell mass ratio

[a]U value of the Mann-Whitney test

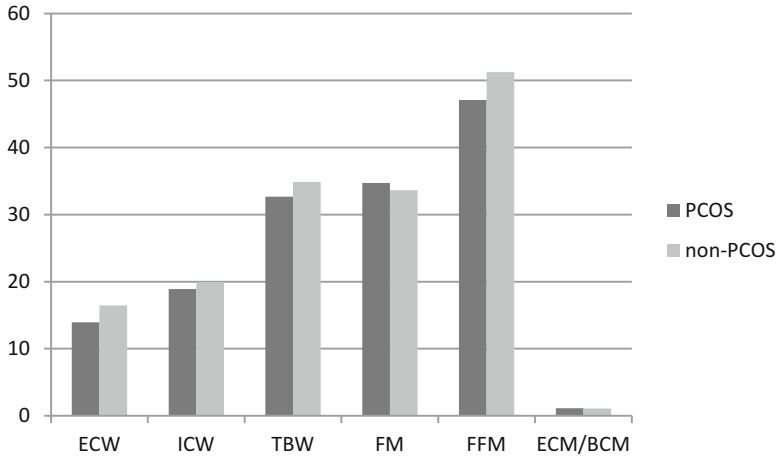

Fig. 1 Bioimpedance measurements (results are presented as mean values) in PCOS and non-PCOS adolescents. *PCOS* polycystic ovary syndrome, *ECW* extra cellular water, *ICW* intra cellular water, *TBW* total body water, *FM* fat mass, *FFM* fat-free mass, *ECM/BCM* extra cellular mass to body cell mass ratio

In the literature, results are conflicting regarding the body composition of women with PCOS, of which some support an increase in body fat [27, 28] and others [29–31] suggest an absence of significant alterations in the body composition profile in women with PCOS compared to controls. More specifically, Aydin et al., found that lean young women with PCOS had similar body composition assessed by BIA, with healthy women [30]. In the study of Churchill et al., adult PCOS women assessed by BIA were found to be comparable for percent body fat to control women, whereas, Attlee et al., also using BIA analysis, found that fat-free mass, percent body fat and visceral fat area did not differ significantly between adolescent and young women with PCOS and non-PCOS controls [29, 31]. Three studies [32–34] that used MRI to

assess body fat distribution in lean, normal weight and obese adults, failed to report preferential visceral fat accumulation in PCOS cases against controls. Moreover, the results of Dolfing et al., demonstrated that the content of visceral deposited fat was lower in lean PCOS cases than controls.

In agreement with most studies, our pilot study demonstrated that fat and fat-free mass, as assessed by BIA, were similar between PCOS adolescents and non-PCOS controls. In addition, we demonstrated absence of statistically significant differences in the other metabolic, homeostatic and inflammation markers ECW, ICW, TBW and ECM/BCM, as well, between patients and controls. Increasing evidence suggests that there is an important connection and interaction between pro-inflammatory pathways, hyperinsulinemia, androgen excess and adipose tissue hypertrophy and dysfunction in PCOS [35]. In our study, the lack of differences in the body composition parameters can be explained by the fact that the origin of metabolic abnormalities and inflammation in PCOS has been ascribed to excess abdominal adiposity or frank obesity [36]. Obesity along with age are considered better predictors of metabolic derangement in PCOS than the presence of the syndrome per se [37]. In the present study, PCOS and non-PCOS groups were controlled for BMI and age, obviously limiting the confounding of age and adiposity in body composition assessment.

It is well established that the syndrome is associated with lifestyle [38, 39]. Physical exercise for less than 9 h per week predicts no statistical differences in intracellular (ICW) and extracellular body water (ECW) in adolescents [40]. This is in accordance with our findings, as all participants except one, exercised for less than 9 h weekly.

ECM/BCM ratio is a sensitive marker of malnutrition [41] that has been associated with nutritional indices (i.e. serum albumin levels), pathological entities and/or mortality [42]. In the present study, the ECM/BCM ratio did not differ between study groups, supporting that our findings were free of nutrition confounding.

Limitations of the study include the small sample size, the lack of other hormonal data as well as, that BIA measurements were performed on random days of the adolescents' menstrual cycles. Although ICW, FM and FFM are independent of the phase of the menstrual cycle [43], TBW and ECW may exhibit some variation. A strength of the study is that it involved adolescents, and PCOS has not been studied extensively in this age group.

In conclusion, this study using a simple, non-invasive method demonstrated that adolescent girls with PCOS had similar body composition with non-PCOS controls. Further studies with larger sample sizes and/or more bioimpendance indices are needed to confirm whether adolescents with PCOS actually share similar body composition characteristics with their non-PCOS peers.

References

1. Fauser, B.C., B.C. Tarlatzis, R.W. Rebar, R.S. Legro, A.H. Balen, R. Lobo, E. Carmina, J. Chang, B.O. Yildiz, J.S. Laven, J. Boivin, F. Petraglia, C.N. Wijeyeratne, R.J. Norman, A. Dunaif, S. Franks, R.A. Wild, D. Dumesic, and K. Barnhart. 2012. Consensus on Women's Health Aspects of Polycystic Ovary Syndrome (PCOS): The Amsterdam Eshre/Asrm-Sponsored 3rd PCOS Consensus Workshop Group. *Fertility and Sterility* 97: 28–38.e25.
2. Sirmans, S.M., and K.A. Pate. 2013. Epidemiology, Diagnosis, and Management of Polycystic Ovary Syndrome. *Journal of Clinical Epidemiology* 6: 1–13.
3. Diamanti-Kandarakis, E. 2008. Polycystic Ovarian Syndrome: Pathophysiology, Molecular Aspects and Clinical Implications. *Expert Reviews in Molecular Medicine* 10: e3.
4. Lord, J., R. Thomas, B. Fox, U. Acharya, and T. Wilkin. 2006. The Central Issue? Visceral Fat Mass is a Good Marker of Insulin Resistance and Metabolic Disturbance in Women with Polycystic Ovary Syndrome. *BJOG* 113: 1203–1209.
5. Komarowska, H., A. Stangierski, I. Warmuz-Stangierska, M. Lodyga, K. Ochmanska, R. Wasko, M. Wanic-Kossowska, and M. Ruchala. 2013. Differences in the Psychological and Hormonal Presentation of Lean and Obese Patients with Polycystic Ovary Syndrome. *Neuro Endocrinology Letters* 34: 669–674.
6. Wan, C., L. Ward, J. Halim, M. Gow, M. Ho, J. Briody, K. Leung, C. Cowell, and S. Garnett. 2014. Bioelectrical Impedance Analysis to Estimate Body Composition, and Change in Adiposity, in Overweight and Obese Adolescents: Comparison with Dual-Energy X-ray Absorptiometry. *BMC Pediatrics* 14: 249.
7. Adami, G.F., G. Marinari, P. Gandolfo, F. Cocchi, D. Friedman, and N. Scopinaro. 1993. The Use of Bioelectrical Impedance Analysis for Monitoring Body Composition Changes During Nutritional Support. *Surgery Today* 23: 867–870.
8. Azcue, M., M. Fried, and P.B. Pencharz. 1993. Use of Bioelectrical Impedance Analysis to Measure Total Body Water in Patients with Cystic Fibrosis. *Journal of Pediatric Gastroenterology and Nutrition* 16: 440–445.
9. Chertow, G.M., E.G. Lowrie, D.W. Wilmore, J. Gonzalez, N.L. Lew, J. Ling, M.S. Leboff, M.N. Gottlieb, W. Huang, B. Zebrowski, and Al Et. 1995. Nutritional Assessment with Bioelectrical Impedance Analysis in Maintenance Hemodialysis Patients. *Journal of the American Society of Nephrology* 6: 75–81.
10. Desport, J.C., P.M. Preux, C. Bouteloup-Demange, P. Clavelou, B. Beaufrere, C. Bonnet, and P.P. Couratier. 2003. Validation of Bioelectrical Impedance Analysis in Patients with Amyotrophic Lateral Sclerosis. *The American Journal of Clinical Nutrition* 77: 1179–1185.
11. Fein, P.A., G. Gundumalla, A. Jorden, B. Matza, J. Chattopadhyay, and M.M. Avram. 2002. Usefulness of Bioelectrical Impedance Analysis in Monitoring Nutrition Status and Survival of Peritoneal Dialysis Patients. *Advances in Peritoneal Dialysis* 18: 195–199.
12. Lukaski, H.C., P.E. Johnson, W.W. Bolonchuk, and G.I. Lykken. 1985. Assessment of Fat-Free Mass Using Bioelectrical Impedance Measurements of the Human Body. *The American Journal of Clinical Nutrition* 41: 810–817.
13. Pencharz, P.B., and M. Azcue. 1996. Use of Bioelectrical Impedance Analysis Measurements in the Clinical Management of Malnutrition. *The American Journal of Clinical Nutrition* 64: 485s–488s.
14. Simons, J.P., A.M. Schols, K.R. Westerterp, G.P. Ten Velde, and E.F. Wouters. 1995. The Use of Bioelectrical Impedance Analysis to Predict Total Body Water in Patients with Cancer Cachexia. *The American Journal of Clinical Nutrition* 61: 741–745.
15. Van Marken Lichtenbelt, W.D., K.R. Westerterp, L. Wouters, and S.C. Luijendijk. 1994. Validation of Bioelectrical-Impedance Measurements as a Method to Estimate Body-Water Compartments. *The American Journal of Clinical Nutrition* 60: 159–166.
16. Zarowitz, B.J., and A.M. Pilla. 1989. Bioelectrical Impedance in Clinical Practice. *DICP* 23: 548–555.

17. Jebb, S.A., M. Siervo, P.R. Murgatroyd, S. Evans, G. Fruhbeck, and A.M. Prentice. 2007. Validity of the Leg-to-Leg Bioimpedance to Estimate Changes in Body Fat During Weight Loss and Regain in Overweight Women: A Comparison with Multi-Compartment Models. *International Journal of Obesity* 31: 756–762.
18. Siri, W.E. 1993. Body Composition from Fluid Spaces and Density: Analysis of Methods. 1961. *Nutrition* 9: 480–491; discussion 480, 492.
19. Wang, Z.M., S. Heshka, R.N. Pierson Jr., and S.B. Heymsfield. 1995. Systematic Organization of Body-Composition Methodology: An Overview with Emphasis on Component-Based Methods. *The American Journal of Clinical Nutrition* 61: 457–465.
20. Wang, Z.M., R.N. Pierson Jr., and S.B. Heymsfield. 1992. The Five-Level Model: A New Approach to Organizing Body-Composition Research. *The American Journal of Clinical Nutrition* 56: 19–28.
21. Lohman, T.G. 1986. Applicability of Body Composition Techniques and Constants for Children and Youths. *Exercise and Sport Sciences Reviews* 14: 325–357.
22. Silva, A.M., D.A. Fields, and L.B. Sardinha. 2013. A Prisma-Driven Systematic Review of Predictive Equations for Assessing Fat and Fat-Free Mass in Healthy Children and Adolescents Using Multicomponent Molecular Models as the Reference Method. *Journal of Obesity*. 148696.
23. Withers, R.T., J. Laforgia, R.K. Pillans, N.J. Shipp, B.E. Chatterton, C.G. Schultz, and F. Leaney. 1998. Comparisons of Two-, Three-, and Four-Compartment Models of Body Composition Analysis in Men and Women. *Journal of Applied Physiology (1985)* 85: 238–245.
24. Bray, G.A., J.P. Delany, D.W. Harsha, J. Volaufova, and C.C. Champagne. 2001. Evaluation of Body Fat in Fatter and Leaner 10-Y-Old African American and White Children: The Baton Rouge Children's Study. *The American Journal of Clinical Nutrition* 73: 687–702.
25. Ramirez, E., M.E. Valencia, H. Bourges, T. Espinosa, S.Y. Moya-Camarena, G. Salazar, and H. Aleman-Mateo. 2012. Body Composition Prediction Equations Based on Deuterium Oxide Dilution Method in Mexican Children: A National Study. *European Journal of Clinical Nutrition* 66: 1099–1103.
26. Tsigos, C., C. Stefanaki, G.I. Lambrou, D. Boschiero, and G.P. Chrousos. 2015. Stress and Inflammatory Biomarkers and Symptoms are Associated with Bioimpedance Measures. *European Journal of Clinical Investigation* 45: 126–134.
27. Ezeh, U., M. Pall, R. Mathur, and R. Azziz. 2014. Association of Fat to Lean Mass Ratio with Metabolic Dysfunction in Women with Polycystic Ovary Syndrome. *Human Reproduction* 29: 1508–1517.
28. Graff, S.K., F.M. Mario, B.C. Alves, and P.M. Spritzer. 2013. Dietary Glycemic Index is Associated with Less Favorable Anthropometric and Metabolic Profiles in Polycystic Ovary Syndrome Women with Different Phenotypes. *Fertility and Sterility* 100: 1081–1088.
29. Attlee, A., A. Nusralla, R. Eqbal, H. Said, M. Hashim, and R.S. Obaid. 2014. Polycystic Ovary Syndrome in University Students: Occurrence and Associated Factors. *International Journal of Fertility & Sterility* 8: 261–266.
30. Aydin, K., N. Cinar, D.Y. Aksoy, G. Bozdag, and B.O. Yildiz. 2013. Body Composition in Lean Women with Polycystic Ovary Syndrome: Effect of Ethinyl Estradiol and Drospirenone Combination. *Contraception* 87: 358–362.
31. Churchill, S.J., E.T. Wang, G. Bhasin, C. Alexander, C. Bresee, M. Pall, R. Azziz, R. Mathur, and M.D. Pisarska. 2015. Basal Metabolic Rate in Women with PCOS Compared to Eumenorrheic Controls. *Clinical Endocrinology* 83: 384–388.
32. Barber, T.M., S.J. Golding, C. Alvey, J.A. Wass, F. Karpe, S. Franks, and M.I. Mccarthy. 2008. Global Adiposity Rather Than Abnormal Regional Fat Distribution Characterizes Women with Polycystic Ovary Syndrome. *The Journal of Clinical Endocrinology and Metabolism* 93: 999–1004.

33. Dolfing, J.G., C.M. Stassen, P.M. Van Haard, B.H. Wolffenbuttel, and D.H. Schweitzer. 2011. Comparison of MRI-Assessed Body Fat Content Between Lean Women with Polycystic Ovary Syndrome (PCOS) and Matched Controls: Less Visceral Fat with PCOS. *Human Reproduction* 26: 1495–1500.
34. Manneras-Holm, L., H. Leonhardt, J. Kullberg, E. Jennische, A. Oden, G. Holm, M. Hellstrom, L. Lonn, G. Olivecrona, E. Stener-Victorin, and M. Lonn. 2011. Adipose Tissue has Aberrant Morphology and Function in PCOS: Enlarged Adipocytes and Low Serum Adiponectin, but not Circulating Sex Steroids, are Strongly Associated with Insulin Resistance. *The Journal of Clinical Endocrinology and Metabolism* 96: E304–E311.
35. Dimitriadis, G.K., I. Kyrou, and H.S. Randeva. 2016. Polycystic Ovary Syndrome as a Proinflammatory State: The Role of Adipokines. *Current Pharmaceutical Design* 22 (36): 5535–5546.
36. Gonzalez, F. 2015. Nutrient-Induced Inflammation in Polycystic Ovary Syndrome: Role in the Development of Metabolic Aberration and Ovarian Dysfunction. *Seminars in Reproductive Medicine* 33: 276–286.
37. Echiburu, B., N. Crisosto, M. Maliqueo, F. Perez-Bravo, A.L. De Guevara, P. Hernandez, G. Cavada, C. Rivas, A. Clavel, and T. Sir-Petermann. 2016. Metabolic Profile in Women with Polycystic Ovary Syndrome Across Adult Life. *Metabolism* 65: 776–782.
38. Khademi, A., A. Alleyassin, M. Aghahosseini, L. Tabatabaeefar, and M. Amini. 2010. The Effect of Exercise in PCOS Women Who Exercise Regularly. *Asian Journal of Sports Medicine* 1: 35–40.
39. Norman, R.J., M.J. Davies, J. Lord, and L.J. Moran. 2002. The Role of Lifestyle Modification in Polycystic Ovary Syndrome. *Trends in Endocrinology and Metabolism* 13: 251–257.
40. Quiterio, A.L., E.A. Carnero, A.M. Silva, F. Baptista, and L.B. Sardinha. 2009. Weekly Training Hours are Associated with Molecular and Cellular Body Composition Levels in Adolescent Athletes. *The Journal of Sports Medicine and Physical Fitness* 49: 54–63.
41. Avram, M.M., P.A. Fein, C. Borawski, J. Chattopadhyay, and B. Matza. 2010. Extracellular Mass/Body Cell Mass Ratio is an Independent Predictor of Survival in Peritoneal Dialysis Patients. *Kidney International Supplement* 117: S37–S40.
42. Barbosa-Silva, M.C., and A.J. Barros. 2005. Bioelectric Impedance and Individual Characteristics as Prognostic Factors for Post-operative Complications. *Clinical Nutrition* 24: 830–838.
43. Fruzzetti, F., S. Lello, V. Lazzarini, S. Fratta, M. Orru, R. Sorge, L. Minerba, C. Ricci, A.R. Genazzani, G.B. Melis, and A.M. Paoletti. 2007. The Oral Contraceptive Containing 30 microg of Ethinylestradiol Plus 3 mg of Drospirenone is Able to Antagonize the Increase of Extracellular Water Occurring in Healthy Young Women During the Luteal Phase of the Menstrual Cycle: An Observational Study. *Contraception* 75: 199–203.

Post-Operative Delirium in Elderly People Diagnostic and Management Issues of Post-Operative Delirium in Elderly People

Christina Florou, Dimitrios Theofilopoulos, Styliani Tziaferi, and Maria Chania

Abstract Hippocrates a Greek physician commonly known as the Father of Medicine was the first to describe delirium, 'phrenitis', differencing it from other disorders such as melancholia and mania. Since then, it still remains a clinical problem which is not yet resolved.

Postoperative delirium is a common medical problem that occurs preferentially in surgical patients aged 65 years and older. It is a true medical emergency that requires immediate professional attention and treatment. Faced by health professionals and especially nurses it is often undetected, misdiagnosed, and under-treated as a complication by them during clinical care.

Delaying diagnosis relates to increased mortality and morbidity and affects deleteriously the elderly patients outcome, predisposing higher postoperative complications, prolonged hospital stay, malnutrition, immobility or bed confinement, higher health care costs, possible transfers to nursing homes and there is even higher death rates within 30 days, 6 months and 1 year.

There is a knowledge deficit of the predisposing and causative factors of postoperative delirium occurrence, evaluation and assessment of cognitive status and health professionals, especially nurses need to receive the necessary training and education to provide quality care for the elderly with postoperative delirium.

As postoperative delirium is frequent in the geriatric patients, it is obvious that we are facing a new challenge to find the right combination for the management and treatment for optimal therapeutic outcomes.

Keywords Delirium • Ageing • Geriatrics • Postoperative care • Risk management • Treatment

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1 Introduction

Hippocrates a Greek physician commonly known as the Father of Medicine was the first to describe delirium, 'phrenitis,' differencing it from other disorders such as melancholia and mania. Since then, it still remains a clinical problem which is not yet resolved.

Postoperative delirium is a common medical problem that occurs preferentially in older surgical patients. Morbidity and mortality rates associated with postoperative delirium are far greater than those associated with deep vein thrombosis [1].

Delaying diagnosis is deleteriously effecting the elderly patients outcome, predisposing higher postoperative complications, prolonged hospital stay, malnutrition, immobility or bed confinement, higher health care costs, possible transfers to nursing homes and there is even higher death rates within 30 days, 6 months and 1 year [2, 3].

There is a knowledge deficit of the predisposing and causative factors of postoperative delirium occurrence, evaluation and assessment of cognitive status and health professionals, especially nurses need to receive the necessary training and education to provide quality care for the elderly with postoperative delirium.

2 Definition

Diagnostic criteria and the terminology of delirium have changed over the years. The American Psychiatric Association defines delirium in the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) as a neurocognitive disorder characterized by disturbance in attention, awareness, and cognition that develops over a short period of time and where the symptoms tend to fluctuate in severity [4].

3 Epidemiology

Delirium is a relatively frequent postoperative complication, with advanced age to be a major risk factor for the appearance of the syndrome [5].

Faced by health professionals and especially nurses it is often undetected, misdiagnosed, and under-treated as a complication by them during clinical care.

Between 32 and 67% of the incidents are unrecognized by physicians and 69% by nurses respectively, resulting to inappropriate nursing care and treatment of the hospitalized patient [6, 7].

It is estimated that 10–15% of patients in the wards of general surgery and approximately 30–40% of hospitalized patients over the age 65 have a delirium episode. Postoperatively the elderly are affected in about 15–53%. Rates vary from

a percentage less than 5% in cataract operations up to 60% in total hip arthroplasty. Incidence rates can reach as high as 48% after cardiac surgery and 38% after colectomy surgery [8, 9].

4 Prevention

Delirium is a true medical emergency that requires immediate professional attention and treatment. Solid proof for preventive interventions that consistently prevents delirium don not exist. It is noted that with the appropriate interventions it is often revertible or can decrease its severity and reduce the duration of postoperative delirium development [10, 11]. Nurses are the key role and have a primary responsibility for the detection and treatment of postoperative delirium. We should not rely only on psychiatric assessments; nurses' skills in recognizing postoperative delirium should be improved.

Researches indicate that physicians and nurses do not take the necessary steps or are not precise in detecting delirium [7, 12], it seems that their main concern is to manage the primary health problem.

Thus it is essential that nurses who spend more time than doctors in the care of patients play a key role in the recognition of delirium. They have the ability to improve nursing quality because of frequent and continuous contact with patients, promoting the treatment and prognosis of patients [13]. They may observe better the fluctuations in attention, the level of consciousness and the cognitive function. As a result, the observations of nurses are vital for the early diagnosis of delirium [14].

The delirium prevention strategies are: detection and early diagnosis of high-risk patients, to prevent the appearance, reduce the duration and proper management [15, 16].

5 Diagnosis: Recognition

No laboratory test can diagnose delirium. The diagnosis of delirium is primarily clinical, requiring frequent observation heightened awareness and a high index of suspicion.

Delirium is often misdiagnosed as dementia, depression, mania, psychotic disorders, or a typical response of the aging brain to hospitalization.

Educating nurses to recognize the core symptoms of delirium is a priority that would lead to a more successful management of delirious patients.

5.1 Diagnostic Criteria

According to the American Psychiatric Association [4] in order to diagnose delirium the following features are required: disturbances in attention, change in cognition, disturbances develops over a short period (usually hours to days) and tend to have a fluctuating course and finally there is evidence that the disturbance is caused by a direct physiologic consequence of a general medical condition, an intoxicating substance, medication use, or more than one cause.

5.2 Psychomotor Behavior

Understanding the three psychomotor behavior subtypes helps to recognize the presence of delirium. According to psychomotor behavior there are three subtypes: the "hyperactive" (or agitated, hyperalert), the "hypoactive" (or lethargic, hypoalert) and the "mixed" type with alternating features of both [6, 17].

Hyperactive patients are aggressive, agitated, emotional instable, at risk for violent and abnormal behavior, often experience hallucinations, delusion and potentially in danger of harming themselves or others. By contrast, hypoactive patients are lethargic, present insensitivity, withdrawal or dullness is observed and are often confused with depression. This is the most common subtype with the most complications and higher mortality. Careful diagnosis is required as these patients are frequently misdiagnosed as having depression or dementia. The mixed subtype has the appearance of characteristics of the other two subtypes and it is the most frequently occurring subtype. It has been suggested that each delirium subtype can result from a different pathophysiological mechanism, and that each might carry a different prognosis [18].

5.3 Postoperative Recognition

Postoperative delirium is observed, mainly, in two periods, upon awakening from anesthesia, in a small percentage of patients, particularly in children, showing hyperactivity and on the first or second postoperative day after a period of clarity elderly patients are often hypoactive and therefore, might go unnoticed [18, 19].

5.4 Tools for Evaluation

Nursing notes can be very helpful for documentation of episodes abnormal behavior, disorientation, hallucinations. Staff should not just report "he was confused."

The use of an assessment tool facilitates the detection of delirium in postoperative patients. Delirium is most accurately assessed if it is monitored regularly and nurses have a uniquely strategic position as their frequent contact affords an ideal opportunity to observe and record the fluctuating feature of delirium symptoms. Using an evaluation tool, nurses are facilitated in the detection, management and prevention of delirium. There are a number of validated tools which can be used including the Confusion Assessment Method (CAM) and the Nursing Delirium Screening Scale (Nu-DESC). The mentioned tools are validated and are used in several countries.

5.4.1 Confusion Assessment Method (CAM)

Among the assessment tools that consistently out-performs all others is the Confusion Assessment Method (CAM) with a sensitivity of 95–100% and a specificity of 89–93%. The CAM algorithm relies on the presence of (1) the acute onset of mental status changes and (2) inattention, and either (3) disorganized thinking or (4) an altered level of consciousness. The diagnosis requires features 1 and 2, plus 3 or 4. It has been confirmed in several studies in order to be used as diagnostic tool by non-psychiatric personnel [20, 21].

It has a best combination of ease, data collection, high reliability and without time consuming—it can be performed within 2–5 min [20, 22].

5.4.2 Nursing Delirium Screening Scale (Nu-DESC)

Nursing Delirium Screening Scale (Nu-DESC) is a simple instrument for early detection suitable for widespread clinical use and shows promise as an evaluation tool. It is an observational five-item scale that can be completed quickly, it is psychometrically valid and has a sensitivity and specificity of 85.7 and 86.8%, respectively. The completion of the assessment test lasts 1–2 min. The Nu-Desc can also evaluate the hypoactive variant of delirium by rating unusual psychomotor retardation [23].

6 Etiology: Risk Factors

Management of delirium begins with evaluation and treatment of its causes. It should however be noted that there are multiple causes that precipitate postoperative delirium and which cannot be fully clarified and understood. The characteristics of the elderly, the chronically ill, the type of the surgery and its complications, preexisting psychiatric, psychological problems and the impact of the environment are factors that when combined lead us to the incidence of postoperative delirium [9, 24].

An important step in the prevention of postoperative delirium is the identification of high risk patients. Main risk factors are pre-existing dementia, history of hypertension, alcoholism or illicit drugs abuse and high medical comorbidity.

6.1 Preoperative Factors

Taking into consideration the patient's history, predisposing risk factors for postoperative delirium in elderly surgical patients are: advanced age over 70 years, impaired cognition, functional impairment, high medical comorbidity, abuse of drugs and alcohol, polypharmacy and drug interactions, sex (for male), preexisting dementia, depression and anxiety. Preoperative cognitive impairment is strongly associated with development of postoperative delirium [9, 25, 26].

6.2 Intraoperative/Postoperative Factors

Within the hospital and the surgical environment are the precipitating factors that could lead to postoperative delirium.

When we refer to precipitating factors besides the duration and type of surgery we take into consideration: hypoxemia $SPO_2 < 90\%$, metabolic disorders, hypoxia, hypercapnia, hypoglycemia, electrolyte abnormalities, great intraoperative blood loss, hypovolemia, large volumes of blood transfusion, postoperative Hct < 30, low serum albumin, systemic infections, withdrawal syndromes (hypnotics, alcohol) and medication (pshychotropics, anticholinergics, opiates) [6, 25]. Postoperatively Billota et al. [27] also mentioned: ICU admission, intraoperative hypotension, low cardiac output requiring inotropes infusion, anemia and malnutrition. Furthermore, last but not least, important factors that must be taken into consideration are urinary retention, constipation, indwelling catheters and inadequate pain control [28].

Among the elderly surgical patients, dementia is also one of the most prominent risk factors for delirium. A 25–75% incidence of patients with delirium has dementia and its presence increases the risk of delirium [29, 30].

7 Treatment: Nursing Care

A wide variation in the appearance of postoperative delirium has been reported and still is a feature of care that most health professionals are unaware [31].

For effective management and treatment there must be a multifactorial approach [32]. Geriatric patients who are at high risk should be identified and treated promptly for the best possible result [8]. For the best outcome, a cooperative group of

medical specialists, like pathologists or surgeons, clinical nurses and psychiatrists are necessary.

The basic management and treatment is: identification and treatment of the underlying causes, the use of supportive non-pharmacological interventions (patient friendly hospital environment), rational use of neuroleptic medication and the avoidance of benzodiazepines.

7.1 Non-Pharmacological Treatment

Knowing that biological and psychosocial factors interact, there must be a holistic approach in the treatment of elderly patients undergoing surgery that have to cope a delirium episode too.

The main non-pharmacological therapeutic targets of delirium is a safe and well organized environment in order to ensure patient's safety and treatment of medical problems.

Actively monitoring, identifying the precipitating risk factors, timely management of acute critical incidents (brain stroke, heart attack, renal failure, infectious processes, electrolyte abnormalities, etc.) and elimination of potential harmful and unnecessary medication are basic elements to avoid further deterioration of delirium.

Prevention of delirium should begin preoperatively with psychological support of the geriatric patient, as it may reduce the occurrence of postoperative delirium [33] and subsequently, during the operation maintaining hemodynamic stability, ensuring adequate oxygen supply, managing electrolyte/acid-base balance disorders and appropriate anesthetic application [34].

Inadequate postoperative pain control is a major risk factor that increases the risk of delirium. Proper management of postoperative pain is one of the matters that must be seriously taken into account by the health staff [35, 36]. Avoidance of opioid analgesics but adequate control of pain with local anaesthetic injection or other analgesics (i.e. gabapentin) probably prevent delirium.

Postoperatively, attention must be given to environmental and supportive strategies since the geriatric patient cannot adapt to the environment and nursing interventions in this case are of great importance.

7.1.1 Environmental: Supportive Interventions

The use of single rooms if possible—room changes should be minimized, quiet environment and noise reduction strategies especially at rest times, lighting appropriate to time of day and avoiding medical interventions at night keep the patient calm, reduce anxiety and aid rest. Appropriate environmental stimuli with the use of glasses and hearing aids when needed, the use of clocks, calendars, magazines,

newspaper, music even television or radio help patients to be oriented to time, place and have contact with the outside world [37, 38].

Ensure safety for patients with risk of self harm, early mobilization—out of bed on POD 1, exercise and rehabilitation [35].

Adequate nutritional intake, regulation of bowel/bladder function and skin care for patients with established incontinence are of importance too.

Providing orienting information including name and role of staff members, contact with relatives, tactile sensory stimulation using personal objects, photographs etc. are personalized interventions that may lessen behavioral disturbance and that health staff must have in the daily cycle of patient care [33, 39].

7.1.2 Empathy-Related Interventions

Empathy appears to be another therapeutic intervention and important aspect of patients' well- being. Although the current empathy intervention literature is limited Howe [40] wrote: 'While scientific instruments measure people objectively, empathy understands them subjectively.' Empathy allows us to experience the subjectivity of the other person. Using empathy as an intervention for treating delirium, health care providers and especially nurses have a particular behavior towards their patients.

The purpose is to create a therapeutic relationship between the patient and the nurse for the benefit of the patient. The key to success for this intervention is to hear what their patient are saying, to trust their stories, whether they are excessive and pompous and interact with them in a way that does not offend their dignity and integrity. It is better for the nursing staff to admit that they cannot share their views and that they cannot know everything and have an opinion about them. However, nurses must be careful in order not to scold the patient or make humorous remarks as this might affect them or for them to consider that something humiliating was taken place and thus make them suffer even more [22].

This approach, during delirium, faces the personal and psychological needs of the patient with sensibility, reassurance and support and may lead to immediate relief and a sense of relaxation, but also show the possibility of the patient to doubt his own perspective—delirium behavior and facilitate our other therapeutic interventions. This kind of approach has proven to be effective in elderly patients who are confused and also aggressive [41].

Trained nurses, geriatrics consultation, even skilled nurses in geriatrics and a geriatric psychiatry in the clinical settings are positive assets for the proper treatment of delirium in the elderly [42].

7.2 Pharmacological Treatment

Pharmacologic treatment of delirium, in the elderly, when required needs monitoring and careful consideration between the potential benefits and the possible side effects that may lead to mental deterioration and it is likely to have a prolonged course. It is recommended as a treatment but it is a peculiar challenge for the health care team when it concerns agitated patients, with disruptive behavior that fail to perceive or acknowledge the environment and results in a hallucinatory perception. Strong verbal behavior is not a reason for pharmacological treatment.

It is important to prevent patients harming themselves and protect the physical integrity of the staff. The use of physical restraints for uncooperative patients is the last resort when alternatives have failed because it increases agitation, depression, immobility and whenever used, the reasons for their use is best to be explained frequently to the patient [25].

In these cases haloperidol can be administered, as it remains the first-line treatment. Haloperidol should be used with extreme caution as there is a risk of excessive sedation in the elderly patients as the half-life of the drug may be longer (72 h) [6, 34].

Great attention should be given in benzodiazepines. Benzodiazepines can protect against delirium but also aggravate delirium, particularly in patients with dementia. Benzodiazepines are effective for delirium that is associated with seizures or withdrawal from alcohol or sedatives and occurs 12–48 h after surgery, as well as in patients with Parkinson's disease [15, 33].

Anticholinergics are not recommended in the elderly as they may cause confusion or other effects blurred vision, constipation and urinary retention [43].

Although the results of the research are not clear, the researchers pointed out that the use of second generation antipsychotic agents are with less side effects. Ziprasidone (Zeldox) and quetiapine (Seroquel) have an effect on aggressive and hallucinated than to hypoactive subtype delirium patients [44].

Additionally, researches have shown that lipid-lowering drugs reduce the incidence of delirium, particularly, in the elderly. In any case, a further research is required in this field for the effect of lipid-lowering drugs in delirium [45].

8 Conclusions

Occurrence of postoperative delirium is frequent in the geriatric patients and it is obvious that we are facing a new challenge to find the right combination for the management and treatment for optimal therapeutic outcomes. Often misdiagnosed and untreated postoperative delirium has relevant clinical implications since it relates to increased morbidity and mortality. To select patients that need dedicated perioperative care pathways awareness of the risk factors, early detection and treatment and a well coordinated health team are the main steps for success.

When delirium steps in, we cannot overstress the importance of nursing care. Nurses are the main contact with the patient and must be well-versed in management and treatment procedures.

We are in the right direction but clinical willingness is required for up to date best practice protocols, guidelines and interventions, to provide high quality geriatric care that would include continuous monitoring for symptoms of delirium and the proper adjustments in the treatment of medical conditions and medication use.

References

1. Gustafson, Y., D. Berggren, B. Brannstrom, G. Bucht, A. Norberg, L.I. Hansson, and B. Winblad. 1988. Acute Confusional States in Elderly Patients Treated for Femoral Neck Fracture. *Journal of the American Geriatrics Society* 36 (6): 525–530.
2. Amador, L.F., and J.S. Goodwin. 2005. Postoperative Delirium in the Older Patient. *Journal of the American College of Surgeons* 200 (5): 767–773.
3. Douglas, L.L., Z. Ying, S.T. Bogardus, T.R. Holford, L.S. Leo-Summers, and S.K. Inouye. 2005. Consequences of Preventing Delirium in Hospitalized Older Adults on Nursing Home Costs. *Journal of the American Geriatrics Society* 53 (3): 405–409.
4. American Psychiatric Association. 2013. *Diagnostic and Statistical Manual of Mental Disorders*. 4th ed. DSM-V, Arlington VA: APA.
5. Mentes, J., K. Culp, M. Maas, and M. Rantz. 1999. Acute Confusion Indicators: Risk Factors and Prevalence Using MDS Data. *Research in Nursing and Health* 22 (2): 95–105.
6. Cole, M.G. 2004. Delirium in Elderly Patients. *The American Journal of Geriatric Psychiatry* 12 (1): 7–21.
7. Inouye, S.K., M.D. Foreman, L.C. Mion, K.H. Katz, and L.M. Cooney. 2001. Nurses' Recognition of Delirium and Its Symptoms. *Archives of Internal Medicine* 161: 2467–2473.
8. Inouye, S.K. 2006. Delirium in Older Persons. *The New England Journal of Medicine* 354 (11): 1157–1165.
9. Lagoo-Deenadayalan, S.A., M.A. Newell, and W.E. Pofahl. 2011. Common Perioperative Complications in Older Patients. In *Principles and Practice of Geriatric Surgery*, ed. R.A. Rosenthal, M.E. Zenilman, and M.R. Katlic. New York: Springer.
10. Marcantonio, E.R., J.M. Flacker, M. Michaels, and N.M. Resnick. 2000. Delirium is Independently Associated with Poor Functional Recovery After Hip Fracture. *Journal of the American Geriatrics Society* 48: 618–624.
11. Tsang, L.F., W.Y. Yiu, W.H. Kwok, C.C. Tse, S.W. Ng, et al. 2015. Management of Postoperative Delirium for Geriatric Patients with Hip Fracture: A Quasi-Experimental Study. *Clinical Nursing Studies* 3 (2): 40.
12. Lacko, L., Y. Bryan, C. Dellasega, and F. Salerno. 1999. Changing Clinical Practice Through Research: The Case of Delirium. *Clinical Nursing Research* 8 (3): 235–250.
13. Insel, K.C., and T.A. Badger. 2002. Deciphering the 4 D's: Cognitive Decline, Delirium, Depression and Dementia-A Review. *Journal of Advanced Nursing* 38 (4): 360–368.
14. Inouye, S.K., R.G. Westendorp, and J.S. Saczynski. 2014. Delirium in Elderly People. *Lancet* 383: 911–922.
15. Meagher, D.J. 2001. Delirium: Optimising Management. *BMJ* 322 (7279): 144–149.
16. Young, J., and S.K. Inouye. 2007. Delirium in Older People. *BMJ* 334 (7598): 842–846.
17. Liptzin, B., and S.E. Levkoff. 1992. An Empirical Study of Delirium Subtypes. *The British Journal of Psychiatry* 161: 843–845.
18. Fong, T.G., S.R. Tulebaev, and S.K. Inouye. 2009. Delirium in Elderly Adults: Diagnosis, Prevention and Treatment. *Nature Reviews Neurology* 5 (4): 210–220.

19. Askitopoulou, E., and A. Papaioannou. 2015. Complications and Anaesthesia Morbidity. In *Manual of Anaesthesiology and Perioperative Care*, ed. E. Askitopoulou and A. Papaioannou. Athens: Hellenic Academic Libraries. Available from: http://hdl.handle.net/11419/3786 (Assecced: 01st August 2016) [Greek Language].
20. Inouye, S.K., S.H. van Dyck, C.A. Alessi, C. Balkin, A.P. Siegal, and R.I. Horwitz. 1990. Clarifying Confusion: The Confusion Assessment Method. *Annals of Internal Medicine* 113 (12): 941–948.
21. Wong, C.L., J. Holroyd-Leduc, D.L. Simel, and S.E. Straus. 2010. Does this Patient Have Delirium?: Value of Bedside Instruments. *JAMA* 304 (7): 779–786.
22. Ski, C., and B. O'Connell. 2005. Mismanagement of Delirium Places Patients at Risk. *The Australian Journal of Advanced Nursing* 23 (3): 42–46.
23. Gaudreau, J.D., P. Gangnon, F. Harel, A. Tremblay, and M.A. Roy. 2005. Fast Systematic, and Continuous Delirium Assessment in Hospitalized Patients: The Nursing Delirium Screening Scale. *Journal of Pain and Symptom Management* 29: 368–375.
24. Whitaker, J.J. 1989. Postoperative Confusion in the Elderly. *International Journal of Geriatric Psychiatry* 4 (6): 311–363. doi:10.1002/gps.930040604.
25. Parikh, S.S., and F. Chung. 1995. Postoperative Delirium in the Elderly. *Anesthesia and Analgesia* 80 (6): 1223–1232.
26. Schenning, K.J., and S.G. Deiner. 2015. Postoperative Delirium in the Geriatric Patient. *Anesthesiology Clinics* 33 (3): 505–516.
27. Billota, F., M.P. Lauretta, A. Borozdina, V.M. Mizikov, and G. Rosa. 2013. Postoperative Delirium: Risk Factors, Diagnosis and Perioperative Care. *Minerva Anestesiologica* 79 (9): 1066–1076.
28. Tropea, J., J.A. Slee, C.A. Brand, L. Gray, and T. Snell. 2008. Clinical Practice Guidelines for the Management of Delirium in Older People in Australia. *Australasian Journal on Ageing* 27 (3): 150–156. doi:10.1111/j.1741-6612.2008.00301.x.
29. Elie, L.M., M.G. Cole, F.J. Primeau, et al. 1998. Delirium Risk Factors in Elderly Hospitalized Patients. *Journal of General Internal Medicine* 13: 204–212.
30. McCarthy, M.C. 2003. Situated Clinical Reasoning: Distinguishing Acute Confusion from Dementia in Hospitalised Older Adults. *Research in Nursing and Health* 26 (2): 90–101.
31. Lemiengre, J., T. Nelis, E. Joosten, T. Braes, M. Foreman, C. Gastmans, et al. 2006. Detection of Delirium by Bedside Nurses Using the Confusion Assessment Method. *Journal of the American Geriatrics Society* 54 (4): 685–689.
32. Hshieh, T.T., J. Saczynski, R.Y. Gou, E. Marcantonio, et al. 2016. Trajectory of Functional Recovery After Postoperative Delirium in Elective Surgery. *Annals of Surgery*. doi:10.1097/SLA.0000000000001952.
33. Attard, A., G. Ranjith, and D. Taylor. 2008. Delirium and Its Treatment. *CNS Drugs* 22 (8): 631–644.
34. Jin, F., and F. Chung. 2001. Minimizing Perioperative Adverse Events in the Elderly. *British Journal of Anaesthesia* 87 (4): 608–624.
35. Guenther, U., L. Riedel, and F.M. Radtke. 2016. Patients Prone for Postoperative Delirium: Preoperative Assessment, Perioperative Prophylaxis, Postoperative Treatment. *Current Opinion in Anaesthesiology* 29 (3): 384–390.
36. Vaurio, L.E., L.P. Sands, Y. Wang, E.A. Mullen, and J.M. Leung. 2006. Postoperative Delirium: The Importance of Pain and Pain Management. *Anesthesia and Analgesia* 102 (4): 1267–1273.
37. Inouye, S.K., S.T. Borgadus Jr., P.A. Carpentier, et al. 1999. A Multicomponent Intervention to Prevent Delirium in Hospitalized Older Patients. *The New England Journal of Medicine* 340 (9): 669–676.
38. Lundström, M., B. Olofsson, M. Stenvall, et al. 2007. Postoperative Delirium in Old Patients with Femoral Neck Fracture: A Randomized Intervention Study. *Aging Clinical and Experimental Research* 19 (3): 178–186.
39. Meagher, D.J., D. O'Hanlon, E. O'Mahony, et al. 1996. The Use of Environmental Strategies and Psychotropic Medication in the Management of Delirium. *The British Journal of Psychiatry* 168 (4): 512–515.

40. Howe, D. 2012. *Empathy: What It Is and Why It Matters*. London: Palgrave MacMillan.
41. Fagerberg, I., and M.E. Jonhagen. 2002. Temporary Confusion: A Fearful Experience. *Journal of Psychiatric and Mental Health Nursing* 9 (3): 339–346.
42. Weber, J.B., J.H. Coverdale, and M.E. Kunik. 2004. Delirium: Current Trends in Prevention and Treatment. *Internal Medicine Journal* 34 (3): 115–121.
43. Feinberg, M. 1993. The Problems of Anticholinergic Adverse Effects in Older Patients. *Drugs & Aging* 3 (4): 335–348.
44. Devlin, J.W., R.J. Roberts, J.J. Fong, Y. Skrobnik, R.R. Riker, N.S. Hill, et al. 2010. Efficacy and Safety of Quetiapine in Critically Ill Patients with Delirium: A Prospective, Multicenter, Randomized, Double-Blind, Placebo-Controlled Pilot Study. *Critical Care Medicine* 38 (2): 419–427.
45. Katznelson, R., G.N. Djaiani, M.A. Borger, Z. Friedman, S.E. Abbey, L. Fedorko, et al. 2009. Preoperative Use of Statins is Associated with Reduced Early Delirium Rates After Cardiac Surgery. *Anesthesiology* 110 (1): 67–73.