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Research report

Alterations of mitochondrial function and correlations with personality traits in selected major depressive disorder patients

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Abstract

Background: Increased occurrence of several physical conditions has been reported in patients with depressive disorders. Various physical conditions and depressive disorder have been reported in patients with mitochondrial disorders. The aim of this study was to investigate mitochondrial function in selected depressed patients in search of an aetiological or pathophysiological factor common to both depression and physical symptoms. **Methods:** Muscle biopsy was performed in 28 patients with a lifetime prevalence of major depressive disorder (MDD), and at least three chronic physical conditions that have been reported to be common in depressive as well as in mitochondrial disorders. Morphologic and biochemical investigations including mitochondrial ATP production rate (MAPR) by the bioluminometric method, spectrophotometric analyses of mitochondrial enzymes, and long-PCR and Southern blot techniques to detect mitochondrial DNA (mtDNA) deletions were performed. The Karolinska Scales of Personality (KSP) with 15 scales assessing vulnerability to psychopathology was filled in by 21 patients. **Results:** Decreases of MAPR and enzyme ratios were found in the patients in comparison with controls ($P < 0.01$). Deletions of mtDNA assessed with long-PCR were more frequent in patients than in controls (chi-square test $P < 0.05$). Correlations were found between MAPR and the KSP scales 'Somatic Anxiety', 'Psychasthenia', and 'Suspicion' ($P < 0.01$). **Limitations:** Results cannot be compared with previous studies, and cannot be generalized to all MDD patients. Individually matched controls were not available. **Conclusions:** The results suggest that mitochondrial dysfunction is associated with vulnerability to psychopathology in this selected patient group.

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Keywords: ATP; Hearing loss; KSP; Mitochondrial disorder; Tinnitus; Unipolar depression

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1. Introduction

The disease model of unipolar major depressive disorder (MDD), which has been conceptualized as an acute and isolated illness, has recently been proposed to be more analogous in terms of lifetime chronicity to hypertension than to acute episodic diseases. A quarter of the patients in a prospective MDD study never had an asymptomatic week, i.e. were free of subthreshold depressive symptoms, during follow-up (Judd et al., 1998).

A threefold increased risk for MDD has been reported in subjects with three or more chronic medical conditions (Lindeman et al., 2000). Depression associated with chronic physical problems was found to be one of six depressive subcategories in a multi-center study (Tylee et al., 1999). Moldin et al. (1993) have suggested that the common concomitant presence of physical illnesses in depression implicates research investigating aetiological or pathophysiological factors common to both depression and specific physical illnesses.

Rajala et al. (1995), in considering the increased prevalence of depression in patients with muscular pain in a population study, suggested that their

findings supported the notion of a common biochemical background of both. A higher incidence of muscle biopsy alterations also in non-treated depressed patients has been reported (Ross-Stanton and Meltzer, 1979). Disturbed sensory perception may be a peripherally induced experience in some depressed patients. Lowered auditory sensitivity and hearing loss (Malone and Hemsley, 1977; Herbst and Humphrey, 1980; Yovell et al., 1995), hyperacusis and tinnitus (Carman, 1973; Mathew et al., 1981), alterations of retinal physiology (Lam et al., 1991, 1992; Seggie et al., 1991; Ozaki et al., 1995; Demet et al., 1997; Terman and Terman, 1999) and pupillary responses (Sokolski et al., 2000), and ocular/visual symptoms (Mathew et al., 1981; Friberg and Borrero, 2000) have been reported in non-treated depressed patients.

A short list of studies exploring interrelationships for depression with pain, tinnitus, visual symptoms, and fatigue, is presented in Table 1.

Mitochondrial disorders are examples of clinically heterogeneous multisystem disorders, with psychiatric as well as somatic symptoms. Symptoms may be episodic, or show periodic exacerbations (Fadic and Johns, 1996; Chinnery and Turnbull, 1997). Depres-

Table 1
Prevalence/occurrence of physical symptoms in depression, and of depression in individuals with the same physical symptoms

Pain: population prevalence headaches 14% (Hagen et al., 2000), episodic myalgia 7%, chronic myalgia 14% (Magni et al., 1990)		
Moldin et al., 1993	914 MDD subjects (1628 controls)	Lifetime frequent/severe headaches in 32% (16%)
Rajala et al., 1995	75 MDD subjects (population study, 705 subjects without MDD)	Frequent neck pains in 63% (41%)
Corruble and Guelfi, 2000	150 MDD in-patients at psychiatric admission	Current pain in 92%: headache in 65%, myalgia in 67%
Katon et al., 1985	37 chronic pain patients admitted for treatment	Current MDD in 32%, lifetime MDD in 57%
Mitsikostas and Thomas, 1999	101 mixed headache patients (150 controls without headaches)	Current MDD in 8% (0.6%)
Magni et al., 1990	416 chronic myalgia subjects (population study, 2388 subjects without chronic myalgia)	Current depression in 18% (8%)
Tinnitus: population prevalence frequent tinnitus 14%, severe tinnitus 2.4% (Axelsson and Ringdahl, 1989)		
Mathew et al., 1981	51 not-subclassified unmedicated depressives (51 controls)	Tinnitus in 49% (12%)
Sullivan et al., 1988	40 severe tinnitus patients (14 controls)	Current MDD in 60% (7%), lifetime MDD in 78% (21%)
Colour vision disturbance: population prevalence in females 0.5% (Heim and Morgner, 1997), blurred vision in 95 subjects 14% (Vincent et al., 1989)		
Heim and Morgner, 1997	75 female MDD patients	Disturbed colour vision in 63%
Mathew et al., 1981	51 not-subclassified unmedicated depressives (51 controls)	Blurred vision in 51% (8%)
Fatigue: population prevalence fatigue 24%, chronic fatigue 10–19% (Hotopf and Wessely, 1997)		
Coulehan et al., 1988	87 MDD patients	Fatigue ('low energy') in 63%
Hotopf and Wessely, 1997	Several studies of chronic fatigue disorder patients	Affective disorders in ~50%

Population prevalence estimates of MDD: 7% point prevalence in females (Hällström, 1984), 9% 12-month prevalence (Lindeman et al., 2000), 26% lifetime prevalence (Levitt et al., 2000). Percentages in brackets in the third column refer to findings in controls or populations, see information in brackets in the second column.

sive disorders have been described in case histories of patients with different mitochondrial disorders (Suomalainen et al., 1992; Sweeney et al., 1993; Melberg et al., 1996; Miyaoka et al., 1997; Onishi et al., 1997; Santorelli et al., 1997), and are suggested to be common (Fadic and Johns, 1996; Chinnery and Turnbull, 1997). Deficits in short-term memory and/or spatial cognition have been demonstrated with neuropsychological tests (Turconi et al., 1999). Common somatic manifestations are muscular weakness and pain, headaches, tinnitus and/or sensorineural hearing loss, ocular/visual symptoms, ophthalmoplegia, and fatigue (Fadic and Johns, 1996; Chinnery and Turnbull, 1997; Korres et al., 1999; Chinnery et al., 2000; Nishino et al., 2000).

Mitochondrial disorders may be the results of mutations in nuclear DNA or mitochondrial DNA (mtDNA), causing impaired production of cellular energy, adenosine triphosphate (ATP). The proportion of mtDNA mutations in tissues may correlate with degree of disease (Chinnery et al., 2000). Other factors than the proportion of mutant mtDNA, in particular nuclear-controlled neuronal differences among various CNS regions, seem to contribute to mitochondrial dysfunction (Zhou et al., 1997). Interference of such highly transient cellular events as mitochondrial calcium-handling affecting the membrane potential may be one of the main effects of some mtDNA mutations (Brini et al., 1999).

Symptoms of mitochondrial disease can mimic the chronic fatigue syndrome (CFS). The percentage of CFS patients with mitochondrial dysfunction is unknown (Chinnery and Turnbull, 1997). Muscle aerobic metabolism utilizing morphological and biochemical analyses has been reported in a study of CFS patients with muscle pain and neuropsychiatric symptoms. The results were interpreted to suggest that the CFS patients comprised a heterogeneous group in which more than one mild energy defect may be present (Behan et al., 1999).

The aim of this study was to determine if alterations of muscle mitochondrial function could be detected in MDD patients selected for presence of coexisting muscular pain, audiological, and visual symptoms. The biochemical measurements were correlated with scores of the Karolinska Scales of Personality (KSP), which include scales associated with vulnerability to depressive disorder.

2. Patients and methods

The study was conducted at a specialized psychiatric out-patient unit for clients with coexisting audiological symptoms affiliated to the Psychiatric Clinic at Huddinge University Hospital, in Stockholm, Sweden.

2.1. Patients

Twenty-eight patients, 14 men and 14 women aged 27–61 years (mean 48.2 ± 8.5), were investigated with muscle biopsy. The investigations were performed with the method in use for routine diagnosis of mitochondrial disorders. The patients were investigated due to clinical suspicion of mitochondrial dysfunction. Studies of muscle tissue mtDNA were performed in the first 25 patients included in the study, 12 men and 13 women aged 27–60 (mean 48.6 ± 7.9).

Only patients who gave informed consent, had lifetime MDD diagnosis according to DSM-IV criteria (American Psychiatric Association, 1995), and muscular pain, severe tinnitus or hyperacusia and/or sensorineural hearing loss, and visual symptoms, were accepted in the study (a list describing the severity of the physical symptoms necessary for inclusion in the study is available on request). Psychiatric investigation was performed with several structured clinical interviews in each patient. No patient had a history of alcohol or drug abuse. EEG and KSP were performed routinely as part of the psychiatric investigation. Mood symptoms were no longer pronounced at the time of the muscle biopsy and filling in of the KSP.

2.2. Karolinska Scales of Personality (KSP)

In order to quantify dimensions linked to depression, the Swedish version of the KSP was filled in by 21 patients, 10 males and 11 females. Five patients with poor command of the Swedish language and two patients with prominent neurocognitive symptoms were not asked to fill in the questionnaire partly for ethical reasons. The KSP, developed by Schalling, with 135 items with a four-point response format summed up to 15 scales, are focused on personality traits that are thought to have biological

correlates, with items referring explicitly to longitudinally stable personality traits, and were developed to cover specific areas of importance for research projects in healthy subjects and on groups of psychiatric patients such as patients suffering from depression and anxiety states. The main areas of research utilizing the KSP have been psychobiological studies exploring associations between personality traits and biological markers. With the exception of some of the aggression-related scales, the KSP scales demonstrate stability over time, and construct validity (Gustavsson, 1997). The scores of the patients were compared with normative data transformed to *T*-scores (50 ± 10) which have been obtained from 400 subjects randomly sampled from the Stockholm population and standardized for age and sex (Bergman et al., 1982), and with scores from another selected MDD group, suicide attempters, for whom the same norm group was used (Pendse et al., 1999). The normative data is in use as controls (Nissen et al., 1998).

High KSP scores indicate pathology on all scales except for the Socialization scale, where low scores indicate pathology. In patients with ongoing MDD as well as in patients who have recovered from mood symptoms, high scores have been reported on the Psychasthenia, Muscular Tension, Somatic Anxiety, and Psychic Anxiety scales, and low scores on the Socialization scale (Perris et al., 1984; Ekselius and von Knorring, 1999; Pendse et al., 1999).

2.3. Muscle biopsies, and controls for biochemistry and mtDNA studies

Muscle biopsies were taken from the right anterior tibial muscle in patients and controls. In one patient the light microscopy investigation was performed also in a biopsy from the deltoid muscle. For the biochemical analyses, 10 healthy sedentary controls, three men and seven women aged 29–55 years (mean 46.4 ± 7.8) were investigated. For mtDNA studies, 22 healthy controls, 13 men and nine women aged 23–74 years (mean 48.6 ± 17.1) were investigated. Muscle biopsy studies of controls were approved by the Ethics Committee of the Karolinska Institutet, Stockholm, Sweden.

2.4. Light and electron microscopy

For light microscopy, the biopsies were frozen and cryostat sections prepared. The histochemical staining methods included hematoxylin–eosin (HE), the myofibrillary ATP-ase reaction at three different pH, modified Gomori's trichrome, oil red O, periodic acid Schiff (PAS), NADH-tetrazolium reductase (NADH-TR), succinate dehydrogenase (SDH), and cytochrome *c* oxidase (COX). Other pieces were fixed in buffered glutaraldehyde, postfixed in osmium tetroxide, and epon-embedded for electron microscopy.

2.5. Biochemical analyses in muscle

Mitochondria were isolated from fresh muscle tissue and mitochondrial ATP production rate (MAPR) was luminometrically determined at 25 °C (Wibom and Hultman, 1990). Eight different substrate combinations were used to test the mitochondrial function: (a) pyruvate + palmitoyl-L-carnitine + α -ketoglutarate + malate (PPKM), (b) glutamate + malate, (c) *N,N,N',N'*-tetramethyl-1,4-phenyldiamine (TMPD) + ascorbate, (d) α -ketoglutarate, (e) palmitoyl-L-carnitine + malate, (f) pyruvate + malate, (g) succinate + rotenone, and (h) succinate only. MAPR was expressed in $\text{mmol ATP min}^{-1} \text{kg}^{-1}$ muscle.

After storage at -70 °C, the enzyme activities were spectrophotometrically determined at 25 °C in the isolated mitochondria. An aliquot was freeze-thawed in hypotonic medium according to the procedure by Birch-Machin et al. (1994), and rotenone-sensitive NADH-cytochrome *c* reductase (NCR, complex I+III) and succinate-cytochrome *c* reductase (SCR, complex II+III) were determined according to Sottocasa et al. (1967) and Cooperstein et al. (1950), respectively. Another aliquot was freeze-thawed in the storage medium, and treated with digitonin 2 g l^{-1} before the analysis of cytochrome *c* oxidase (COX, complex IV) (Cooperstein and Lazarow, 1951). Citrate synthase (CS) was determined according to the method by Alp et al. (1976) after permeabilization of the mitochondria in a medium containing Triton X-100 0.05% (v/v),

K_2HPO_4 50 mmol l^{-1} and EDTA 1 mmol l^{-1} , pH 7.5. The activities of NCR, SCR and COX were expressed in relation to the CS activity in the isolated mitochondria (units per unit citrate synthase).

2.6. Mitochondrial DNA in muscle

We searched for the presence of two mitochondrial point mutations (nt1555 A→G and nt7445 A→G), which have been found in families with maternally inherited sensorineural hearing loss with considerable variability between individuals. The 1555-mutation abolishes a *BsmAI* restriction site and the 7445-mutation abolishes an *XbaI* restriction site. DNA from the patients were amplified with PCR technique, digested with *BsmAI* or *XbaI* and separated on an agarose gel (Prezant et al., 1993; Reid et al., 1994).

We used two different methods in the search for deletions in the mtDNA: long-PCR analysis which is a non-quantitative method, and Southern blot analysis which is a quantitative method (Coulter-Mackie et al., 1998). With long-PCR, three overlapping regions between nt4336 and nt946 of the mtDNA were amplified, resulting in a product of 5.5 kilobasepairs (kbp) encompassing the mtDNA 4977 base pairs 'common deletion', a 9.2 kbp product encompassing nt4336–13 532, and a 9.5 kbp product encompassing nt7976–946. We used Southern blot to analyse the full length mtDNA. Total DNA from patients and controls was digested with *PvuII*, separated on a 0.8% agarose gel and transferred to a nylon membrane. The membrane was hybridized with ^{32}P -labeled probes corresponding to mtDNA nt1591–3651 and nt1086–946 and exposed to an X-ray film. Deletion levels above 1% of total mtDNA have been demonstrated with this method.

2.7. Statistics

Group results are presented as means \pm S.D. The significance level was set at $P < 0.01$ since multiple *t*-tests and *F*-tests were performed. Chi-square analysis was performed to determine differences between patients and controls with respect to the number of individuals harbouring mtDNA deletions. Significance level for chi-square tests was set at $P < 0.05$.

Relationships between variables were evaluated with the Spearman rank correlation test and were considered significant when they were higher/lower than ± 0.45 (limit for $P < 0.05$). The following precautions were added to decrease the risk of chance findings: rank correlations had to remain stable when each individual had been excluded in turn (repeated resampling, a simplified version of the bootstrap validation used for large sample sizes, see Linnet (2000)), and remained higher/lower than ± 0.40 when the material was randomly divided (split-half technique, see Ågren and Wide (1982)). The random order of patients for the split divisions was changed with each calculation.

3. Results

3.1. Clinical characteristics

During mood episodes, anhedonia and/or irritable mood was more pronounced than low mood in 21 patients (75%). Severely depressed mood with melancholic features was only observed in three patients. No patient had had manic features or elevated mood. All patients complained of chronic concentration difficulties and mental fatigue causing marked distress and interference with social and/or occupational functioning. Paroxysmal or pronounced increase of low frequency EEG-activity was seen on visually interpreted EEG in 15/27 patients (56%). Twenty-five patients (89%) have been medicated with antidepressants at some occasion or on a continual basis, and five (18%) with neuroleptics. At the time of the muscle biopsy, seven patients (25%) were taking antidepressant medications (two patients tricyclics, five patients serotonin reuptake inhibitors). No patient was taking any neuroleptic agent.

3.2. KSP results

The response rate to KSP was 100%. Four patients missed occasional items on the inventory, but were included in the analyses for the parts they had filled in adequately. Results are presented in Table 2. The scales are ranked, with the most deviant mean scores in the patients in comparison to the norm group at

Table 2
Results of the Karolinska Scales of Personality questionnaire

Karolinska Scales of Personality	Patients' scores N=21	Major depressive disorder ^a N=23	P-value
Psychasthenia ^b	71±11	63±16	<0.05
Muscular Tension ^c	69±16	66±14	NS
Somatic Anxiety ^d	68±16	65±15	NS
Socialization ^e	35±13	34±12	NS
Psychic Anxiety ^f	61±11	62±13	NS
Suspicion	58±14	59±13	NS
Guilt	56±9	57±10	NS
Irritability	56±10	56±11	NS
Inhibition of Aggression	54±10	56±12	NS
Monotony Avoidance	54±8	57±8	NS
Indirect Aggression	53±11	55±10	NS
Social Desirability ^g	48±9	44±8	<0.05
Verbal Aggression	49±9	49±10	NS
Impulsiveness	49±12	52±12	NS
Detachment	50±13	52±13	NS

The norm group (N=400) used as controls, normal score 50±10.

^a In the third column from the left, KSP scores for other patients with MDD, used by permission of B. Pendse, M.D., are presented. The P-values in the fourth column refer to comparisons between the patients in this study, and the other MDD patient group. NS, not significant.

^b High scorers are described as easily fatigued, feeling uneasy when urged to speed up and when facing new tasks.

^c High scorers are described as tense and stiff, not relaxed.

^d High scorers are described as having autonomic disturbances, restless, panicky.

^e Low scores can be interpreted as reflecting general dissatisfaction and resentment over childhood and present life circumstances.

^f High scorers are described as worrying, anticipating, lacking self-confidence, hypersensitive.

^g High scorers are described as socially conforming, friendly, helpful, or 'faking good' (Gustavsson, 1997).

the top of the table. Scores on the Psychasthenia, Muscular Tension, Somatic Anxiety, Socialization, and Psychic Anxiety scales ranged from one to two standard deviations from the norm group, i.e. the same characteristic KSP score deviations as reported in other MDD studies were found. In comparisons with another selected MDD group (Pendse et al., 1999), Psychasthenia scores in the patients in this study were higher and more aberrant in comparison to the norm group, and Social Desirability scores were higher and more normal in comparison to the norm group.

3.3. Muscle morphology results

The light microscopy investigation showed unspecific muscle cell alterations in 23 patients (82%). A normal mosaic pattern with predominance for fibre type I, as to be expected in the tibial muscle, was detected in 26 patients (93%). There was complete

type I fibre predominance in one patient. Increase of central nuclei was detected in 14 patients (50%), deficiency of stain for COX in seven patients (25%, in one patient found in the deltoid but not in the tibial muscle), and increase of stain for lipid in six patients (21%). Ragged-red fibres (RRF) or increase of stain for glycogen was not found in any patient. Electron microscopy showed minor subsarcolemmal accumulations of mitochondria with normal structure in 11 patients (39%). No light microscopy or ultrastructural changes were detected in three patients (11%).

3.4. Biochemical differences between patients and controls

Mean±S.D. for MAPR and enzyme ratios are presented in Table 3. Significant differences between patients and controls were detected with MAPR using α -ketoglutarate and succinate as substrates.

Table 3
Mean±S.D. for MAPR with different substrates and enzyme ratios

	Patient mean±S.D. N=28	Control mean±S.D. N=10	P-value
Mitochondrial ATP production rate (MAPR) tested with addition of different substrates			
PPKM	6.56±1.73	8.10±1.26	0.019
Glutamate + malate	6.27±1.45	7.38±1.41	0.043
TMPD + ascorbate	5.53±1.08	5.85±1.21	0.444
α-Ketoglutarate	3.71 ± 1.28*	5.10±1.07	0.004
Palmitoyl-L-carnitine + malate	3.48±1.12	4.02±0.64	0.179
Pyruvate + malate	2.43±0.69	2.62±0.63	0.464
Succinate + rotenone	2.06±0.49	2.43±0.42	0.037
Succinate	0.86±0.30*	1.23±0.29	0.002
Mitochondrial respiratory chain enzymes			
NCR/CS	0.77±0.22	0.92±0.20	0.065
SCR/CS	0.46±0.12	0.53±0.09	0.112
COX/CS	1.64±0.18	1.48±0.30	0.057
NCR/SCR	1.77±0.64	1.78±0.51	0.948
NCR/COX	0.47 ± 0.13*	0.64±0.18	0.004
SCR/COX	0.28 ± 0.07*	0.37±0.08	0.005

MAPR using PPKM and palmitoyl-L-carnitine + malate as substrates were performed in nine controls. CS is a mitochondrial matrix enzyme used to assess mitochondrial yield. Significant differences with controls in **bold italics**. For assay units, see Section 2. *The mean difference is significant at the $P < 0.01$ level (two-tailed).

Fig. 1 depicts individual MAPR results for patients and controls. The results of the patient group were spread more widely than the results of the control group for several substrates. None of these wider dispersals was significant by F -tests (MAPR using palmitoyl-L-carnitine + malate $P = 0.053$, remaining $P > 0.10$). ATP production above control range was found in the patient with complete predominance of oxidative type I fibres on muscle histochemistry. When this patient was excluded, the difference between patients and controls in MAPR using PPKM as substrate ($P = 0.019$) became significant ($P = 0.007$). Significant differences between patients and controls were found in the NCR/COX and SCR/COX ratios.

No statistically significant differences on the biochemical analyses were detected between patients with and without antidepressant medication (all $P > 0.10$).

3.5. mtDNA nt1555 and 7445 point mutations

The mtDNA nt1555 and 7445 point mutations were not detected in any patient.

3.6. mtDNA deletions in patients and controls

Deletions were found in 17 patients (68%) and eight controls (36%) using the 9.2 kbp long-PCR amplification. The difference was significant, chi-square = 4.7, $df = 1$, $P = 0.030$. No significant differences were found using the 5.5 kbp or the 9.5 kbp amplifications (deletions were found in one patient and two controls, and 19 patients (76%) and 12 controls (55%), respectively). Southern blot analysis revealed the presence of an mtDNA deletion in one patient. Further analysis of this single deletion has been published elsewhere (Houshmand et al., 1999).

3.7. Correlations between KSP scores and biochemistry

All significant correlations for scores on the KSP scales with mitochondrial biochemistry are presented in Table 4. Negative correlations were found for the KSP scales Psychasthenia, Somatic Anxiety, and Suspicion, with MAPR assessments. A positive correlation was found for Socialization with a MAPR assessment.

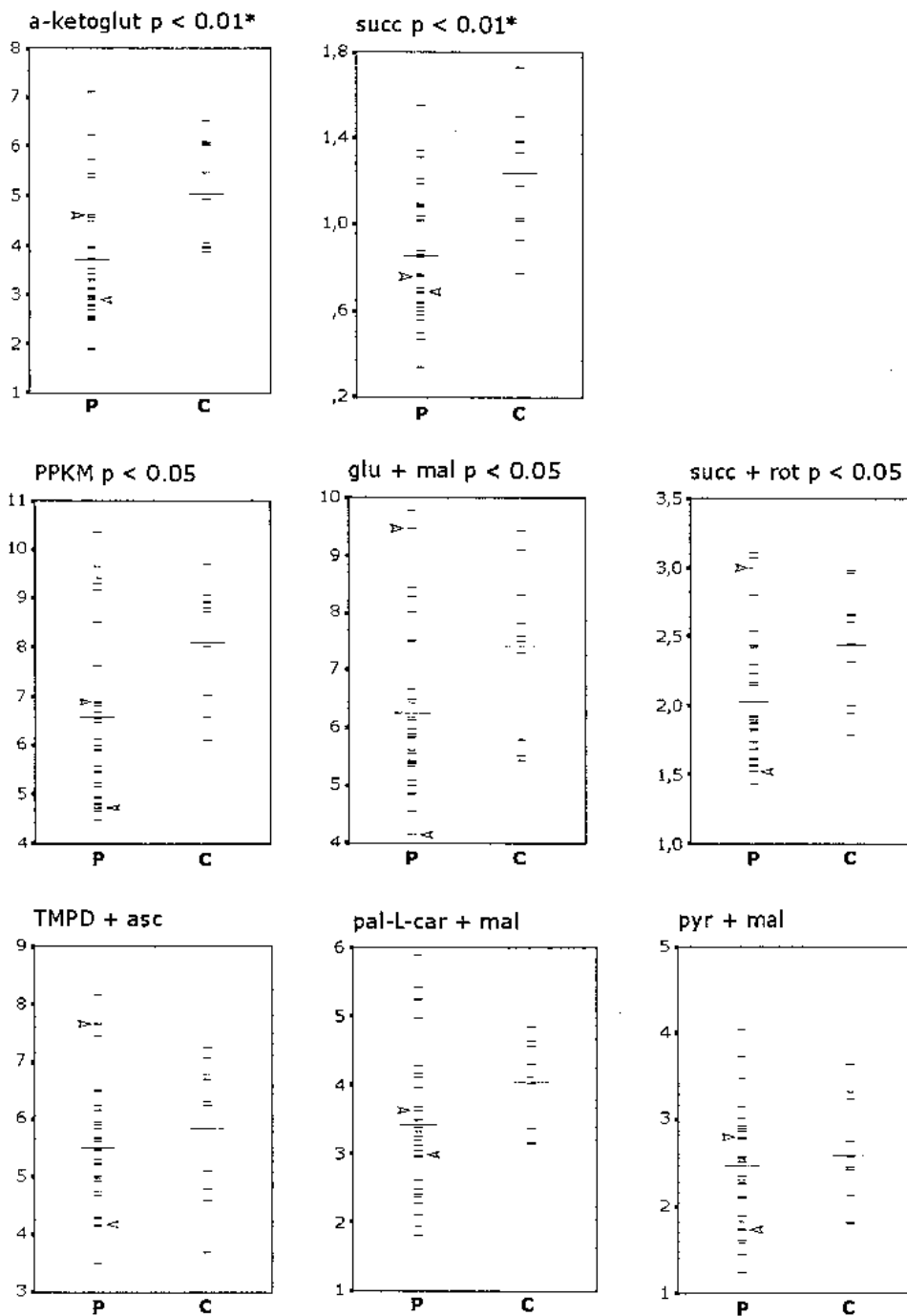


Fig. 1. Individual values for mitochondrial ATP production rates (MAPRs) with different substrates in patients (P) and controls (C). Substrate abbreviations; a-ketoglut, α -ketoglutarate; succ, succinate; glu + mal, glutamate + malate; succ + rot, succinate + rotenone; asc, ascorbate; pal-L-car + mal, palmitoyl-L-carnitine + malate; pyr + mal, pyruvate + malate. Arrowheads; left, results from a patient with MAPRs both above and below control range; right, results from a patient in whom only deleted mtDNA was demonstrated in COX deficient fibres (Houshmand et al., 1999). *Significant mean difference.

Table 4
Correlations between KSP scores and mitochondrial biochemistry

Karolinska Scales of Personality (KSP)	Mitochondrial ATP production rate (MAPR)		
	PPKM	Pal-L-car + malate	Succinate + rotenone
Psychasthenia	−0.57 (0.007)	−0.57 (0.007)	
Somatic Anxiety	−0.62 (0.003)		
Socialization			0.54 (0.011)
Suspicion		−0.57 (0.007)	

Spearman rank correlations (with *P*-values in parentheses) for correlations for KSP with biochemistry. Only correlations considered as significant, see Statistics, are presented. All correlations reflect associations between KSP scores indicating vulnerability to psychopathology with low biochemical measurements.

4. Discussion

This is the first study to examine associations between depression and mitochondrial function. Significant decreases of mitochondrial ATP production rates and mitochondrial enzyme ratios compared to controls were found in MDD patients selected for coexisting specific physical conditions. Significant correlations between scores on rating scales associated with vulnerability to depression, and biochemistry, add a further support for relationships between the biochemical alterations and psychopathology.

4.1. Depressive symptomatology and KSP results in the patients

The course of depressive disorder in the patients is chronic with interspersed mood episodes. The mean KSP scores were found to be different from another selected MDD group (Pendse et al., 1999) on two scales. The mean Psychasthenia score in the patients in this study was higher and even more aberrant from the norm group. The patients in this study may represent a subgroup of unipolar MDD characterized by central fatigue related to difficulties with mental functioning such as attention and concentration, and effortful cognition. The mean Social Desirability score was higher than in the other selected MDD group, and less aberrant from the norm group. The Social Desirability mean score level in the patients in this study was similar to mean score levels in depressed patients in primary care (Ekselius and von

Knorrning, 1999). The difference with the other selected MDD group may be related to the fact that scores in the patients in this study were obtained when mood symptoms were no longer pronounced, or to a difference in this personality trait between these groups.

4.2. Results of the morphological, biochemical, and genetic investigations

At the light- and electron microscopy studies, unspecific changes were detected in the majority of the patients. Similar alterations have been reported previously in patients with affective (Ross-Stanton and Meltzer, 1979, 1981) and psychotic disorders (Borg et al., 1987). Typical muscle morphological alterations suggestive of mitochondrial disease with RRF or structurally altered subsarcolemmal mitochondria, which have been detected in many but not all previously reported patients with mitochondrial disorders (Chinnery and Turnbull, 1997), were not found in any patient in this study. COX-deficient fibres, which are common in mtDNA disorders (Chinnery et al., 1999), were found in a quarter of the patients. However, COX-deficient fibres may be secondary to several disease processes. No morphologic abnormalities in muscle were found in healthy controls in a study of CFS patients. In the CFS group there was increase of stain for lipid in a quarter, and mitochondrial accumulations in almost a fifth, of the patients (Behan et al., 1999).

Significant MAPR decreases were found in the

patients with the substrates α -ketoglutarate and succinate indicating decreased activity in enzyme complexes before complex IV. MAPR has been shown to be highly correlated to mitochondrial oxygen consumption in preparations of isolated mitochondria (Tonkonogi and Sahlin, 1997).

Significant decreases were found in the NCR/COX (complex I+III/complex IV) and SCR/COX (complex II+III/complex IV) enzyme ratios in the patients, lending support to the MAPR findings. No significant imbalance between respiratory chain enzymes has been suggested to occur as a result of normal physiological ageing. Absolute respiratory chain enzyme values have been suggested to be of limited value when diagnosing partial defects of respiratory chain enzymes (Chretien et al., 1994). Medication did not seem to influence the biochemical results, as no difference between unmedicated and medicated patients was found.

Alterations of oxidative metabolism on biochemical examination of muscle homogenate are specific for mitochondrial disorders, but the examination is not sensitive enough for definite diagnosis in many cases since mitochondrial cytopathies may only be expressed in a minority of fibres. Even rather low levels of mutated mtDNA may cause a pathological phenotype since mtDNA mutations affect individual muscle fibres. Even low degrees of single mtDNA deletions in muscle have been demonstrated to result in biochemical abnormalities indicating absence of a well-defined threshold (Schröder et al., 2000).

Wider but non-significant dispersals of MAPR results in the patients in this study compared to controls suggest that the patients may comprise a heterogeneous group with more than one type of mild energy defect. In CFS patients, the dispersal of lactate/pyruvate production ratios in myoblast cultures was found to be significantly increased, suggesting heterogeneity of aerobic defects with some cases of mild defects in mitochondrial respiration as well as of pyruvate dehydrogenase deficiency (Behan et al., 1999).

Long-PCR revealed multiple short bands in several patients and controls. Deletions of mtDNA were found mainly in the older individuals, as to be expected. A significant difference was found, with more patients than controls found to harbour mtDNA deletions using the 9.2 kbp amplification.

An expanding number of autosomal diseases has been associated with mtDNA multiple deletions. These disorders have been classified as defects of intergenomic communication because mutations of the nuclear DNA are thought to disrupt the normal cross-talk that regulates the integrity of mtDNA (Hirano and Vu, 2000). Since its advent, long-PCR has been adopted as a screening tool for mtDNA deletions, and has been used in many studies (Coulter-Mackie et al., 1998). However, the method has recently been criticized as unreliable when used for diagnostic purposes (Kajander et al., 1999; Lightowers et al., 1999).

In this study, long-PCR was used to demonstrate differences between groups, rather than as a diagnostic tool. The unreliability of long-PCR was demonstrated by the fact that no deletions were found with this method in the only patient in whom deletions were found with the Southern blot analysis. One or both the primers that were used in each PCR reaction lost its site because of the deletion, and only non-deleted mtDNA was amplified. In this patient, only deleted mtDNA was detected in isolated COX-deficient fibres (Houshmand et al., 1999). Analysis of mtDNA in isolated muscle fibres has not been performed in any other patient.

The significant decreases of ATP production and mitochondrial respiratory chain enzyme ratios that were detected in the patients, and the significantly increased proportion of patients harbouring mtDNA deletions, indicate mitochondrial dysfunction that may represent the final common path for several events including mutations in mtDNA or nuclear genes, or other events affecting mitochondrial function such as enzyme co-factor levels.

4.3. *Correlations between biochemistry and KSP*

Bonferroni adjustment for multiple tests were not used in the correlation assessments, in accordance with the suggestions by Perneger (1998), and Damberg et al. (2000). Other precautions were undertaken to exclude chance findings due to a few extreme cases contributing to significant correlations (see Statistics). Significant correlations were found for four of the 15 KSP scales with biochemistry. The correlations were all in line with the expected effect pattern. High scores on Psychasthenia and Somatic

Anxiety were associated with low ATP production. In a twin study utilizing five of the 15 KSP scales, genetic factors were shown to influence individual differences on the Psychasthenia and Somatic Anxiety scales, but not the Muscular Tension, Psychic Anxiety, or inhibition of Aggression scales (Gustavsson et al., 1996). The negative and positive correlations that were found in this study with Suspicion and Socialization, respectively, may reflect associations between difficulties in social interactions and low ATP production.

The significant correlations that were found for KSP scales with biochemistry are a further support of a pathophysiological significance of the biochemical alterations, and for relationships between skeletal muscle intracellular milieu, and brain cell intracellular milieu.

5. Conclusion

The patients in this study were selected such that all patients had a lifetime diagnosis of major depressive disorder and chronic physical conditions that have been reported previously in patients with depressive as well as mitochondrial disorders. Significant differences between patients and healthy controls were found on muscle mitochondrial biochemistry. A significantly higher proportion of patients in comparison to healthy controls were found to harbour mtDNA deletions. Significant correlations for biochemistry with scores on the Karolinska Scales of Personality scales suggest a pathophysiological importance of the biochemical alterations. The clinical presentation of the coexisting conditions in the patients has been designated COMP for cochlear and mild ocular, muscular and psychiatric symptoms. Further studies are warranted to elucidate if mitochondrial dysfunction is found in other patients with the COMP symptomatology, and to compare the findings with those from other mood disorder patients without chronic medical conditions.

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