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**Mitochondrial dysfunction and alterations of
brain HMPAO SPECT in depressive disorder -
perspectives on origins of 'somatization'**

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'Moralistic' labels such as---somatization---enable the physician to believe that he/she knows more about the patient than is warranted by actual experience with them.---Moralistic labels encourage the physician to overlook or dismiss complaints about symptoms, perform a more cursory physical exam, [and] omit the ordering of laboratory tests.---This will lead the physician to miss a medical diagnosis because 'it has been superseded by a metaphorical one'.

(Howard F Stein: American Medicine as Culture. Boulder: Westview Press. 1990, p. 71).

To Stig, Robin and Isobel

The present thesis is based on the following studies

- I. Gardner A, Johansson A, Wibom R, Nennesmo I, von Döbeln U, Hagenfeldt L, Hällström T. Alterations of mitochondrial function and correlations with personality traits in selected major depressive disorder patients. *J Affect Disord* 2003; 76: 55-68.
- II. Houshmand M, Gardner A, Hällström T, Müntzing K, Oldfors A, Holme E. Different tissue distribution of a mitochondrial DNA duplication and the corresponding deletion in a patient with a mild mitochondrial encephalomyopathy: deletion in muscle, duplication in blood. *Neuromuscul Disord* 2004; 14: 195-201.
- III. Gardner A, Pagani M, Wibom R, Nennesmo I, Jacobsson H, Hällström T. Alterations of rCBF and mitochondrial dysfunction in major depressive disorder: a case report. *Acta Psychiatr Scand* 2003; 107: 233-239.
- IV. Gardner, Hällström T. High somatic distress with high long-term stability in selected patients with chronic depression: a 3-year follow-up of ratings with Karolinska Scales of Personality (KSP). *Nord J Psychiatry*: in press.
- V. Gardner A, Pagani M, Jacobsson H, Lindberg G, Larsson SA, Wägner A, Hällström T. Differences in resting state rCBF assessed with ^{99m}Tc-HMPAO SPECT and brain atlas matching between depressed patients with and without tinnitus. *Nucl Med Commun* 2002; 23: 429-439.
- VI. Gardner A, Pagani M, Beier H, Jacobsson H, Larsson SA, Hällström T. ^{99m}Tc-HMPAO distribution at SPECT is associated with succinate-cytochrome *c* reductase (SCR) activity in subjects with psychiatric disorders. *Nucl Med Biol* 2004; 31: 277-282.

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Abstract

Gardner, A. 2004. Mitochondrial dysfunction and alterations of brain HMPAO SPECT in depressive disorder - perspectives on origins of 'somatization'. Karolinska Institutet at Karolinska University Hospital in Huddinge, Neurotec Institution, Division of Psychiatry, SE-141 86 Stockholm

A range of somatic symptoms are more common in patients with major depression than in the general population. Similar somatic symptoms and depression have been described in mitochondrial disorders, in which decreased production of adenosine triphosphate (ATP) is found. The aim of the present thesis was to investigate mitochondrial functions, regional distribution of the radiotracer HMPAO at brain SPECT with 3-dimensional interpretation, and results of Karolinska Scales of Personality (KSP) in patients with chronic depression and somatic symptoms. Relationships between measures were explored.

The thesis is based on six papers:

- I. ATP production and other investigations including mitochondrial DNA (mtDNA) studies in muscle were analyzed in 28 patients, of whom 21 patients filled in the KSP.
- II. Case history and in situ-hybridization of muscle mtDNA in one patient from study I.
- III. Case history and results of muscle biopsy, KSP and HMPAO SPECT in another patient from study I.
- IV. KSP was filled in by 84 patients. A follow-up rating after 3.5 years was obtained in 65 patients. Comparisons were performed with depressed patients in primary care.
- V. Comparisons of HMPAO SPECT results between depressed patients, 27 with and 18 without tinnitus.
- VI. Relationships between the activity of succinate-cytochrome c reductase (SCR, enzyme complex II + III in the mitochondrial respiratory chain) in muscle and the HMPAO distribution at SPECT in 20 unmedicated patients with chronic psychiatric disorders, 16 with depression and four with schizophrenia.

Significantly lower ATP production and respiratory chain enzyme ratios, and increased prevalence of deleted mtDNA, were found in a group of depressed patients with somatic symptoms in comparisons with healthy controls. Low ATP production correlated with high KSP scores for somatic symptoms. Psychiatric symptoms were the first overt disease presentation in two patients also presented individually. Deleted mtDNA correlated with phenotypic expression in one patient with mtDNA rearrangements in blood and muscle. Stable and significantly increased KSP scores were found for somatic symptoms in a large patient group compared with depressed patients in primary care. Significant differences at HMPAO distribution at SPECT were found between depressed tinnitus and non-tinnitus patients in some brain regions previously found to be involved in tinnitus perception. Significant relationships between SCR and HMPAO distribution in associative sensory regions in patients with psychiatric disorders indicate that mitochondrial functions may contribute to SPECT alterations in these disorders.

Biological alterations could be demonstrated in patients with chronic depression and somatic symptoms using novel investigatory methods. Relationships were found between biological alterations and symptom presentation. Various origins of somatic symptoms in depression have been proposed including 'somatization' reflecting a psychological mechanism rather than biological abnormality. The results of the thesis indicate that chronic depression, at least when associated with somatic symptoms, is a systemic disorder with a disturbance at the level of a cell organelle, and entail a different perspective on the etiology of 'somatization'.

Keywords: 99mTc-d,l-hexamethylpropyleneamine oxime, clinical rating scales, hearing loss, mtDNA, muscle biopsy, PCR, single photon emission computed tomography, tinnitus, unipolar depression.

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ABBREVIATIONS

3-D	three-dimensional
ADP	adenosine diphosphate
ATP	adenosine triphosphate
ANT	adenine nucleotide translocator
bp	base pair
CBA	computerized brain atlas
COX	cytochrome <i>c</i> oxidase, complex IV
DNA	deoxyribonuclei acid
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, 4 th Edition
FADH ₂	reduced flavin adenine dinucleotide
GSH	reduced glutathione
KSP	Karolinska Scales of Personality
MADRS-S	The self-report version of the Montgomery-Åsberg Depression Rating Scale; rating of depression severity
MAPR	mitochondrial ATP production rate
MRS	(proton) magnetic resonance spectroscopy
MSBP	Münchausen syndrome by proxy; purportedly involves a parent (usually the mother) who deliberately induces or exaggerates illness in a child to gain attention from the medical community
mtDNA	mitochondrial DNA
NADH	reduced nicotinamide adenine dinucleotide
NCR	NADH-cytochrome <i>c</i> reductase, complex I + III
nDNA	nuclear DNA
OXPPOS	oxidative phosphorylation
³¹ P	phosphorous-31
PCR	polymerase chain reaction
PPKM	pyruvate + palmitoyl-L-carnitine + α -ketoglutarate + malate
rCBF	regional cerebral blood flow
RNA	ribonuclei acid
ROI	region of interest
RRF	ragged-red fibres, proliferated mitochondria
rRNA	ribosomal RNA
SCR	succinate-cytochrome <i>c</i> reductase, complex II + III
SD	standard deviation
SDH	succinate dehydrogenase, complex II
SOE	singlet oxygen energy
SPECT	single photon emission computed tomography
^{99m} Tc-HMPAO	^{99m} Technetium - <i>d,l</i> - hexamethylpropylene amine oxime
TMPD	N,N,N ¹ ,N ¹ -tetramethyl-1,4-phenyldiamine
tRNA	transfer RNA
VOI	volume of interest

INTRODUCTION

The Mood Disorders, in which a mood disturbance is considered to be the most predominant feature, are divided into Depressive Disorders (‘unipolar depression’), Bipolar Disorders, and two disorders based on etiology – Mood Disorders Due to a General Medical Condition, and Substance-Induced Mood Disorder. Bodily aches and pains, decreased energy, tiredness or sustained fatigue without physical exertion, concentration difficulties, distractability, impaired memory, and anxiety and excessive worry over physical health, are listed as associated features of Major Depressive Episodes in the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) (APA, 1995).

The origin of the associated somatic symptoms in depressive disorder is unknown. Similar somatic symptoms are common in mitochondrial disorders, i.e., conditions where the origin of the symptoms is known. In this section, somatic symptoms in depression, and psychiatric and somatic symptoms in mitochondrial disorders, are described. Previous studies of mitochondrial functions in psychiatric disorders, and functional brain imaging in patients with mitochondrial and depressive disorders, are reviewed. ‘Psychic’ and ‘somatic’ anxiety, and a rating scale assessing these different anxiety components, are presented at the end of the section.

Somatic complaints in depression

Mathew et al (1981) administered a physical-symptom questionnaire assessing a variety of somatic complaints to 51 unmedicated depressed patients and 51 matched healthy controls. No patient had any known medical illness. Twenty-one of the 27 symptoms listed on the questionnaire were reported more frequently in patients than controls. No relationship was found between physical symptoms and severity of depression.

Audiological, ophthalmological, pain and other symptoms and findings in depression

Audiological symptoms are common in patients with depressive disorders. Tinnitus (an adverse auditory perception appearing in the absence of external sound, and lacking content or meaning) may occur in subjects with normal as well as impaired hearing. Tinnitus has been reported in 49% of unmedicated depressed patients (Mathew et al, 1981). Hyperacusia (sound intolerance) may occur in depression (Carman, 1973) and hearing impairment (Katzenell and Segal, 2001). A relationship between hearing loss and depression in middle-aged and aged subjects has been reported (Herbst and Humphrey, 1980; Yovell et al, 1995).

Blurred vision has been reported in 51% of unmedicated depressed patients (Mathew et al, 1981). Other ocular symptoms in depression are alterations of pupillary responses (Sokolski et al, 2000), decreased perception of ambient light (Friberg and Borrero, 2000), and prolongation of the reaction time of saccadic eye movements (Mahlberg et al, 2001). Increased prevalences of muscular pain (Rajala et al, 1995; Corruble and Guelfi, 2000), fatigue (‘low energy’) (Coulehan et al, 1988), migraine/cluster headaches (Moldin et al, 1993), and irritable bowel syndrome (Dewsnap et al, 1996), have also been reported.

A population prevalence of 14% has been reported for tinnitus (Axelsson and Ringdahl, 1989), chronic myalgia (Magni et al, 1990), headaches (Hagen et al, 2000), and irritable bowel syndrome (Drug et al, 2002). The same figure of 14% was also found for blurred vision in a large control group (Vincent et al, 1989). Population prevalences of 10-19% have been reported for chronic fatigue (Hotopf and Wessely, 1997). A longitudinal prevalence rate of 12% for 'atypical depression' has been reported in a population sample. 'Atypical depression' includes features such as severe lethargy and fatigue (Angst et al, 2002). It is not known if there is overlap between the subjects in the population reporting tinnitus, chronic myalgia, headaches, irritable bowel syndrome, blurred vision, and fatigue, and if there is overlap with subjects with depressive symptoms.

Findings indicating that brains of unmedicated patients with treatment-refractory depression use an energy source other than glucose have been reported suggesting increased metabolism of other energy substrates (Lambert et al, 2000). Other indices of disturbed carbohydrate metabolism in depressed patients are an increased prevalence of type II diabetes (Eaton et al, 1996; Grandinetti et al, 2000), and increased intra-abdominal fat deposition (Thakore et al, 1997). Unmedicated patients with depression and generalized anxiety have been found to have increased levels of cholesterol and triglycerides (Sevincok et al, 2001). An increase of daytime body temperature of unknown origin has been reported in unmedicated depressed patients (Rausch et al, 2003).

Mitochondrial disorders: background

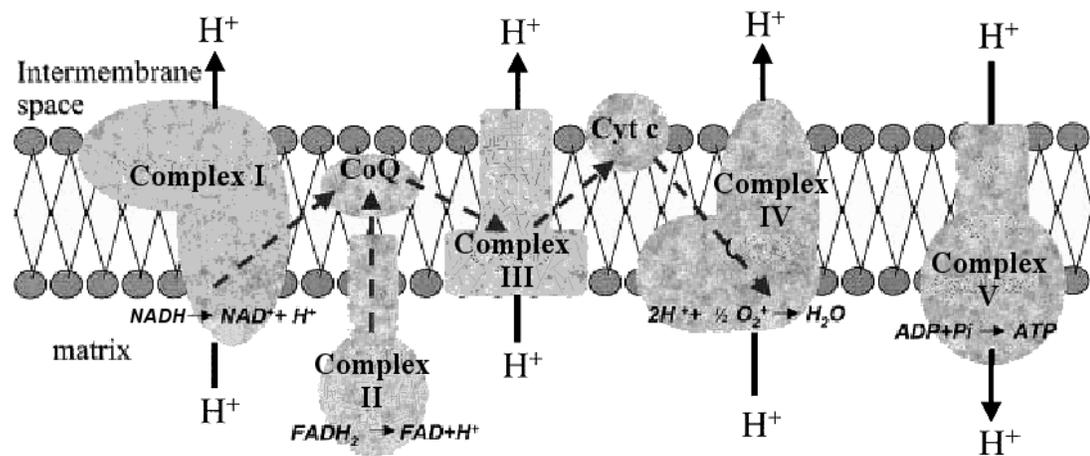
Mitochondrial disorders are examples of clinically heterogeneous multisystem disorders with psychiatric as well as somatic symptoms. Mitochondrial disease with non-thyroidal hypermetabolism and increased body temperature due to 'loose coupling' between mitochondrial respiration and phosphorylation was first reported in a patient investigated at Karolinska Hospital in Stockholm over 40 years ago (Luft et al, 1962; Smeitink, 2003). Although only two cases with the same disease presentation have been described in the literature, the clinical description and biochemical studies paved the way for clinical and pathological research on patients with suspected mitochondrial disease. Patients were classified into groups based upon the pattern of clinical involvement, histological and ultrastructural abnormalities of mitochondria, and biochemical assays of mitochondrial function. It was clear that there were clinical similarities among some patients, allowing the definition of specific syndromes, but it was recognised that there was considerable phenotypic diversity and that many patients did not fit neatly into a specific diagnostic group.

Mitochondria are cell organelles, subcompartments of the cell surrounded by two membranes. They may be 'cigar' shaped structures (see Figure 1, next page), or make up fusing networks similar to the endoplasmic reticulum. They play a part in intra-cellular signalling and apoptosis, and in the metabolism of amino acids, lipids, cholesterol, steroids, and nucleotides. The most fundamental function for mitochondria may be their role in cellular energy metabolism. This includes fatty acid β -oxidation, the urea cycle, and the final common pathway for production of adenosine triphosphate (ATP) in the respiratory chain (Chinnery and Schon, 2003).



Figure 1. A schematic image of a mitochondrion with outer and inner membranes.

The mitochondrial respiratory chain consists of five enzyme complexes situated on the inner mitochondrial membrane. Each complex is composed of multiple subunits, the largest being complex I with at least 45 polypeptide components, of which seven are encoded by mitochondrial DNA (mtDNA) and the remainder by nuclear DNA (nDNA) (Smeitink, 2003). The overall process is called oxidative phosphorylation (OXPHOS) and is schematically presented in Figure 2.



Complex subunits:

mtDNA-encoded	7	0	1	3	2
nDNA-encoded	38	4	10	10	12

Figure 2. The picture (modified from Ben-Shachar, 2002) shows the respiratory chain complexes, with their rough spatial configurations, embedded in the mitochondrial inner membrane. Reduced cofactors (NADH and FADH₂) generated from the intermediary metabolism of carbohydrates, proteins, and fats, donate electrons to complex I and II. These electrons flow between the complexes down an electrochemical gradient, shuttled by complexes III and IV and by two mobile electron carriers, ubiquinone (coenzyme Q10) and cytochrome c. The liberated energy is used by complexes I, III, and IV to pump protons (H⁺) out of the matrix, the mitochondrial centre, into the intermembrane space, the space between the outer and inner membranes. This proton gradient, which generates the bulk of the mitochondrial membrane potential (the asymmetrical distribution of ions across the inner membrane makes up the “chemical” portion of the gradient), is harnessed by complex V to synthesise ATP from adenosine diphosphate (ADP) and inorganic phosphate (Pi).

ATP is the high energy source used for essentially all active metabolic processes within the cell, and it must be released from the mitochondrion in exchange for cytosolic ADP. Thus the respiratory chain is an elaborate system that must respond to the energy requirements of the cell. While these requirements may be constant (e.g., in hepatocytes), they may also change dramatically over short periods of time (e.g., in muscle cells and neurons).

In the early 1960s it was discovered that mitochondria contain their own DNA, i.e., mtDNA. The human mtDNA was sequenced in 1981 and has been found to code for 13 essential subunits of respiratory chain complexes, two ribosomal RNA (rRNA) genes and 22 transfer RNA (tRNA) genes that are needed for intramitochondrial protein synthesis. Nuclear genes code for the majority of mitochondrial respiratory chain polypeptides. These polypeptides are synthesised in the cytoplasm with a mitochondrial targeting sequence that directs them through the translocation machinery spanning the outer and inner membranes of mitochondria (Chinnery and Schon, 2003).

Mitochondrial disorders may thus arise due to mutations in two distinct genetic systems. Some patients appear to be sporadic cases, whereas others are clearly familial. Identification of pathogenic mtDNA mutations that could be linked to specific disorders was reported in 1988. MtDNA is inherited in the maternal line, and a maternal inheritance pattern has been established for some mtDNA point mutations. Failure to eliminate paternal mtDNA has been observed in abnormal embryos (St John et al, 2000). Paternal inheritance of mtDNA in a human has been reported only once (Schwartz and Vissing, 2003). Over 150 different pathogenic point mutations and a larger number of different rearrangements (partial deletions and duplications) of mtDNA have been associated with disease. Various nuclear genes have also been identified that are fundamentally important for mitochondrial homeostasis, and when these genes are disrupted, they cause autosomally inherited mitochondrial disease (Chinnery and Schon, 2003). An alteration of cell surface receptors has been suggested to cause the mitochondrial dysfunction observed at disturbance of an extracellular constituent, a matrix protein found in skeletal muscle, skin and cartilage (Irwin et al, 2003).

While most human cells contain two copies of nuclear DNA, they contain many more copies of mtDNA (from 1000 to 100 000, depending on the cell type). The expression of the whole mtDNA consisting of a ~16 600 base pair circle of double stranded DNA is essential for mitochondrial bioenergetic function, whereas only ~7% of the nuclear genome of ~ 10^9 base pairs is ever expressed at any particular differentiated stage (Ozawa, 1997).

MtDNA molecules are all identical in a healthy individual at birth (homoplasmy). By contrast, patients harbouring pathogenic mtDNA defects often have a mixture of mutated and normal, or 'wild-type', mtDNA (heteroplasmy). The percentage of mutated mtDNA can vary widely among different patients also from the same maternal line, and within the same individual also from organ to organ, and even between cells. Clinical expression of mitochondrial disorders is influenced by heteroplasmy, mtDNA background, nuclear genes, and their interaction with the environment (Chinnery and Schon, 2003).

Energy consumption in the brain and selective vulnerability

ATP derived from mitochondrial oxidative phosphorylation of glucose is the main source of chemical energy in the brain. Approximately 50 - 60% of the energy consumed is spent in maintaining ionic gradients across the membranes necessary for neuron excitability. Approximately 30% of the energy is used for transmitter turnover, which includes several energy requiring processes such as transmitter synthesis, packing into vesicles, flux of vesicles, and transmitter uptake. The maintenance of brain structure may require about 10 – 20% of the ATP production. Dendritic metabolism has been suggested to make the largest single contribution to the metabolic activity of the brain. The majority of synapses are made in dendrites, which constitute the largest surface area of the neuron, and contain the highest reported mitochondrial areal density that has been reported so far, more than 60%. Dendrites almost always stain high for oxidative enzymes, e.g., complex IV (cytochrome *c* oxidase, COX). Neuronal cell bodies can stain low, moderate or high. Certain cortical interneurons, receiving both excitatory and inhibitory synapses, stain high which may reflect the energy demand for ion pumping following depolarizing potentials (Wong-Riley, 1989; Douglas and Martin, 1998; Ledberg, 2001).

Dendritic terminals forming symmetric synapses, which are presumed to be inhibitory and to have greater tonic (sustained high-frequency firing) activity than most excitatory terminals, stain the highest for COX (Wong-Riley, 1989). Tonic neurons, terminals and synapses have a higher mitochondrial density than their phasic counterparts (Ishihara et al, 1997; Nguyen et al, 1997; Brodin et al, 1999) suggesting a higher energy expenditure, and a higher vulnerability to decrements of energy production, in tonic as compared to phasic neuronal elements.

Findings of unevenly distributed activities of mitochondrial electron transport chain enzymes in various brain regions and subcellular compartments, and of unequal proportions with other electron transport chain enzymes between brain regions, suggest selective brain distribution of mitochondrial components (Battino et al, 1991). The 'selective vulnerability' concept refer to the phenomenon that a cellular subpopulation may be selectively more affected by a generalized impairment. Selective vulnerability has been suggested to reflect selective distribution of mitochondrial components involved in ATP production in the vulnerable as compared to the non-vulnerable brain cells (Blass, 1999).

Personality, cognition and mtDNA

MtDNA can be included among the multitude of biological components with behavioural effects. Scores in the pathological range on a questionnaire assessing Hypochondriasis, Hysteria and Paranoia have been found in about half of mothers of children with mitochondrial disorders. The elevated scores may reflect intrinsic personality features somehow linked to maternal carrier status for mitochondrial disorder (Varvogli and Waisbren, 1999). MtDNA has been found to be associated with IQ in humans (Skuder et al, 1995) and cognition in mice (Robertoux et al, 2003).

Depressive and neuropsychological symptoms in mitochondrial disorders

Depressive disorders have been described in case histories of patients with different mitochondrial disorders (Stewart and Naylor, 1990; Suomalainen et al, 1992; Sweeney et al, 1993; Melberg et al, 1996; Miyaoka et al, 1997; Onishi et al, 1997; Santorelli et al, 1997; Campos et al, 2001; Jaksch et al, 2001; Berio and Piazzini, 2002; Siciliano et al, 2003).

Depression was listed as one of the manifestations of mitochondrial disorders by Fadic and Johns (1996). Other psychiatric manifestations considered to be manifestations of mitochondrial disorders by these authors are psychosis, and non-specific anxiety and personality disorders. Chinnery and Turnbull (1997), in another review article about mitochondrial disorders, write that 'psychiatric complications of mitochondrial disease are common. These usually take the form of a reactive depression [---]. We have seen a number of cases of severe depression and attempted suicide *before* [authors' italic] diagnosis.'

Deficits in attention and abstraction/flexibility suggesting frontal lobe involvement, and deficits in short-term memory and spatial cognition, have been demonstrated at neuropsychological investigations in adults with mitochondrial disorders. There were no signs of general cognitive decline (Turconi et al, 1999; Bosbach et al, 2003).

Somatic symptoms in mitochondrial disorders

Tissues and organs that are heavily dependent upon oxidative phosphorylation are affected the most in mitochondrial disorders. Mitochondrial disorders, because of the widespread cellular distribution of mitochondria, may lead to all kinds of clinical signs and symptoms ranging from mild myopathic complaints to neonatal death and virtually everything in between (Smeitink, 2003). The manifestations of mitochondrial disorders have been presented in review articles (e.g., Fadic and Johns, 1996; Chinnery and Turnbull, 1997; Cohen and Gold, 2001; Schmiedel et al, 2003).

Audiological symptoms with tinnitus, and/or sensorineural hearing loss of various age-of-onset and severity, are among the most common manifestations of mitochondrial disorders (Fadic and Johns, 1996; Korres et al, 1999; Chinnery et al, 2000; Deschauer et al, 2001).

Visual and ocular symptoms include blurred vision, photophobia (hypersensitivity to light), nyctalopia (night blindness), corneal edema or opacities, slowing of saccadic eye movements, external ophthalmoplegia, ptosis, and visual impairment or blindness due to cataracts, optic atrophy, pigmentary retinopathy, and macular degeneration (Fadic and Johns, 1996; Yen et al, 1996; Chinnery and Turnbull, 1997; Smith et al, 1999; Boonstra et al, 2002).

Exercise intolerance, which may be the sole manifestation of a mitochondrial disorder, and muscular weakness and pain, are common. Rapid heartbeat and a sense of breathlessness may be provoked even by trivial exercise in patients with mitochondrial disorders without evidence of impaired heart conduction or cardiomyopathy. Exaggerated cardiovascular and ventilatory responses during exercise have been suggested to be sensitive indicators of an underlying mitochondrial impairment (Taivassalo et al, 2003). Painful muscle stiffness and myoclonia may occur (Deschauer

et al, 2001). Gross muscle wasting and severe weakness are rare (Fadic and Johns, 1996; Chinnery and Turnbull, 1997).

Asymptomatic peripheral neuropathy with loss of sensation, or severe neuropathic pain, may occur (Fadic and Johns, 1996; Chinnery and Turnbull, 1997; Cohen and Gold, 2001).

The symptoms of mitochondrial disorders can mimic the chronic fatigue syndrome. It is not known what percentage of patients with chronic fatigue have mitochondrial dysfunction, but they are suspected to be rare (Chinnery and Turnbull, 1997; Cohen and Gold, 2001).

Cerebral symptoms may be migraine-like headaches, stroke-like episodes and early strokes, progressive encephalopathy with periodic exacerbations leading to seizures and dementia, and cerebellar ataxia (Fadic and Johns, 1996; Chinnery and Turnbull, 1997).

Cardiac manifestations include conduction abnormalities which are common, and severe dysrhythmias that may necessitate cardiac pacemaker. Cardiomyopathy that may necessitate heart transplantation is a serious but uncommon complication (Santorelli et al, 2002; Holmgren et al, 2003; Schmiedel et al, 2003).

Gastrointestinal symptoms with episodic nausea and vomiting, gut dysmotility with chronic diarrhoea, or chronic constipation that may progress to intestinal pseudo-obstruction in a minority of cases, are also common in mitochondrial disorders (Chinnery and Turnbull, 1997; Nishino et al, 2000; Cohen and Gold, 2001).

Hyperlipidaemia, and diabetes type II and thyroid dysfunction that may include organ specific antibodies, are other common manifestations of mitochondrial disorders (Fadic and Johns, 1996; Chinnery and Turnbull, 1997; Finsterer et al, 2001).

Apart from sideroblastic anaemia and transient elevations of serum alanine transaminase, clinically significant haematological or hepatological complications are rarely seen in mitochondrial disorders in adults. Renal symptoms may occur due to complications of neuropathic urinary retention (Chinnery and Turnbull, 1997).

Smeitink (2003) propose the consideration of mitochondrial disorder in every chronic, intermittent or progressive disorder with single system or multisystem involvement, and writes 'Among the different groups of inborn errors of metabolism, mitochondrial disorders are the most frequent, with an estimated incidence of at least 1 in 10 000 live births. This incidence figure [---] is certainly an underestimation if one takes into account the high number of genes that are expected to be involved in mitochondrial biogenesis and maintenance.' The proposal is supported by the findings of a recent study of mtDNA sequence variation in hearing-impaired patients with maternal relatives with hearing impairment. An increase of mtDNA variants was found in the patients suggesting a mildly deleterious effect of mtDNA variants previously considered as probable neutral polymorphisms (Lehtonen et al, 2003).

Diagnostic problems in mitochondrial disorders

As a general rule, cells in mitochondrial diseases are disabled, but often do not die. Muscle necrosis, inflammatory reaction or connective tissue infiltration is usually absent in mitochondrial myopathies. Blood levels of muscle creatine kinase and lactate may be increased, but are often normal. Investigations have to be performed at specialist centres, where measurements of the individual respiratory chain complexes are

performed, usually in muscle biopsies. However, the muscle may be both morphologically and histochemically normal (Chinnery and Turnbull, 1997; Schon et al, 1997). Respiratory chain enzymes in patients with mtDNA mutations may be within the normal range (Sciacco et al, 2001). A mitochondrial disorder may result in a functional defect that is not detectable in the laboratory. There is no one single test that will prove or disprove whether a patient has a mitochondrial disorder (Chinnery and Schon, 2003). 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).

As seen from the presentation of symptoms in mitochondrial disorders above, many symptoms are unspecific. The symptoms may also show an episodic course, or periodic exacerbations (Fadic and Johns, 1996; Chinnery and Turnbull, 1997). Spontaneous recovery of vision even after years of blindness has been reported in patients with a mitochondrial optic neuropathy (Stone et al, 1992, Yamada et al, 1997). Considering these observations, and that mitochondrial disorders are still unknown by many doctors, it is not surprising that such symptoms as photophobia, vertigo and increasing fatigue in a child who eventually was shown to have a mitochondrial disorder initially were considered as psychosomatic (Nørby et al, 1994). Chinnery and Turnbull (1997) note that breathlessness, muscle weakness and chronic fatigue in depressed patients with mitochondrial disorders 'were dismissed as being functional for many years'.

Muscle and mitochondrial studies in affective disorders

An elevation of skeletal muscle creatine kinase in blood, and morphological and histochemical alterations of a diverse nature in muscle, were reported in 1973 in more than half of inpatients with affective disorders in whom schizoaffective disorder was excluded. The alterations were only found in patients considered to have psychotic, but not neurotic, depressive symptoms. In a follow-up study, no effects of antidepressant medications were found on creatine kinase levels or muscle cell alterations. Standard methods were used for histochemistry and electron microscopy. The detected abnormalities were excessive scattered atrophy, fibre type grouping, central core or targetoid fibres, cytoplasmic or rod bodies, Z-band streaming and myofibrillar disruption (Meltzer, 1973; Ross-Stanton and Meltzer, 1979).

In a study of gene expression in post-mortem frontal cortex samples from subjects with depression and schizophrenia, altered gene expression was found in seven of 74 assays. Six of the seven assays were altered in the group of depressed subjects, and four of the seven assays in the group of schizophrenic subjects. No information about depressive subtypes or medication was provided for the depressed subjects. Further analysis for identification of the genes whose relative transcript levels were altered was conducted in samples from the schizophrenic patients and indicated that the altered gene expression derived from mtDNA. The results of the study were interpreted to suggest altered mitochondrial gene expression in affective disorder and schizophrenia (Whatley et al, 1996).

Excess maternal transmission that may be due to various phenomena, including mtDNA mutations, has been reported for bipolar disorder in two studies (McMahon et al, 1995; Gershon et al, 1996).

An increase of homoplasmic mtDNA sequence variants (polymorphisms) at bp 5178 and 10398 (positions are numbered anticlockwise on the ~16 600 bp circular mtDNA molecule) has been found in bipolar disorder that was suggested to indicate risk factors for the disorder. The polymorphisms cause amino acid substitutions in two mtDNA-encoded subunits of complex I (Kato et al, 2000; 2001). The gene expression level of an nDNA-encoded complex I subunit located to chromosome 18p11 has been found to be decreased in leucocytes in bipolar disorder. Previous reports suggest the 18p11 position as a susceptibility locus for bipolar disorder. Altered intracellular calcium signaling due to a decrease in the mitochondrial membrane potential are among the effects of inhibition of complex I (Washizuka et al, 2003).

ATP is released by the mitochondrion in exchange for cytosolic ADP. This is carried out by the adenine nucleotide translocator (ANT) embedded in the inner membrane, which has various tissue specific isoforms. Mutations in the nuclear gene ANT1 cause secondary mtDNA deletion formation (Chinnery and Schon, 2003). The ANT1 protein is a structural part of the mitochondrial permeability transition pore involved in the regulation of cellular calcium levels. A mutation in the ANT1 gene located to chromosome 4q34-35 has been reported in a family with an autosomal pattern of bipolar disorder in several generations. Other symptoms that were found in family members harbouring the ANT1 mutations were old-age hearing loss, ptosis, ophthalmoparesis, migraine-like headaches, bilateral facial hypokinesia, exercise intolerance, and peripheral neuropathy. Muscle biopsies from two affected subjects showed alterations compatible with mitochondrial disorder with reduced stain for COX in both, and presence of ragged-red fibres (RRF, compensatory proliferated mitochondria) in one subject. Multiple mtDNA deletions detected with Southern blot accounting for 28% of total mtDNA were detected in the biopsy with RRF that was available for such studies (Siciliano et al, 2003).

Conflicting results depending on method sensitivity have been obtained in studies investigating mtDNA deletion levels in post-mortem brain cortex samples from subjects with bipolar depression, suicide victims, and normal controls. With the Southern blot method, which is expected to reveal mtDNA deletions constituting more than 5% of total mtDNA, there was no increase of mtDNA deletions compared to controls (Stine et al, 1993). In a follow-up study of the same subjects utilizing PCR, a significant increase of 'the common deletion' encompassing ~30% of the mtDNA molecule, was found only in bipolar patients. The highest deletion level in the study was found in a suicide victim. Southern blot analysis has a much lower sensitivity ($1/10^2$) for the detection of 'the common deletion' compared with quantitative polymerase chain reaction (PCR) methods ($1/10^8$) (Kato et al, 1997).

The muscle studies of patients with depressive disorders mentioned in the beginning of this chapter were conducted with standard methods of the time, and before the advent of 'mitochondrial medicine'. Similar unspecific muscle cell alterations as found in these studies have been reported in mitochondrial disorders, e.g., rod bodies and Z-band streaming (Uemura et al, 1987). The results of the above mentioned of studies of mitochondrial functions and mtDNA indicate a mitochondrial involvement in cases of bipolar disorder, but do not support a mitochondrial involvement in unipolar depressive disorder.

Functional brain imaging: background

Functional brain imaging techniques are considered to measure aspects of 'neuronal activity'. 'Neuronal activity' is a concept that may refer to spiking activity or local synaptic activity, and is associated with changes in several physiological variables such as energy consumption, glucose utilization, oxygen consumption, and regional cerebral blood flow (rCBF).

Many details in the coupling between neuronal activity and metabolic and hemodynamic events are still unknown. The brain receives 15% of the cardiac output but constitutes only 2% of the body weight. There are large local blood flow variations due to the heterogeneity of the brain, with lower blood flow in white compared to grey matter. The oxygen extraction from the blood is around 30%, of which nearly all is used in the mitochondrial electron transport chain. The average brain glucose extraction from the blood is around 10%. Glucose is the sole substrate for cerebral energy metabolism during normal physiological conditions. Non-oxidated glucose that is not used in the mitochondrial electron transport chain constitutes around 16%, of which the largest part is probably involved in the synthesis of amino acids and other chemical substances. Increases of brain energy demands have for more than a century been believed to increase brain blood flow, but the precise nature of the relationship remains unknown (Clarke and Sokoloff, 1994; Ledberg, 2001).

Many studies lend support to the concept of a differential energy metabolism among brain regions. The brain can be considered to be a metabolic architecture (Friede, 1966; Borowsky and Collins, 1989; Hevner et al, 1995). In humans, the frontal lobes are among the regions with the highest metabolic demands (Roland and Friberg, 1985; Roland et al, 1987).

Early data provide support for direct coupling between neuronal activity and glucose metabolism (Sharp et al, 1977; Kadekaro et al, 1985). Global and regional blood flow is easier to measure than glucose metabolism, and measurements of blood flow have a higher temporal resolution. Positive correlations have been shown between capillary density and metabolic rate and blood flow. There is evidence for a coupling between blood flow and synaptic activity even in circumstances when there is a lack of coupling between blood flow and metabolism. Various mediators have been suggested between neuronal activity and blood flow coupling, and it seems clear that blood flow and metabolism can vary quite independently. Interpreting the magnitude of rCBF changes as changes in the magnitude of neuronal, and specifically synaptic, activity may be reasonable foremost during normal circumstances (Ledberg, 2001).

Functional brain imaging in mitochondrial and affective disorders

Functional brain imaging techniques include single photon emission computed tomography (SPECT), positron emission tomography (PET), functional magnetic resonance imaging (fMRI), and proton magnetic resonance spectroscopy (MRS) utilizing signals from, e.g., phosphorous-31 (^{31}P) allowing studies of high energy phosphate metabolism.

Spectroscopy with ^{31}P -MRS of patients with mitochondrial disorders have revealed abnormal indices of brain energy metabolism (Barbirolli et al, 1999; Lodi et al, 2002).

Decreased high energy phosphates have been reported at brain ^{31}P -MRS in bipolar disorder (Kato et al, 1994; 1995). Frontal lobe concentrations were found to be lower in severe compared to mildly affected mixed unipolar and bipolar depressed patients implying that alterations in energy metabolism might be related to the severity of depression (Kato et al, 1992). Indices for ATP levels at ^{31}P -MRS studies were found to be lower in unipolar depressed patients compared to healthy subjects in the frontal lobe (Volz et al, 1998) and basal ganglia (Moore et al, 1997). In a review article, it was suggested that these and related results indicate a dysfunction of mitochondrial energy transduction in affective disorders that possibly may be involved in the pathogenesis (Moretti et al, 2003).

$^{99\text{m}}\text{Tc}$ -*d,l*-hexamethylpropyleneamine oxime ($^{99\text{m}}\text{Tc}$ -HMPAO) is a commonly used radiotracer at SPECT investigations. Intracellular trapping of lipophilic *d,l*- $^{99\text{m}}\text{Tc}$ -HMPAO and its conversion to hydrophilic form has been considered to be the basis of fixation of the tracer (Neirinckx et al, 1987; Babich et al, 1991). The conversion has been related to the cellular content of reduced glutathione (GSH), a non-protein thiol, which is present at high intracellular concentrations (Ballinger et al, 1988; Neirinckx et al, 1988). The tissue uptake for the *d,l*- $^{99\text{m}}\text{Tc}$ -HMPAO-isomer in brain, heart and liver, where conversion to the retainable (non-diffusible) form is large enough, has been suggested to reflect blood flow (Neirinckx et al, 1988), and thus brain $^{99\text{m}}\text{Tc}$ -HMPAO SPECT is often considered to reflect rCBF.

Other phenomena than intracellular GSH content may contribute to fixation of $^{99\text{m}}\text{Tc}$ -HMPAO in brain (Jacquier-Sarlin et al, 1996; Sasaki et al, 1998; Fujibayashi et al, 1998). In a study of the $^{99\text{m}}\text{Tc}$ -HMPAO metabolism in brain homogenate, the mitochondrial fraction showed a two-fold higher $^{99\text{m}}\text{Tc}$ -HMPAO activity compared to the cytosol fraction. This finding may reflect an involvement of non-protein thiols with higher reductive activity than glutathione in mitochondria, with ensuing increased mitochondrial $^{99\text{m}}\text{Tc}$ -HMPAO fixation. Hyperfixation of $^{99\text{m}}\text{Tc}$ -HMPAO in the brain has been suggested to indicate damaged mitochondria (Fujibayashi et al, 1998).

Since the SPECT technique is a semi-quantitative method, the concept 'distribution' is a more correct term than 'uptake' or 'retention' for measurements of radiotracer concentration.

$^{99\text{m}}\text{Tc}$ -HMPAO SPECT results in clinical examinations are usually given in qualitative values, i.e., the reading physician bases the image interpretation on subjective evaluation performed by, e.g., visually comparing the intensity of the tracer distribution between the hemispheres. Digital data are necessary for investigations where statistical analyses may be necessary in order to reveal small differences as in some psychiatric disorders, and are sought for research studies. Digital analyses of SPECT data may be performed with different manual, semi-automatic and automatic methods. Standardization software with three-dimensional (3-D) coordinates and processing have been applied in $^{99\text{m}}\text{Tc}$ -HMPAO SPECT studies of depressive disorders, schizophrenia, and Alzheimers disease; e.g., Statistical Parametric Mapping (SPM) (Friston, 1995; Wright et al, 1995; Cho et al, 2002), the Human Brain Atlas (HBA) (Roland et al, 1994, Ito et al, 1996; Imran et al, 1999), and the Computerized Brain Atlas (CBA) (Greitz et al, 1991; Jonsson et al, 2000; Pagani et al, 2001).

$^{99\text{m}}\text{Tc}$ -HMPAO SPECT brain studies in patients with mitochondrial disorders have shown only decreased $^{99\text{m}}\text{Tc}$ -HMPAO distribution in frontal and other regions

(Grünwald et al, 1990; 1991; Lien et al, 2001), increases and decreases (Watanabe et al, 1998; Peng et al, 2000), as well as normal findings (Inagaki et al, 1997).

Brain SPECT utilizing ^{99m}Tc -HMPAO in 11 studies of 218 unmedicated depressed patients showed decreased ^{99m}Tc -HMPAO distribution in frontal regions compared to controls to be the most common finding (Yazici et al, 1992; Maes et al, 1993; Lesser et al, 1994; Fischler et al, 1996; Mozley et al, 1996; Hornig et al, 1997; Tutus et al, 1998a; 1998b; Kowatch et al, 1999; Milo et al, 2001; Navarro et al, 2001). Increased ^{99m}Tc -HMPAO distribution in frontal and other regions was reported in four of the studies (Hornig et al, 1997; Tutus et al, 1998b; Kowatch et al, 1999; Milo et al, 2001). In two of the studies there were also regions with decreased ^{99m}Tc -HMPAO regional distribution (Kowatch et al, 1999; Milo et al, 2001). In one study there was no difference with controls (Maes et al, 1993).

Relationships between depression severity and ^{99m}Tc -HMPAO distribution in the frontal lobes have been found to be inverse (Yazici et al, 1992, Iidaka et al, 1997), absent (Fischler et al, 1996), and positive at omission of items for negative symptoms (Galynker et al, 1998).

Comparisons between results from the studies summarized above are difficult due to the great methodological variability. The spatial resolution of SPECT cameras, choice of reference region, size of the regions of interest (ROIs), kind of statistical approach used, and having the analysis of data in 2-D instead of 3-D, varied between studies. The use of smaller ROIs and a 3-D approach is more likely to catch significant differences. This has particular importance when altered tracer distribution is limited to small regions.

It is yet not clear if alterations of tracer distribution at SPECT reflect 'trait' phenomena that are present prior to onset of overt depression, or 'state' phenomena. It is also unknown if abnormalities of tracer distribution are pathophysiologically involved in the evolution of depression, or are an additional expression of an as yet unknown etiological factor of the disease (Bonne and Krausz, 1997).

Functional brain imaging with ^{31}P -MRS can show abnormalities that are directly linked to mitochondrial dysfunction. The origin of the alterations of brain ^{99m}Tc -HMPAO SPECT in patients with mitochondrial and depressive disorders is unknown. It is likely that the abnormal results that have been found with this brain imaging technique in these groups of patients reflect various pathological phenomena including mitochondrial dysfunction.

Somatic and psychic anxiety components and the Karolinska Scales of Personality

Results of factor analyses have indicated a 'psychic' and a 'somatic' component of anxiety. 'Somatic anxiety' includes sensory somatic symptoms, muscular somatic symptoms, cardiovascular, respiratory, gastro-intestinal, genito-urinary and general autonomic symptoms. Asthenic symptoms and difficulties in concentration have also been included as features of anxiety (Schalling et al, 1973). In the study by Schalling et al (1973), observed and reported anxiety ratings of several clinical groups, including inpatients with depression, showed that psychic and somatic anxiety were significantly correlated but that the shared variance was only 40%. The authors write 'it is doubtful whether Muscular Tension [defined by the authors as awareness of muscular tension and contraction, and difficulties in muscular relaxation] should be included in Psychic

or Somatic Anxiety'. Fatigue was considered to be too general a phenomenon to constitute a core item in an anxiety scale.

Somatic Anxiety, Psychic Anxiety, Muscular Tension and Psychasthenia (fatigueability) were included as different 10-items scales at the self-report questionnaire Karolinska Scales of Personality (KSP) that was developed and finished by Schalling in 1975. The Somatic Anxiety and Muscular Tension scales are considered to reflect the 'nervous tension and distress' dimension of anxiety proneness, and to be more specific scales assessing facets of the broader Psychasthenia construct. The KSP has been utilized in more than 200 studies including original articles and book chapters, reviews, reports, and student's theses. The main areas of research utilizing the KSP have been descriptions of various patient groups and psychobiological studies exploring associations with biological markers (Gustavsson, 1997).

Increased scores on the Somatic Anxiety, Muscular Tension and Psychasthenia scales have been reported in depressed patients (Perris et al, 1984; Pendse et al, 1999; Ramklint and Ekselius, 2003). In a patient group with health complaints scoring high on scales assessing depression and 'somatization', increased KSP scores were only found on the Psychasthenia scale (Österberg et al, 2002).

AIMS

The present study proposes an aberrant mitochondrial function behind the somatic symptoms, and possibly the mood symptoms, in patients with chronic depression and somatic symptoms. The methods used in the study were to perform a series of investigations in patients with this disease presentation in order to study:

- O mitochondrial functions as assessed in muscle biopsies
 - ◆ Group differences compared to healthy controls
 - ◆ Individual results in two patients

- O levels and temporal stability of measures of somatic types of anxiety

- O regional brain metabolism as assessed by ^{99m}Tc -HMPAO SPECT
 - ◆ Differences between patients with and without tinnitus

- O relationships between
 - ◆ Mitochondrial functions and measures of personality traits
 - ◆ Regional brain metabolism and measures of psychic anxiety
 - ◆ Regional brain metabolism and a mitochondrial enzyme complex.

METHODS

The Ethics committee of the Karolinska Institutet approved the studies. The aims and possible adverse effects of the investigations were explained to the patients. Each included subject gave their informed written consent to participate. Most methods employed have been presented in doctoral theses, e.g., mitochondrial ATP production rate (MAPR) in Paper I + III (Wibom, 1991), mtDNA analyses and in situ hybridization in Paper II (Houshmand, 1999), KSP in Paper I, III, IV + V (Gustavsson, 1997), and SPECT with CBA interpretations in Paper III, V + VI (Pagani, 2000).

General design

The present thesis comprises six papers. Mitochondrial functions and measures of different personality traits including somatic types of anxiety were studied in patients with chronic depression and concomitant audiological and other somatic symptoms in Papers I – III. The level and temporal stability of somatic types of anxiety were studied in patients with chronic depression and somatic symptoms in Paper IV. Regional brain metabolism in patients with chronic depression with and without the audiological symptom tinnitus, and differences in the relationships between regional brain metabolism and measures of psychic anxiety between patients with and without tinnitus, were studied in Paper V. Relationships between regional brain metabolism and a mitochondrial enzyme complex were explored in Paper VI.

Subjects: patients

A total number of 103 patients, 37 men and 66 women, were included in the papers. All but two of the patients attended a specialized tertiary psychiatric service, the Psychiatric Outpatient Unit for Patients with Hearing Disorders affiliated to the Clinic of Psychiatry at the Huddinge University Hospital in Stockholm, on a regular basis and were seen by the author of this thesis. Deaf patients in need of sign language communication, patients with other audiological symptoms, and relatives of the patients in need of psychiatric care, are accepted at the service. Seven patients (including two patients with schizophrenia in Paper VI) were living outside of the Stockholm county. A list of all patients is presented in Table A. Fifty-two patients participated in one study, 36 patients in two studies, seven patients in three studies, and eight patients in four studies. Two of the patients participating in four studies are described in the Case presentations (Paper II + III).

Of the 28 patients included in Paper I, the first 25 patients were selected from 26 consecutive patients attending the specialized out-patient service fulfilling the inclusion criteria, and for whom research source funding was obtained for muscle biopsy studies. One patient refused to participate. Three patients were included later as funding for muscle biopsy studies in them was obtained from the referring unit.

The 84 patients included in Paper IV were selected from 85 consecutive depressed patients with a score of ≥ 20 at the self-report version of the Montgomery-Åsberg Depression Rating Scale (MADRS-S) (Montgomery and Åsberg, 1979), and with enough command of the Swedish language to be able to fill in the KSP, attending the

specialized out-patient service during a two-year period. A patient later found to have postpolio syndrome with persistent polio virus in the cerebrospinal fluid was excluded.

The 45 patients included in Paper V were recruited from 53 patients with chronic depression investigated with ^{99m}Tc -HMPAO SPECT who also filled in the KSP. Two patients had refused participation due to fear of claustrophobia at the SPECT examination. Eight patients were excluded from the study, two patients because of atypical clinical features (cyclic vomiting in one and pronounced personality disorder in the other), and six young patients in order to obtain a good age matching with available healthy controls for ^{99m}Tc -HMPAO SPECT. There was no funding for ^{99m}Tc -HMPAO SPECT investigations of further patients.

The 20 patients included in Paper VI were all unmedicated patients out of 34 patients with chronic depression or schizophrenia who had been investigated with both muscle biopsy and ^{99m}Tc -HMPAO SPECT.

The 99 depressed patients in this thesis are not representative of patients with major depression in the general population, or of depressed patients with severe early hearing impairment using sign language communication. Only 16 of the 99 patients (16%) used sign language. Seventy-four of the remaining 83 patients had other audiological symptoms for which they had sought medical consultation ranging from severe tinnitus and/or hyperacusia with normal hearing to uni- or bilateral hearing impairments of various severity, with or without tinnitus and/or hyperacusia.

Subjects: controls

For the biochemical analyses in Paper I + III, 10 healthy sedentary controls were investigated, three men and seven women aged 29 – 55 years (mean age 46.4 ± 7.8). For mtDNA studies, 22 healthy controls were investigated, 13 men and nine women aged 23 – 74 years (mean age 48.6 ± 17.1). Muscle biopsy studies of controls were approved by the Ethics Committee of the Karolinska Institutet, Stockholm.

KSP scores in Paper I, III + IV were compared with normative data transformed to T-scores (50 ± 10) obtained from 400 subjects randomly sampled from the Stockholm population and standardized for gender and age (Bergman et al, 1982). KSP scores in Paper I + IV were compared with other groups of patients with major depression for whom the same normative group was used (Pendse et al, 1999, Ekselius and von Knorring, 1999).

In order to obtain normal data for comparisons, SPECT results from a large control group of subjects (Pagani et al, 2002) were selected. The controls for Paper III were 21 age-matched male subjects (mean age 59 ± 10 years), and for Paper V, 26 subjects, 10 men and 16 women (mean age 49 ± 6 years) comprising the youngest subjects included in the large control group. The controls were free of psychiatric, neurological, cardiovascular or pulmonary disorders. Swedish versions of the cognitive screening test Mini-Mental State Examination (MMSE) (Folstein et al, 1979) and the depression rating scale MADRS (Montgomery and Åsberg, 1979) were performed with normal results. SPECT studies of controls were approved by the Ethics Committee and the Radiation Protection Committee of the Karolinska Institutet and the Karolinska Hospital, Stockholm.

Clinical measures (All studies)

DSM-IV criteria for Major Depressive Disorder were fulfilled in 99 patients, and for Schizophrenia in four patients included in Paper VI. The diagnoses were based on repeated clinical interviews by the author and after medical records had been scrutinized. Ongoing drug or alcohol abuse were exclusion criteria for the studies. For 91 of the depressed patients (92%), scores at ≥ 18 considered to reflect Major Depressive Disorder were obtained at one or more occasions at the MADRS-S. MADRS-S scores are presented in Table A. For those patients in whom an even higher MADRS-S score was obtained after the completion of the studies, this higher score is listed. For seven of the eight patients without listed MADRS-S scores, depressive symptoms had been more severe in the past at a time they were not asked to fill in the MADRS-S. One patient was unable to fill in the self-report because of poor command of the Swedish language.

The patients in Paper I were selected by criteria designed for the study (see APPENDIX: Table B). The criteria describe audiological, ophthalmological, muscular, psychiatric and other symptoms that are common among the patients attending the specialized out-patient service according to clinical experience, and in mitochondrial disorders (see INTRODUCTION: Depressive and neuropsychological symptoms in mitochondrial disorders, and Somatic symptoms in mitochondrial disorders, and references therein, page 14).

A somatic investigation including routine laboratory analyses and blood pressure examination was performed in most patients. Minor blood laboratory alterations were found in several patients that were not considered to cause the depressive symptoms.

Morphological, biochemical and mtDNA studies in muscle (Paper I, III + VI)

Muscle biopsy was performed in the right anterior tibial muscle. For light microscopy, muscle tissue samples 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 COX. Other pieces were fixed in buffered glutaraldehyde, postfixed in osmium tetroxide, and epon-embedded for electron microscopy.

Mitochondria were isolated from fresh muscle tissue and MAPR was luminometrically determined using firefly luciferase according to the method described by Wibom and Hultman (1990). Eight different substrate combinations were used to test the mitochondrial function: 1) pyruvate + palmitoyl-L-carnitine + α -ketoglutarate + malate (PPKM), 2) glutamate + malate, 3) N,N,N¹,N¹-tetramethyl-1,4-phenyldiamine (TMPD) + ascorbate, 4) α -ketoglutarate, 5) palmitoyl-L-carnitine + malate, 6) pyruvate + malate, 7) succinate + rotenone, and 8) succinate only.

The enzyme activities were spectrophotometrically determined 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) and succinate-cytochrome *c* reductase (SCR) 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 COX (Cooperstein and Lazarow, 1951). Citrate synthase (CS)

was determined according to the method by Alp et al. (1976) after permeabilization of the mitochondria.

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, was determined using restriction site methodologies (Prezant et al, 1993; Reid et al, 1994). Deletions of the mtDNA were analyzed using long-PCR and Southern blot according to the methods as described and referred to by Coulter-Mackie et al. (1998).

Karolinska Scales of Personality (Paper I, III, IV + V)

A short description of the KSP questionnaire is presented above (see INTRODUCTION: Somatic and psychic anxiety components and the Karolinska Scales of Personality, page 20). The KSP consists of 135 items with a four-point response format summed up to 15 scales. The KSP scales were conceived to be quantifications of some crucial personality or temperament dimensions representing qualities of the information processing and arousal systems of the individual. The items refer explicitly to longitudinally stable personality traits and were formulated on the basis of assumptions regarding the main vulnerability dimensions underlying various mental disorders. Apart from some aggression-related scales, the scales are stable over time regarding absolute (mean level) and relative (the consistency of individual differences within samples over time) stability in healthy subjects and ulcerative colitis patients (Gustavsson, 1997; Weinryb et al, 1992). High scores indicate pathology on all scales except for the Socialization scale, for which low scores indicate pathology.

The patients were given the KSP questionnaire at an appointment, or received it by post before their first appointment, accompanied by a prepaid stamped envelope with the address for the author of the thesis written upon it. The questionnaires were then filled in by the patients in their homes without assistance, and returned by postal mail.

Good command of the Swedish language is necessary in order to complete the questionnaire. For this reason, the questionnaire was only handed out to patients with good Swedish language command, which is the reason why some patients with early deafness, for whom the Swedish language is their second language and very cumbersome to achieve proficiency of, were not asked to fill in the questionnaire. A few patients with severe concentration difficulties filled in the questionnaire but complained of developing a headache while doing so. They and some other patients with similar symptoms were not asked to fill in the questionnaire a second time, and some patients not at all, for ethical reasons.

MtDNA studies and in situ hybridization (Paper II)

Total DNA was isolated from lymphocytes of the patient, her mother and brother, and from frozen muscle tissue obtained from a right anterior tibial muscle biopsy of the patient, according to a standard method (Larsson et al, 1990). Southern blot analysis was used to identify mtDNA deletions and duplications. Two restriction enzymes (Pvu II and BamH I) were used to linearize the circular mtDNA. Wild type mtDNA and mutated mtDNA were separated by electrophoresis and transferred to a nylon membrane. Hybridization was then performed with a radioactive method using ³²P-labelled mtDNA, which is more sensitive than the fluorescence method. Long extension

PCR was used to amplify 8-12 kb fragments to identify the duplicated part of mtDNA. Several primer pairs 60-80 bp apart in the normal mtDNA were used to obtain the duplicated fragment.

Staining for COX and SDH of muscle tissue was performed as described by Oldfors et al. (1989). In situ hybridization was performed to study the distribution of wild type, deletion and duplication of mtDNA in differentially stained tissue (Oldfors et al, 1992). MtDNA fragments, each hybridizing to a unique part of DNA/RNA, were used to create five probes that were used to detect wild type and duplicated but not deleted, and duplicated/deleted, mtDNA.

^{99m}Tc-HMPAO SPECT (Paper III, V + VI)

SPECT brain imaging was performed using a three-headed gamma camera (TRIAD XLT 20, Trionix Research Laboratory Inc., Twinsburg, OH, USA) equipped with low-energy ultrahigh-resolution (LEUHR) collimators. ^{99m}Tc-HMPAO (Ceretek[®], Amersham International Plc, Little Chalfont, UK) was injected after 30 minutes rest in a quiet dim lighted room. The SPECT data were analyzed by the CBA, a commercially available software tool (Applied Medical Imaging AB, Uppsala, Sweden) (Greitz et al, 1991; Thurffjell et al, 1995). The CBA allows for identification of anatomical brain structures, and assessment of their relative radiopharmaceutical uptake. For normalization, the upper 13% of all voxels in the SPECT matrix was applied as reference (Jonsson et al, 1998; 2000; Pagani et al, 2001; 2002). Normalized tracer uptake for volumes of interest (VOIs) was automatically assessed by the CBA. Each VOI was built up automatically by CBA summing the corresponding ROIs in consecutive sections. Tracer uptake was separately calculated for each VOI.

^{99m}Tc-HMPAO SPECT in individual patients

Individual ^{99m}Tc-HMPAO SPECT results for 13 patients with chronic depressive symptoms, of whom seven patients are included in the papers, are presented at the end of RESULTS in order to show the magnitude of alterations that may be discernible with CBA interpretations and comparisons with healthy controls. Results of the patient included in Paper II, and six other female patients with audiological symptoms and six daughters of these patients, of whom five daughters have no audiological symptom, are presented in order to show that altered ^{99m}Tc-HMPAO distributions can be found in patients with and without audiological symptoms. The investigations were approved by the Ethics Committee of the Karolinska Institutet, and the Radiation Protection Committee of the Karolinska Hospital, Stockholm. All patients gave informed consent.

Statistical analyses

All variables were summarized using standard descriptive statistics (mean), and standard deviation (SD). Student's *t*-test was applied for mean comparisons of two groups in Paper I + IV, and paired samples *t*-test in Paper IV. Two-tailed tests were used. In Paper V, three-group mean differences were assessed with ANCOVA controlled for age and gender followed by two-group comparisons using Bonferroni post-hoc tests.

Chi-square analysis was performed to determine differences between patients and controls with respect to the number of individuals with mtDNA deletions in Paper I, and between the number of individuals with extreme distributions of ^{99m}Tc -HMPAO SPECT in Paper V. The Kruskal-Wallis chi-two test was used for four-group comparisons of extreme ^{99m}Tc -HMPAO SPECT distributions in Paper V. Wilcoxon signed ranks test was used in the comparison of the distribution of correlation coefficients in Paper V.

Relationships between variables were evaluated with the Spearman rank correlation test in Paper I + VI which included variables of different logical dimensions, and with the Pearson correlation test in Paper IV for scores at self-rating questionnaires. In Paper V, partial correlations controlled for age and gender were used to evaluate relationships between KSP scores and ^{99m}Tc -HMPAO SPECT distribution.

Significance levels differed between the studies. Correction for multiple comparisons was applied in Paper IV + VI. Precautions were undertaken to decrease the risk of chance findings for some analyses using a significance level of $p < 0.05$ in Paper I + V. Repeated resampling and split-half-technique were added to the correlation tests in Paper I. Findings in the available subsample of females were added to post hoc test findings in Paper V.

RESULTS AND COMMENTS

Mitochondrial dysfunction in patients with chronic depression (Paper I, II + III)

The study presented in Paper I was designed to investigate mitochondrial functions and relations with 'personality traits' in patients fulfilling criteria designed for the study (see APPENDIX: Table B).

The morphological and histochemical studies of muscle revealed unspecific alterations in the majority (89%), with COX deficient fibres in a quarter, of the patients (n = 28). At the biochemical studies there were significant ($p < 0.01$) decreases in two of the eight MAPR assessments (MAPRs), non-significant decreases ($p < 0.05$) in three MAPRs, and significant decreases of the ratios between the activities of the respiratory chain enzymes NCR and COX, and SCR and COX, in comparisons with healthy controls (n = 10) (see Table 1).

Table 1.

	Patients	Controls	<i>p</i> -value
mean age	48.2 ± 8.5	46.4 ± 7.8	
MAPR with succinate	0.86 ± 0.30	1.23 ± 0.29	0.002
MAPR with α-ketoglutarate	3.71 ± 1.28	5.10 ± 1.07	0.004
MAPR with PPKM	6.56 ± 1.73	8.10 ± 1.26	0.019
MAPR with succinate + rotenone	2.06 ± 0.49	2.43 ± 0.42	0.037
MAPR with glutamate + malate	6.27 ± 1.45	7.38 ± 1.41	0.043
NCR/COX	0.47 ± 0.13	0.64 ± 0.18	0.004
SCR/COX	0.28 ± 0.07	0.37 ± 0.08	0.005

An mtDNA deletion in muscle detected with the Southern blot method was found in one of the investigated patients (n = 25). There was a significant increase ($p < 0.05$) of the proportion of patients with mtDNA deletions detected with the long-PCR method although no deletions were found in the patient with the Southern blot-detected deletion, and 23% of the controls (n = 22) were older than the patients. No patient was older than 60 years. Five older controls (≥ 70 years) were recruited in order to achieve a similar mean age for controls as for patients (mean age 48.6 ± 17.1 and 48.6 ± 7.9 years, respectively). All these older controls had mtDNA deletions, as to be expected in older subjects. Two point mutations in mtDNA that have been found in families with maternally inherited hearing loss were absent in all patients.

'Personality traits' were assessed using the KSP. Mean scores near or above 2 SDs compared with the normative group were only found for the Somatic Anxiety, Muscular Tension and Psychasthenia scales in the patients (n = 21). The Psychasthenia score, considered to reflect psychic fatiguability, was increased at comparisons with another group of patients with major depression, suicide attempters (Pendse et al, 1999) (see Table 2, next page).

Table 2.

Karolinska Scales of Personality	Chronic Depression n = 21	Major Depressive Disorder n = 23	<i>p</i> - value
Normal score 50 ± 10			
Somatic Anxiety	68 ± 16	65 ± 15	not sign
Muscular Tension	69 ± 16	66 ± 14	not sign
Psychasthenia	71 ± 11	63 ± 16	< 0.05

Significant correlations at the $p < 0.01$ level were only found between MAPRs and Somatic Anxiety (see Figure 3), Psychasthenia, and Suspicion.

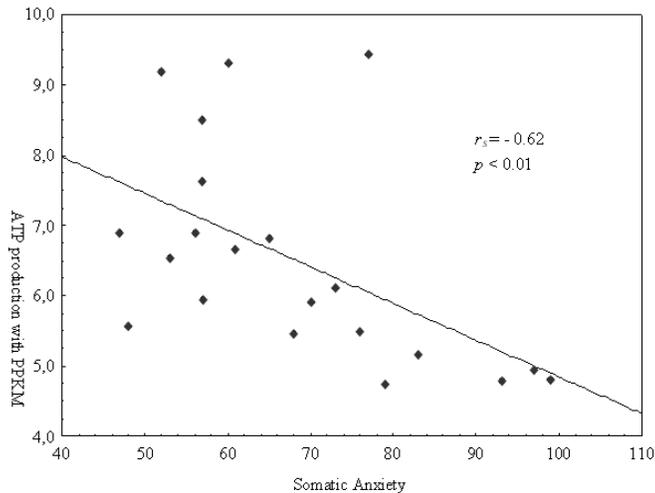


Figure 3. The relationship between Somatic Anxiety scores, considered to reflect autonomic disturbances, restlessness and panic proneness, and muscle ATP production assessed with PPKM. The figure is presented in Paper IV: Figure 1 with further explanations. The patient with a Somatic Anxiety score of 77 and the highest ATP production of the patients had an atypical muscle fibre type composition with an increase of oxidative fibres. At exclusion of this patient, Spearman correlations at the $p < 0.01$ level were found for Somatic Anxiety, Muscular Tension and Psychasthenia ($r_s = -0.74, -0.57,$ and $-0.63,$ respectively), but not for any of the other 12 KSP scales.

The results of study I were interpreted to indicate mitochondrial dysfunction that may represent the final common path for several events including mutations in mtDNA or nDNA. The correlations between MAPRs and KSP scales with mean score increases compared to the normative group suggest that mitochondrial dysfunction may be associated with vulnerability to depression in these selected patients with chronic depression. Since this is the first study of patients with a psychiatric disorder using the standard method for diagnosis of mitochondrial disorder with muscle biopsy, results cannot be compared with other studies.

Individual results from two patients included in Paper I are presented in Paper II + III. Psychiatric symptoms were the first disease manifestations in the patients, a female age 57 and a male age 60. In Paper II, an mtDNA duplication found in lymphocytes in the female patient, that was not found in the patient's mother or brother, is described (see Figure 4, next page). The duplication might generate the mtDNA deletion that was

the only mtDNA species found in COX deficient muscle fibres at in situ hybridization. There was no indication of generally impaired transcription or depletion of mtDNA in COX deficient muscle fibres (see Figure 5, next page).

Although a pathogenic effect of duplicated mtDNA, which does not lack any gene, cannot be excluded (Dunbar et al, 1993), available data indicate the accumulating corresponding deleted mtDNA. A correlation between accumulation of deleted but not duplicated mtDNA, and accumulation (proliferation, increased number) of mitochondria and occurrence of COX negativity, have previously been reported in a patient suggesting that it is the deletion that is associated with phenotypic expression (Manfredi et al, 1997). Presence of a low level of duplicated mtDNA was found at reanalysis of all patients with a multisystem disorder with mtDNA deletions (Poulton et al, 1993). A decreasing level of duplicated mtDNA, and a concomitant increase of deleted mtDNA with time, has been documented (Poulton et al, 1995). MtDNA deletions may thus be generated by duplicated mtDNA, or may be the result of nDNA mutations causing secondary mtDNA deletion formation (Chinnery and Schon, 2003).

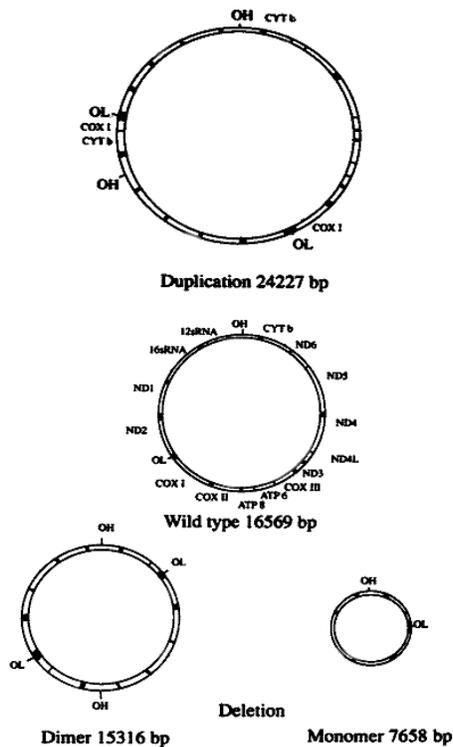


Figure 4. The figure shows the four types of mtDNA molecules found in the patient. The middle circle labelled 'Wild type' depicts normalized double-stranded circular mtDNA. OH (at 12 o'clock) is the origin of replication for the guanine-rich 'heavy strand', and OL (8 o'clock) the origin for the cytosine-rich 'light strand'. A 24227 bp duplicated mtDNA molecule with double copies of OH (12 and 8.30 o'clock) and OL (5 and 9.30 o'clock), and of the COX I (5 and 9 o'clock) and cytochrome b (CYT b) (1 and 9 o'clock) genes, is shown at the top. Deletion species (an uncut 15316 bp dimer and a 7658 bp monomer) containing OH and OL permitting replication of these molecules, are shown at the bottom. The figure is presented in Paper II: Figure 1, with positions for cleavage sites for the restriction enzymes, and extensions of predicted hybridization patterns for the five probes, used in the study.

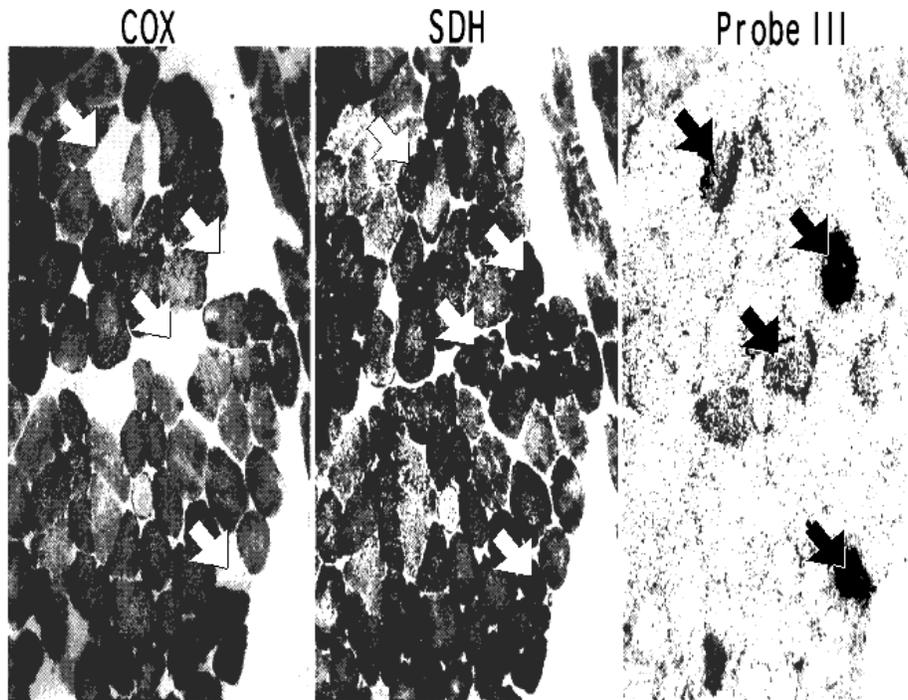


Figure 5. COX deficient muscle fibres are indicated by white arrows. No decrease of SDH-staining is found in COX deficient fibres indicated by white arrows. SDH is encoded by nDNA. In situ hybridization with probe III, which detects wild type, duplicated and deleted mtDNA, shows that COX deficient muscle fibres, indicated by black arrows, have accumulated mtDNA.

Scores 2 SDs above the normative group at the KSP scales Somatic Anxiety, Psychic Anxiety, Muscular Tension and Psychasthenia were found in the female patient and are presented in Paper IV: Table 4. Low MAPRs compared with healthy controls were found and are indicated in Paper I: Figure 1. The ^{99m}Tc -HMPAO SPECT with CBA interpretation in the lateral brain aspects in this patient is presented below at the end of this section (see: ^{99m}Tc -HMPAO SPECT in individual patients, Figure 11, page 39). The CBA interpretation showed increased ^{99m}Tc -HMPAO distribution in VOIs in the right frontal lobe, the left anterior cingulate and insular region, the bilateral temporal lobes, putamen and caudate. Decreased ^{99m}Tc -HMPAO distribution was only found in the left hippocampus.

In the male patient presented in Paper III, there were four MAPRs below 2 SDs and four MAPRs below 1 SD compared with healthy controls, and KSP scores at 4 and 2 SDs above the normative group on Somatic Anxiety, Muscular Tension, and Psychasthenia. The disease course started in the third decade with depressive episodes, fatigability, and photophobia, with subsequent onset of hearing impairment, tinnitus, myalgia, diabetes mellitus, and chronic depression. Decreased ^{99m}Tc -HMPAO distribution was found at SPECT with CBA interpretation in VOIs in the left dorsolateral frontal lobe, the anterior cingulate, and the superior temporal lobes. These alterations have also been described in other ^{99m}Tc -HMPAO SPECT studies of major depression (Milo et al, 2001; Navarro et al, 2001; Conca et al, 2000). Decreases were also found in the bilateral insular regions, the left posterior cingulate, and the right thalamus. Increased ^{99m}Tc -HMPAO distribution was found in VOIs in the bilateral

superior parietal lobes, and the right frontal and occipital lobes. The extent of the altered distributions compared to healthy male controls are shown below (see Figure 6) according to a scheme (see Figure 7).

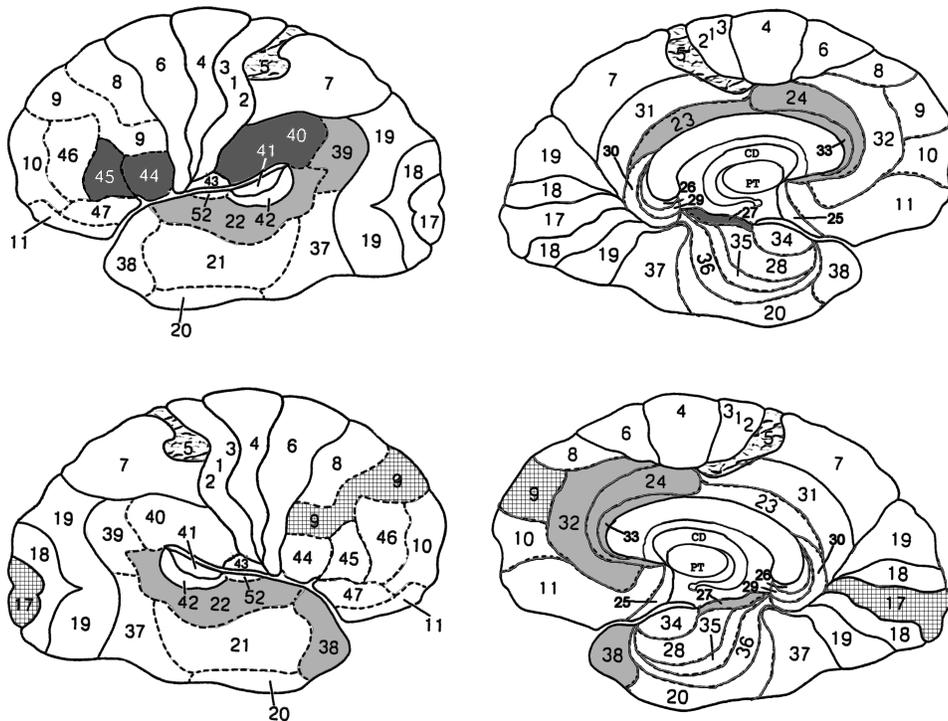


Figure 6. Top row: the left lateral brain aspect to the left, and the left medial aspect to the right. Bottom row: corresponding aspects for the right hemisphere. Regional numeration according to Brodmann.

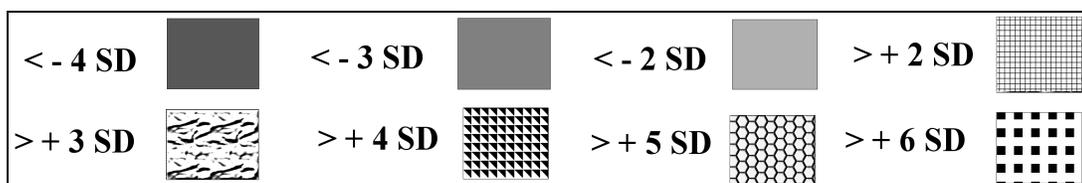


Figure 7. Decreased ^{99m}Tc -HMPAO distribution is indicated by grey shades, and increased distributions by patterns.

Levels and stability of 'somatic' types of anxiety (Paper IV)

The aim of the study was to investigate mean levels and long-term stability of the KSP scales Somatic Anxiety, Psychic Anxiety, Muscular Tension, Psychasthenia, and Socialization, in patients with chronic depressive symptoms. The inclusion criterion for the study was a minimum score of 20 at the MADRS-S. Mean scores in the patients ($n = 84$, mean age 47 ± 10 years) on Somatic Anxiety, Muscular Tension and Psychasthenia were 2 SDs above the normative group. Psychic Anxiety and Socialization scores were within ± 2 SD for the normative group. Sixty-five of the patients filled in the KSP after

a mean interval of 3.5 years. There was no significant difference between the scores on the five scales at first KSP and follow-up. High significant correlations ($p < 0.001$) were found between the test occasions.

Significantly higher ($p < 0.001$) scores for Somatic Anxiety, Muscular Tension, and Psychasthenia were found for patients assessed twice in comparisons with scores from a large group of untreated depressed patients in primary care, of whom 93% responded to antidepressant treatment (Ekselius and von Knorring, 1999) (see Table 3).

Table 3.

	Chronic depression n = 65		Depressed patients in primary care n = 163		Suicide attempters n = 26	
Male/female (%)	29/71		29/71		19/81	
	First KSP	3.5-year follow-up	Before treatment	After 24 w. treatment	During admission	5-year follow-up
Mean age	46 ± 10	50 ± 11	48		36 ± 11	
Somatic Anxiety	72 ± 12	70 ± 12	64 ± 13	56 ± 10	63 ± 15	52 ± 11
Muscular Tension	75 ± 12	72 ± 12	65 ± 14	59 ± 10	64 ± 17	55 ± 13
Psychasthenia	75 ± 11	74 ± 11	61 ± 11	55 ± 10	58 ± 11	55 ± 13

Mean scores in the chronically depressed patients at follow-up after 3.5 years, in the depressed primary care patients after 24 weeks of antidepressant treatment, and in suicide attempters after five years (Öjehagen et al, 2003), showed decreases in all three patient groups on Somatic Anxiety (2, 8 and 11 scores, respectively), Muscular Tension (3, 6 and 9 scores), and Psychasthenia (1, 6 and 3 scores). The mean score decreases were less pronounced in the chronically depressed patients indicating higher temporal stability of symptoms assessed with these scales in this patient group.

Moderate significant correlations ($p < 0.001$) were found at follow-up in the chronically depressed patients between MADRS-S and Somatic Anxiety, Psychic Anxiety and Muscular Tension ($r_p = 0.49, 0.47$ and 0.49 , respectively). These results indicate a moderate relationship between high depression rating scores and high KSP scores on these scales in the whole patient group. The results should not be interpreted to suggest covariation in individual patients, since MADRS-S and KSP scores were not assessed simultaneously at several occasions in individual patients.

The results of the study were interpreted to suggest that patients with chronic depression may comprise a depressive subtype characterized by high and stable 'somatic distress' symptoms as assessed by the KSP scales Somatic Anxiety, Muscular Tension and Psychasthenia. In the light of the findings reported in Paper I, it was suggested that the conceptualization of 'somatic distress' symptoms as 'personality traits' ought to be discussed, since such symptoms may reflect common manifestations of mitochondrial dysfunction.

^{99m}Tc-HMPAO SPECT in depressed patients with and without tinnitus (Paper V)

Differences in the distribution of ^{99m}Tc-HMPAO at SPECT were explored between chronically depressed patients with and without severe tinnitus in Paper V. Patients who filled in the KSP (n = 45), of whom 60% (n = 27) complained of severe long-standing tinnitus, and healthy subject (n = 26), were investigated. There was an equal proportion of males (38%) and similar mean ages in patients and controls (49 ± 8 and 49 ± 6 years,

respectively). Age and gender were entered as covariates at the the three-group comparisons of the distribution of ^{99m}Tc -HMPAO in 34 bilateral VOIs since age and gender effects were found for controls, and separation of patients into tinnitus and non-tinnitus groups resulted in unequal gender proportions. Significant differences ($p < 0.01$) were found for eight of the 34 VOIs. Post hoc tests were performed and showed decreased ^{99m}Tc -HMPAO distribution in tinnitus compared to non-tinnitus patients in three VOIs (see Table 4, and Figure 8). Involvement of these VOIs in tinnitus perception, with activation or deactivation at brain imaging studies, has previously been reported (Mirz et al, 1999; Andersson et al, 2000).

Table 4.

n =	Controls	All tinnitus patients	All non-tinnitus patients	ANCOVA (df = 2,66)		Post-hoc tests (df = 2,66)		
				F-value	p-value	Controls vs. tinnitus	Controls vs. non-tinnitus	Tinnitus vs. non-tinnitus
n =	26	27	18					
Age mean	49 ± 6	50 ± 8	48 ± 7					
Male/female	10/16	15/12	2/16					
Right area 45	44.1 ± 1.6	44.7 ± 2.3	46.4 ± 1.8	7.621	0.001		0.001	0.014
Left area 39	41.6 ± 1.2	39.8 ± 1.8	41.8 ± 1.7	8.976	0.000	0.001		0.003
Left area 18	42.4 ± 1.6	40.9 ± 2.1	42.4 ± 2.0	5.500	0.006	0.010		0.034

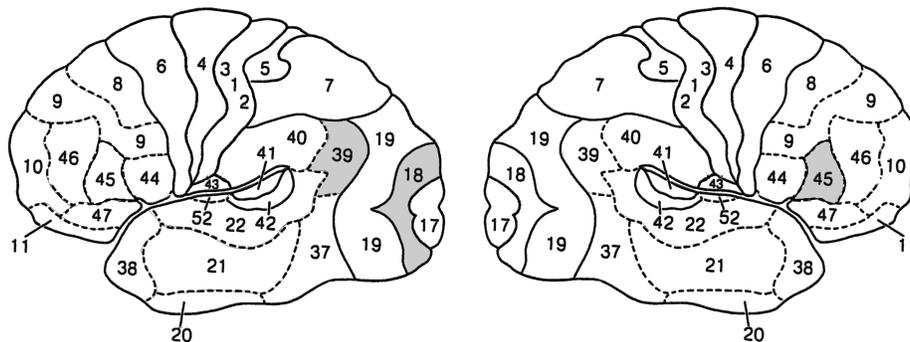


Figure 8. The lateral brain aspects, with the left lateral aspect shown to the left. Regional numeration according to Brodmann. Decreased ^{99m}Tc -HMPAO distribution was found in depressed tinnitus compared to depressed non-tinnitus patients in the left parietal multisensory cortex (area 39), the left visual association cortex (area 18) and the right prefrontal dorsolateral cortex (area 45).

Two-thirds of the male and female tinnitus patients were found to have extreme ^{99m}Tc -HMPAO distribution, i.e., alterations above or below gender-matched control group mean ± 2 SD, in VOIs located in the bilateral superior temporal lobe, i.e., the primary auditory cortex and regions participating in the processing of auditory stimuli. Involvement of these VOIs has been reported in tinnitus perception (Lockwood et al, 1998; Girard et al, 1999; Mirz et al, 1999; Andersson et al, 2000; Mirz et al, 2000). The proportions of tinnitus patients with extreme distributions in these VOIs were significantly increased compared to gender-matched controls ($p \leq 0.003$) and non-

tinnitus patients ($p < 0.05$). The difference in the directions of the altered ^{99m}Tc -HMPAO distributions in VOIs related to auditory phenomena explain why differences between patients and healthy subjects were not found at the mean comparisons.

Significant positive correlations controlled for age and gender ($r = 0.51 - 0.61$, $p \leq 0.01$) were found in tinnitus patients between the KSP scale Psychic Anxiety measuring 'trait anxiety' and ^{99m}Tc -HMPAO distribution in brain structures that are part of, or receive input from, limbic structures, i.e., in VOIs in the bilateral anterior cingulate, and in the right caudate. Non-significant mostly negative correlations were found in non-tinnitus patients. The differential correlations were in support of a previous suggestion of altered functional connectivity in tinnitus patients possibly accounting for the emotional associations with tinnitus (Lockwood et al, 1998). The origin of the increased ^{99m}Tc -HMPAO distribution relating to high 'trait anxiety' in the limbic and limbic-related structures is unknown.

Inconsistent results have been reported between previous neuroimaging studies of depression (Conca et al, 2000). The results of the study were interpreted to suggest that subdivision of depressed patients into subpopulations with shared audiological characteristics may yield more uniform results. Tinnitus has been reported in almost half of depressed patients (Mathew et al, 1981). Since this is the first ^{99m}Tc -HMPAO SPECT study of depressed patients divided according to tinnitus presence, and of depressed patients utilizing the CBA, results cannot be compared with other studies.

The increased ^{99m}Tc -HMPAO distribution found in five of eight assessed right frontal lobe VOIs in the depressed patients compared to the healthy subjects (Paper V: Table 3) was not discussed in the paper. The female and male patient presented in Paper II + III, respectively, were both as previously described (see this section, end of: Mitochondrial dysfunction in patients with chronic depression (Paper I, II + III), page 32) investigated with ^{99m}Tc -HMPAO SPECT. The results of the patients were compared with age- and gender-matched control groups, each comprising 21 subjects. Increased ^{99m}Tc -HMPAO distribution in right frontal lobe VOIs was found in both patients. Some regional involvements were present in both patients, i.e., of Brodmann area 24 in the left anterior cingulate, the left insular region, and area 22 in the right middle and superior temporal gyri. In these VOIs with altered ^{99m}Tc -HMPAO distributions in both patients, there was increased distribution in the female patient, and decreased distribution in the male patient.

The origin and relevance of the different directions, increased and decreased ^{99m}Tc -HMPAO distribution, is unknown. The different directions of ^{99m}Tc -HMPAO distributions in the patients in Paper II + III cannot, at least not with present-day knowledge, be linked to the clinical presentations. The somatic symptoms in the patients were quite similar. Both patients were severely hearing impaired with tinnitus. The male patient experienced his tinnitus as very severe, while the female patient 'had decided tinnitus is not severe'. Higher scores at aggression-related KSP scales were found in the female compared with the male patient (Indirect Aggression: 81 and 39, Irritability: 80 and 58, Suspicion: 90 and 57, respectively). Only the male patient, in whom no mtDNA studies were performed, has had depressive episodes necessitating hospitalization. A difference in the origin of mitochondrial dysfunction in these patients may, hypothetically, influence depression subtype, and brain tissue fixation of ^{99m}Tc -HMPAO.

^{99m}Tc-HMPAO SPECT and relations with a respiratory chain enzyme (Paper VI)

Relations between ^{99m}Tc-HMPAO distribution at SPECT and SCR activity were explored in unmedicated patients (n = 20, mean age 41 ± 13 years), chronic depression (n = 16) and schizophrenia (n = 4), in Paper VI. SCR is comprised of the respiratory chain enzyme complexes II + III. SCR activity was measured in isolated mitochondria obtained from muscle biopsies taken from the right anterior tibial muscle. No tissue-specific isoforms have been reported for the subunits of complex II (Rustin and Rotig, 2002) and III (Mourmans et al, 1997). SCR activity has previously been shown to be unevenly distributed in mitochondrial pellets from various brain regions (Battino et al, 1991).

Significant negative correlations were found between the ^{99m}Tc-HMPAO distribution in four of the 42 assessed VOIs, and SCR activity. Results for these four VOIs and the contralateral VOIs are presented below (see Table 5 and Figure 9).

Table 5.

Brodmann area		r_s	p -value
Left	21	-.60	0.005
Right	21	-.77	0.00008*
Left	22	-.70	0.0006*
Right	22	-.57	0.009
Left	39	-.59	0.006
Right	39	-.78	0.00004*
Left	19	-.51	0.022
Right	19	-.68	0.001*

r_s : Spearman correlation coefficient, *significant with Bonferroni corrections.

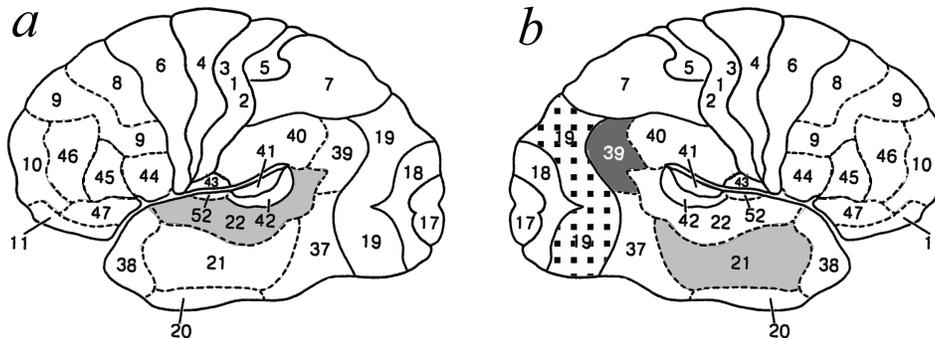


Figure 9. *a*, The left lateral brain aspect. *b*, The right lateral aspect. Regional numeration according to Brodmann. Significant negative correlations were found for three Brodmann areas, light grey: auditory association cortex, dark grey: multisensory association cortex, chequered: visual association cortex.

The strongest negative correlation ($r_s = -0.78$) was found for a multisensory association area in the right parietal lobe (see Figure 10, next page). The correlations were pronounced irrespective of gender (11 males $r_s = -0.61$; 9 females $r_s = -0.65$) and clinical diagnosis (16 depressed patients $r_s = -0.76$, four schizophrenia patients $r_s = -1.00$).

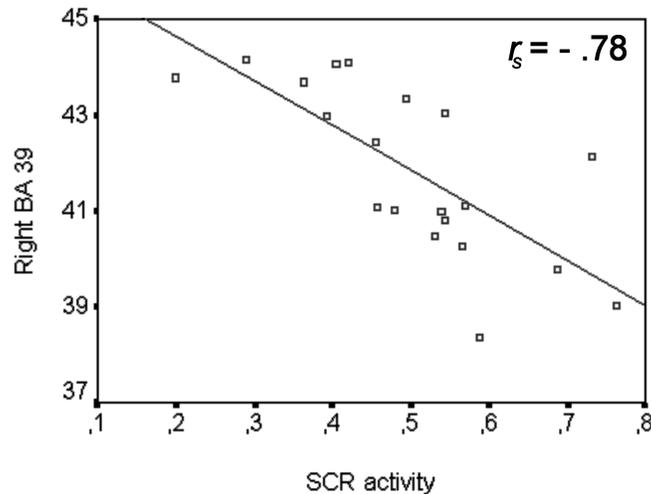


Figure 10. Scattergram showing a negative relation between the ^{99m}Tc -HMPAO distribution in the right Brodmann area (BA) 39 and SCR activity in isolated muscle mitochondria.

The significant negative relationships between ^{99m}Tc -HMPAO distribution and SCR activity in some associative sensory cortices might reflect altered ^{99m}Tc -HMPAO fixation related to events produced by differences in SCR activity. The highest cortical staining for complex II has been reported to be located in sensory and associative sensory areas (Friede 1960; 1966) indicating dependency of normal complex II activity for these brain regions. The 'selective vulnerability' concept refers to the phenomenon that a subpopulation of neurons may be relatively selectively affected by a generalized insult (Blass, 1999). A mild decrement of SCR activity may be present in at least some subjects with psychiatric disorders causing ^{99m}Tc -HMPAO hyperfixation in those brain regions that are most dependent upon normal SCR activity for normal function. Lower muscle SCR activity was found in the female patients in this study ($n = 9, 0.41 \pm 0.11$) compared with the healthy females ($n = 7, 0.51 \pm 0.11$) in Paper I, but the difference did not reach significance in this small sample ($p = 0.089$). No comparison was performed between the male patients ($n = 11$) and the healthy males ($n = 3$) in Paper I.

Intracellular trapping of ^{99m}Tc -HMPAO and its conversion to hydrophilic form has been considered to be the basis of brain fixation of the tracer (Neirinckx et al, 1987; Babich, 1991). The conversion has been related to the cellular content of GSH (Neirinckx et al, 1988). Other phenomena than intracellular GSH content have been suggested to contribute to fixation of ^{99m}Tc -HMPAO in brain. A hyper-reduced state in the extracellular space has been linked to increased extracellular ^{99m}Tc -HMPAO conversion to hydrophilic ^{99m}Tc -HMPAO (Jacquier-Sarlin et al, 1996). The hyper-reduced state in the extracellular space is in principle applicable even to the intracellular space. An intracellular hyper-reduced state caused by electron excess due foremost to impaired complex III activity may result in ^{99m}Tc -HMPAO hyperfixation. The production of reactive oxygen species indicating electron excess or decrease of electron transfer is considered to be highest within complex III (Gille et al, 2001), but complex II may also contribute (Lenaz, 2001).

The results of the study support a previous observation of an association between ^{99m}Tc -HMPAO fixation and mitochondrial function by Fujibayashi et al. (1998), and

prompted a suggestion of a relationship between the regional distribution of ^{99m}Tc -HMPAO and mitochondrial functions affecting the redox state in psychiatric patients. Further studies are necessary before any conclusions can be drawn.

^{99m}Tc -HMPAO SPECT in individual patients

^{99m}Tc -HMPAO SPECT results are presented below for 13 female patients with chronic depressive symptoms. The mtDNA mutations in Patient I are described in Paper II. The other patients are all six mother-daughter pairs seen by the author of this thesis in whom SPECT was performed in both. Mothers are designated []:a and daughters []:b. Five mothers are hearing impaired, and mother VI:a has episodic hyperacusia and tinnitus. An audiological symptom was only present in one of the daughters, Patient II:b with mild tinnitus. There was an earlier age onset of depressive symptoms in the daughters compared to in their mothers.

Altered ^{99m}Tc -HMPAO distribution was observed at the visual interpretations of the SPECT scans in six patients. CBA interpretations, with the left lateral brain aspect to the left, and the right aspect to the right, are presented in Figures 11 – 17. Controls for the middle-aged patients are 21 healthy females aged 58 ± 11 years. Available controls for daughters are seven healthy females aged 38 ± 4 years. Since the control group for daughters is small, only alterations above or below control mean ± 3 SD are presented for them. Altered ^{99m}Tc -HMPAO distribution is indicated according to the scheme below (see Figure 7). VOIs with altered distributions reflect averaging between spots with altered ^{99m}Tc -HMPAO uptake, and the uptake in the remaining volume. One patient (VII:b) was treated with antidepressant medication at the time of SPECT.

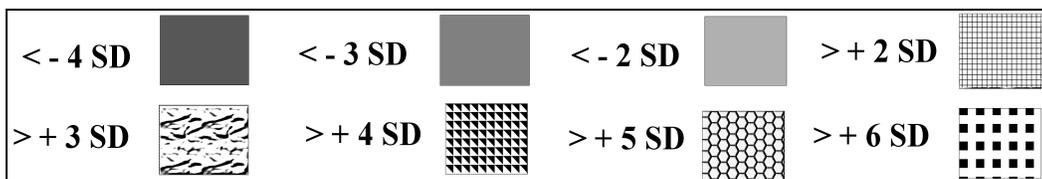


Figure 7. Decreased ^{99m}Tc -HMPAO distribution is indicated by grey shades, and increased distributions by patterns. The figure was presented previously on page 33.

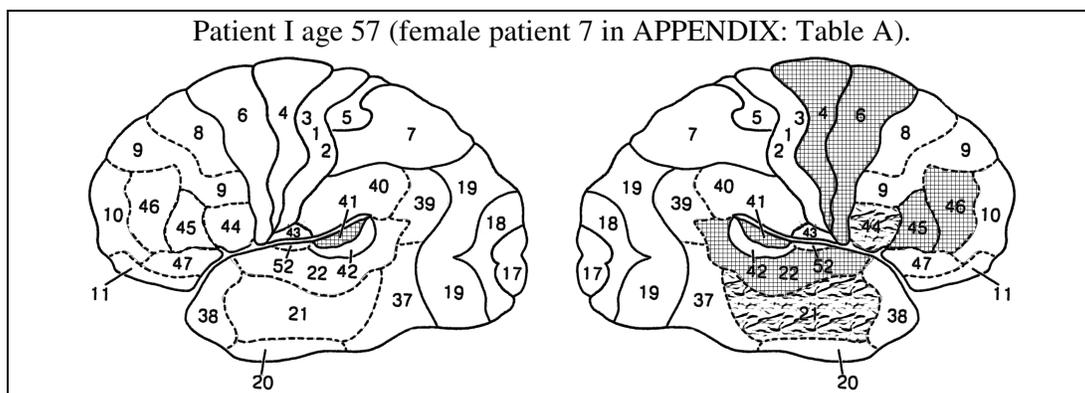


Figure 11.

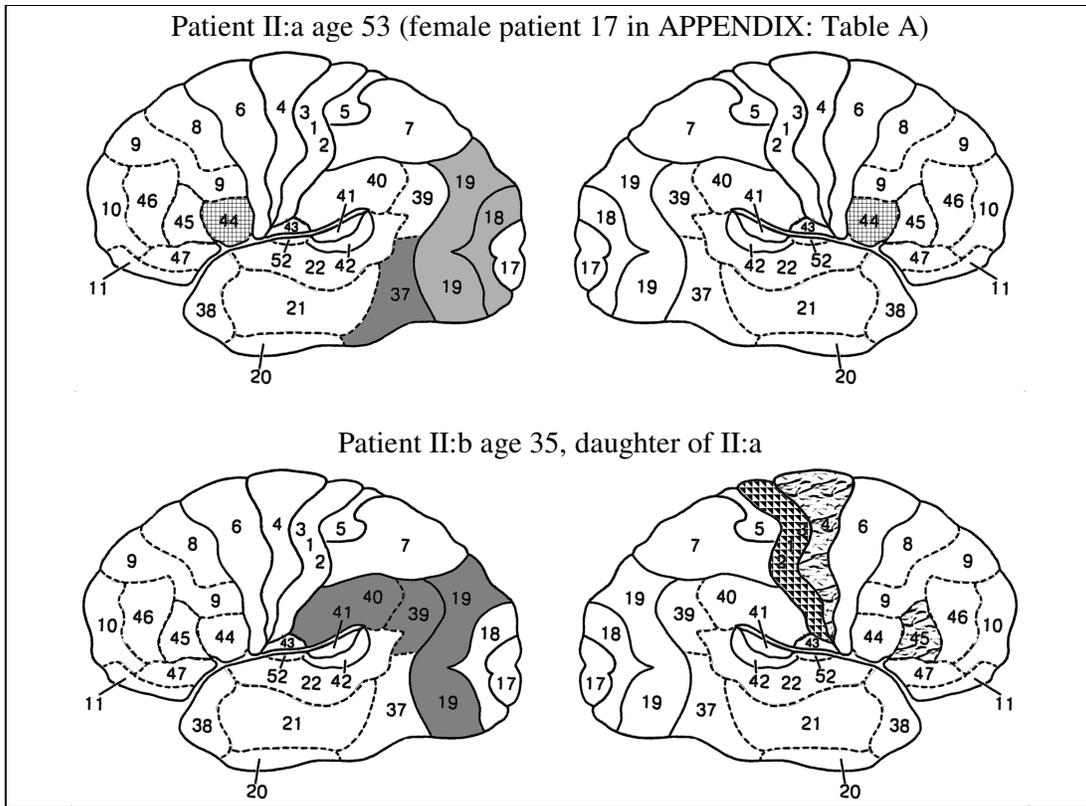


Figure 12.

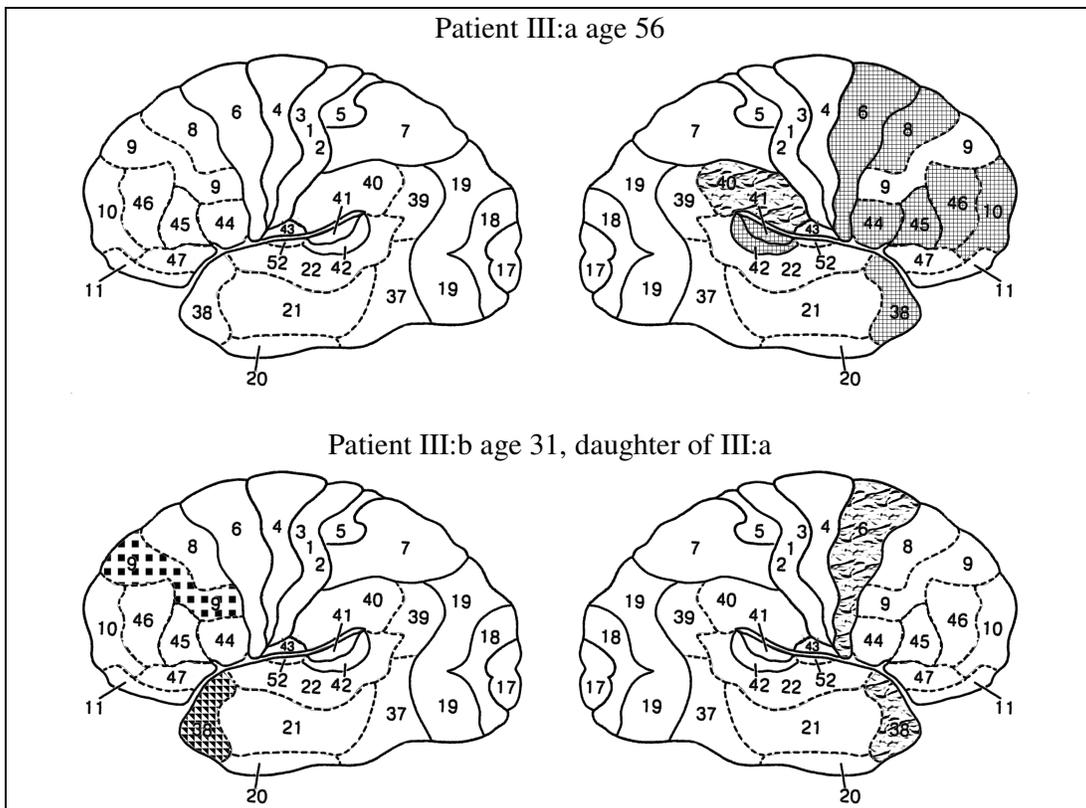


Figure 13.

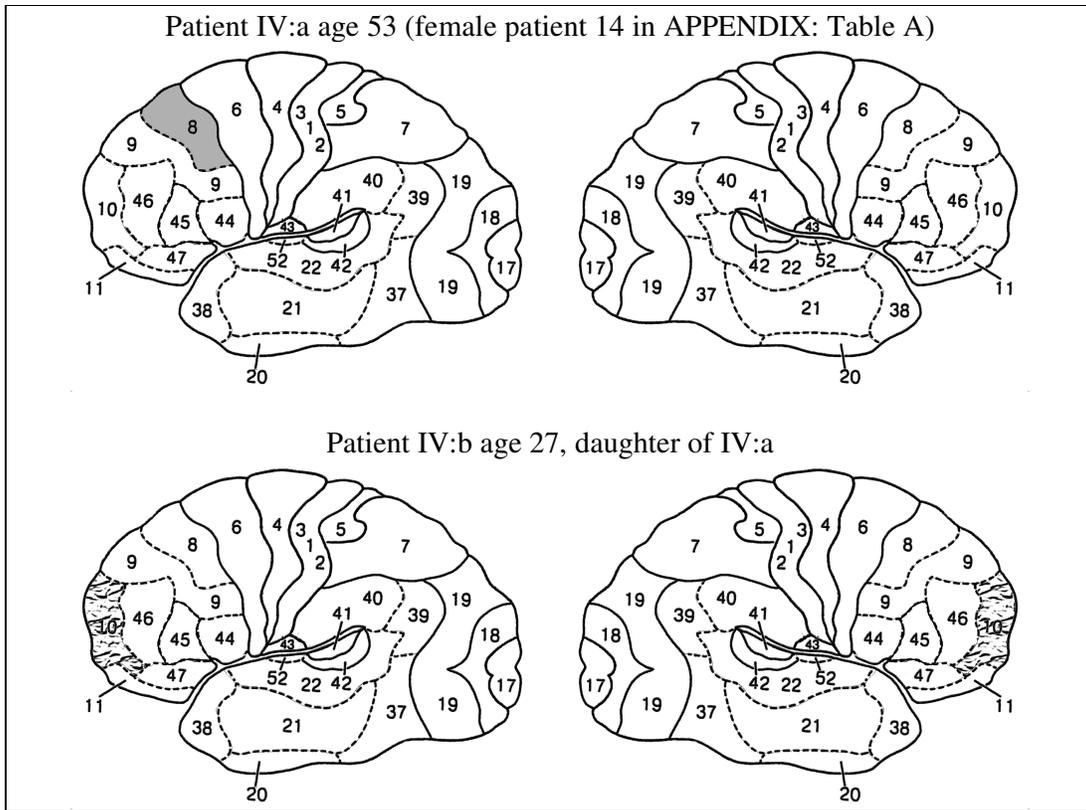


Figure 14.

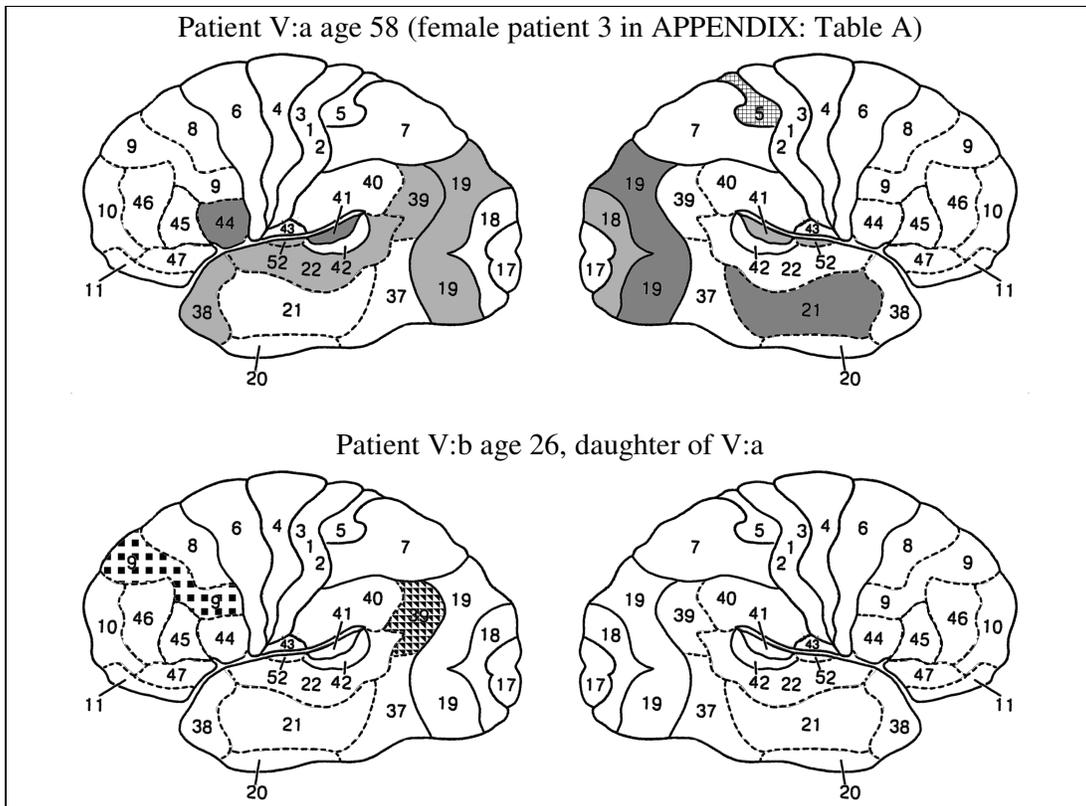


Figure 15.

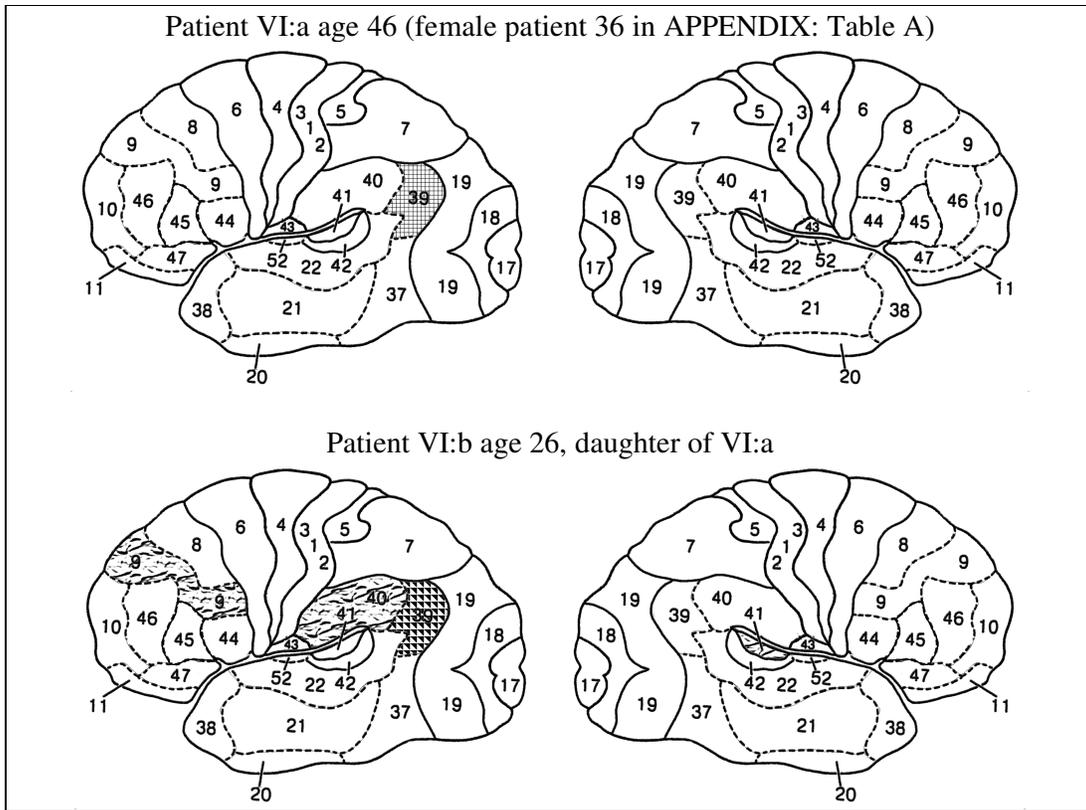


Figure 16.

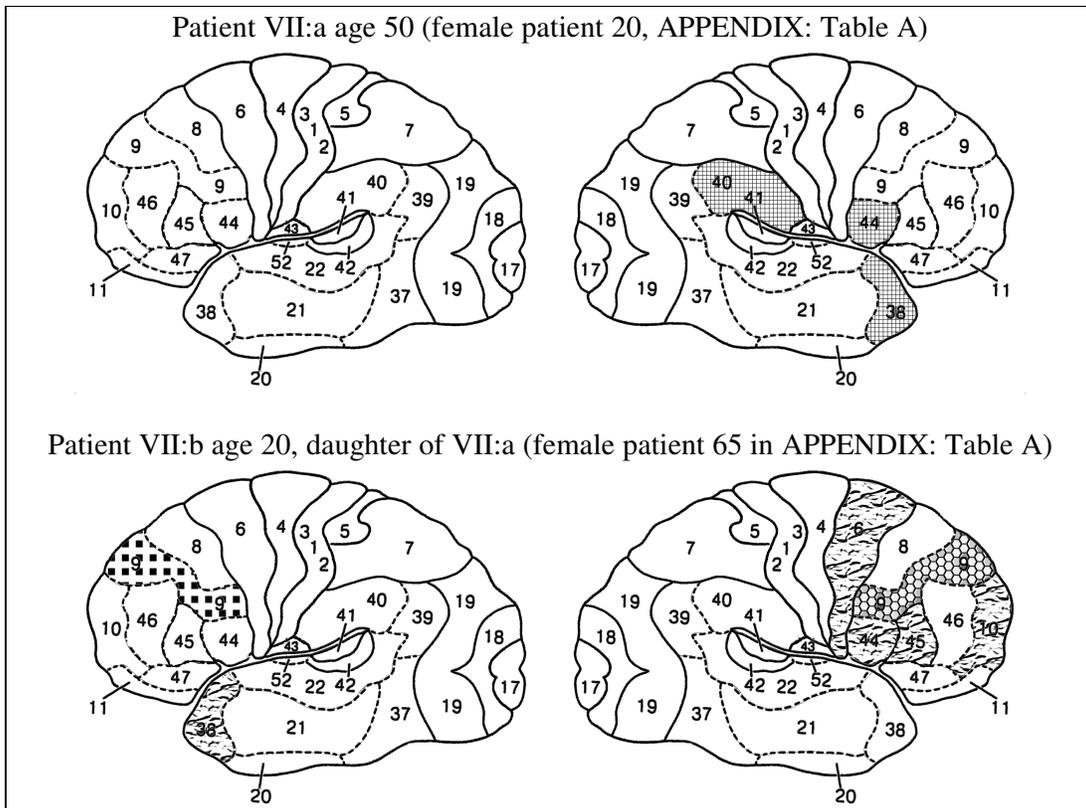


Figure 17.

Results of rating scales/questionnaires in 12 of the 13 patients are presented in Table 6.

Table 6.

Patient mother:a daughter:b	Highest MADRS-S score	KSP (normal score 50 ± 10)				
		Somatic Anxiety	Psychic Anxiety	Muscular Tension	Psych- asthenia	Irritability
I	24	79	73	73	75	80
II:a	-	68	64	61	60	44
II:b	18	63	69	72	84	54
III:a	22	-	-	-	-	-
III:b	43	82	69	88	95	97
IV:a	-	-	-	-	-	-
IV:b	36	80	74	88	87	69
V:a	23	47	44	48	66	51
V:b	11	80	72	88	79	76
VI:a	31	65	30	75	67	54
VI:b	20	58	41	71	66	69
VII:a	30	73	61	71	66	58
VII:b	47	84	74	84	82	62

MADRS-S scores ≥ 18 are considered to indicate major depression. MADRS-S was filled in at one or more occasions in most patients, but not at heights of depressive mood in several of them. Two patients with lack of good command of the Swedish language did not fill in the KSP. Not performed: - .

Individual patterns of altered ^{99m}Tc -HMPAO distributions were found in the patients. In daughter II:b, there was decreased distribution in Brodmann area 9 in the left frontal lobe ($<$ control mean $- 2.5$ SD, not shown since it did not reach $- 3$ SD) indicating that the increased distribution in four of the daughters ($>$ control mean $+ 6$ SD in three daughters) in this area was not the result of spurious sampling of the healthy controls used for the daughters.

Increases of the ^{99m}Tc -HMPAO distributions in the left and right frontal lobes were found in six and eight, respectively, of the 13 patients. In depressed patients included in Paper V (Table 3), there were mean increases above control mean $+ 1$ SD in the right frontal lobe Brodmann areas 8, 9, 10, 45 and 46. Increase of right frontal lobe ^{99m}Tc -HMPAO distribution in depressed patients has only occasionally been reported (Tutus et al, 1998b). Increases of right frontal lobe ^{99m}Tc -HMPAO distribution may reflect a fairly uncommon neurobiological alteration in depression, may be less common in acutely depressed hospitalized patients which often are investigated in neuroimaging studies, or may only be overt with 3-D interpretation of subregions of the frontal lobe.

Decreased ^{99m}Tc -HMPAO distribution in the left visual cortex was found in mother-daughter pair II. In Patient V:a, who spontaneously complained of inability in recognizing faces (prosopagnosia), there was decreased ^{99m}Tc -HMPAO distribution in the left multimodal association area (BA) 39 in the parietal lobe, and the bilateral visual cortex. In her daughter V:b who also has difficulties in recognizing faces, and severe difficulties with perception of movement and depth confirmed at an ophthalmological investigation, there was increased ^{99m}Tc -HMPAO distribution in the left BA 39, but no alterations in the visual cortex.

A hereditary metabolic alteration with predilection for, i.e., conferring selective vulnerability for, the left visual cortex, or BA 39, cannot be excluded in mother-daughter pairs II and V, respectively (see INTRODUCTION: Energy consumption in the brain and selective vulnerability, page 13). However, since the fathers of the daughters have not been investigated with SPECT, speculations about a possible maternal hereditary influence on the ^{99m}Tc -HMPAO distribution can only be tentative.

Nine of the 11 patients who filled in the KSP had elevated (≥ 1 SD) scores on the three KSP scales Somatic Anxiety, Muscular Tension and Psychasthenia. Elevated scores on these scales may reflect mitochondrial dysfunction (see Discussion in Paper IV). Alterations were found at the muscle biopsies which were obtained in five of the 13 patients. MAPRs below control range were found in Patients I, II:a and IV:a who are included in Paper I, and in Patient II:b. COX deficient muscle fibres were observed in Patients I, II:a and VI:a.

Antidepressant medications had been without effects, or only had marginal effects, in daughters III:b, IV:b, VI:b and VII:b. Daughters II:b and V:b had never received such medication. Psychogenic origins of the depressive symptoms were presumed by psychiatrists or psychologists at other services for Patients I, III:a, VI:a, VII:a, II:b, III:b, IV:b, VI:b, and VII:b. Six of these nine patients have received longterm psychodynamic psychotherapies without effects on symptomatology (further discussed below, see GENERAL DISCUSSION: *^{99m}Tc -HMPAO SPECT with automatic 3-D interpretation in individual patients*, page 50).

GENERAL DISCUSSION

The present thesis consists of six studies, mainly of patients with a chronic type of depressive illness with co-occurrence of audiological and other somatic symptoms (n = 90), and of some patients with chronic depression with somatic symptoms, but no audiological symptom severe enough to fulfill the criteria listed in APPENDIX: Table B (n = 9), and a few patients with schizophrenia (n = 4). The main aims were to study signs of mitochondrial dysfunction and relations with 'personality traits' in patients with chronic depression and somatic symptoms, differences at ^{99m}Tc -HMPAO brain SPECT between depressed patients with and without tinnitus, and relations between a mitochondrial respiratory chain enzyme and ^{99m}Tc -HMPAO distribution at SPECT in patients with psychiatric disorders.

Significant differences indicating mitochondrial dysfunction were found between patients and healthy subjects. Significant differences at ^{99m}Tc -HMPAO SPECT were found between patients with and without tinnitus, and between patients and healthy subjects. Significant correlations were found between the ^{99m}Tc -HMPAO distribution in some functionally related brain regions and a mitochondrial respiratory chain enzyme.

Main conclusion of the studies

Patients with chronic depression and somatic symptoms exhibit abnormalities of mitochondrial ATP production and the ^{99m}Tc -HMPAO distribution at SPECT. Consequently, chronic depression associated with somatic symptoms may be a systemic disorder also affecting the brain. The findings of biochemical alterations in isolated mitochondria indicate a disturbance at the level of a cell organelle.

Some methodological issues

The question of cause and effect

An important question is if biological alterations are primary or secondary to disease-related factors such as medication, drug abuse, and physical inactivation. No difference between medicated and unmedicated patients were found at the biochemical investigations of mitochondrial functions in Paper I, or at the study of ^{99m}Tc -HMPAO SPECT in Paper V. The relationship between a mitochondrial respiratory chain enzyme and ^{99m}Tc -HMPAO distribution was only explored in unmedicated patients. No signs of ongoing drug or alcohol abuse were present in any patient. However, analyses to detect presence of the medications, or drugs, in the patients were not conducted.

Physical inactivation may influence muscle mitochondrial functions. The patients in Paper I, II, III + VI were out-patients participating in activities of normal daily living necessary when living outside of an institution, but not in any regular sports activities. Several of the patients took long daily walks as this was one of the few activities they felt comfortable while doing. The healthy control subjects for the study of muscle mitochondrial biochemistry were chosen to be 'sedentary', meaning that they did not partake in any regular sports activities. The increased proportion of patients with mtDNA deletions argue against a primarily inactivation-induced decrease of mitochondrial functions.

As for the ^{99m}Tc -HMPAO SPECT findings, it is difficult to envisage a scenario in which brain metabolism in depressed tinnitus patients was differently affected in connection with the investigation compared to depressed non-tinnitus patients. As for the differences that were found between patients and healthy subjects, motivations for participation were different. Whether such motivational differences may exert an influence on the distribution of ^{99m}Tc -HMPAO is unknown.

The issue of whether disturbed brain metabolism is the origin of psychiatric symptoms, or if psychiatric symptoms per se cause disturbed brain metabolism, is as yet a matter of different beliefs. The author of this thesis believes in the former position. This issue is further commented below in this section (see The concept of mind, and logical types and levels invoked in this thesis, page 51).

Medication and inactivity can be ruled out as the main reason for the biological findings in the patients. The findings thus seem to be intrinsic to the disease.

The sample sizes

The issue of power has been a matter of consideration in all studies. This question consists of different issues, the mode and basis of recruitment of subjects, the number and characteristics of drop-outs, and the problem of multiple statistical analyses on a restricted number of patients.

The mode of recruitment and drop-outs provide information on whether the sample is representative of the population studied. The present samples of patients with chronic depression were consecutively recruited, i.e., whenever a patient passing inclusion criteria was seen at a scheduled appointment at the specialized service by the author of the thesis, he/she was asked to participate. Few patients declined participation (one patient in study I, two patients in study V), or did not return a follow-up KSP (eight patients in study IV). The high participation rate, which may be due to that the patients knew they would later be informed about their results and the results of the whole study, assured the representativity of the sample. Since the patients were recruited from a specialized psychiatric out-patient service, they are only representative of patients attending this service.

Several statistical analyses were performed on data with relatively small groups. There were 10 control subjects for the biochemical analyses in Paper I, 18 non-tinnitus patients in paper V, and 20 patients in Paper VI. A power level of $p < 0.01$ was used for the biochemical analyses in Paper I and group comparisons in Paper V. Correction for multiple comparisons was used in Paper VI. Precautions to decrease the risk of Type-I errors were added when possible when a power level of $p < 0.05$ was used. In addition, the data have to be interpreted considering the biological plausibility of the findings, as suggested by Perneger (1998).

The COMP criteria

The criteria listed in APPENDIX: Table B were designed for selection of patients in Paper I. E criteria 3 + 4 (concerning EEG and ECG results) were found to be unnecessary and can be omitted. Of the 99 depressed patients in this thesis, 78 fulfilled the criteria (see APPENDIX: Table A) attesting to that this combination of symptoms is common among the patients attending the specialized tertiary psychiatric service for patients with hearing disorders.

The specificity of the findings

In the present studies, most patients had concomitant audiological symptoms. Therefore, it is not possible to have a well-grounded opinion on the specificity of the findings, i.e., if they apply only to this subgroup of chronic depression, or if they may be applicable also to chronic depression in general, or even to other groups of patients such as schizophrenia. The findings cannot be considered to have a diagnostic specificity for patients with chronic depression with audiological symptoms. The disparate biological alterations that were found, however, suggest that somatic symptoms such as tinnitus, muscular stiffness and pain, and fatigue, have a true biological background and cannot be considered to be due to 'Somatization Disorder'. The labelling of the somatic symptoms as 'functional' is discussed in the next chapter.

Clinical implications

The concepts of 'somatization' and 'functional' disorder

The construct of Somatoform Disorders, of which Somatization Disorder is a subcategory, is found in international psychiatric classification systems and denote a pattern of recurring, multiple, and clinically significant somatic complaints for which there are no laboratory tests to support the subjective complaints. The unexplained somatic symptoms are not considered to reflect intentionally feigned or produced symptoms (APA, 1995).

In a prospective community study, 'highly somatizing' 13 to 16-year-old adolescents were found to be at increased risk of major depression four years later. The association could not be explained by detectable emotional disorder at baseline (Zwaigenbaum et al, 1999). In another prospective study, somatic complaints in females were found to predict symptoms of depression five years later (Terre et al, 2003).

Katon et al (1982) have suggested that depression is 'one of the most common causes of somatization', and define somatization as 'the selective perception and focus on the somatic manifestations of depression with denial or minimization of the affective and cognitive changes'. The authors suggest that the origin of somatization is 'communication of distress', and further write 'Because the hypochondriacal patterns many patients use to cope with dysphoric affect and social stress [---], eventually secondary gain occurs that further sustains these symptoms. Secondary gain commonly takes the form of disability payments and change in the family systems that enable patients to avoid stressful situations like work and to fulfill dependency needs by becoming passive recipients of care by others'.

The somatization construct has been criticized as originating in an artificial separation of bodily and psychological symptoms (Epstein et al, 1999; Merskey, 2000). According to Epstein et al (1999), 'the conceptual and theoretical basis of the concept of somatization as a diagnostic entity is frequently unhelpful in understanding patients' distress and guiding effective therapy'. According to Merskey (2000), 'somatization disorder' has replaced the concept of 'hysteria' used earlier. Other terms in use for unexplained somatic symptoms are 'non-specific, stress-induced symptoms' (Mathews et al, 1981), 'hypochondriacal symptoms' (Katon et al, 1982), and 'functional symptoms' (Epstein et al, 1999).

The term 'functional disorder' was presented in a textbook of diseases of the brain and nerves by Reynolds published in 1855. Reynolds assumed that the symptoms of functional disorders were due either to a decrease or an inappropriate increase in functional activity. Subsequently, the terms organic and functional came to be used by his successors simply to distinguish between conditions in which somatic pathology had and had not been demonstrated. In another textbook published in 1893, Parkinson's disease, chorea, torticollis, epilepsy, and narcolepsy, were classified as functional disorders simply because at the time there was no visible lesion to justify regarding them as organic (Kendell, 2001).

Many of the physical symptoms in the patients included in this thesis have been considered to be due to 'somatization', or have been termed 'functional' by other investigators in referral notes or medical files. The biological findings in the patients indicate that their symptoms are linked to alterations of tissue functions rather than 'communication of distress', 'hypochondriacal patterns', or 'secondary gain'. The term 'functional', if used in the original sense, is probably a correct conception of the kind of tissue disturbance that is present. However, the term has come to indicate a psychogenic origin (Kendell, 2001), and should perhaps, therefore, be abandoned along with the somatization concept for such symptoms as exhibited by patients when present in the context of symptom occurrence from several ATP-demanding tissues.

In a study of the experiences of patients afflicted by two disorders without any known etiology, fibromyalgia and chronic fatigue syndrome, Åsbring and Närvänen (2002) concluded that such patients often perceived themselves to be challenged concerning their morality, and often felt that their symptoms were psychologized. The psychologizing of the health problems were described as especially stigmatizing by the patients. In a follow-up study of perspectives of physicians about such patients (Åsbring and Närvänen, 2003), the authors note that physicians may be sceptical concerning the disease status of the conditions, and may interpret patients in moralising terms.

'Abnormal illness behaviour' such as 'Somatising disorder' in parents has been suggested to be a risk factor for 'Münchausen syndrome by proxy' (MSBP) abuse (Meadow, 2000). Scores in the pathological range on Hypochondriasis and other personality variables in about half of mothers of children with mitochondrial disorders (Varvogli and Waisbren, 1999) (see INTRODUCTION: Personality, cognition and mtDNA, page 13), and the unspecific and sometimes intermittent symptoms of the disorders and lack of alterations at routine laboratory tests supporting disease in their children, may explain why some mothers have been accused of MSBP abuse (Boles, 2000; English, 2003). Helen Hayward-Brown, the author of a PhD thesis about experiences by parents with children with chronic illnesses (Hayward-Brown, 1999), believes that there is a very high risk that parents with children with mitochondrial disorders may be accused of MSBP abuse (Hayward-Brown, personal communication Oct 2003).

In the experience of the author of this thesis, some patients including the patient in Paper II, have been more traumatized by having had the reality of their physical symptoms called into question than by the symptoms themselves. This phenomenon, and the risk for parents of children with mitochondrial disorders of being accused of MSBP abuse, testify to the necessity of knowledge about mitochondrial disorders in the medical community.

Mood symptoms

Features of 'atypical depression' such as reactivity of mood (i.e., the capacity to be cheered up by positive events), severe lethargy and fatigue, rejection sensitivity (excessive reaction to perceived rejection in social relationships), and reverse neuro-vegetative symptoms (over-sleeping and/or over-eating, or weight gain) (Angst et al, 2002) were common in the depressed patients included in this thesis.

The correlations that were found between MAPRs and KSP scales in Paper I indicate a relationship between mitochondrial functions and vulnerability to depression, but not that mitochondrial dysfunction per se causes depression. Several environmental factors seem to have a negative impact on mood in the patients according to the experience of the author of this thesis. Among these factors are increased levels of mental work, which may include participation in activities conceived as positive by patients, and sudden negative psychological impacts including perceived rejection in social relationships. In addition to antidepressant medications for those patients who can tolerate them, treatment of the patients have included teaching them a vocabulary in which they can formulate their experiences of different neurocognitive symptoms, and making them aware of the connection between increased mental work load even if enjoyable, and the sometimes ensuing increased symptomatology. Other contents in the treatment strategies are to teach the patients to 'listen to the signs from the brain and the rest of the body', and to discuss changes of life style that can lead to increased self-management and self-control of activity levels.

The most important environmental factor is seasonal effects in the experience of the author of this thesis. While increase of symptoms in spring time was quite rare, most patients complained of an increase of mood symptoms, over-sleeping or fragmented sleep, tinnitus, muscular pain and headaches, and fatigue, in the late autumn and early winter months. Pronounced seasonal effects for mitochondrial energy decoupling, with maxima in the spring months and December, have been reported (Starkov et al, 1997). Many patients reported surprise at the immediate positive effects, amelioration of mood symptoms, tinnitus, pains, and fatigue, when visiting countries at lower latitudes. Such improvements usually declined immediately at the return to Sweden, even if the patient was looking forward to it, and the return was during the summer months. This common presumed 'latitude effect' is further commented below.

Treatment of mitochondrial disorders

A number of different vitamins and co-factors are used in the treatment of patients with mitochondrial disease. Subjective and objective improvement has been documented in isolated cases (Chinnery and Turnbull, 1997). Relatively small increases of ATP production may change the threshold for disease expression and allow clinical improvement. For this reason, the currently available 'metabolic therapies', a concept used by Wallace (1992), may be most effective in patients with relatively mild disease manifestations according to Shoffner and Wallace (1994).

'Metabolic therapies' for mitochondrial disease are intended to augment energy production as well as reduce the production of free radicals and other toxic metabolites that further limit the generation of cellular energy. A list of commonly used treatments for mitochondrial disease, with dose ranges, has been presented by Gold and Cohen (2001). The authors write that it is very unlikely that there will be class 1 proof of efficiency of these treatments for various reasons including the clinical variability

between conditions, the variable and unpredictable natural course, and the problem of identifying which of the various symptoms or signs to evaluate. Providing the treatments as part of an individual trial in which the patient serves as his/her own control was suggested to be a reasonable approach.

Forty-seven of the 99 depressed patients included in the papers in this thesis (47%) are being treated on a long-term basis with vitamins and co-factors as suggested for mitochondrial disease due to subjective improvement in these patients, with ensuing reduced need for medications to alleviate various symptoms. Objective measures (ECG, blood laboratory analyses) improved in a few cases. One of the difficult issues of 'metabolic therapies' is the length of the time period before evaluation. If improvement is reported by the patient after about six months, some further gradual improvement is likely to occur for several years.

The patient included in this thesis with the most pronounced seasonal effect, necessitating hospital stays during early winter, has reported subjective improvement after having started inhaling air that has been illuminated by singlet oxygen energy (SOE). SOE is produced by a photosensitization process and is believed to involve energy that has been emitted as photons at an energy level in the visible red light region produced from singlet oxygen relaxing to the ground state, and transferred to the media. The theoretical background, and the commercially available SOE production devices, have been described in a thesis by Lundberg (2002). SOE delivered by direct illumination, or by light-illuminated air that has been bubbled through the media, has been shown to decrease measurements reflecting production of reactive oxygen species in human monocytes. It was concluded that SOE treatment may have a wide range of medical applications in conditions involving oxidative stress (Hultén et al, 1999). SOE delivered by direct illumination has been shown to be a powerful method for preservation of ATP in ischemic and reperfused muscle tissue. Future studies on SOE effects on the cellular and organ levels were suggested (Lundberg, 2002; Lundberg et al, 2002; Lindgård et al, 2003). A study to investigate if placebo effects may explain influence of SOE-illuminated air reported by various individuals is in preparation (Torsten Norlander, personal communication Dec 2003).

Solar irradiance, the number of photons falling on a ground area during a time period, varies with the latitude, season, and cloud level. Seasonal biological effects by light are believed to act through the retinal influence on the suprachiasmatic nucleus (Lincoln et al, 2003). In non-mammalian species such as reptiles and birds, seasonal effects are produced also by photons penetrating into extraretinal photoreceptors in deep encephalic neurons (Foster and Soni, 1998). Whether the solar irradiance at lower latitudes exerts effects on biological processes similar to the effect produced by photosensitization in the SOE device for air inhalation is unknown. Treating energy deficiency disorders with energy seems an attractive approach.

^{99m}Tc-HMPAO SPECT with automatic 3-D interpretation in individual patients

^{99m}Tc-HMPAO SPECT with CBA interpretations were presented above for 13 female patients with chronic depressive symptoms (see RESULTS AND COMMENTS: ^{99m}Tc-HMPAO SPECT in individual patients, page 39).

Six of the patients, all of whom had alterations of the ^{99m}Tc-HMPAO distribution, have received longterm psychodynamic psychotherapies without effects on symptomatology. The clinical presentation of the depressive disorder in most of these

patients is characterized by garrulity or rumination reminiscent of psychomotor agitation rather than retardation with slow mentation, which may be one of the reasons why the symptoms in the patients have been considered to have a psychogenic origin by other psychiatrists or psychologists in more than half of the patients who have had such contacts. It is difficult for an observer to conciliate the claims of the patients about depressed mood and fatiguability with their observable liveliness and increase of thought processes. Attesting thought content rather than the mode of presentation may not be of use for patients. Garrulity or rumination may indicate, e.g., deficiencies in inhibitory neurobiological mechanisms (see INTRODUCTION: Energy consumption in the brain and selective vulnerability, page 13). Selecting psychotherapeutic strategies for patients according to their mode of clinical presentation, and possibly after a SPECT investigation with automatic 3-D interpretation, may be more efficacious than selecting strategies only or mainly according to the patients' thought content.

The concept of mind, and logical types and levels invoked in this thesis

The materialist philosopher Gilbert Ryle, in discussing the conception of the separateness of mental and physical existence, has suggested that the origin of this conception rests on a common aversion to a mechanistic assumption of the mind, and a category-mistake with a belief that Mind and Matter are terms of the same logical type. 'Reduction' of the material world to mental states and processes, as well as the 'reduction' of mental states and processes to physical states and processes, is thus logically impossible (Ryle, 1983).

Christian de Duve, a Nobel prize laureate for work on the structural and functional organization of the cell, in his book on the emergence and development of life on Earth including the role of the ATP molecule (de Duve, 1995), concluded that mind research is still in an embryonic stage. According to de Duve, mind can be thought of as a special manifestation of matter organized in a special fashion, similar to other manifestations of matter such as life, gravitation, and electromagnetism.

Eric Kandel, who initially planned to become a psychoanalyst before choosing a career in research, and a Nobel prize laureate for his work on signal transduction in the nervous system, has outlined the beginnings of a new intellectual framework for psychiatry derived from current biological thinking about the relationship of mind to brain. He advocates a greater knowledge of the structure and functioning of the brain in the professional requirements for future psychiatrists than currently available in most training programs for psychiatrists (Kandel, 1998; 1999).

This thesis invokes observations and concepts derived from 12 logical types or levels, which cannot be 'reduced' to observations and concepts derived from the other levels.

1. Communications with the patients in order to fit their symptoms into present-day psychiatric diagnoses
2. self-report questionnaires filled in by the patients themselves
3. brain imaging with measurements reflecting metabolic properties of brain tissue
4. structural and metabolic muscle cell properties observed by light microscopy

5. even smaller structural muscle cell properties observed by electron microscopy
6. in situ hybridization for muscle tissue localization of specific mtDNA molecules
7. ATP production in isolated muscle mitochondria measured with a bioluminometric method
8. muscle mitochondrial enzyme activities measured by spectrophotometric methods
9. presence of muscle tissue mtDNA deletions using the Southern blot method
10. presence of muscle tissue mtDNA deletions using PCR methodology
11. presence of mtDNA point mutations using restriction site methodologies.

This extreme disparity of observational levels and methods ranging from the two-person dialogue level to the level of single mtDNA bp's, has to be reconciled with that observations were mainly made on patients with a more similar symptom presentation than generally achieved in studies of depression. Also, the observational levels were chosen for logical reasons and not haphazardly. Any inferences concerning mood symptoms, and not only the somatic symptoms of depression, drawn from these disparate logical levels (in particular from levels 3 – 10 to levels 1 and 2), can only be tentative due to the very 'embryonic stage' of our understanding of mind-brain relations.

The majority of the investigatory methods used in this study cannot be applied in clinical psychiatry on a routine basis. For the clinical psychiatrist, understanding of the background of symptoms in individual patients may be enhanced by familiarity with current thinking of mind-brain relations, and of the biological underpinnings of behaviour such as, e.g., mitochondrial functions. Routine use of rating scales and functional brain imaging with the SPECT method with automatic interpretation in 3-D space can be applied in psychiatric investigations.

CONCLUSIONS

The main results and conclusions of the thesis may be summarized as follows

1. Signs of dysfunction of mitochondrial energy production in muscle were present in patients with chronic depression and audiological and other somatic symptoms to a much greater extent than in controls. The findings represent the final common path for several events such as primary involvement or secondary impacts on the mitochondrial respiratory chain. The findings indicate a disturbance at the level of a cell organelle.
2. Relationships between measurements of mitochondrial energy production and clinical dimensions assessed by a questionnaire indicate that mitochondrial dysfunction is associated with somatic symptoms that are common in depression. Increased and more stable somatic symptoms were found compared to depressed patients in primary care.
3. Signs of mitochondrial dysfunction in muscle, and regional alterations of brain metabolism, and relations between the activity of a mitochondrial respiratory chain enzyme and regional brain metabolism indicate that chronic depression, at least when associated with somatic symptoms, is a systemic disorder with brain manifestations.
4. Mitochondrial dysfunction seems to be associated with vulnerability to mood symptoms. Additional impacts may be necessary for appearance of pronounced mood symptoms.

Future research

There are several possible future directions of research on the basis of the results of this study. A study of the neuropsychological profile of patients also investigated with muscle biopsy and brain imaging is ongoing, and of signs of childhood and present Attention Deficit Hyperactivity Disorder in all patients. Effects of therapies designed to ameliorate mitochondrial dysfunction ought to be investigated. Studying mitochondrial functions in other groups of depressed patients compared to matched healthy controls is another future direction.

SAMMANFATTNING PÅ SVENSKA

Mitokondriell störning och avvikelser vid HMPAO SPECT i hjärna vid depressionssjukdom – perspektiv på orsaker till ”somatisering”

Somatiska (kroppsliga) symtom som hörsel- och synsymtom, muskel- och huvudvärk och trötthet är vanligare hos obehandlade patienter med depression än hos friska individer. De somatiska symtomen har bland annat föreslagits kunna orsakas av ”somatisering” (att uttrycka sig i kroppsliga snarare än psykologiska termer) och stresssymtom, eller vara uttryck för hypokondri (rädsla för sjukdom eller inbillade symtom). Begreppet ”funktionella symtom” används också och anses ofta innebära en störning utan påvisbar biomedicinsk avvikelse.

Depression, och hörsel-, syn/ögon-, och muskelsymtom, har beskrivits hos patienter med s.k. mitokondriella sjukdomar, tillstånd vid vilka sänkt produktion av cellenergi, adenosin trifosfat (ATP), i den mitokondriella andningskedjan anses bidra. Avhandlingen avsåg att undersöka om minskad ATP-produktion kan påvisas hos patienter med depression och samtidig förekomst av ovannämnda somatiska symtom. Patienter har utretts med muskelbiopsi för att undersöka mitokondriefunktionen, ”hjärnblodflöde” för att undersöka fördelningen av spårämnet HMPAO med gammakamera (SPECT) och utvärdering med standardiserad hjärnatlas, och besvarat frågeformuläret Karolinska Scales of Personality (KSP). Samband mellan KSP-delskalor och ATP-produktion eller HMPAO-fördelning, och mellan ett mitokondriellt andningskedjeenzymkomplex och HMPAO-fördelning, har undersökts.

Nedan förtecknade delprojekt ingår i avhandlingen:

- I. Prov från en främre underbensmuskel från 28 patienter med depression analyserades avs. ATP-produktion och andningskedjeenzym, deletioner i mitokondriellt DNA (mtDNA), och ljus- och elektronmikroskopi. 21 patienter besvarade KSP.
- II. Fallbeskrivning av en patient från delprojekt I: karakterisering av mtDNA-mutationer och förekomst i enskilda muskelfibrer (in situ hybridisering).
- III. Fallbeskrivning av en annan patient från delprojekt I: redovisning av sjukhistorien över tid samt resultat från muskelprov, HMPAO SPECT och KSP.
- IV. KSP besvarades av 84 patienter med depression varav alla även hade somatiska symtom. 65 patienter besvarade KSP vid uppföljning efter 3 eller 4 år.
- V. HMPAO SPECT och samband med KSP-poäng jämfördes mellan depressionspatienter med och utan tinnitus.
- VI. Samband mellan ett andningskedjeenzym i muskel och HMPAO-fördelningen vid SPECT undersöktes hos 20 obehandlade patienter, 16 med depression och fyra med schizofreni.

Resultat. I delprojekt I visas att en depressionsgrupp, där alla individer hade samtidig tinnitus och/eller hörselnedsättning och lindriga syn/ögon- och muskelsymtom, hade sänkt ATP-produktion och sänkta kvoter mellan andningskedjeenzym i muskel i jämförelse med 10 friska individer. Patientgruppen hade ökad förekomst av små mtDNA-deletioner (avsaknad av delar av molekylen) i muskel jämfört med friska individer. Smärre ospecifika muskelcellsförändringar vid ljus- och/eller elektronmikroskopi sågs hos 89% av patienterna. Låga värden för ATP-produktion hade samband med höga poäng på KSP-skalorna Somatisk Ångest, Psykasteni och Misstänksamhet. Resultaten talar för att störningar av mitokondriefunktionen med olika bakgrund förekom i patientgruppen, och att sådana störningar kan bidra till sårbarhet för depression.

Resultat från två patienter från delprojekt I redovisas i delprojekt II och III. För bägge patienter debuterade psykiatriska symtom före somatiska symtom. En kvinnlig patient visades ha förlängda mtDNA-molekyler i 30% av totalt mtDNA i lymfocyter. Deleterade mtDNA-molekyler med klyvningspunkter motsvarande de förlängda mtDNA-molekylerna påvisades i muskelfibrer med låg färgbarhet för ett andningskedjeenzym. Normallånga eller förlängda mtDNA-molekyler kunde knappast påvisas alls i dessa muskelfibrer. De förlängda mtDNA-molekylerna förmodas generera de deleterade mtDNA-molekylerna, som kan orsaka störning av mitokondriefunktionen. Hos en manlig patient med låg ATP-produktion förelåg avvikande HMPAO-fördelning vid SPECT med ett liknande mönster som tidigare beskrivits vid depression. Patienten hade mycket förhöjda poäng på KSP-delskalorna Somatisk Ångest, Muskelspänning och Psykasteni i jämförelse med normalbefolkningen.

I delprojekt IV redovisas resultat från fem KSP-delskalor som besvarats med ett medelintervall av 3,5 år av 65 depressionspatienter med olika somatiska symtom, varav c:a hälften behandlades med antidepressiva läkemedel. Ingen skillnad sågs mellan testtillfällena. I jämförelse med 163 obehandlade depressionspatienter i primärvård hade patientgruppen ännu mer avvikande (högre) poäng på Somatisk Ångest, Muskelspänning och Psykasteni. För Psykisk Ångest och Socialisation sågs ingen skillnad. Det föreslogs att den selekterade depressionsgruppen med somatiska symtom karakteriseras av hög och stabil "somatic distress". Begreppet "personality trait", som används som övergripande beteckning för symtom som mäts av KSP-delskalor, föreslås diskuteras vad gäller uttalade "somatic distress"-symtom, då sådana är vanliga vid och kan vara direkta uttryck för mitokondriella sjukdomar.

I delprojekt V beskrivs skillnader i HMPAO-fördelning vid SPECT mellan depressionspatienter, 27 med tinnitus och 18 utan tinnitus, i hjärnregioner som tidigare visat förändringar vid tinnitussupplevelse. Sambanden mellan två "ångestskalor" från KSP och HMPAO-fördelningen i några hjärnregioner visade skillnader mellan tinnitus- och icke-tinnitus-patienter, vilket tolkades som möjligt uttryck för skillnader i neural aktivitet.

Starka samband mellan låga nivåer av andningskedjeenzymet SCR (komplex II+III) i muskel och hög HMPAO-fördelning i sensorisk associationshjärnbark hos obehandlade patienter med kroniska psykiatriska tillstånd redovisas i delprojekt VI. Färgbarhet för komplex II har tidigare visats vara högst i sensorisk hjärnbark. SCR-nivåer kan vara likartade i muskel och hjärna hos en individ eftersom inga skillnader mellan subenzym av SCR rapporterats i olika vävnader. Ökad produktion av fria elektroner och/eller reaktiva syreradikaler vid låga SCR-nivåer skulle kunna bidra till en ökad konvertering av HMPAO till den form som ansamlas i hjärnceller och mäts vid SPECT. Resultaten ger en ny infallsvinkel till möjliga orsaker till de avvikelser av HMPAO-fördelningen vid SPECT som rapporterats vid psykiatriska tillstånd.

Slutsats. Resultaten från denna avhandling visar att biomedicinska avvikelser som kan orsaka symtom från inneröron, ögon, muskel och hjärna kunde påvisas med hjälp av nya undersökningsmetoder i en depressionsgrupp med sådana somatiska symtom. Depressionsjukdomen i denna patientgrupp manifesteras i hela kroppen och får ett av sina tydligaste kliniska uttryck från hjärnans funktioner. En gemensam faktor bakom fynden kan vara störd mitokondriefunktion. En primär genetisk störning i mtDNA kunde bara säkerställas för den enda patient för vilken en högt specialiserad utredning utfördes. Fynden av biomedicinska avvikelser talar för att de kroppsliga symtomen ej bör betraktas som "somatisering" eller "funktionella symtom". Projektet har drivits i samarbete med specialiserade laboratorier vid Karolinska universitetssjukhusen i Huddinge och Solna och Sahlgrenska sjukhuset, och har involverat ett stort antal medarbetare.

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REFERENCES

Alp P, Newsholme E, Zammit V. Activities of citrate synthase and NAD⁺-linked and NADP⁺-linked isocitrate dehydrogenase in muscle from vertebrates and invertebrates. *Biochem J* 1976; 154: 689-700.

Andersson G, Lyttkens L, Hirvelä C, Furmark T, Tillfors M, Fredriksson M. Regional cerebral blood flow during tinnitus: a PET case study with lidocaine and auditory stimulation. *Acta Otolaryngol* 2000; 120: 967-972.

Angst J, Gamma A, Sellaro R, Zhang H, Merikangas K. Toward validation of atypical depression in the community: results of the Zurich cohort study. *J Affect Disord* 2002; 72: 125-138.

APA: Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, International Version. Washington, DC, American Psychiatric Association, 1995, p. 329, 331.

Åsbring P, Närvänen AL. Women's experiences of stigma in relation to chronic fatigue syndrome and fibromyalgia. *Qual Health Res* 2002; 12: 148-160.

Åsbring P, Närvänen AL. Ideal versus reality: physicians perspectives on patients with chronic fatigue syndrome (CFS) and fibromyalgia. *Soc Sci Med* 2003; 57: 711-720.

Axelsson A, Ringdahl A. Tinnitus – a study of its prevalence and characteristics. *Br J Audiology* 1989; 23: 53-62.

Babich JW. Technetium-99m-HMPAO and the role of glutathione: the debate continues. *J Nucl Med* 1991; 32: 1681-1683.

Ballinger JR, Reid RH, Gulenchyn KY. Technetium-99m HM-PAO stereoisomers: differences in interaction with glutathione. *J Nucl Med* 1988; 29: 1998-2000.

Barbiroli B, Iotti S, Lodi R. Improved brain and muscle mitochondrial respiration with CoQ. An in vivo study by 31P-MR spectroscopy in patients with mitochondrial cytopathies. *Biofactors* 1999; 9: 253-260.

Battino M, Bertoli E, Formiggini G, Sassi S, Gorini A, Villa RF, Lenaz G. Structural and functional aspects of the respiratory chain of synaptic and nonsynaptic mitochondria derived from selected brain regions. *J Bioenerg Biomembr* 1991; 23: 345-363.

Ben-Shachar D. Mitochondrial dysfunction in schizophrenia: a possible linkage to dopamine. *J Neurochem* 2002; 83: 1241-1251.

- Bergman H, Bergman I, Engelbrekson K, Holm L, Johannesson K, Lindberg S. Psykologhandbok, Del I (Psychological Manual, Part I). Magnus Huss Klinik, Stockholm, 1982.
- Berio A, Piazzzi A. A case of Kearns-Sayre syndrome with autoimmune thyroiditis and possible Hashimoto encephalopathy. *Panminerva Med* 2002; 44: 265-269.
- Birch-Machin MA, Briggs HL, Saborido AA, Bindoff LA, Turnbull DM. An evaluation of the measurement of the activities of complexes I-IV in the respiratory chain of human skeletal muscle mitochondria. *Biochem Med Metabol Biol* 1994; 51: 35-42.
- Blass JP. Conference summary: mitochondria, neurodegenerative diseases, and selective neuronal vulnerability. *Ann NY Acad Sci* 1999; 893: 434-439.
- Boles RG. Cyclic vomiting in mitochondrial disease. *The Official Newsletter of the CVSA-USA/Canada* 2000; 8(1). <http://www.cvsasonline.org/cvsmitocodev.htm>
- Bonne O, Krausz Y. Pathophysiological significance of cerebral perfusion abnormalities in major depression-trait or state marker? *Eur Neuropsychopharmacol* 1997; 7: 225-233.
- Boonstra F, Claerhout I, Hol F, Smit G, Collenburg v V, Meire F. Corneal decompensation in a boy with Kearns-Sayre syndrome. *Ophthalmic Genet* 2002; 23: 247-251.
- Borowsky IW, Collins RC. Metabolic anatomy of brain: a comparison of regional capillary density, glucose metabolism, and enzyme activities. *J Comp Neurol* 1989; 288: 401-413.
- Bosbach S, Kornblum C, Schröder R, Wagner M. Executive and visuospatial deficits in patients with chronic progressive external ophthalmoplegia and Kearns-Sayre syndrome. *Brain* 2003; 126: 1231-1240.
- Brini M, Pinton P, King MP, Davidson M, Schon EA, Rizzuto R. A calcium signaling defect in the pathogenesis of a mitochondrial DNA inherited oxidative phosphorylation deficiency. *Nat Med* 1999; 5: 951-954.
- Brodin L, Bakeeva L, Shupliakov O. Presynaptic mitochondria and the temporal pattern of neurotransmitter release. *Phil Trans R Soc Lond B* 1999; 354: 365-372.
- Campos Y, Garcia A, Eiris J, Fuster M, Rubio JC, Martin MA, del Hoyo P, Pintos E, Castro-Gago M, Arenas J. Mitochondrial myopathy, cardiomyopathy and psychiatric illness in a Spanish family harbouring the mtDNA 3303C > T mutation. *J Inherit Metab Dis* 2001; 24: 685-687.
- Carman JS. Imipramine in hyperacusis depression [letter]. *Am J Psychiatry* 1973; 130: 937.

- Chinnery PF, Turnbull DM. Mitochondrial medicine. *Quart J Med* 1997; 90: 657-667.
- Chinnery PF, Elliot C, Green GR, Rees A, Coulthard A, Turnbull DM, Griffiths TD. The spectrum of hearing loss due to mitochondrial DNA defects. *Brain* 2000; 123: 82-92.
- Chinnery PF, Schon EA. Mitochondria. *J Neurol Neurosurg Psychiatry* 2003; 74: 1188-1199.
- Cho MJ, Lyoo IK, Lee DW, Kwon JS, Lee JS, Lee DS, Jung JK, Lee MC. Brain single photon emission computed tomography findings in depressive pseudodementia patients. *J Affect Disord* 2002; 69: 159-166.
- Clarke DD, Sokoloff L. Circulation and energy metabolism of the brain. In: Siegel GJ, Agranoff BW, Albers RW, Molinoff PB (editors). *Basic neurochemistry*. Raven Press, New York, fifth edition, 1994, pp. 645-680.
- Cohen BH, Gold DR. Mitochondrial cytopathies in adults: what we know so far. *Cleveland Clin J Med* 2001; 68: 625-642.
<http://www.ccjm.org/pdf/files/COHEN701.PDF>
- Conca A, Fritzsche H, Peschina W, Konig P, Swoboda E, Wiederin H, Haas C. Preliminary findings of simultaneous 18F-FDG and 99mTc-HMPAO SPECT in patients with depressive disorders at rest: differential correlates with ratings of anxiety. *Psychiatry Res* 2000; 98: 43-54.
- Cooperstein SJ, Lazarow A, Kurfess NJ. A microspectrophotometric method for the determination of succinic dehydrogenase. *J Biol Chem* 1950; 186: 129-139.
- Cooperstein SJ, Lazarow A. A microspectrophotometric method for the determination of cytochrome oxidase. *J Biol Chem* 1951; 189: 665-670.
- Corruble E, Guelfi JD. Pain complaints in depressed inpatients. *Psychopathology* 2000; 33: 307-309.
- Coulehan JL, Schulberg HC, Block MR, Zettler-Segal M. Symptom patterns of depression in ambulatory medical and psychiatric patients. *J Nerv Ment Dis* 1988; 176: 284-288.
- Coulter-Mackie MB, Applegarth DA, Toone JR, Gagnier L. A protocol for detection of mitochondrial DNA deletions: characterization of a novel deletion. *Clin Biochem* 1998; 31: 627-632.
- De Duve C. *Vital dust. Life as a cosmic imperative*. New York: Basic Books, 1995, p. 259.

Deschauer M, Muller T, Wieser T, Schulte-Mattler W, Kornhuber M, Zierz S. Hearing impairment is common in various phenotypes of the mitochondrial DNA A3243G mutation. *Arch Neurol* 2001; 58: 1885-1888.

Dewsnap P, Gomborone J, Libby G, Farthing F. The prevalence of symptoms of irritable bowel syndrome among acute psychiatric inpatients with an affective diagnosis. *Psychosomatics* 1996; 37: 385-389.

Douglas R, Martin K. Neocortex. In: Shepherd GM (editor). *The synaptic organization of the brain*. Oxford University Press, New York, fourth edition, 1998, pp. 459-509.

Drug VL, Costea F, Ciochina AD, Bradatan O, Tarasi I, Mitrica D, Stanciu C. Functional digestive disorders and the relationship with psychiatric diseases [article in Romanian]. *Rev Med Chir Soc Med Nat Iasi* 2002; 107: 307-310.

Dunbar DR, Moonie PA, Swingler RJ, Davidson D, Roberts R, Holt IJ. Maternally transmitted partial direct tandem duplication of mitochondrial DNA associated with diabetes mellitus. *Hum Mol Genet* 1993; 2: 1619-1624.

Eaton WW, Armenian H, Gallo J, Pratt L, Ford DE. Depression and risk for onset of type II diabetes. A prospective population-based study. *Diabetes Care* 1996; 19: 1097-1102.

Ekselius L, von Knorring L. Changes in personality traits during treatment with sertraline or citalopram. *Br J Psychiatry* 1999; 174: 444-8.

English B. Mortal heritage. *Boston Globe Magazine* 2003.
<http://www.boston.com/globe/magazine/2003/0727/disease.htm>

Epstein RM, Quill TE, McWhinney IR. Somatization reconsidered. Incorporating the patient's experience of illness. *Arch Intern Med* 1999; 159: 215-222.

Fadic R, Johns DR. Clinical spectrum of mitochondrial diseases. *Semin Neurol* 1996; 16: 11-20.

Finsterer J, Jarius C, Eichberger H. Phenotype variability in 130 adult patients with respiratory chain disorders. *J Inherit Metab Dis* 2001; 24: 560-576.

Fischler B, D'Haenen H, Cluydts R, Michiels V, Demets K, Bossuyt A, Kaufman L, De Meirleir K. Comparison of 99m Tc HMPAO SPECT scan between chronic fatigue syndrome, major depression and healthy controls: an exploratory study of clinical correlates of regional cerebral blood flow. *Neuropsychobiology* 1996; 34: 175-183.

Folstein MF, Folstein SE, McHugh PR. 'Mini-mental state', a practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* 1975; 12: 189-198.

- Foster RG, Soni BG. Extraretinal photoreceptors and their regulation of temporal physiology. *Rev Reprod* 1998; 3: 145-150.
- Friberg TR, Borrero GJ. Diminished perception of ambient light: a symptom of clinical depression? *J Affect Disord* 2000; 61: 113-118.
- Friede RL. A comparative study of cytoarchitectonics and chemoarchitectonics of the cerebral cortex of the guinea pig. *Z Zellforsch Mikrosk Anat* 1960; 52: 482-493.
- Friede RL. *Topographic brain chemistry*. New York: Academic Press, 1966, p. 16-131, 492-500.
- Friston KJ. Commentary and opinion: II. Statistical parametric mapping: ontology and current issues. *J Cereb Blood Flow Metab* 1995; 15: 361-370.
- Fujibayashi Y, Taniuchi H, Waki A, Yokoyama A, Ishii Y, Yonekura Y. Intracellular metabolism of ^{99m}Tc-d,l-HMPAO in vitro: a basic approach for understanding the hyperfixation mechanism in damaged brain. *Nucl Med Biol* 1998; 25: 375-378.
- Galynker II, Cai J, Ongseng F, Finestone H, Dutta E, Sersen D. Hypofrontality and negative symptoms in major depressive disorder. *J Nucl Med* 1998; 39: 608-612.
- Gershon ES, Badner JA, Detera-Wadleigh SD, Ferraro TN, Berrettini WH. Maternal inheritance and chromosome 18 allele sharing in unilineal bipolar illness pedigrees. *Am J Med Genet* 1996; 67: 202-207.
- Gille L, Nohl H. The ubiquinol/bc1 redox couple regulates mitochondrial oxygen radical formation. *Arch Biochem Biophys* 2001; 388: 34-38.
- Giraud AL, Chéry-Croze S, Fischer G, Fischer C, Vighetto A, Gregoire MC, Lavenne F, Collet L. A selective imaging of tinnitus. *NeuroReport* 1999; 10: 1-5.
- Gold DR, Cohen BH. Treatment of mitochondrial cytopathies. *Semin Neurol* 2001; 21: 309-325.
- Grandinetti A, Kaholokula JK, Chang HK. Delineating the relationship between stress, depressive symptoms and glucose intolerance [letter]. *Diabetes Care* 2000; 23: 1443-4.
- Greitz T, Bohm C, Holte S, Eriksson L. A computerized brain atlas: construction, anatomical content, and some applications. *J Comp Assist Tomogr* 1991; 15: 26-38.
- Grünwald F, Zierz S, Broich K, Schumacher S, Bockisch A, Biersack H-J. HMPAO-SPECT imaging resembling Alzheimer-type dementia in mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS). *J Nucl Med* 1990; 31: 1740-1742.

Grünwald F, Zierz S, Broich K, Dewes W, Böker T, Biersack H-J. Brain SPECT imaging with Tc-99m HMPAO in ophthalmoplegia plus. *Clin Nucl Med* 1991; 1: 20-23.

Gustavsson JP. Stability and validity of self-reported personality traits. Contributions to the evaluation of the Karolinska Scales of Personality [thesis]. Department of Clinical Neuroscience and the Institute for Environmental Medicine, Karolinska Institutet, Stockholm, Sweden, 1997.

Hagen K, Zwart JA, Vatten L, Stovner LJ, Bovim G. Head-HUNT, validity and reliability of a headache questionnaire in a large population-based study in Norway. *Cephalalgia* 2000; 20: 244-251.

Hayward Brown H. False and highly questionable allegations of Munchausen syndrome by proxy. Bathurst: Charles Sturt University, 1999.
<http://signup.pnc.com.au/~heleneli/>

Herbst KG, Humphrey C. Hearing impairment and mental state in the elderly living at home. *BMJ* 1980; 281: 903-905.

Hevner RF, Liu S, Wong-Riley MT. A metabolic map of cytochrome oxidase in the rat brain: histochemical, densitometric and biochemical studies. *Neuroscience* 1995; 65: 313-342.

Holmgren D, Wåhlander H, Eriksson BO, Oldfors A, Holme E, Tulinius M. Cardiomyopathy in children with mitochondrial disease. Clinical course and cardiological findings. *Eur Heart J* 2003; 24: 280–288.

Hornig M, Mozley PD, Amsterdam JD. HMPAO SPECT brain imaging in treatment-resistant depression. *Prog Neuropsychopharmacol Biol Psychiatry* 1997; 21: 1097-1114.

Hotopf M, Wessely S. Depression and chronic fatigue syndrome. In: Robertson MM and Katona CLE (Eds), *Depression and physical illness*. John Wiley & Sons Ltd, England, 1997, pp. 499-522.

Houshmand M. Mitochondrial DNA mutations, pathogenicity and inheritance [thesis]. Institute of Laboratory Medicine, Department of Clinical Chemistry and Transfusion Medicine, Göteborg University, Sahlgrenska University Hospital, Gothenburg, Sweden, 1999.

Hultén LM, Holmström M, Soussi B. Harmful singlet oxygen can be helpful. *Free Radic Biol Med* 1999; 27: 1203-1207.

Iidaka T, Nakajima T, Suzuki Y, Okazaki A, Maehara T, Shiraishi H. Quantitative regional cerebral blood flow measured by Tc-99m HMPAO SPECT in mood disorder. *Psychiatry Res: Neuroimaging* 1997; 68: 143-154.

- Imran MB, Kawashima R, Sato K, Kinomura S, Ono S, Qureshy A, Fukuda H. Detection of CBF deficits in neuropsychiatric disorders by an expert system: a ^{99m}Tc-HMPAO brain SPET study using automated image registration. *Nucl Med Commun* 1999; 20: 25-32.
- Inagaki T, Ishino H, Seno H, Ohguni S, Tanaka J, Kato Y. Psychiatric symptoms in a patient with diabetes mellitus associated with a point mutation in mitochondrial DNA. *Biol Psychiatry* 1997; 42: 1067-1069.
- Irwin WA, Bergamin N, Sabatelli P, Reggiani C, Megighian A, Merlini L, Braghetta P, Columbaro M, Volpin D, Bressan GM, Bernardi P, Bonaldo P. Mitochondrial dysfunction and apoptosis in myopathic mice with collagen VI deficiency. *Nat Genet* 2003; 35: 367-371.
- Ishihara A, Hayashi S, Roy RR, Tamada Y, Yokoyama C, Ohira Y, Edgerton VR, Iyata Y. Mitochondrial density of ventral horn neurons in the rat spinal cord. *Acta Anat* 1997; 160: 248-253.
- Ito H, Kawashima R, Awata S, Ono S, Sato K, Goto R, Koyama M, Sato M, Fukuda H. Hypoperfusion in the limbic system and prefrontal cortex in depression: SPECT with anatomic standardization technique. *J Nucl Med* 1996; 37: 410-414.
- Jacquier-Sarlin MR, Polla BS, Slosman DO. Oxido-reductive state: the major determinant for cellular retention of technetium-99m-HMPAO. *J Nucl Med* 1996; 37: 1413-1416.
- Jaksch M, Lochmuller H, Schmitt F, Volpel B, Obermaier-Kusser B, Horvath R. A mutation in mt tRNA^{Leu}(UUR) causing a neuropsychiatric syndrome with depression and cataract. *Neurology* 2001; 57: 1930-1931.
- Jonsson C, Pagani M, Ingvar M, Thurfjell L, Kimiaei S, Jacobsson H, Larsson SA. Resting state rCBF mapping with single-photon emission tomography and positron emission tomography: magnitude and origin of differences. *Eur J Nucl Med* 1998; 25: 157-165.
- Jonsson C, Pagani M, Johansson L, Thurfjell L, Jacobsson H, Larsson SA. Reproducibility and repeatability of normal resting state ^{99m}Tc-HMPAO rCBF SPET using brain atlas matching. *Nucl Med Commun* 2000; 21: 9-18.
- Kadekaro M, Crane AM, Sokoloff L. Differential effects of electrical stimulation of sciatic nerve on metabolic activity in spinal cord and dorsal root ganglion in the rat. *Proc Natl Acad Sci USA* 1985; 82: 6010-6013.
- Kandel ER. A new intellectual framework for psychiatry. *Am J Psychiatry* 1998; 155: 457-469.

Kandel ER. Biology and the future of psychoanalysis: a new intellectual framework for psychiatry revisited. *Am J Psychiatry* 1999; 156: 505-524.

Kato T, Takahashi S, Shioiri T, Inubushi T. Brain phosphorous metabolism in depressive disorders detected by phosphorous-31 magnetic resonance spectroscopy. *J Affect Disord* 1992; 27: 53-60.

Kato T, Takahashi S, Shioiri T, Murashita J, Hamakawa H, Inubushi T. Reduction of brain phosphocreatine in bipolar II disorder detected by phosphorous-31 magnetic resonance spectroscopy. *J Affect Disord* 1994; 31: 125-133.

Kato T, Shioiri T, Murashita J, Hamakawa H, Takahashi Y, Inubushi T, Takahashi S. Lateralized abnormality of high energy phosphate metabolism in the frontal lobes of patients with bipolar disorder detected by phase-encoded 31P-MRS. *Psychol Med* 1995; 25: 557-566.

Kato T, Stine OC, McMahon FJ, Crowe RR. Increased levels of a mitochondrial DNA deletion in the brain of patients with bipolar disorder. *Biol Psychiatry* 1997; 42: 871-875.

Kato T, Kunugi H, Nanko S, Kato N. Association of bipolar disorder with the 5178 polymorphism in mitochondrial DNA. *Am J Med Genet* 2000; 96: 182-186.

Kato T, Kunugi H, Nanko S, Kato N. Mitochondrial DNA polymorphisms in bipolar disorder. *J Affect Disord* 2001; 62: 151-164.

Katon W, Kleinman A, Rosen G. Depression and somatization: a review. *Am J Med* 1982; 72: 127-135.

Katzenell U, Segal S. Hyperacusis: review and clinical guidelines. *Otol Neurotol* 2001; 22: 321-326.

Kendell RE. The distinction between mental and physical illness [editorial]. *Br J Psychiatry* 2001; 178: 490-493.

Korres SG, Manta PB, Balatsouras DG, Papageorgiou CT. Audiological assessment in patients with mitochondrial myopathy. *Scand Audiol* 1999; 28: 231-240.

Kowatch RA, Devous Sr MD, Harvey DC, Mayes TL, Trivedi MH, Emslie GJ, Weinberg WA. A SPECT HMPAO study of regional cerebral blood flow in depressed adolescents and normal controls. *Prog Neuropsychopharmacol Biol Psychiatry* 1999; 23: 643-656.

Lambert G, Johansson M, Ågren H, Friberg P. Reduced brain norepinephrine and dopamine release in treatment-refractory depressive illness: evidence in support of the catecholamine hypothesis of mood disorders. *Arch Gen Psychiatry* 2000; 57: 787-793.

Larsson NG, Holme E, Kristiansson B, Oldfors A, Tulinius M. Progressive increase of the mutated mitochondrial DNA fraction in Kearns-Sayre syndrome. *Pediatr Res* 1990; 28: 131-136.

Ledberg A. Measuring brain functions: statistical tests for neuroimaging data [thesis]. Department of Neuroscience, Karolinska Institutet, Stockholm, Sweden, 2001.

Lehtonen MS, Moilanen JS, Majamaa K. Increased variation in mtDNA in patients with familial sensorineural hearing impairment. *Hum Genet* 2003; 113: 220-227.

Lenaz G. The mitochondrial production of reactive oxygen species: mechanisms and implications in human pathology. *IUBMB Life* 2001; 52: 159-164.

Lesser IM, Mena I, Boone KB, Miller BL, Mehringer CM, Wohl M. Reduction of cerebral blood flow in older depressed patients. *Arch Gen Psychiatry* 1994; 51: 677-686.

Lien LM, Lee HC, Wang KL, Chiu JC, Chiu HC, Wei YH. Involvement of nervous system in maternally inherited diabetes and deafness (MIDD) with the A3243G mutation of mitochondrial DNA. *Acta Neurol Scand* 2001; 103: 159-165.

Lincoln GA, Andersson H, Loudon A. Clock genes in calendar cells as the basis of annual timekeeping in mammals--a unifying hypothesis. *J Endocrinol* 2003; 179: 1-13.

Lindgård A, Lundberg J, Rakotonirainy O, Elander A, Soussi B. Preservation of rat skeletal muscle energy metabolism by illumination. *Life Sci* 2003; 72: 2649-2658.

Lockwood AH, Salvi RJ, Coad ML, Towsley ML, Wack DS, Murphy BW. The functional neuroanatomy of tinnitus. Evidence for limbic system links and neural plasticity. *Neurology* 1998; 50: 114-120.

Lodi R, Carelli V, Cortelli P, Iotti S, Valentino ML, Barboni P, Pallotti F, Montagna P, Barbiroli B. Phosphorus MR spectroscopy shows a tissue specific in vivo distribution of biochemical expression of the G3460A mutation in Leber's hereditary optic neuropathy. *J Neurol Neurosurg Psychiatry* 2002; 72: 805-807.

Luft R, Ikkos D, Palmieri G, Ernster L, Afzelius B. A case of severe hypermetabolism of nonthyroid origin with a defect in the maintenance of mitochondrial respiratory control: a correlated clinical, biochemical and morphological study. *J Clin Invest* 1962; 41: 1776-1804.

Lundberg J. Energy metabolism in skeletal muscle during ischemia and reperfusion. Experimental and clinical aspects [thesis]. Department of Plastic Surgery, Institute of Surgical Sciences, Sahlgrenska University Hospital, Gothenburg, Sweden, 2002.

Lundberg J, Lindgård A, Elander A, Soussi B. Improved energetic recovery of skeletal muscle in response to ischemia and reperfusion injury followed by in vivo ³¹P-magnetic resonance spectroscopy. *Microsurgery* 2002; 22: 158-164.

Maes M, Dierckx R, Meltzer HY, Ingels M, Schotte C, Vandewoude M, Calabrese J, Cosyns P. Regional cerebral blood flow in unipolar depression measured with Tc-99m-HMPAO single photon emission computed tomography: negative findings. *Psychiatry Res* 1993; 50: 77-88.

Magni G, Caldieron C, Rigatti-Luchini S, Merskey H. Chronic muskuloskeletal pain and depressive symptoms in the general population. An analysis of the 1st National Health and Nutrition Examination survey data. *Pain* 1990; 43: 299-307.

Mahlberg R, Steinacher B, Mackert A, Flechtner KM. Basic parameters of saccadic eye movements--differences between unmedicated schizophrenia and affective disorder patients. *Eur Arch Psychiatry Clin Neurosci* 2001; 251: 205-210.

Manfredi G, Vu T, Bonilla E, Schon EA, DiMauro S, Arnaudo E, Zhang L, Rowland LP, Hirano M. Association of myopathy with large-scale mitochondrial DNA duplications and deletions: which is pathogenic? *Ann Neurol* 1997; 42: 180-188.

Mathew RJ, Weinman ML, Mirabi M. Physical symptoms of depression. *Br J Psychiatry* 1981; 139: 293-296.

McMahon FJ, Stine OC, Meyers DA, Simpson SG, DePaulo JR. Patterns of maternal transmission in bipolar affective disorder. *Am J Hum Genet* 1995; 56: 1277-1286.

Meadow R. The dangerousness of parents who have abnormal illness behaviour. *Child Abuse Review* 2000; 9: 62-67.

Melberg A, Arnell H, Dahl N, Stålberg E, Raininko R, Oldfors A, Bakall B, Lundberg PO, Holme E. Anticipation of autosomal dominant progressive external ophthalmoplegia with hypogonadism. *Muscle Nerve* 1996; 19: 1561-1569.

Meltzer HY. Skeletal muscle abnormalities in patients with affective disorders. *J Psychiat Res* 1973; 10: 43-57.

Merskey H. Beware somatization [Guest editorial]. *Eur J Pain* 2000; 4: 3-4.

Milo TJ, Kaufman GE, Barnes WE, Konopka LM, Crayton JW, Ringelstein JG, Shirazi PH. Changes in regional cerebral blood flow after electroconvulsive therapy for depression. *J ECT* 2001; 17: 15-21.

Mirz F, Stødkilde-Jørgensen H, Pedersen CB. Evidence of cortical networks subserving the perception of tinnitus: a fMRI study. *Neuroimage* 1999; 9: S794.

Mirz F, Gjedde A, Ishizu K, Pedersen CB. Cortical networks subserving the perception of tinnitus – a PET study. *Acta Otolaryngol* 2000; Suppl 543: 241-243.

Miyaoka H, Suzuki Y, Taniyama M, Miyaoka Y, Shishikura K, Kamijima K, Atsumi Y, Matsuoka K. Mental disorders in diabetic patients with mitochondrial transfer RNA^{Leu(UUR)} mutation at position 3243. *Biol Psychiatry* 1997; 42: 524-526.

Moldin SO, Scheftner WA, Rice JP, Nelson E, Knesevich MA, Akiskal H. Association between major depressive disorder and physical illness. *Psychol Med* 1993; 23: 755-761.

Montgomery SA, Åsberg M. A new depression scale designed to be sensitive to change. *Br J Psychiatry* 1979; 134: 382-389.

Moore CM, Christensen JD, Lafer B, Fava M, Renshaw PF. Lower levels of nucleoside triphosphate in the basal ganglia of depressed subjects: a phosphorous-31 magnetic resonance spectroscopy. *Am J Psychiatry* 1997; 154: 116-118.

Moretti A, Gorini A, Villa RF. Affective disorders, antidepressant drugs and brain metabolism. *Mol Psychiatry* 2003; 8: 773-785.

Mourmans J, Wendel U, Bentlage HA, Trijbels JM, Smeitink JA, de Coo IF, Gabreëls FJ, Sengers RC, Ruitenbeek W. Clinical heterogeneity in respiratory chain complex III deficiency in childhood. *J Neurol Sci* 1997; 149: 111-117.

Mozley PD, Hornig-Rohan M, Woda AM, Kim HJ, Alavi A, Payer F, Amsterdam JD. Cerebral HMPAO SPECT in patients with major depression and healthy volunteers. *Prog Neuropsychopharmacol Biol Psychiatry* 1996; 20: 443-458.

Navarro V, Gasto C, Lomena F, Mateos JJ, Marcos T. Frontal cerebral perfusion dysfunction in elderly late-onset major depression assessed by 99mTc-HMPAO SPECT. *Neuroimage* 2001; 14: 202-205.

Neirinckx RD, Canning LR, Piper IM, Nowotnik DP, Pickett RD, Holmes RA, Volkert WA, Forster AM, Weisner PS, Marriott JA, Chaplin SB. Technetium 99m d,l-HM-PAO: a new radiopharmaceutical for SPECT imaging of regional cerebral blood perfusion. *J Nucl Med* 1987; 28: 191-202.

Neirinckx RD, Burke JF, Harrison RC, Forster AM, Andersen AR, Lassen NA. The retention mechanism of technetium-99m-HM-PAO: intracellular reaction with glutathione. *J Cereb Blood Flow Metab* 1988; 8: S4-S12.

Nguyen PV, Marin L, Atwood HL. Synaptic physiology and mitochondrial function in crayfish tonic and phasic motor neurons. *J Neurophysiol* 1997; 78: 281-294.

Nishino I, Spinazzola A, Papadimitriou A, Hammans S, Steiner I, Hahn CD, Connolly AM, Verloes A, Guimaraes J, Maillard I, Hamano H, Donati MA, Semrad CE, Russell

JA, Andreu AL, Hadjigeorgiou GM, Vu TH, Tadesse S, Nygaard TG, Nonaka I, Hirano I, Bonilla E, Rowland LP, DiMauro S, Hirano M. Mitochondrial neurogastrointestinal encephalomyopathy, an autosomal recessive disorder due to thymidine phosphorylase mutations. *Ann Neurol* 2000; 47: 792-800.

Nørby S, Lestienne P, Nelson I, Nielsen IM, Schmalbruch H, Sjo O, Warburg M. Juvenile Kearns-Sayre syndrome initially misdiagnosed as a psychosomatic disorder. *J Med Genet* 1994; 31: 45-50.

Öjehagen A, Johnsson E, Träskman-Bendz L. The long-term stability of temperament traits measured after a suicide attempt. A 5-year follow-up of ratings of Karolinska Scales of Personality (KSP). *Nord J Psychiatry* 2003; 57: 125-130.

Oldfors A, Sommerland H, Holme E, Tulinius M, Kristiansson B. Cytochrome c oxidase deficiency in infancy. *Acta Neuropathol (Berl)* 1989; 77: 267-275.

Oldfors A, Larsson NG, Holme E, Tulinius M, Kadenbach B, Droste M. Mitochondrial DNA deletions and cytochrome c oxidase deficiency in muscle fibres. *J Neurol Sci* 1992; 110: 169-177.

Onishi H, Kawanishi C, Iwasawa T, Osaka H, Hanihara T, Inoue K, Yamada Y, Kosaka K. Depressive disorder due to mitochondrial transfer RNA^{Leu(UUR)} mutation. *Biol Psychiatry* 1997; 41: 1137-1139.

Österberg K, Karlson B, Orbaek P. Personality, mental distress, and risk perception in subjects with multiple chemical sensitivity and toxic encephalopathy. *Scand J Psychol* 2002; 43: 169-175.

Ozawa T. Genetic and functional changes in mitochondria associated with aging. *Physiological Reviews* 1997; 77: 425-464.

Pagani M. Advances in brain SPECT. Methodological and human investigations [thesis]. Departments of Radiology and Hospital Physics, Section for Nuclear Medicine, Karolinska Hospital and Karolinska Institutet, Sweden, 2000.

Pagani M, Salmaso D, Ramström C, Jonsson C, Lundqvist R, Thurffjell L, Schnell PO, Wägner A, Jacobsson H, Larsson SA. Mapping pathological ^{99m}Tc-HMPAO uptake in Alzheimer's disease and frontal lobe dementia with SPECT. *Dement Geriat Cogn Disord* 2001; 12: 177-184.

Pagani M, Salmaso D, Jonsson C, Hatherly R, Jacobsson H, Larsson SA, Wägner A. Regional cerebral blood flow as assessed by principal component analysis and (99m)Tc-HMPAO SPET in healthy subjects at rest: normal distribution and effect of age and gender. *Eur J Nucl Med Mol Imaging* 2002; 29: 67-75.

Pendse B, Westrin A, Engstrom G. Temperament traits in seasonal affective disorder, suicide attempters with non-seasonal major depression and healthy controls. *J Affect Disord* 1999; 54:55-65.

Peng NJ, Liu RS, Li JY, Tsay DG, Kong KW, Kwok CG, Strauss HW. Increased cerebral blood flow in MELAS shown by Tc-99m HMPAO brain SPECT. *Neuroradiology* 2000; 42: 26-29.

Perneger TV. What's wrong with Bonferroni adjustments. *BMJ* 1998; 316: 1236-1238.

Perris C, Eisemann M, von Knorring L, Perris H. Personality traits in former depressed patients and in healthy subjects without past history of depression. *Psychopathology* 1984; 17: 178-186.

Poulton J, Deadman ME, Bindoff L, Morten K, Land J, Brown G. Families of mtDNA re-arrangements can be detected in patients with mtDNA deletions: duplications may be a transient intermediate form. *Hum Mol Genet* 1993; 2: 23-30.

Poulton J, Morten KJ, Marchington D, Weber K, Brown KG, Rötig A, Bindoff L. Duplications of mitochondrial DNA in Kearns-Sayre syndrome. *Muscle Nerve* 1995; Suppl 3: S154-158.

Prezant TR, Agapian JV, Bohlman MC, Bu X, Öztas S, Qiu W-Q, Arnos KS, Cortopassi GA, Jaber L, Rotter JI, Shohat M, Fischel-Ghodsian N. Mitochondrial ribosomal RNA mutation associated with both antibiotic induced and non-syndromic deafness. *Nat Genet* 1993; 4: 289-294.

Rajala U, Keinanen-Kiukaanniemi S, Uusimaki A, Kivela SL. Musculoskeletal pains and depression in a middle-aged Finnish population. *Pain* 1995; 61: 451-457.

Ramklint M, Ekselius L. Personality traits and personality disorders in early onset versus late onset major depression. *J Affect Disord* 2003; 75: 35-42.

Rausch JL, Johnson ME, Corley KM, Hobby HM, Shendarkar N, Fei Y, Ganapathy V, Leibach FH. Depressed patients have higher body temperature: 5-HT transporter long promoter region effects. *Neuropsychobiology* 2003; 47: 120-127.

Reid FM, Vernham GA, Jacobs HT. A novel mitochondrial point mutation in a maternal pedigree with sensorineural deafness. *Hum Mutat* 1994; 3: 243-247.

Roland PE, Friberg L. Localization of cortical areas activated by thinking. *J Neurophysiol* 1985; 53: 1219-1243.

Roland PE, Eriksson L, Stone-Elander S, Widen L. Does mental activity change the oxidative metabolism of the brain? *J Neurosci* 1987; 7: 2373-2389.

Roland PE, Graufelds CJ, Wählin J, Ingelman L, Andersson M, Ledberg A, Pedersen J, Åkerman S, Dabringhaus A, Zilles K. Human brain atlas: for high-resolution functional and anatomical mapping. *Hum Brain Mapp* 1994; 1: 173-184.

Ross-Stanton J, Meltzer HY. Skeletal muscle morphology of depressed patients after medication [letter]. *Muscle Nerve* 1979; 2: 239.

Roubertoux PL, Sluyter F, Carlier M, Marcet B, Maarouf-Veray F, Cherif C, Marican C, Arrechi P, Godin F, Jamon M, Verrier B, Cohen-Salmon C. Mitochondrial DNA modifies cognition in interaction with the nuclear genome and age in mice. *Nat Genet* 2003; 35: 65-69.

Rustin P, Rotig A. Inborn errors of complex II--unusual human mitochondrial diseases. *Biochim Biophys Acta* 2002; 1553: 117-122.

Ryle G. The concept of mind. Penguin Book Ltd, Middlesex, England, 9th Edition, 1983.

Santorelli FM, Tanji K, Sano M, Shanske S, El-Shahawi M, Kranz-Eble P, DiMauro S, De Vivo DC. Maternally inherited encephalopathy associated with a single-base insertion in the mitochondrial tRNA^{Trp} gene. *Ann Neurol* 1997; 42: 256-260.

Santorelli FM, Gagliardi MG, Dionisi-Vici C, Parisi F, Tessa A, Carozzo R, Piemonte F, Pfeiffer K, Schagger H, Bertini E. Hypertrophic cardiomyopathy and mtDNA depletion. Successful treatment with heart transplantation. *Neuromuscul Disord* 2002; 12: 56-59.

Sasaki T, Fujibayashi Y, Senda M. Distribution of glutathione and technetium-99m-meso-HMPAO in normal and diethyl maleate-treated mouse brain mitochondria. *J Nucl Med* 1998; 39: 2178-2183.

Schalling D, Cronholm B, Åsberg M, Espmark S. Ratings of psychic and somatic anxiety indicants. Interrater reliability and relations to personality variables. *Acta Psychiatr Scand* 1973; 49: 353-368.

Schmiedel J, Jackson S, Schäfer J, Reichmann H. Mitochondrial cytopathies. *J Neurol* 2003; 250: 267-277.

Schon EA, Bonilla E, DiMauro S. Mitochondrial DNA mutations and pathogenesis. *J Bioenerg Biomembr* 1997; 29: 131-149.

Schwartz M, Vissing J. New patterns of inheritance in mitochondrial disease. *Biochem Biophys Res Commun* 2003; 310: 247-251.

Sciacco M, Prella A, Comi GP, Napoli L, Battistel A, Bresolin N, Tancredi L, Lamperti C, Bordoni A, Fagiolari G, Ciscato P, Chiveri L, Perini MP, Fortunato F, Adobbati L, Messina S, Toscano A, Martinelli-Boneschi F, Papadimitriou A, Scarlato G, Moggio M.

- Retrospective study of a large population of patients affected with mitochondrial disorders: clinical, morphological and molecular genetic evaluation. *J Neurol* 2001; 248: 778-788.
- Sevincok L, Buyukozturk A, Dereboy F. Serum lipid concentrations in patients with comorbid generalized anxiety disorder and major depressive disorder. *Can J Psychiatry* 2001; 46: 68-71.
- Sharp FR, Kauer JS, Shepherd GM. Laminar analysis of 2-deoxyglucose uptake in olfactory bulb and olfactory cortex of rabbit and rat. *J Neurophysiol* 1977; 40: 800-813.
- Shoffner JM, Wallace DC. Oxidative phosphorylation diseases and mitochondrial DNA mutations: diagnosis and treatment. *Annu Rev Nutr* 1994; 14: 535-568.
- Siciliano G, Tessa A, Petrini S, Mancuso M, Bruno C, Griecod GS, Malandrini A, DeFlorio L, Martini B, Federico A, Nappi G, Santorelli FM, Murri L. Autosomal dominant external ophthalmoplegia and bipolar affective disorder associated with a mutation in the ANT1 gene. *Neuromuscul Disord* 2003; 13: 162-165.
- Skuder P, Plomin R, McClearn GE, Smith DL, Vignetti S, Chorney MJ, Chorney K, Kasarda S, Thompson LA, Detterman DK, Petrill SA, Daniels J, Owen MJ, McGuffin P. A polymorphism in mitochondrial DNA associated with IQ? *Intelligence* 1995; 21: 1-11.
- Smeitink JA. Mitochondrial disorders: clinical presentation and diagnostic dilemmas. *J Inherit Metab Dis* 2003; 26: 199-207.
- Smith PR, Bain SC, Good PA, Hattersley AT, Barnett AH, Gibson JM, Dodson PM. Pigmentary retinal dystrophy and the syndrome of maternally inherited diabetes and deafness caused by the mitochondrial DNA 3243 tRNA(Leu) A to G mutation. *Ophthalmology* 1999; 106: 1101-1108.
- Sokolski KN, Nguyen BD, DeMet EM. Decreases in dilated pupil size in depressed patients with age may reflect adrenergic changes. *Psychiatry Res* 2000; 94: 267-272.
- Sottocasa GL, Kuylenstierna B, Ernster L, Bergstrand A. An electron-transport system associated with the outer membrane of liver mitochondria. A biochemical and morphological study. *J Cell Biol*. 1967; 32: 415-438.
- Starkov AA, Simonyan RA, Dedukhova VI, Mansurova SE, Palamarchuk LA, Skulachev VP. Regulation of the energy coupling in mitochondria by some steroid and thyroid hormones. *Biochim Biophys Acta* 1997; 1318: 173-183.
- Stewart JB, Naylor GJ. Manic-depressive psychosis in a patient with mitochondrial myopathy—A case report. *Med Sci Res* 1990; 18: 265-266.

St John J, Sakkas D, Dimitriadi K, Barnes A, Maclin V, Ramey J, Barratt C, De Jonge C. Failure of elimination of paternal mitochondrial DNA in abnormal embryos [letter]. *Lancet* 2000; 355: 200.

Stine OC, Luu S-U, Zito M, Casanova M. The possible association between affective disorder and partially deleted mitochondrial DNA. *Biol Psychiatry* 1993; 33: 141-142.

Stone EM, Newman NJ, Miller NR, Johns DR, Lott MT, Wallace DC. Visual recovery in patients with Leber's hereditary optic neuropathy and the 11778 mutation. *J Clin Neuroophthalmol* 1992; 12: 10-14.

Suomalainen A, Majander A, Haltia M, Somer H, Lönnqvist J, Savontaus M-L, Peltonen L. Multiple deletions of mitochondrial DNA in several tissues of a patient with severe retarded depression and familial progressive external ophthalmoplegia. *J Clin Invest* 1992; 90: 61-66.

Sweeney MG, Bunday S, Brockington M, Poulton KR, Winer JB, Harding AE. Mitochondrial myopathy associated with sudden death in young adults and a novel mutation in the mitochondrial DNA leucine transfer RNA^(UUR) gene. *Quart J Med* 1993; 86: 709-713.

Taivassalo T, Jensen TD, Kennaway N, DiMauro S, Vissing J, Haller RG. The spectrum of exercise tolerance in mitochondrial myopathies: a study of 40 patients. *Brain* 2003; 126: 413-423.

Terre L, Poston WS, Foreyt J, St Jeor ST. Do somatic complaints predict subsequent symptoms of depression? *Psychother Psychosom* 2003; 72: 261-267.

Thakore JH, Richards PJ, Reznick RH, Martin A, Dinan TG. Increased intra-abdominal fat deposition in patients with major depressive illness as measured by computed tomography. *Biol Psychiatry* 1997; 41: 1140-1142.

Thurfjell L, Bohm C, Bengtsson E. CBA--an atlas-based software tool used to facilitate the interpretation of neuroimaging data. *Comput Methods Programs Biomed* 1995; 4: 51-71.

Turconi AC, Bent R, Castelli E, Pochintesta S, Felisari G, Comi G, Gagliardi C, Del Piccolo L, Bresolin N. Focal cognitive impairment in mitochondrial encephalomyopathies, a neuropsychological and neuroimaging study. *J Neurol Sci* 1999; 170: 57-63.

Tutus A, Kibar M, Sofuoglu S, Basturk M, Gönül AS (a). A technetium-99m hexamethylpropylene amine oxime brain single-photon emission tomography study in adolescent patients with major depressive disorder. *Eur J Nucl Med* 1998; 25: 601-606.

Tutus A, Simsek A, Sofuoglu S, Nardali M, Kugu N, Karaaslan F, Gönül AS (b). Changes in regional cerebral blood flow demonstrated by single photon emission

- computed tomography in depressive disorders: comparison of unipolar vs. bipolar subtypes. *Psychiatry Res* 1998; 83: 169-177.
- Uemura A, Osame M, Nakagawa M, Nakahara K, Sameshima M, Ohba N. Leber's hereditary optic neuropathy: mitochondrial and biochemical studies on muscle biopsies. *Br J Ophthalmology* 1987; 71: 531-536.
- Varvogli L, Waisbren SE. Personality profiles of mothers of children with mitochondrial disorders. *J Inher Metab Dis* 1999; 22: 615-622.
- Vincent AJ, Spierings EL, Messinger HB. A controlled study of visual symptoms and eye strain factors in chronic headache. *Headache* 1989; 29: 523-527.
- Volz H-P, Rzanny R, Riehemann S, May S, Hegewald H, Preussler B, Hubner G, Kaiser WA, Sauer H. ³¹P magnetic resonance spectroscopy in the frontal lobe of major depressed patients. *Eur Arch Psychiatry Clin Neurosci* 1998; 248: 289-295.
- Wallace DC. Diseases of the mitochondrial DNA. *Annu Rev Biochem* 1992; 61: 1175-1212.
- Washizuka S, Kakiuchi C, Mori K, Kunugi H, Tajima O, Akiyama T, Nanko S, Kato T. Association of mitochondrial complex I subunit gene NDUFV2 at 18p11 with bipolar disorder. *Am J Med Genet* 2003; 120B: 72-78.
- Watanabe Y, Hashikawa K, Moriwaki H, Oku N, Seike Y, Kodaka R, Ono J, Uehara T, Kusuoka H, Nishimura T. SPECT findings in mitochondrial encephalomyopathy. *J Nucl Med* 1998; 39: 961-964.
- Weinryb RM, Gustavsson JP, Åsberg M, Rössel RJ. Stability over time of character assessment using a psychodynamic instrument and personality inventories. *Acta Psychiatr Scand* 1992; 86: 179-84.
- Whatley SA, Curti D, Marchbanks RM. Mitochondrial involvement in schizophrenia and other functional psychoses. *Neurochem Res* 1996; 21: 995-1004.
- Wibom R. Studies of mitochondrial ATP-production rate in skeletal muscle [thesis]. Department of Clinical Chemistry II, Karolinska Institutet, Huddinge University Hospital, Huddinge, Sweden, 1991.
- Wibom R, Hultman E. ATP production rate in mitochondria isolated from microsamples of human muscle. *Amer J Physiol* 1990; 259: E204-E209.
- Wong-Riley MTT. Cytochrome oxidase: an endogenous metabolic marker for neuronal activity. *TINS* 1989; 12: 94-101.

Wright IC, McGuire PK, Poline JB, Travers JM, Murray RM, Frith CD, Frackowiak RS, Friston KJ. A voxel-based method for the statistical analysis of gray and white matter density applied to schizophrenia. *Neuroimage* 1995; 2: 244-252.

Yamada K, Mashima Y, Kigasawa K, Miyashita K, Wakakura M, Oguchi Y. High incidence of visual recovery among four Japanese patients with Leber's hereditary optic neuropathy with the 14484 mutation. *J Neuroophthalmol* 1997; 17: 103-107.

Yazici KM, Kapucu O, Erbas B, Varoglu E, Gulec C, Bekdik CF. Assessment of changes in regional cerebral blood flow in patients with major depression using the ^{99m}Tc-HMPAO single photon emission tomography method. *Eur J Nucl Med* 1992; 19: 1038-1043.

Yen MY, Wei YH, Liu JH. Stargardt's type maculopathy in a patient with 11778 Leber's optic neuropathy. *J Neuroophthalmol* 1996; 16: 120-123.

Yovell Y, Sakeim HA, Epstein DG, Prudic J, Devanand DP, McElhiney MC, Settembrino JM, Bruder GE. Hearing loss and asymmetry in major depression. *J Neuropsychiatry* 1995; 7: 82-89.

Zwaigenbaum L, Szatmari P, Boyle MH, Offord DR. Highly somatizing young adolescents and the risk of depression. *Pediatrics* 1999; 103: 1203-1209.

APPENDIX

Table A. Gender, year of birth, inclusion in four of the Papers, presence of COMP symptomatology, and audiological characteristics of the patients in the thesis. The investigations were performed during 1994 – 2003.

Patient number S = Schizophrenia	year of birth	highest MADRS-S score	included in Paper				fulfills COMP criteria	uses sign language	no audiological symptom
			I	IV	V	VI			
Male patients									
1	1934	27	•	•	•	•	•		
2	1938	25	•	•	•		•		
3	1939	42		•	•		•		
4	1940	30		•					•
5	1940	30		•	•				•
6	1940				•				
7	1941	40		•	•				
8	1943	24		•			•		
9	1943	21	•	•	•		•		
10	1943	29	•	•	•	•	•		
11	1944	36	•	•	•		•		
12	1944	21		•			•		
13	1945	38		•			•		
14	1945	22		•			•		
15	1945	21		•	•		•		
16	1945		•		•	•	•	•	
17	1946	22		•	•		•		
18	1946	23	•	•	•		•		
19	1947	27		•			•		
20	1948	38		•			•		
21	1949	35		•	•		•		
22	1950		•		•		•		
23	1952		•				•	•	
24	1954	32		•				•	
25	1954	21	•			•	•	•	
26	1954	41	•			•	•		
27	1955		•			•	•	•	
28	1959	33	•	•	•	•	•		
29	1963	37		•			•		
30	1964	44		•	•		•		
31	1967	18	•			•	•	•	
32	1968	33		•			•		
33 S	1972					•			•
34	1973	20		•					
35 S	1974					•	•		
36 S	1976					•			
37	1980	20		•					•
Female patients									
1	1936	20	•				•	•	
2	1938	23	•	•			•		
3	1939	23		•	•		•		
4	1939	18	•				•		
5	1940	29		•	•		•		
6	1940	26	•	•	•	•	•		
7	1940	24	•	•	•	•	•		
8	1941	38		•			•		
9	1943	26		•	•				
10	1943	26		•			•		
11	1943	34		•			•		
12	1943	22	•	•	•		•		

Patient number S = Schizophrenia	year of birth	highest MADRS-S score	included in Paper				fulfills COMP criteria	uses sign language	no audiological symptom
			I	IV	V	VI			
13	1943	33		•			•		
14	1943		•			•	•	•	
15	1944	26	•	•	•	•	•		
16	1944	36		•			•		
17	1944		•			•	•	•	
18	1945	24	•	•			•		
19	1946	22		•	•		•		
20	1946	30		•	•		•		
21	1946	22		•	•		•		
22	1947	31	•	•	•	•	•		
23	1947	24		•	•				•
24	1947	33		•	•		•		
25	1947	33		•			•		
26	1947	35		•	•		•		
27	1948	42		•			•		
28	1948	33		•			•		
29	1949	39		•			•		
30	1950	39		•			•		
31	1951	32		•			•		
32	1951	34		•			•		
33	1951	50		•	•		•		
34	1952	19			•		•		
35	1953	23		•			•		
36	1953	31		•	•		•		
37	1954	33		•				•	
38	1954	30		•			•		
39	1955	31	•	•	•		•		
40	1955	43		•			•		
41	1955	29		•	•				
42	1956	20		•	•				
43	1956	33		•			•		
44	1957	32		•			•		
45	1957	36		•			•		
46	1957	38		•				•	
47	1957	31		•	•		•		
48	1957	49		•	•				•
49	1957	25		•	•		•		
50	1958	31		•	•		•		
51	1959	30		•			•		
52	1959	20		•	•				•
53	1959	27		•			•		
54	1960				•				•
55	1961	20		•	•				•
56	1962	27	•	•	•	•	•		
57	1963	18	•			•	•		
58	1965	24		•			•	•	
59	1965	25		•			•	•	
60	1968	33		•					
61	1968	28		•					
62	1969	24		•			•	•	
63	1969	32		•			•	•	
64	1976	20		•			•	•	
65	1977	47		•					•
66 S	1977					•			•
Number of patients		91	28	84	45	20	78	17	11

Table B. Criteria for COMP – cochlear and mild ocular, muscular and psychediatric symptoms. At least 6 of the 17 criteria must be fulfilled with at least one criterion from each of the categories A-E.

- A) Affliction of the inner ear** (or affliction of central auditory pathways, the presumed cause should not be taken into account)
- 1) sensorineural hearing impairment verified by audiogram investigation
 - 2) tinnitus or hyperacusia distressing enough to have caused the patient to seek medical consultation.
- B) Affliction of ocular tissues**
- 1) photophobia (glare sensitivity), the patient may not have sought medical consultation but uses sun glasses or sun shade in strong sunshine, or has noted difference compared to others (chooses weaker light bulbs, other contrast on the television screen)
 - 2) any of the following symptoms: ocular fatigue or itch rendering lengthy visual concentration difficult not caused by lack of eye glass correction for refractory errors, attacks of blurred vision, colour vision differences between the eyes, bulbar injection or spontaneous scleral hematomae
 - 3) ocular muscle affliction (strabismus, episodic spasms, orbitomyopathy).
- C) Affliction of skeletal muscles or neuropathy**
- 1) muscle pain, cramps or stiffness of sufficient severity to have caused the patient to seek medical consultation or physiotherapy, or almost daily pain or stiffness in the neck region since at least half a year for which the patient has not sought medical advice if these symptoms have been suggested to be secondary to 'neck muscle strain in connection with hearing impairment'
 - 2) muscle fatigue at exertion (for example when walking stairs, riding a bike) representing a decline from a previous level of functioning
 - 3) painful calf cramps at least once a month for at least half a year not in connection with pregnancy
 - 4) decreased sensation or pain in feet and/or in hands with clumsiness.
- D) Psychiatric symptoms**
- 1) any of the following subjective symptoms that must cause marked distress or impairment in occupational areas or when participating in social groups with several members: diminished tolerance for mental strain, concentration difficulties, memory impairment, increased mental fatigue or increased irritability
 - 2) mental fatigue after work, necessitating rest during leisure time
 - 3) pronounced increase of flight of ideas during conversation, inability to keep to the topic of conversation, must be brought back to the topic (the patient may be unaware of this symptom)
 - 4) psychiatric symptoms severe enough to have caused the patient to seek medical consultation.
- E) Other symptoms, results of laboratory investigations**
- 1) pronounced general fatigue and feeling of illness which has caused the patient to seek medical consultation, or pronounced general fatigue rendering full-time work impossible
 - 2) migraine attacks or frequent headaches for a period of at least three months
 - 3) EEG alterations with paroxysmal or pronounced increase of low frequency activity
 - 4) at least five episodes of chest palpitations, or aberrations on ECG investigation.