



KAIJA HUUHKA

## Electroconvulsive Therapy

Association of genetic polymorphisms with  
treatment resistant depression and treatment response



ACADEMIC DISSERTATION

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# List of original publications

The present dissertation is based on the following original publications, referred to in the text by their Roman numerals I-VI. Some additional data is also presented.

I Huuhka K, Anttila S, Huuhka M, Leinonen E, Rontu R, Mattila KM, Lehtimäki T (2007): Brain-Derived Neurotrophic Factor (BDNF) polymorphisms G196A and C270T are not associated with the response to electroconvulsive therapy in major depressive disorder. *Eur Arch Psychiatry Clin Neurosci* 257:31-35.

II Anttila S, Huuhka K, Huuhka M, Rontu R, Hurme M, Leinonen E, Lehtimäki T (2007): Interaction between 5-HT1A and BDNF genotypes increases the risk of treatment-resistant depression. *J Neural Transm* 114:1065-1068.

III Anttila S, Huuhka K, Huuhka M, Rontu R, Mattila KM, Leinonen E, Lehtimäki T (2007): Interaction between TPH1 and GNB3 genotypes and electroconvulsive therapy in major depression. *J Neural Transm* 114:461-468.

IV Huuhka K, Kampman O, Anttila S, Huuhka M, Rontu R, Mattila K M, Hurme M, Lehtimäki T, Leinonen E (2008): RGS4 polymorphism and response to electroconvulsive therapy in major depressive disorder. *Neurosci Lett* 437:25-28.

V Anttila S, Huuhka K, Huuhka M, Illi A, Rontu R, Leinonen E, Lehtimäki T (2008): Catechol-O-methyltransferase (COMT) polymorphisms predict treatment response in electroconvulsive therapy. *Pharmacogenomics J* 8:113-116.

VI Huuhka K, Anttila S, Huuhka M, Hietala J, Huhtala H, Mononen N, Lehtimäki T, Leinonen E (2008): Dopamine 2 receptor C957T and catechol-o-methyltransferase Val158Met polymorphisms are associated with treatment response in electroconvulsive therapy. *Neurosci Lett* 448:79-83.



# Abbreviations

ACTH	Adenocorticotrophic hormone
AD	Antidepressant
APA	American Psychiatric Association
APOE	Apolipoprotein E
BDNF	Brain derived neurotrophic factor
cAMP	Cyclic adenosine monophosphate
CBT	Cognitive behavioral therapy
CGI	Clinical Global Impression scale
CI	Confidence interval
COMT	Catechol-o-methyltransferase enzyme
CREB	cAMP response-element binding protein
CRF	Corticotrophin releasing factor
CRH	Corticotrophin releasing hormone
CSF	Cerebrospinal fluid
CT	Cognitive therapy
DAT	Dopamine transporter
DBS	Deep brain stimulation
DEX/CRH	Dexamethasonesuppression/ CRH test
DLPFC	Dorsolateral prefrontal cortex
DMPFC	Dorsomedial prefrontal cortex
DNA	Deoksyribonucleid acid
DSM-IV-TR	Diagnostic and statistical manual of mental disorders, fourth edition, text revision
DSM-IV	Diagnostic and statistical manual of mental disorders, fourth edition
DRD2	Dopamine receptor D2
DST	Dexamethasonesuppression test
ECG	Electrocardiogram
ECS	Electroconvulsive shocks
ECT	Electroconvulsive therapy
EEG	Electroencephalography
fMRI	Functional magnetic resonance imaging
GABA	$\gamma$ -aminobutyric acid
GNB3	G protein beta 3 subunit
GR	Glucocorticoid receptors
5-HIAA	5-hydroxyindoleacetic acid
HPA	Hypothalamic-pituitary-adrenal
HRSD	Hamilton Rating Scale for Depression

5-HT	5-Hydroxytryptamine, Serotonin
5-HTTLPR	Serotonin transporter promoter
HVA	Homovanillic acid
ICD-10	International classification of diseases
IPT	Interpersonal psychotherapy
MADRS	Montgomery and Åsberg Depression Rating Scale
MAO	Monoamine oxidase
MAOI	Monoamine oxidase inhibitor
MAPK	Mitogen activated protein kinase
MDD	Major depressive disorder
MDE	Major depressive episode
MHPG	3-methoxy-4-hydroxyphenylglycol
MMSE	Mini Mental State Examination Scale
MR	Mineralocorticoid receptors
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleid acid
NET	Norepinephrine transporter
NPY	Neuropeptide Y
NGF	Nerve growth factor
NT3	Neurotrophin 3
OR	Odds ratio
PCR	Polymerase chain reaction
PET	Positron emission tomography
QIDS-C <sub>16</sub>	16-item Quick Inventory of Depressive Symptomatology-Clinician-rated
RGS	Regulator of G protein signaling
rTMS	Repetitive transcranial magnetic stimulation
SERT	Serotonin transporter
SNP	Single nucleotide polymorphism
SPECT	Single photon emission computed tomography
SNRI	Serotonin and norepinephrine reuptake inhibitor
SSRI	Serotonin selective reuptake inhibitor
STAR*D	Sequenced Treatment Alternatives to Relieve Depression
TCA	Tricyclic antidepressant
TMS	Transcranial magnetic stimulation
TPH	Tryptophan hydroxylase
TRD	Treatment resistant depression
TrkB	Tyrosine kinase B
VEGF	Vascular endothelial growth factor
VGF	Neuropeptide VGF
VLPFC	Ventrolateral prefrontal cortex
VMPFC	Ventromedial prefrontal cortex
VNS	Vagus nerve stimulation
WHO	World Health Organization

# Abstract

**Background.** Major Depressive Disorder is common in general population affecting in their lifetime about 16 % of population. It is one leading cause of early retirement. It affects women more often than men and middle-aged more often than younger. Treatment of depression is challenging. Not all patients improve with the first antidepressant trial and switching to another antidepressant the outcomes may still be poor. Treatment resistant depression is thus a severe problem. The patient does not benefit from an adequate antidepressant treatment and this may lead to chronicity and cause severe problems in the patient's life. Nowadays the research is moving towards the basic unresolved mechanisms underlying this serious and multifactorial disease and its various forms. Moreover, more effective treatment methods are being evaluated.

Electroconvulsive therapy is an effective somatic treatment in major depression. It has been used since the 1930s. Its technique has been established in clinical practice. Some negative attitudes may still relate to this treatment method but it remains the fastest and most effective treatment method for major depression. In treatment resistant depression up to 50-60 % of recipients benefit from electroconvulsive therapy.

Susceptibility to Major Depressive Disorder has been found to be partly inherited. The inheritance is multifactorial, both genes and environment affecting the risk. The Human Genome Project was concluded in 2003. In every somatic cell 25,000 human genes are arranged on 46 chromosomes. Every gene has its own locus which contains an identical or slightly different form of gene, called an allele. Genes encode proteins. Nuclear deoxyribonucleic acid (DNA) is nearly 99.9 % identical in any two humans. The small fraction of DNA sequence different among individuals is responsible for the genetically determined variability among individuals. When a variant is found in more than 1% of chromosomes in the general population it is called a genetic polymorphism. In addition to the more rare disease-causing mutations, variants common enough to be polymorphisms are also known to predispose to severe illnesses. Most common of all polymorphisms are Single Nucleotide Polymorphisms (SNPs). SNPs have only two alleles corresponding to the two different bases occupying a particular location in the genome. SNPs are common and occur on average once every 1,000 base pairs, which means that there is an average of 1,500,000 differences between any two human genomes. They are essential tools in studying the heritability of multifactorial diseases.

**Aims.** The association between treatment resistant depression and treatment response to electroconvulsive therapy was examined with BDNF, 5-HT1A, TPH1, GNB3, RGS4, COMT and DRD2 genetic polymorphisms.

**Subjects and methods.** All 119 patients in this study were hospitalized due to treatment resistant depression. They had failed two adequate antidepressant trials and had been treated with electroconvulsive therapy based on clinical criteria. The controls were 383-398 healthy blood donors. Genomic DNA was extracted from peripheral blood leucocytes and the samples were coded. Patients were evaluated before treatment and immediately after treatment with the Montgomery and Åsberg Depression Rating Scale.

**Results.** BDNF polymorphism together with 5-HT1A polymorphism was associated with treatment resistant depression. TPH1 polymorphism and GNB3 polymorphism were associated with treatment resistant depression both alone and together. The polymorphisms associated with the treatment response to electroconvulsive therapy were BDNF alone, TPH1 and GNB3 together, COMT alone and together with DRD2.

**Conclusions.** Some of the genetic polymorphisms studied were associated with treatment resistant depression and treatment response to electroconvulsive therapy. In light of these findings it could be hypothesized that treatment resistant depression could be associated more with serotonergic regulation linked genetic polymorphisms and the variation in the response to electroconvulsive therapy could be associated more with dopamine regulation linked genetic polymorphisms. In the future it may be possible to better understand the development and the genetic basis of severe treatment resistant depression. It may also be possible to individually choose more effective treatment method based on the patient's genotype and thereby reducing individual suffering and the risk of chronicity.

# Tiivistelmä

**Tausta.** Vaikea-asteinen masennus on yleinen väestössä. Se aiheuttaa ongelmia ja kärsimystä yksilötasolla ja on vakava kansanterveydellinen ongelma. Se on yksi keskeisimpiä varhaiseen eläköitymiseen johtavia syitä. Vaikea masennus koskettaa eniten naisia ja on yleisintä keski-ikäisillä. Vain osa potilaista hyötyy ensimmäisestä hoitoyrityksestä ja aina siirryttäessä tehottomaksi osoittautuneesta masennuslääkityksestä toiseen, hoidosta hyötyvien osuus pienenee. Hoitoresistentissä masennuksessa potilas ei hyödy riittävästi asianmukaisesta hoidosta. Pitkittynyt hoitoresistentti masennus johtaa sairauden kroonistumiseen ja vaikuttaa negatiivisesti potilaan ja hänen perheensä elämään. Tämän vuoksi tutkimuksia on suunnattu tämän monitekijäisen sairauden syntymekanismeihin. Toisaalta pyritään kehittämään entistä tehokkaampia masennuksen hoitomenetelmiä.

Psykiatrissa sähköhoitoa on käytetty jo 1930-luvulta lähtien. Sähköhoidon tekniikka on kehittynyt ja menetelmänä se on vakiintunut kliinisessä työssä. Sen käyttöön saattaa vielä liittyä negatiivisia mielikuvia, mutta siitä huolimatta se on edelleen tehokkain ja nopein hoitomuoto vakavassa masennuksessa. Hoitoresistenteistä masennuspotilaista 50 - 60 % saa vasteen sähköhoidosta.

Tutkimuksissa on selvitetty masennuksen olevan monitekijäisesti periytyvää, sekä geenit että ympäristö vaikuttavat sairastumisalttiuteen. Ihmisen perimä selvitettiin kokonaan vuonna 2003. Perimä rakentuu noin 25000 geenistä, joissa on yhteensä noin kolme miljardia DNA-kirjainta eli nukleotidia. Jokaisen solun tumassa geenit ovat järjestäytyneet kromosomeiksi, joita ihmisellä on 46. Jokaisessa kromosomissa on eri määrä genejä, jokaisella geenillä on oma paikka, locus. Jokaisessa locusessa voi olla joko identtinen tai hiukan erilainen muoto geenistä, jota kutsutaan alleeliksi, vastingeeniksi. Geenien tehtävänä on koodata proteiineja ja on tärkeää, että yksittäiset geenit toimivat oikeissa kudoksissa oikeaan aikaan. Ihmisen DNA sekvenssi on 99.9 % yhteinen yksilöiden kesken. Perimässä esiintyy kuitenkin yhden nukleotidin muutoksia, joita kutsutaan geneettiseksi monimuotoisuudeksi (single nucleotide polymorphism, SNP). Monimuotoiseksi kutsutaan DNA:n kohtaa, jossa vähintään kahden vaihtoehdoisen alleelin eli vastingeenin esiintymistiheys on väestössä yli 1 %. SNP vaihtelut ovat keskeinen työkalu haettaessa perimävaihteluiden yhteyttä monitekijäisiin sairauksiin. Vaikea masennus on monitekijäinen sairaus, sairastumisalttiuteen vaikuttavat sekä perimä, että ympäristötekijät. Myös masennuksen hoitovasteen vaihtelun taustalla lienee geneettisiä tekijöitä. Viimevuosina masennuksen genetiikkaa on tutkittu paljon. Osassa tutkimuksia on löydetty yhteys geneettisen monimuotoisuuden ja sairastumisalttiuden välillä ja yhteyttä on havaittu myös geneettisen

monimuotoisuuden ja masennuksen hoitovasteen suhteen. Osa tuloksista on kuitenkin ollut negatiivisia tai ristiriitaisia.

**Tavoitteet.** Tässä tutkimuksessa selvitettiin hoitoresistentin masennuksen ja psykiatrisen sähköhoidon vasteen yhteyttä BDNF, 5-HT1A, TPH1, GNB3, RGS4, COMT ja DRD2 geenien polymorfismeihin.

**Potilaat ja menetelmät.** Kaikki potilaat olivat sairaalahoidossa vaikean hoitoresistentin masennuksen vuoksi. He eivät olleet hyötynet kahdesta asianmukaisesta eri masennuslääkehoidosta tämän sairausjakson aikana. Kaikki potilaat saivat psykiatrisen sähköhoidon ja valikoituivat siihen kliinisin perustein. Tutkimuksessa oli 119 potilasta ja verrokkeina 383 - 398 tervettä verenluovuttajaa. Genominen DNA eristettiin perifeerisen veren valkosoluista ja DNA näytteet genotyyppitettiin. Ennen sähköhoidon aloittamista ja välittömästi hoidon päättymisen jälkeen potilaat arvioitiin standardoidulla Montgomeryn ja Åsbergin depression arviointiasteikolla. Tutkimuksessa tutkittiin geenimuunteluiden vaikutusta hoitoresistentin masennuksen vaaraan verrattuna verrokeihin ja samojen geenimuunteluiden vaikutusta psykiatrisen sähköhoidon vasteeseen.

**Tulokset.** Yhteys hoitoresistenttiin masennukseen havaittiin BDNF polymorfismilla yhdessä 5-HT1A polymorfismin kanssa. TPH1 polymorfismi ja GNB3 polymorfismi olivat yhteydessä hoitoresistenttiin masennukseen sekä erikseen, että yhdessä. Sähköhoidon hoitovasteeseen vaikuttivat BDNF polymorfismi, TPH1 ja GNB3 polymorfismit yhdessä, COMT polymorfismi yksin ja yhdessä DRD2 polymorfismin kanssa.

**Johtopäätökset.** Geenimuunteluilla on yhteyttä sekä hoitoresistenttiin masennukseen, että sähköhoidon hoitovasteeseen. Näiden tutkimusten valossa näyttää siltä, että hoitoresistentin masennus liittyy enemmän serotoniinin säätelyyn aivoissa osallistuviin polymorfismeihin ja sähköhoidon vaste dopamiinin säätelyyn osallistuviin polymorfismeihin. Tulevaisuudessa tavoitteena on ymmärtää paremmin vakavan ja hoitoresistentin masennuksen syntymekanismeja ja perinnöllisyyttä yleensä ja hoitovasteen geneettistä vaihtelua. Siten voi olla mahdollista suunnitella hoitomuotoja yksilöllisemmin ja näin vähentää sairauden aiheuttamaa pitkäaikaista haittaa ja kroonistumisen riskiä.



# Introduction

Major Depressive Disorder (MDD) is a disabling disease affecting in their lifetime up to 16 % of the population, more women than men, peaking in middle-age (Kessler et al. 2005, Pirkola et al. 2005). It is one of the leading causes of early retirement and a major concern in public health. Besides individual suffering, it causes a major social burden and financial losses. MDD is often recurrent and may also have a chronic course. With standard antidepressant (AD) treatment only around 30 % of patients achieve remission (Trivedi et al. 2006b).

Treatment resistant depression (TRD) is a severe and remarkably common form of MDD (Souery et al. 1999, Sackeim 2001a, Fava 2003a). This is defined as nonremission after two adequate AD trials. The percentual share of remitters diminishes at every step when one unsuccessful antidepressant treatment is changed to another (Rush et al. 2006b). This is a major concern in psychiatry leading to a need for effective treatments and studies focusing on the complex pathogenesis of MDD. TRD includes the risk of chronicity, suicidality and several somatic diseases.

Electroconvulsive therapy (ECT) has been used since the 1930s. There may still be some negative attitudes toward this treatment (Dowman et al. 2005). However, it is the most effective and fastest treatment in MDD. The technique in this treatment is nowadays well established. In TRD up to 50-60 % of patients have been reported to benefit from ECT (Devanand et al. 1991, Prudic et al. 1996).

The Human Genome Project was concluded in 2003. The human genome contains approximately 25,000 genes, units of genetic information (International Human Genome Sequencing Consortium 2004). The sequence of nuclear deoxyribonucleic acid (DNA) is nearly 99.9% identical between any two humans (Venter et al. 2001). The small fraction of DNA sequence differing between individuals is responsible for the genetically determined variability among humans. A phenotype is the biochemical, physiological and morphological characteristics of an individual which is determined by genotype and the environment. When a gene variant is found in more than 1% of chromosomes in general population it is called a genetic polymorphism. In addition to the more rare disease-causing mutations, variants common enough to be polymorphisms are also known to predispose to severe illnesses. The most common of all polymorphisms are Single Nucleotide Polymorphisms (SNPs). SNPs usually have only two alleles corresponding to the two different bases occupying a particular location in the genome. SNPs are common and occur on average once every 1,000 base pairs, which means that there

is an average of 1,500,000 differences between any two human genomes. They are essential tools in studying the heritability of multifactorial diseases.

MDD is a multifactorial inherited syndrome, both genes and environment affecting the risk (Kendler et al. 1995, Caspi et al. 2003). The variability in the treatment response between individuals may also be partly related to genetic variation. There are several studies suggesting either association between genetic polymorphisms and MDD or its treatment response to ADs. However, many studies have also reported conflicting or negative results. The data on genetic variability in treatment response to ECT is scanty.

In this dissertation, both the association with TRD and the treatment response to ECT were studied in relation to some brain derived neurotrophic factor (BDNF), serotonin 1A receptor (5-HT1A), tryptophan hydroxylase 1 (TPH1), G protein beta 3 subunit (GNB3), regulator of G protein signaling 4 (RGS4), catechol-o-methyltransferase (COMT) and dopamine receptor D2 (DRD2) polymorphisms. A longterm goal would be to improve the understanding of the genetics of TRD and MDD in general and treatment response in particular and thus rationalize the choice of treatment options.

# 1. Review of the literature

## 1.1 Major Depressive Disorder

MDD is a common disorder with high lifetime rate, particularly in women. It causes disability and social burden and also death both by suicide and due to increased occurrence of physical diseases. Many cases remain undiagnosed and treatment may often be inadequate. Early recognition is important to prevent individual suffering. The development of effective treatment methods to prevent chronicity is a main goal of research.

TRD, which is defined as nonremission after two adequate AD treatments, is a growing problem and effective treatment methods should also be available in outpatient clinics. The use of ECT should be considered earlier, not only in hospitalized patients suffering the most severe forms of the disorder but also in the earlier course of MDD. Patients who have failed trials of ADs may have suffered many years before being treated with ECT. Recent studies have aimed to understand the complex background of MDD and to create more effective treatments. Genetic studies have suggested some polymorphisms concerning both the risk of MDD and its treatment response. In future it may be possible to choose an appropriate treatment method based on the patient's genotype and thus minimize prolonged individual suffering and prevent resistance and chronicity in MDD.

### 1.1.1 Diagnosis

MDD is characterized as low mood (sadness) and loss of pleasure (anhedonia). Changes of mood are normally experienced in everyday life in response to life events but when sad mood becomes distressing and prolonged the diagnosis can be made according to diagnostic criteria. The MDD patient is incapable of experiencing a lifting of mood as a result of rewarding events, negative thoughts are present, possibly suicidal ideations and also disturbances in sleep, appetite and sexual behavior. Activities of daily living may be disrupted, likewise concentration and memory.

Mood problems vary with age, gender, culture and medical condition resulting in a need for a valid classification of mood disorders. There are two major classification systems which include reliable and valid diagnostic criteria for MDD, Diagnostic and Statistical Manual of Mental Disorders, fourth edition, text revision

(DSM-IV-TR, American Psychiatric Association, APA 2000a) and International Classification of Diseases, ICD-10 (World Health Organization, WHO 1992). According to DSM-IV MDD is characterized by one or more major depressive episodes (MDE) lasting at least two weeks when either depressive mood or the loss of interest or pleasure is present as a core symptom (APA 2000a). In addition, at least four associated symptoms, such as significant weight change, insomnia or hypersomnia, psychomotor agitation or retardation, fatigue or loss of energy, feelings of worthlessness or extreme guilt, impaired ability to think or concentrate, and suicidal ideation or thoughts of death are present. DSM-IV also lists three levels of severity of MDD: mild, moderate or severe (with or without psychotic features). DSM-IV codes and criteria are mostly compatible with ICD-10, and the diagnosis of MDD is basically similar in both classifications. Compared with DSM-IV, ICD-10 splits one criterion (feelings of worthlessness and unreasonable guilt), requires one symptom less for diagnosis, and also includes fatigue or loss of energy among the core symptoms. The ICD-10 also includes somatic symptoms as a defining symptom cluster, whereas the DSM-IV-TR does not (Joska and Stein 2008, WHO 1992). MDD research uses the DSM-IV-TR classification rather than ICD-10 because it includes more detailed descriptions of the symptoms than the ICD-10.

## 1.1.2 Subtypes

MDD has been divided into various subtypes according to symptoms or their severity. Only the subtypes concerning this series of studies are presented here.

### 1.1.2.1 *Treatment resistant Major Depressive Disorder*

A major concern among MDD patients is treatment resistance, a failure to achieve remission with a second adequate trial of AD from different pharmacological classes (Souery et al. 1999, Fava 2003, Suomen Psykiatriyhdistys 2004), although in clinical practice the definition of treatment resistance may vary (Berlim and Turecki 2007). According to Thase and Rush (1997) TRD can be divided into five stages:

Stage I: Failure of at least one adequate trial of one major class of AD

Stage II: Stage I resistance plus failure of an adequate trial of distinctly different class of AD than used in stage I

Stage III: Stage II resistance plus failure of an adequate trial of tricyclic antidepressant (TCA)

Stage IV: Stage III resistance plus failure of an adequate trial of monoamine oxidase inhibitor (MAOI)

Stage V: Stage IV resistance plus failure of a course of bilateral ECT

TRD is a heterogeneous and multifactorial issue (Thase and Rush 1997). Many factors can be considered to affect the resistance. The onset, the adequacy of dose and the duration of given AD treatments may be different. Sometimes prolonged trials

lasting over 10 weeks are needed to achieve a therapeutic response (Rush et al. 2006b). The steady state concentrations of different ADs are achieved about 5 to 12 days after initiation of each dosage and this should also be taken into account when the adequate duration of dose titration is assessed (Berlim and Turecki 2007). Nonadherence to treatment may also account for as much as 20 % of cases of TRD (Fagiolini and Kupfer 2003). Misdiagnosis should also be considered (Thase and Rush 1997). In the Sequenced Treatment Alternatives to Relieve Depression (STAR\*D) study in the first treatment step (up to 14 weeks with citalopram alone), fewer than one third of patients remitted (Trivedi et al. 2006b). Only 50 to 55% of the depressed patients achieved remission after a second AD trial, the rest having more or less chronic course and up to 20% have insufficient response to all AD medications (Hussain and Cochrane 2004, Huynh and McIntyre 2008). Sixty-five per cent of the subjects in a Finnish study (Viinamäki et al. 2006) were still depressed at two-year follow-up. This percentage was lower (28 %) in a Dutch one year follow-up study (Spijker et al. 2002). Patients who not fully recover and have residual symptoms are prone to relapse and the lengthening of the episode may decrease the likelihood to remit (Rush et al. 2006a).

Medical comorbidity has been found to be a predictor of treatment resistance in MDD (Iosifescu et al. 2004). More prior depressive episodes also predispose to treatment resistance. Greater severity of MDD, psychotic symptoms, chronicity, psychiatric and general medical comorbidity have been found to be characteristic to those patients who require more treatment steps (Rush et al. 2006b, Souery et al. 2007). Age at onset of MDD has been suggested to be a risk factor for TRD, and patients over 60 years have been considered to have an increased risk because they usually have more psychotic symptoms, vascular brain changes and more medical comorbidity (Kornstein et al. 2001). A family history of depression may be predictive for TRD (Keller 2005). A family history has also been related to early onset of MDD and with chronicity, both of which have also been linked to TRD (Fava and Davidson 1996). Thus age is a risk factor at both ends, early and late onset. Clinically a family history of TRD may also be associated with poorer prognosis of MDD (Berlim and Turecki 2006).

#### *1.1.2.2 Psychotic Major Depressive Disorder*

When psychotic symptoms like nihilistic or somatic delusions together with or without auditory hallucinations are present in patients with MDD, this state is defined as psychotic depression. Psychotic depression is a severe form of MDD and may cause chronicity and treatment resistance. It usually requires inpatient treatment because it is accompanied by significant functional impairment, distress and patients with psychotic depression are also at high risk for suicide (Schaffer et al. 2008). About five percent of patients with MDD have psychotic features (Gaudiano et al. 2008). In the Finnish Health 2000 survey 3.4 % of MDD patients (4.3 % of males and 2.9 % of females) were estimated to have psychotic depression (Pirkola et al. 2005). Psychotic depression affects roughly 20% of hospitalized patients with MDD

(Flores et al. 2006). It is more likely to recur and relatives of these patients are especially prone to MDD. Long-term outcome is generally poorer than in nonpsychotic MDD (Flint and Rifat 1998), however, ECT may protect against relapse (Birkenhäger et al. 2005).

### *1.1.2.3 Late onset Major Depressive Disorder*

MDD often goes unrecognized and untreated in elderly people (Bruce et al. 2002, Jongenelis et al. 2004). The symptoms of depression in the elderly may differ from those in younger patients (Glasser and Gravdal 1997). Elderly depressive patients may complain of symptoms such as neurocognitive impairment (pseudodementia) (Plotkin et al. 1985, Koskinen 1991), somatic symptoms and hypochondriasis. Agitated behavior and verbal aggressiveness may also be related to depression in the elderly (Fountoulakis et al. 2003). The first MDE in the old age may predict dementia (Jorm 2001, Leinonen et al. 2004, Barnes et al. 2006). Such an early age as 45 to 50 years at first episode of depression has been previously used as a cut-point indicating late-onset MDD. (Krishnan et al. 1996, Zubenko et al. 1996, Fishman et al. 2001, Huuhka et al. 2005, Chen et al. 2006).

Vascular disease can increase the risk for depression in later life (Alexopoulos et al. 1997, Alexopoulos 2006). Recent studies have suggested that patients with later onset MDD have greater intima-media thickness compared to controls, a marker of systemic atherosclerosis (Chen et al. 2006, Smith et al. 2009). It has been suggested that poorer vascular health results in greater white matter damage, dysregulation of the frontal-striatal systems in brain (Vataja 2005, Alexopoulos et al. 2008).

### **1.1.3 Epidemiology**

The 12-month prevalence of depression is 3.1-10.1 % in Europe (Wittchen and Jacobi 2005) and 6.6% in the United States (Kessler 2003), lifetime prevalence is estimated at about 16 % in the United States (Kessler et al. 2003, Kessler et al. 2005). In Finland the 12-month prevalence of MDD is 4.9 % and any depressive disorder 6.5 % respectively (Pirkola et al. 2005).

According to this Finnish Health 2000 survey depressive disorders are most common in the age group of 45 to 54 years, although the peak in females is in the age group of 30-44 years and in males 45-54 respectively (Pirkola et al. 2005). The burden of this disease is thus clustered in the middle-aged group and diminishes in later life. The mean age of onset of MDD is around 30 (Kessler et al. 2005). MDD affects females twice as often as males (Pirkola et al. 2005, Kessler and Walters 1998). Other risk factors for developing MDD are: a family history of depression, childhood and other trauma, stress, non-marriage, divorce, low socioeconomic status (Brown et al. 1993, Brown et al. 1996, Kendler 1998, Pirkola et al. 2005), negative life-events and life stressors (Mandelli et al. 2007, Paykel 2003). Life stressors are

possibly more likely to be associated with onset of mood disorders among females than males (Nazroo et al. 1997, Mandelli et al. 2007). A family history of MDD is associated with early age at onset, longer length and more comorbid anxiety disorders and also more suicide attempts (Husain et al. 2009). MDD is estimated to rise to second place in the International Burden of Disease ranking by 2020 (WHO 2009).

#### 1.1.4 Etiology and genetics

The concept of heritability defines the role of genetic differences in determining variability in phenotype and is therefore a measure of the extent to which different alleles at various loci are responsible for the variability in a given trait seen across a population. Multifactorial inheritance is responsible for diseases which have a genetic and also environmental component. MDD is a multifactorial, clinically heterogeneous disorder with many possible etiological factors (Kendler et al. 2002, 2006b). Previously genetic, biological, developmental and environmental risk factors were seen to be unrelated, but nowadays these risk factors are assumed to be related and interacting (Goodyer et al. 2000, Kendler et al. 2002, 2004, 2006b, Caspi et al. 2003). The Human Genome Project was completed in 2003. Knowledge of the complete sequence of DNA allows the identification of all genes and their variations and also how these variations contribute to health and diseases. The development of diagnostic tools, preventive measures and therapeutic methods will be based on knowledge of the genome.

##### *1.1.4.1 Basics of human genetics*

The genome in the nucleus of human somatic cells consists of 46 chromosomes, arranged in 23 pairs. Of those 23 pairs, 22 are alike in males and females and called autosomes. The remaining pair comprises the sex chromosomes. The Human Genome Project determined the deoxyribonucleic acid (DNA) sequence of the entire human genome. The products of genes are proteins. Many genes are capable of generating multiple different proteins by using alternative coding segments or by biochemical modification of the encoded protein. The 25,000 human genes may possibly encode a million different proteins (International Human Genome Sequencing Consortium, 2004). DNA is organized into chromosomes in the nucleus of each cell. Each chromosome carries a different subset of genes that are arranged linearly along its DNA. Members of a pair of chromosomes carry matching genetic information and they have the same genes in the same sequence. At any specific locus, however, they may have either identical or slightly different forms of the same gene, called alleles. An allele is one of two alternative versions of a gene or DNA sequence at a given locus. The sequence of nuclear DNA is nearly 99.9% identical between any two humans (Venter et al. 2001). A mutation is defined as a change in the nucleotide sequence or arrangement of DNA. When a variant is found in more than 1% of chromosomes in general population it is called as a genetic

polymorphism. In addition to the more rare disease-causing mutations, variants common enough to be polymorphisms are also known to predispose to various illnesses. Most common of all polymorphisms are single nucleotide polymorphisms (SNPs). SNPs have only two alleles corresponding to the two different bases occupying a particular location in the genome. SNPs are common and occur on average once every 1,000 base pairs, which means that there is an average of 1,500,000 differences between any two human genomes. Polymorphisms are essential tools in studying the heritability of multifactorial diseases.

DNA contains within its structure the genetic information needed for embryogenesis, development, growth, metabolism, and reproduction in human. DNA is a polymeric nucleic acid macromolecule composed of three types of units: a five-carbon sugar, deoxyribose, which is a nitrogen-containing base, and a phosphate group. The bases are of two types, purines and pyrimidines. In DNA, there are two purine bases, adenine (A) and guanine (G), and two pyrimidine bases, thymine (T) and cytosine (C). Nucleotides composed of a base, a phosphate and a sugar moiety, polymerized into long polynucleotide chains by 5'-3' phosphodiester bonds formed between adjacent deoxyribose units. These polynucleotide chains are in the form of a double helix and are hundreds of millions of nucleotides long. The double helix is formatted by hydrogen bonds between pairs of bases: A of one chain paired with T, and G with C. A phenotype is the biochemical, physiological and morphological characteristics of an individual which is determined by genotype and the environment. Some DNA sequence differences have little or no effect on phenotype, whereas other differences are directly responsible for causing disease. Rare variants can cause illnesses, more common variants can increase the susceptibility to diseases and the most common variation in the population may have no known effects on diseases.

#### *1.1.4.2 Genetic risk of Major Depressive Disorder*

Risk of MDD is suggested to be only partly genetic and nongenetic factors are also important (Fava and Kendler 2000). In family studies first degree relatives of patients with recurrent MDD had a 2-4 times higher risk of depression than controls and the heritability of MDD in twin studies has been found to be approximately 31-42 % (Sullivan et al. 2000, Kendler et al. 2006a). A hospital-based twin study suggested a heritability of 48-75 % (McGuffin et al. 1996).

No specific gene for MDD has been found and many genes are probably involved. In a recent meta-analysis statistically significant evidence was found for six MDD susceptibility genes: Apolipoprotein E (APOE), DRD4, GNB3, MTHFR, SLC6A3 and SLC6A4 (Lopez-Leon et al. 2008). It is obvious that MDD is a heterogenic syndrome and future studies will not find a universal mechanism for developing MDD.



SLC6A4 gene is mapped to chromosome 17q11.1-q12 and contains an insertion/deletion (I/s) variant in the promoter region, serotonin transporter promoter (5-HTTLPR) polymorphism. Alleles with the deletion are coded s for the short form of the allele, and those with the insertion are coded l for the long form. Short s allele has been associated with lower function of serotonin transporter (SERT) while the long l allele is associated with better function. The frequencies of these alleles have racial differences: Caucasians have s/s genotype about 25% and Asians about 58%. For the s/l genotype the frequencies were about 47% for Caucasians and about 35% for Asians. The frequencies for the l/l genotype were about 28% for Caucasians and about 7% for Asians (Smits et al. 2004).

Repeated suicide attempts were associated with 5-HTTLPR s allele carrying (Courtet et al. 2005). 5-HTTLPR s allele was also found to be more common in patients with MDD than in healthy controls in a sample of 466 MDD patients and 836 controls ( $p=0.006$ , odds ratio (OR) =1.26) (Hoefgen et al. 2005). Short s allele also predisposed to more depressive symptoms and suicidality in relation to stressful life events (Caspi et al. 2003). This was a striking finding when published. An association with s allele and exposure to stressful life events at the onset of MDD was also found in a study by Mandelli et al. (2007). However, in a recent meta-analysis Risch et al. (2009) reported that 5-HTTLPR genotype addition did not improve the prediction of the risk of depression. The samples, study designs, measures and analyses were contradictory across these replication studies.

Another SERT gene polymorphism, variable number of tandem repeats (VNTR) in the second intron (intron 2) of SERT gene (SERT-in2) has also been associated with MDD in a mixed Croatian sample of 114 MDD patients and 120 healthy volunteers as a control population, s allele was more common in patients than in controls ( $p=0.04$ ) (Bozina et al. 2006).

5-HT<sub>1A</sub> receptors are expressed presynaptically on 5-hydroxytryptamine (5-HT, serotonin) neurons in the raphe and postsynaptically on the pyramidal neurons, some  $\gamma$ -aminobutyric acid (GABA) -ergic interneurons, astrocytes and some glia in the limbic area and neocortex (Azmitia et al. 1996). 5-HT<sub>1A</sub> receptor gene polymorphism C1019G G allele may regulate 5-HT<sub>1A</sub> gene expression by derepression of the 5-HT<sub>1A</sub> promoter in presynaptic raphe neurons, leading to overexpression of presynaptic 5-HT<sub>1A</sub> autoreceptors and thus may lead to reduction in serotonergic neurotransmission (Lemondé et al. 2003). In that study 5-HT<sub>1A</sub> C1019G polymorphism was associated with MDD and completed suicide in a Caucasian sample of 129 MDD patients and 134 healthy controls and 102 suicide completers and 116 healthy controls. Carrying G allele predisposed to both ( $p=0.0006$  and  $p=0.00008$  respectively). In MDD patients the GG genotype was twice as common compared to controls ( $p=0.017$ ). Parsey et al. (2006) found a trend-like replication in a small sample of 28 MDD patients compared to 42 controls. G allele tended to be more common ( $p=0.059$ ) in MDD patients. A polymorphism in the promoter region of another serotonin receptor 5-HT<sub>2A</sub> gene (-

1438A/G) G allele frequency was higher in Korean MDD patients (n=189) than in healthy controls (n=148) (p=0.007, OR=1.52) (Choi et al. 2004).

TPH, a rate-limiting enzyme of serotonin synthesis has so far identified two isoforms; the TPH2 is found mainly in the raphe nuclei, where the majority of the 5-HT producing neurons are (Zill et al. 2007). TPH1 is also expressed in peripheral tissue, it exceeds the TPH2 in hypothalamus and amygdala and equal amounts of both isoforms have been found in cortex, thalamus, hippocampus and cerebellum in postmortem human brains (Zill et al. 2007). TPH2 gene may thus be mainly responsible for the amount of TPH and serotonin synthesis in the raphe nucleus.

An association of TPH2 SNP rs1386494 G allele with MDD was found in 300 Caucasian patients compared to healthy controls (n=265) (p=0.0012, OR=0.60). A marginal relation with SNP rs1843809 T allele and MDD was found (p=0.0496, OR=1.38, although insignificant after Bonferroni correction) (Zill et al. 2004a). Zill et al. (2004b) in another report also found an association between the same TPH2 SNP (rs1386494), G allele associated with completed suicide (n=263, controls 266, p=0.004, OR=0.62). Tsai et al. (2009b) found an association with TPH2 polymorphism rs17110747 GG genotype and MDD (n=508, controls 463, p=0.002, OR=1.75).

TPH1 A218C (rs1800532) polymorphism A allele may be associated with milder symptomatology in MDD (AA vs. CC, p<0.0001, AA vs. AC, p<0.0007) in a Caucasian small sample (n=51) (Mann et al. 1997). Of these patients 29 had attempted suicide and paradoxically A allele was also found to be associated with suicidal behavior (p<0.009). Serretti and coworkers (2001c) also found a possible association in males, A allele associating with milder symptomatology in MDD compared to C allele (n=511 MDD patients, 318 controls, p=0.016). By contrast, TPH1 polymorphisms A218C AA genotype and A-6526G AA genotype were associated with suicide attempt during one year follow-up (p=0.002 and p=0.001 respectively) in MDE patients (n=343) (Galfalvy et al. 2009). In a haplotype analysis the presence of four A alleles predicted suicide attempt during one year follow-up (p=0.043) compared to those with no risk alleles. The sample consisted of mixed ethnicity with both bipolar disorder and MDD patients. TPH1 A218C A allele and a dose-dependent association with suicidal behavior compared to CC genotype in caucasians was found in a meta-analysis (Bellivier et al. 2004). The patients in these studies, however, had various psychiatric diagnoses. An association of TPH1 SNP A779C (rs1799913) A allele and MDD was found in a Caucasian sample of 228 MDD patients compared to 253 healthy controls (p=0.0013, preserved after correction for multiple testing) (Gizatullin et al. 2006).

In BDNF gene functional polymorphism G196A (Val66Met) (rs6265) A (Met) allele was associated with lower secretion of BDNF in hippocampal neurons (Egan et al. 2003). MDD has been found to be associated with reduced BDNF activity and lower serum BDNF protein levels have been detected with MDD patients compared

to healthy controls (Shimizu et al. 2003, Aydemir et al. 2006, Huang et al. 2008, Piccinni et al. 2008, Sen et al. 2008, Matrisciano et al. 2009).

Met/Met (AA) genotype associated with geriatric MDD in a sample of 110 patients compared to 171 controls, both of Asian origin ( $p=0.003$ ,  $OR=2.49$ ) (Hwang et al. 2006). A (Met) allele was also associated with suicidal behavior in MDE patients of Caucasian origin ( $n=170$ ,  $n=68$  for suicide attempters), although the result remained insignificant after correcting for multiple testing and the sample included also bipolar depression (Sarchiapone et al. 2008). Moreover, Met (A) allele predisposed especially men to MDD according to a meta-analysis including 14 studies (2,812 patients and 10,843 controls of Caucasian and Asian origin,  $p=0.001$ ) (Verhagen et al. 2008). A (Met) allele has been suggested to be related to anxiety disorder together with MDD ( $n=24$ ) rather than MDD alone ( $n=23$ ) (Jiang et al. 2005). This suggestion should, however, be interpreted cautiously because of small sample. Schüle et al. (2006) found an association with BDNF Val66Met polymorphism and stress hormone response in patients ( $n=187$ ) with MDE (bipolar depression was also included). Met/Met (AA) genotype carrying patients had higher hypothalamic-pituitary-adrenal (HPA) axis activity during the dexamethasone suppression / corticotrophin releasing hormone (DEX/CRH) test than patients carrying the Val/Val (GG) genotype ( $p=0.015$ ) and a trend-like association was found in the comparison of Met/Met (AA) versus Val/Met (GA) carrying ( $p=0.076$ ). This may indicate an association between HPA axis dysregulation and neurotrophic system in the pathophysiology of depression.

COMT gene polymorphism Val158Met Met allele has been found to be associated with the onset of mood disorder after stressful life-events compared to Val/Val genotype in a sample of 686 patients, which also included 363 with bipolar depression ( $p=0.0019$ ) (Mandelli et al. 2007). In these patients also 5-HTTLPR s allele containing genotypes associated with the onset of depression compared to ll genotype ( $p=0.0097$ ). The interaction between 5-HTTLPR and COMT on stressors at onset (patients genotyped for both of these polymorphisms  $n=334$ ) was significant ( $p=0.00053$ ), patients carrying both of these risk alleles (s for 5-HTTLPR and Met for COMT Val158Met) were found to have the highest incidence of life stressors at the onset of mood disorder. On the other hand, COMT Val158Met polymorphism Val/Val genotype was suggested to be associated with early onset MDD ( $n=378$ ) compared to controls ( $n=628$ ,  $p=0.01$ ,  $OR=2.07$ ) (Massat et al. 2005). Val allele also showed an association with a risk of affective disorders in general ( $n=112$  of which 82 bipolar disorder, 30 MDD, 467 controls, all of Caucasian origin,  $p=0.018$ ,  $OR=1.43$ ) (Funke et al. 2005). Moreover, COMT Val158Met Val allele was associated with greater severity of TRD in a Caucasian sample of 104 TRD patients ( $p=0.024$ ) (Domschke et al. 2009). However, in a population based study no association was found with COMT Val158Met polymorphism and depression or anxiety (Baekken et al. 2008). The G allele of another COMT polymorphism rs165599 and the A allele of COMT SNP -278A/G showed a trend for significance in risk of affective disorder ( $n=112$ , 82 bipolar depression and 30 MDD, 468 controls,  $p=0.039$ ,  $OR=1.38$  and  $p=0.062$ ,  $OR=1.34$ , respectively). COMT -278A/G

A allele was marginally associated with MDD (n=30, 468 controls, p=0.046, OR=1.79) (Funke et al. 2005).

In GNB3 C825T polymorphism T allele predicted depressive mood in young, healthy adults (Exton et al. 2003). In a Korean population an association of T allele with MDD was found (n=106, 133 controls, p=0.012, OR=2.19) and T allele carrying MDD patients also had more severe symptoms than patients with CC genotype (p<0.05) (Lee et al. 2004). T allele was associated with MDD also in Caucasian patients in two separate studies (n=78, 111 controls, p=0.011, OR=1.79 and n=201, 161 controls, p=0.035, OR=1.61 respectively) (Zill et al. 2000, Bondy et al. 2002)

T-182C polymorphism of the norepinephrine transporter (NET) gene was associated with MDD in a Korean sample (n=112, controls 136), lower frequency of TT genotype was found in patients than in controls (p=0.019) (Ryu et al. 2004).

Cyclic adenosine monophosphate (cAMP) response-element binding protein (CREB) is associated with several neurotrophic factors. Alterations in CREB expression have been reported in MDD patients and in animal models of depression (Nestler et al. 2002). CREB1 gene, which encodes CREB protein may be a susceptibility gene for MDD especially among females (Zubenko et al. 2002, Zubenko et al. 2003).

Associations of some polymorphisms with MDD are presented in Table 1.

Table 1. Gene polymorphisms associated with MDD

<b>Gene</b>	<b>Variant</b>	<b>Association</b>	<b>Study reference</b>	<b>N Ethnicity</b>	<b>P (OR)</b>
<b>SERT</b>	5-HTTLRP	s allele associated with MDD	Hoefgen et al. 2005	patients 466, controls 836, Caucasian	0.006 (1.26)
	SERTin2	s allele associated with MDD	Bozina et al. 2006	patients 114, controls 120, Caucasian	0.04
<b>5-HT1A</b>	C1019G	G allele associated with MDD G allele associated with increased risk for suicide	Lemondé et al. 2003	patients 129, suicide completers 102, controls 116, Caucasian	0.0006 0.00008
<b>5-HT2A</b>	-1438A/G	G allele associated with MDD	Choi et al. 2004	patients 189, controls 148, Asian	0.007 (1.52)
<b>BDNF</b>	Val66Met	Met allele associated with MDD in males	Verhagen et al. 2008	meta-analysis of 14 studies patients 2,812, controls 10,843, Caucasian, Asian	0.001
		Met/Met genotype associated with MDD in elderly patients	Hwang et al. 2006	patients 110, controls 171, Asian	0.003 (2.49)
<b>TPH1</b>	A218C	A allele may be associated with milder symptoms in MDD in males	Serretti et al. 2001c	patients 511, controls 318, Caucasian	0.016
		A allele associated with milder symptoms in MDD A allele associated with suicidal behavior in MDE	Mann et al. 1997	patients 51, (non-attempters n=22, attempters n=29), Caucasian	0.0007 0.009
	A779C	A allele associated with MDD	Gizatulin et al. 2006	patients 228, controls 253, Caucasian	0.0013

<b>Gene</b>	<b>Variant</b>	<b>Association</b>	<b>Study reference</b>	<b>N Ethnicity</b>	<b>P (OR)</b>
<b>TPH2</b>	rs1386494	G allele associated with MDD	Zill et al. 2004a	patients 300, controls 265, Caucasian	0.0012 (0.60)
		G allele associated with increased risk for suicide	Zill et al. 2004b	patients 263, controls 266, Caucasian	0.004 (0.62)
	rs17110747	GG genotype associated with MDD	Tsai et al. 2009b	patients 508, controls 463, Asian	0.002 (1.75)
<b>GNB3</b>	C825T	T allele associated with MDD	Lee et al. 2004	patients 106, controls 133, Asian	0.012 (2.19)
			Bondy et al. 2002	patients 201, controls 161, Caucasian	0.035 (1.61)
			Zill et al. 2000	patients 78, controls 111, Caucasian	0.011 (1.79)
<b>COMT</b>	Val158Met	Val/Val genotype associated with early onset MDD	Massat et al. 2005	patients 378, controls 628	0.01 (2.07)
		Val/Val genotype associated with higher severity of TRD	Domschke et al. 2009	patients 104, Caucasian	0.024
<b>NET</b>	T-182C	lower frequency of TT genotype associated with MDD	Ruy et al. 2004	patients 112, controls 136, Asian	0.019

## 1.1.5 Biological theories

### *1.1.5.1 The monoamine theory*

The principal hypothesis in the biological etiology of depression is based on monoamine neurotransmitter deficiency (monoamine theory). This hypothesis was formulated in the mid 1960s based on the antidepressant effects of the tricyclic antidepressants (TCA), monoamine oxidase (MAO) inhibitors and the depressive effects of reserpine, a monoamine depletor. However, no monoamine-related factor has been found that is diagnostic for depression (Bellmaker 2008). The monoamine neurotransmitters in the brain are serotonin (5-hydroxytryptamine, 5-HT), norepinephrine and dopamine. Impaired function of e.g. serotonin is suggested to be associated with clinical depression (Leyton et al. 2000). The monoamine hypothesis is supported by the mechanism of the action of AD drugs by boosting one or more of these neurotransmitters (Delgado 2000). ADs acutely increase the availability of neurotransmitters at the synapse, either inhibiting their intraneuronal reuptake or metabolism, or increasing their release (Elhwuegi 2004). Despite this it takes 6 to 10 weeks to achieve full effects in AD therapy. This indicates that depression is more complex than a mere insufficiency in these neurotransmitters alone (Higgins and George 2007). The neurotransmitter itself or the agonist can induce a downregulation of its receptors (the number of receptors is decreased). An antagonist can speed up the rate of synthesis of receptors (upregulation). These slow changes in receptor synthesis can modify neurotransmission at the synapses, signal transduction in postsynaptic neurons and consequently the gene expression.

Serotonin is synthesized from aminoacid tryptophan in the serotonergic neuron by the enzymes TPH, a rate-limiting enzyme and aromatic aminoacid decarboxylase (Szabo et al. 2004). It is then stored in presynaptic vesicles by monoamine vesicular transporter and released to the synapse. MAOB enzyme degrades serotonin in the neuron but the main degradation process is done by MAOA in the synaptic cleft. MAO enzymes metabolize serotonin to 5-hydroxyindoleacetic acid (5-HIAA). Decreased 5-HIAA in the cerebrospinal fluid (CSF) has been associated with violent suicide, aggression and impulsive behavior (Åsberg et al. 1976). Low CSF 5-HIAA is associated with short-term suicide risk in male mood disorder inpatients (Jokinen et al. 2009). Serotonin is taken back from the synapse into the presynaptic neuron by the SERT and restored in presynaptic vesicles for reuse in neurotransmission. Drugs blocking SERT increase serotonin and its action in the synapse. Presynaptic serotonin receptors regulate serotonin release and impulse flow. Presynaptic 5-HT<sub>1B/D</sub> receptor is a terminal autoreceptor located on the presynaptic axon terminal. It detects serotonin in the synapse and causes a blockage of further serotonin release. The drugs affecting these autoreceptors can thus promote serotonin release. Postsynaptic 5-HT<sub>1A</sub> receptors inhibit cortical pyramidal

neurons, regulate hormones and may play a role in depression, anxiety and cognition. 5-HT<sub>2A</sub> receptors excite the cortical pyramidal neurons, increase glutamate release, decrease dopamine release and may affect sleep and hallucinations. When 5-HT<sub>1A</sub> presynaptic receptors inhibit serotonin release the 5-HT<sub>2A</sub> postsynaptic receptors cannot be activated and the inhibitory action of serotonin on dopamine is lost (disinhibition) while dopamine release is enhanced. The presynaptic somatodendritic 5-HT<sub>1A</sub> autoreceptors are thus dopamine accelerators. 5-HT<sub>2C</sub> receptors regulate dopamine and norepinephrine release and play a role in obesity, mood and cognition. 5-HT<sub>3</sub> receptors regulate inhibitory interneurons in the brain and mediate vomiting through the vagal nerve. 5-HT<sub>6</sub> receptors regulate the release of brain derived neurotrophic factor (BDNF) and affect long-term memory. 5-HT<sub>7</sub> receptors may be involved in circadian rhythms, mood and sleep.

Dopamine is synthesized in dopaminergic neurons from amino acid tyrosine, which is converted into dopamine by enzyme tyrosine hydroxylase and dopamine decarboxylase (Szabo et al. 2004). Dopamine is taken into the synaptic vesicles in the presynaptic neuron by vesicular monoamine transporter and stored there until it is used in neurotransmission. A reuptake pump, dopamine transporter (DAT), specific to dopamine, inactivates dopamine in the synapse and returns it to the presynaptic vesicles for reuse. In the prefrontal cortex DATs are sparse and dopamine elimination is done by other mechanisms. Dopamine can also be transported by NET as a false substrate. Extracellularly in the synapse COMT enzyme and MAOA destroy dopamine. Intracellularly in the presynaptic neuron MAOA and MAOB eliminate it. Dopamine D<sub>2</sub> autoreceptor regulates the release of dopamine from the presynaptic neuron. Of the postsynaptic receptors the dopamine D<sub>2</sub> receptors are best understood because almost all antipsychotics and dopamine agonists for Parkinson's disease bind to these receptors. Other postsynaptic dopamine receptors are D<sub>1</sub>, D<sub>3</sub>, D<sub>4</sub> and D<sub>5</sub>.

Norepinephrine is synthesized from tyrosine in the noradrenergic neuron (Szabo et al. 2004). It is converted into dopa by tyrosine hydroxylase enzyme, a rate-limiting enzyme. Dopa is converted into dopamine by dopa decarboxylase, which is converted into norepinephrine by dopamine beta hydroxylase. Norepinephrine is then stored in the presynaptic vesicles via the vesicular monoamine transporter in the presynaptic neuron and released from there into the synapse in neurotransmission. The action of norepinephrine is terminated by MAOA or B in the presynaptic neuron and COMT and MAOA in the synapse. The NET on the presynaptic noradrenergic nerve terminal also prevents norepinephrine from acting in the synapse by taking it back to the neuron. Norepinephrine can be restored for reuse. Presynaptic alpha 2 receptors regulate norepinephrine release (autoreceptors). When they recognize norepinephrine they turn off its further release. Other noradrenergic receptors are postsynaptic, alpha 1, 2A, 2B, 2C, beta 1, 2 and 3.

The monoamine theory is based on the acute mechanism of different antidepressants increasing the synaptic levels of monoamines, leading to the



suggestion of deficiency in monoamines in the limbic regions of the brain in depressed patients. However, the levels of monoamines are increased immediately after the initiation of AD treatment but their therapeutic response comes after several weeks. Moreover, monoamine depletion rarely causes depression in healthy individuals. Further on this has led to adaptive plasticity models where the molecular and cellular adaptations to AD treatment underlie the subsequent therapeutic response. Prolonged stress may affect these adaptive processes, exacerbate depression and also be a risk factor of it.

#### *1.1.5.2 Intracellular signal transduction*

Neurotransmission occurs in presynaptic axon, synapse and postsynaptic neuron (Szabo et al. 2004). The genomes of both pre- and postsynaptic neurons are involved and communication between these genomes occurs in both directions, from the genome of the presynaptic neuron to the genome of the postsynaptic neuron and also in the reverse direction. Neurotransmission signal transduction cascades end at the final molecule to influence gene transcription. Signal transduction cascades in the presynaptic neuron begin with the transcription of a gene into protein. In the postsynaptic neuron, the formation of a second messenger is based on the neurotransmission received from the presynaptic neuron and further on the transcription of genes is triggered in the genome also based in this neurotransmission.

Psychotropic drugs target the transporters of a neurotransmitter, receptors coupled to G proteins, ligand-gated ion channels, voltage-sensitive ion channels and various enzymes in order to affect neurotransmission. The first messenger is the neurotransmitter, which activates the production of the chemical second messenger in the postsynaptic neuron. In a G protein coupled signal transduction, a neurotransmitter released from the presynaptic neuron binds to its G protein coupled receptor in the postsynaptic neuron cell membrane. The neurotransmitter transforms the receptor so that it can bind to the G protein which is a signal transducer. G protein then binds to an enzyme capable of synthesizing the second messenger. For example, G protein binds to the adenylate cyclase and synthesizes cAMP which acts as a second messenger. This signal transduction cascade is used by dopamine, serotonin, norepinephrine, acetylcholine (muscarinic), glutamate (metabotropic), GABA B and histamine neurotransmitters.

In another signal cascade, the first messenger binds to receptors which are proteins or protein complexes that contain ion channels, i.e. the ligand-gated ion channels. The binding of the neurotransmitter to the receptor opens an ion channel to allow e.g. calcium to enter the neuron inducing synaptic potential and/or activating signal transduction pathways. Calcium is a second messenger. Glutamate (ionotropic), acetylcholine (nicotinic), GABA A and serotonin (5-HT<sub>3</sub>) neurotransmitters use this second messenger system.

Second messengers, e.g. cAMP, activate the third messengers, e.g. enzyme kinases which add phosphate groups to fourth messenger proteins to create phosphoproteins. These are able to trigger gene expression and synaptogenesis. The second messenger, e.g. calcium, can activate enzyme phosphatases which remove phosphate groups from fourth messenger phosphoproteins and can reverse the actions of the third messenger enzyme kinase. The balance of kinase and phosphatase activity, phosphorylation and dephosphorylation, is regulated by neurotransmitters activating these enzymes. The phosphorylation may be activating for some phosphoproteins. However, the dephosphorylation may also be activating for others. The activity between the neurotransmitters determines the downstream chemical activity. Activation of fourth messenger phosphoproteins can change the synthesis of neurotransmitters, alter their release, change the conductance of ions and maintain the chemical neurotransmission ready or silent. In the cell nucleus fourth messengers activate genes by phosphorylating CREB. CREB has been suggested to be associated with neuronal plasticity, cognition and long term memory (Weeber and Sweatt 2002). Increased CREB activity in the hippocampal dentate gyrus by injection of a viral vector encoding CREB leads to an antidepressant-like effect in animal models of depression (Chen et al. 2001). This could be related to CREB's association with long term memory. CREB is reported to have synergistic interactions with nuclear estrogen receptors (Lazennec et al. 2001, McEwen 2001, Tremblay and Giguere 2001) and this may be associated with sex-specific patterns of gene expression and further on to the sex-specificity of the susceptibility locus for mood disorders (Zubenko et al. 2002). The BDNF gene is induced in vitro and in vivo by CREB (Conti et al. 2002).

Hormones can enter the neuron and bind to their receptors in the neuron to form a hormone-nuclear receptor complex. In the cell nucleus this complex can interact with hormone response elements and trigger the activation of specific genes. The neurotrophin system activates a series of kinase enzymes to trigger gene expression which may control synaptogenesis and neuronal survival and plasticity.

#### *1.1.5.3 Hypothalamic-pituitary-adrenal axis, the stress-cortisol theory*

A second major hypothesis regarding depression has been the stress-cortisol hypothesis. Excessive glucocorticoid activity may be important in depression. In a stressful event the HPA axis responds to stress increasing the release of corticotrophin releasing factor (CRF) which stimulates the release of adenocorticotrophic hormone (ACTH) from the pituitary. ACTH causes glucocorticoid release from the adrenal gland, which feeds back to the hypothalamus and inhibits CRF release and the stress response is terminated. In chronic stress CRF, ACTH and glucocorticoids remain elevated and glucocorticoids may cause hippocampal atrophy and thus prevent the hippocampal inhibition of the HPA axis leaving the stress hormones chronically elevated. This may be associated with the onset of MDD or anxiety disorder. Hippocampal volume has been reported to be decreased in MDD patients, possibly due the repeated episodes (Videbeck and

Ravnskilde 2004) and the normal nerve growth may be disrupted. The recovery of the HPA axis during the treatment of depression with fluoxetine is mediated via restoration of glucocorticoid negative feedback on ACTH levels (Inder et al. 2001). This is mediated by corticosteroid receptors, Type 1 mineralocorticoid receptors (MR) and type 2 or glucocorticoid receptors (GR). MRs mediate and possibly control the low basal circadian levels of circulating glucocorticoids and the GRs mediate the effects of high stress levels of glucocorticoids and are responsible for the negative feedback of glucocorticoids on the HPA system (Ratka et al. 1989, Funder 1994). Cortisol binds to GRs in a cell cytoplasm and the hormone-receptor complex can travel to the cell nucleus and trigger transcription of glucocorticoid genes. Glucocorticoid antagonists compete with cortisol at the GRs and inhibit glucocorticoid binding and prevent the expression of glucocorticoid genes. In abnormal stress response the persistent CRF action at HPA CRF1 receptors leads to glucocorticoid elevation. The blocking of these receptors with CRF1 antagonists may reverse the damaging stress response. Vasopressin acts via Vasopressin1b receptors in the HPA axis and regulates the ACTH release in stress reactions.

However, blood cortisol levels are not diagnostic for depression (Bellmaker 2008). In the Dexamethasone Suppression Test (DST) (Carroll et al. 1976, Carroll et al. 1981) dexamethasone is given in the afternoon to provide feedback inhibition of cortisol production by the adrenal cortex and serum cortisol levels are measured the following day. The value of the cortisol level indicates how readily the pituitary-adrenal-cortical axis can be suppressed. Normally cortisol level decreases with DST. Hyperactivity at any point between the hypothalamus and the adrenal cortex can be associated with failure of DST and the cortisol levels are thus higher afterwards (nonsuppression). In around 30-50 % of patients with MDD DST is pathologic (Carroll 1982, Arana et al. 1985, Miller and Nelson 1987, Nelson and Davis 1997) and the test is also unspecific. However, patients with psychotic depression have the highest rates of nonsuppression on the DST, around 65 % has been suggested. (Schatzberg et al. 1985, Schatzberg et al. 1988, Nelson and Davis 1997). Nonsuppression of DST has been reported to be associated with risk of suicide in male depressive inpatients and dysregulation of the HPA axis seems to be a long-term suicide predictor (Jokinen et al. 2009).

Another laboratory test combines the DST and corticotropin-releasing hormone (CRH) challenge test, the dexamethasone/CRH (DEX/CRH) test (Holsboer et al. 1987, von Bardeleben and Holsboer 1989). An oral dexamethasone and intravenous human CRH are given. Plasma concentrations of ACTH and cortisol are measured. The DEX/CRH test has been suggested to be more closely associated with the activity of the HPA system than the standard DST in healthy and depressed subjects (Deuschle et al. 1998). HPA axis response to the DEX/CRH test is enhanced in depressed patients compared to controls. Up to 80 % specificity for MDD has been reported (Heuser et al. 1994). Severity of depression has also been suggested to correlate with this test. The treatments of depression (ADs, ECT) reduced the levels of ACTH and cortisol and this reduction was greater in ECT treated patients (Kunugi et al. 2006).

#### 1.1.5.4 Neurogenesis and neuroprotection, the neurotrophic theory

Impaired neurogenesis has been hypothesized to be related to the pathophysiology of MDD (Duman 2004). In adult hippocampus precursor cells are produced, migrated and differentiated into new functioning neurons. Neurogenesis is stimulated through learning, psychotherapy, exercise, endogenous growth factors and also by antidepressants and ECT. Neurotrophins and growth factors promote neurogenesis, synaptic plasticity and neuronal survival. Hippocampus is vulnerable to stress, aging and diseases but can restore itself. The neuroplasticity in hippocampus is impaired in MDD (D'Sa and Duman 2002). The hippocampal hypofunction can lead to hypoactivity in prefrontal cortex because the hippocampus regulates its function and together they regulate explicit memory.

Hippocampal synaptic plasticity has been suggested to be an important mechanism of hippocampus-dependent memory formation (Malenka and Bear 2004). This is associated with the function of the hippocampus and the medial temporal lobe (Squire et al. 2004). Explicit memory deficit is included in the symptoms of MDD (Zakzanis et al. 1998). The hippocampus is thus assumed to have a role in the pathogenesis of MDD, although it is not obvious if the changes in hippocampal volumes are the reason for MDD or a consequence of the disease via stress related toxicity (Frodl et al. 2002).

BDNF acts by multiple mechanisms and influences both the early and late phases of synaptic plasticity, in both the presynaptic and the postsynaptic cells (Cao et al. 2004). Long-term potentiation is the strengthening (or potentiation) of the connection between two neurons. It lasts for an extended period of time (typically minutes to hours *in vitro* and hours to days and months *in vivo*). Long term potentiation can be induced experimentally by a sequence of short, high frequency stimulation to afferent fibers (or presynaptic nerve cell). Another form of synaptic plasticity is homosynaptic long-term depression (Citri and Malenka 2008). These mechanisms may converge at the level of specific phosphoproteins.

Increased BDNF leads to increased neurogenesis. Stress and genetic vulnerability decreases BDNF in the central nervous system (CNS). Effective treatments (AD, ECT, psychotherapies) can reverse this process and BDNF production and the neuronal growth increases (D'Sa and Duman 2002, Castren 2004). Exposure to stress and thus reduced BDNF expression and prevailing MDD can lead to functional and morphological and also structural changes in the brain (Warner-Schmidt and Duman 2006). Treatments of depression are thus proposed to be neuroprotective. Serum BDNF levels are lower in patients with MDD and the BDNF levels are elevated following a course of antidepressant treatment (Aydemir et al. 2006, Piccinni et al. 2008, Sen et al. 2008). Antidepressant drugs increase tyrosine kinase B (TrkB), receptor of BDNF, and BDNF signaling in cerebral cortex and this induces the formation and stabilization of the synaptic connectivity (Saarelainen et al. 2003, Castren 2004). Fluoxetine has been found to restore plasticity in adult visual system in rats and these effects were accompanied by

reduced intracortical inhibition and increased expression of BDNF (Maya Vetencourt et al. 2008).

Neurotrophin-3 (NT3) is a protein that regulates neuronal survival, synaptic plasticity, and neurotransmission. It is expressed in the hippocampus and affects hippocampal plasticity by regulating neurogenesis. Infusion of NT3 increases the level of BDNF messenger ribonucleic acid (mRNA) expression in the cerebral cortex and produces BDNF-like effects that induce cortical TrkB phosphorylation. It may also be related to serotonin and norepinephrine modulation according to findings in animal studies (Pae et al. 2008).

There are several other neural growth factors which may be associated with mood or its treatment response. BDNF induces neuropeptides VGF, neuropeptide Y (NPY), substance P, and nociceptin (Alder et al. 2003, Ring et al. 2006). In a recent study of an animal model of depression a correlation between inflammation and lower mRNA expression of nerve growth factor (NGF) in the hippocampus was found (Song et al. 2009). NGF has also been reported to be reduced by chronic stress (Alfonso et al. 2004). VGF enhances the DNA synthesis in hippocampus and promotes the differentiation of neuronal cells (Thakker-Varia et al. 2007, Malberg and Monteggia 2008). VGF influences synaptic plasticity and metabolism and in a recent study it was reported to produce an antidepressant response in mice (Hunsberger et al. 2007). Moreover, its expression has been found to be decreased in two experimental models of depression (Thakker-Varia et al. 2007).

The proliferation of new hippocampal neurons is regulated by vascular endothelial growth factor (VEGF) (Cao et al. 2004) and survival by BDNF (Sairanen et al. 2005). VEGF (like BDNF) activates the mitogen-activated protein kinase (MAPK) pathway. MAPK cascade has been suggested to be related to the stress and antidepressant treatment response. CREB can be activated by MAPK (Giovannini 2006). CREB induces effector genes and contributes to the stabilization of synaptic plasticity (Kandel 2001). Activation of CREB promotes neurogenesis and blocking of CREB function decreases neurogenesis (Nakagawa et al. 2002a, Nakagawa et al. 2002b).

#### *1.1.5.5 Mood related neuroanatomical structures*

In the post-mortem tissue of MDD patients a loss of neurons in size and amount, decrease in cortical thickness and neuronal and glial density in dorsolateral prefrontal cortex (DLPFC) and orbitofrontal cortex have been detected (Rajkowska et al. 1999, Cotter et al. 2002, Rajkowska et al. 2007). The density of glia and oligodendrocytes was lower in MDD patients than controls in the amygdala (Hamidi et al. 2004). The volume of basal ganglia was reduced in MDD patients (Bauman et al. 1999).

The orbital and inferior mesial regions in the frontal cortex are associated with mood. The DLPFC regulates cognitive functions like concentration, attention, working memory and mood. The ventromedial prefrontal cortex (VMPFC) is involved in affect, social behavior, personality and sensitivity to environmental influences. A hyperactivity of VMPFC and the orbitofrontal cortex is associated with anhedonia (Gorwood 2008). The temporal lobe also regulates memory and emotion. In the mesial temporal region is the amygdala, which combines emotion and memory. The VMPFC and amygdala together regulate emotions such as sadness and happiness. From the hippocampus the widespread connections project to the cortical areas, the prefrontal cortex, anterior thalamic nuclei, amygdala, basal ganglia, and hypothalamus. All these areas are associated with mood regulation (Soares and Mann 1997). Basal ganglia integrate emotion, executive functions, motivation and motor activity. By negative feedback the hippocampus modulates the HPA stress hormone axis and hippocampal dysfunction may affect the abnormal stress response in MDD. The hippocampus also regulates the nucleus accumbens and the ventral tegmental area (Lisman and Grace 2005). Nucleus accumbens has a central role in the mechanisms of natural reward and its dysregulation is suggested to be related to symptoms of anhedonia in depression (Dunn et al. 2002, Nestler and Carlezon 2006, Gorwood 2008). The hypothalamus regulates internal homeostasis and physiological reactions to emotional stimuli.

#### 1.1.5.6 *Neuroimaging studies in Major Depressive Disorder*

Structural and functional imaging studies *in vivo* have shown abnormalities in specific regions of the brain in MDD patients. In prefrontal cortex, in the anterior cingulate and in the orbitofrontal cortex, a bilateral volume reduction in gray matter has been found with magnetic resonance imaging (MRI) (Ballmaier et al. 2004). Smaller anterior and posterior cingulate volumes were found with MRI in MDD patients compared to controls; in remitted patients the left anterior part of the cingulum was still reduced compared to controls (Caetano et al. 2006). Hippocampal atrophy has also been found in MDD (Bremner et al. 2000, Frodl et al. 2002, Videbech and Ravnkilde 2004). The number of neurons and glial cells is not diminished but the neurons are smaller and the packing density is increased (Stockmeier et al. 2004). Amygdala volume has been found to be increased in MDD patients (Lange and Irle 2004, van Eijndhoven 2009). Patients with MDD also showed a decrease in gray matter density compared to controls in hippocampus, anterior cingulum, left amygdala, and right dorsomedial prefrontal cortex (DMPFC) (Frodl et al. 2008). Those who remitted during the three-year follow-up had less volume decline than nonremitted patients in the left hippocampus, left anterior cingulum, left DMPFC, and bilaterally in the DLPFC.

In a recent study by Zou et al. (2008) microstructural changes in the white matter were detected with MRI in MDD patients. Decreased gray matter volume was found in an MRI study by Kim et al. (2008) in women with MDD compared to controls in the caudate nucleus and the thalamus.

The resting activity in the left amygdala and left prefrontal cortex is increased in MDD patients compared to healthy controls (Drevets et al. 1992). DLPFC regulates cognitive functions such as concentration and attention and according to functional magnetic resonance imaging (fMRI) study depressed patients need greater activation in this region during working memory tasks to reach the same level of performance than healthy controls (Harvey et al. 2005). During tasks of attention and performance monitoring reduced activation in anterior cingulate gyrus and right lateral prefrontal cortex have been detected in event-related fMRI in adult MDD first episode drug naïve patients (Halari et al. 2008). In the ventral striatal regions, including the nucleus accumbens, decreased activity has been demonstrated with fMRI in response to positive stimuli in depressed subjects (Epstein et al. 2006).

A failure of activation and functioning of the hippocampus and anterior cingulate have also been detected in MDD in a positron emission tomography (PET) study (Bremner et al. 2004). In the prefrontal, cingulate and parietal regions in both hemispheres and in the temporal region on the right a PET study found decreased glucose metabolism, increased metabolism in the occipital pole, vermis, cerebellum, dorsal-frontal, central convexity areas and basal ganglia in both hemispheres in MDD patients compared to controls (Fujimoto et al. 2008). Increased activity in the hippocampus and cerebellum has been found in MDD patients compared to the healthy controls (Videbech et al. 2001). The metabolic rates were also increased in the orbital part of the frontal lobe and decreased in the DLPFC and parietal cortex of MDD patients studied with PET (Biver et al. 1994).

## 1.2 Treatment of Major Depressive Disorder

The choice of the treatment method depends on clinical and practical factors (APA 2000b, Suomen Psykiatriyhdistys 2004). ADs can be used as an initial treatment option, likewise psychotherapy alone with mild to moderate MDD. If a patient prefers psychotherapeutic approaches this should be considered. Also, if there are significant psychosocial stressors, interpersonal difficulties or a comorbid axis II disorder, psychotherapy may be a first line treatment. Also with moderate to severe MDD the combination of ADs and effective psychotherapy may be the initial treatment. Combined treatment can be considered even when a previous single treatment modality has provided only a partial response. ADs should be used for moderate to severe MDD. A combination of antipsychotic and AD medications or ECT should be used for psychotic depression. ECT should be used in very severe MDD with functional impairment or when psychotic symptoms or catatonia are present or with patients who are suicidal or refuse food and drink and need a response urgently.

### 1.2.1 Psychotherapies

Various psychotherapies affect the chemistry and physiology of the brain because they cause a stimulus that leaves a memory trace. The influences of psychotherapies have been found to affect synaptic plasticity in the brain (Liggan and Kay 1999). Cognitive-behavioral therapy (CBT) and interpersonal psychotherapy (IPT) are established as effective treatments for major depression (APA 2000b, Suomen psykiatriyhdistys 2004). Although both are equally effective, CBT may be preferred in severe depression (Luty et al. 2007).

Cognitive therapy (CT) is based on the fact that depressed patients have negative thoughts and in 20 or fewer sessions a patient can actively learn to brake away from negative thoughts by writing these down and organizing them, recognizing patterns in depressive thinking and thus re-determine their performance and situation. CBT combines behavioral techniques for CT providing patient skills in dealing with negative thinking. CBT is well established for acute therapy of MDD (Friedman and Thase 2006, Suomen Psykiatriyhdistys 2004). CBT can be as effective as ADs, even in outpatients with severe MDD but this efficacy is associated with the therapist's experience (DeRubeis et al. 2005). CBT lessens relapse and recurrence in MDD and the effect has been reported to persist for several years (Paykel 2007). CBT alone and in combination with citalopram were as effective in MDD patients as second-step antidepressive treatment after one unsuccessful AD trial (Thase et al. 2007b). In CBT remission was achieved 3 weeks later than with AD treatment. CBT can also be proposed as an alternative for patients who have not responded to ADs (Fava et al. 1997, Thase et al. 2001, McPherson et al. 2005).

Interpersonal psychotherapy (IPT) focuses on interpersonal crisis. In IPT depression is seen as a treatable medical illness and the patient is relieved of guilt and self-blame. It relates the depressive episode to life events or a life change in a vulnerable individual. The treatment focuses on resolving this current interpersonal crisis (Weissmann et al. 2000, Weissmann et al. 2007). IPT is also a time-limited acute treatment, 12 to 16 weekly sessions, and focuses on current life problems, but affectively and interpersonally focused, not cognitively and behaviorally. Intensive combined AD and IPT have been reported to have better acute and long-term effects than AD and standard treatment in inpatients with chronic MDD (Schramm et al. 2008). Combination of AD and IPT instead of AD or IPT alone reduced depressive symptoms better in MDD outpatients (Blom et al. 2007). Maintenance IPT was found to be a good method in relapse prevention even at a frequency of only one session per month for women with MDD who were remitted with IPT alone (Frank et al. 2007). However, in those women with MDD who required a combination of IPT and AD the IPT alone was less effective in maintenance. Recurrence rates in that study were 26 % and 50 %. Furthermore, in women who achieved remission with IPT and AD in combination but still reported sleeping problems, maintenance with IPT alone was not sufficient to prevent recurrence (Dombrovski et al. 2008).



Psychodynamic psychotherapy is based on developmental principles of adult relationships coming from unconscious patterns from childhood; unconscious meanings, conflicts and desires create distress. These patterns are brought into the therapeutic relationship. Psychodynamic psychotherapy focuses on transference, countertransference and resistance along with unconscious conflict and internal object relation. This therapy is indicated when a patient has for example a strong motivation to understand, capacity for insight (psychological mindedness), tolerance for frustration, intact reality testing and meaningful object relations. Psychodynamic psychotherapy has not been adequately studied in MDD (APA 2000b) but it can be useful as a brief therapy in mild to moderate MDD (Suomen psykiatriyhdistys 2004). In a recent study by Kronström et al. (2009) short term psychodynamic psychotherapy was found to alleviate the symptoms of depression in general. Those patients with immature defenses seemed to benefit more from psychotherapy than medication in that study.

The use of psychotherapy in the continuation and maintenance phases prevents recurrences (APA 2000b, Nierenberg et al. 2003, Suomen psykiatriyhdistys 2004). Frequency of visits usually diminishes in the maintenance phase. A combination of psychotherapy and medication is recommended for those with psychosocial/interpersonal problems, or comorbid axis II disorder together with moderate to severe MDD. Poor adherence to treatments may also warrant a combination of treatment modalities (APA 2000b). Combined AD treatment and psychosocial treatment is suggested to be associated with a higher improvement rate than pharmacotherapy alone (Pampallona et al. 2004).

## 1.2.2 Antidepressive medication

AD treatment consists of an acute phase, a continuation phase and a maintenance phase (APA 2000b, Suomen psykiatriyhdistys 2004). MDE should be treated for 4-9 months after remission (continuation phase). As maintenance AD treatment should be considered to prevent recurrences of MDEs when there are prior episodes, comorbid conditions, residual symptoms, suicidality, psychotic features, severe functional impairments and patient's preference. The dose in continuation and maintenance phases should be the same as in the acute phase (APA 2000b, Suomen psykiatriyhdistys 2004).

In the late 1950s TCAs (e.g. amitriptyline, clomipramine, doxepin, imipramine, nortriptyline and trimipramine) were discovered. They inhibit the reuptake of norepinephrine or both norepinephrine and serotonin (Glowinski and Axelrod 1964, Carlsson et al. 1968). They are effective drugs in treating depression but they have also many undesirable effects. Nowadays they are third-line drugs in the treatment of depression but still used in difficult-to-treat patients. Undesirable adverse effects like sedation, weight gain (blockage of histamine 1 receptors), dry mouth, blurred vision, urinary retention, constipation (blockage of M1 muscarinic cholinergic receptors), orthostatic hypotonia, dizziness (blockage of alpha 1 adrenergic

receptors) and interference with insulin action (blockage of M3 cholinergic receptors) are associated with these drugs. In overdose they are fatal. Weak blocking of sodium channels in the heart and brain results in seizures and coma due to CNS actions and arrhythmias, cardiac arrest and death due to peripheral cardiac actions.

In the late 1980s the serotonin selective reuptake inhibitors (SSRIs: citalopram (and later escitalopram), fluoxetine, fluvoxamine, paroxetine, sertraline, zimelidine) were introduced. They soon became widely used because they had fewer adverse effects and were safer than TCAs. They increase serotonin in synapses by blocking the reuptake (inhibition of the SERT). Serotonin increases in the synaptic clefts in the somatodendritic area of the serotonin neuron and less in the axon terminals. The somatodendritic 5-HT<sub>1A</sub> autoreceptors desensitize and downregulate and the serotonin is released from the axon terminals as a consequence. This is not a fast process and may in part explain the delay in antidepressant effect. Then the increased amount of serotonin causes postsynaptic receptor downregulation. This may also affect tolerance of the adverse effects. Many adverse effects of SSRIs are associated with the acute stimulation of serotonin receptors subtypes (5-HT<sub>2A</sub>, 5-HT<sub>2C</sub>, 5-HT<sub>3</sub> and 5-HT<sub>4</sub> postsynaptic receptors) all over the body. The main acute adverse effects are nausea, vomiting, dyspepsia, diarrhoea, abdominal pain, rash, sweating, agitation, anxiety, tremor, insomnia. In most cases they alleviate or diminish over time. Later on in SSRI treatment sexual dysfunction may be a major problem.

Serotonin and norepinephrine reuptake inhibitors (SNRIs: venlafaxine, duloxetine, milnacipran) block the reuptake of both serotonin and norepinephrine (NET inhibition). They boost serotonin and norepinephrine in the brain and also dopamine in prefrontal cortex. Dopamine is boosted because in prefrontal cortex its actions are terminated by NET (uptake) and inhibition of NET also increases synaptic dopamine. If the SSRI treatment does not help the patient, SNRI should be considered. In addition to serotonin-linked adverse effects, blood pressure and heart rate fluctuation may occur due to acute stimulation of noradrenergic receptors. Stimulation of noradrenergic receptors in the sympathetic nervous system can cause a reduction in parasympathetic cholinergic tone and later pseudoanticholinergic symptoms like constipation, dry mouth and urinary retention. These symptoms are milder than with TCAs, directly blocking muscarinic cholinergic receptors.

Norepinephrine and dopamine reuptake inhibitor bupropione inhibits DAT and NET. It has no significant serotonergic component and thus does not cause serotonergic adverse effects. It can be augmented with SSRI or SNRI. It is activating and has no sexual adverse effects. It may cause seizures associated with peak plasma concentrations. It is not licenced for MDD in Finland but could be effective in some patients not responding to SSRIs or SNRIs.

Alpha 2 receptor blockers (mianserin, mirtazapine) disinhibits norepinephrine and serotonin release. This blockage results in an increase in both serotonergic and noradrenergic neurotransmission. The increased serotonin release is partly also

mediated via 5-HT<sub>1A</sub> receptors. Moreover, serotonin stimulation of 5-HT<sub>1A</sub> receptors results in release of dopamine. This could improve depression and cognition. The action of mirtazapine is synergistic with SNRIs actions and can sometimes be combined with these in patients who have not responded to SNRIs alone. In addition, mirtazapine blocks postsynaptic 5-HT<sub>2C</sub>, 5-HT<sub>2A</sub> and 5-HT<sub>3</sub> receptors and 5-HT<sub>2A/C</sub> antagonism may have anxiolytic, sleep-restoring and antidepressant effects. Mirtazapine causes no significant sexual dysfunction. 5-HT<sub>2C</sub> antagonism and antihistamine H<sub>1</sub> action may cause weight gain. Mianserin also has potent alpha 1 antagonist properties and enhances predominantly noradrenergic neurotransmission.

The effectiveness of different ADs is comparable and the selection of an antidepressant will largely be based on its profile of side-effects, interactions with other medications and patient's preference (APA 2000b, Suomen Psykiatriyhdistys 2004). TCAs have been suggested to be slightly more effective in hospitalized patients (Anderson et al. 2000). Accordingly, venlafaxine has been suggested to be slightly more effective than SSRI in treating MDD (Smith et al. 2002). However, in a recent meta-analysis of 12 different new generation ADs, it was suggested that escitalopram and sertraline might be the best choice at initiation of treatment in moderate or severe MDD because they have the best balance between efficacy and tolerability (Cipriani et al. 2009).

### 1.2.3 Electroconvulsive therapy

ECT treatment has been used since the 1930s. In ECT an electric current is passed through a patient's brain during general anesthesia and muscle relaxation to produce a convulsion. The clinical practice of ECT is well established, although the complete mechanism of action is still not known. ECT is not used in clinical practice as a first line therapy, except in life-threatening cases when a patient is stuporous, refuses to eat or drink or in cases of attempted suicide. There are well established indications for the clinical use of ECT including the most severe and psychotic forms of MDD. ECT is used mainly in hospitalized patients and its use increases with age. The use of ECT varies across countries and may not be performed according to the guidelines. In Belgium it is underused (Sienaert et al. 2006). In Japan its use is also low (Chanpattana et al. 2005) but in the United States the decline in its use ended in the 1980s (Thompson et al. 1994).

Without any active treatment almost all patients relapse 6 months after ECT (Sackeim et al. 2001b). This relapse rate with placebo was found to be 84 %. A nortriptyline-lithium combination after ECT was found to be effective in preventing relapse. The relapse rate was 39 % with this combination whereas it was 60 % with nortriptyline alone in 24-month follow-up. TRD, female gender and more severe MDD but not psychotic features predicted more rapid relapse.

### 1.2.3.1 *Mechanism of action*

ECT induces many acute and long term physiological changes in levels of neurotransmitter, neuroendocrinological and neurotrophin systems. ECT increases the blood levels of epinephrine and norepinephrine (Weinger et al. 1991) which are associated with the ECT dosage (Mann et al. 1990), however, ECT does not seem to cause consistent changes in CSF, plasma, or urinary levels of the major monoamine metabolites. Findings have been contradictory, either decrease, increase or no change after ECT have been reported in homovanillic acid (HVA), 3-methoxy-4-hydroxyphenylglycol (MHPG), or 5-hydroxyindoleacetic acid (5-HIAA) (Lerer and Belmarker 1982, Linnoila et al. 1984, Devanand et al. 1989, Lykouras et al. 1990, Hofmann et al. 1996). According to a recent study HVA, 5-HIAA and NPY concentrations in CSF were elevated after ECT, but CRH was lowered in TRD patients (Nikisch and Mathé 2008). It was hypothesized that the enhancement of NPY may play a role in AD response. Conversely, it has been suggested that if response to ECT is not associated with major modifications in central serotonergic or dopaminergic responsivity, only moderate increase in 5-HT<sub>1A</sub> receptor responsivity may occur (Markianos et al. 2002b). However, in the study by Okamoto et al. (2008) plasma HVA but not MHPG levels were found to be reduced after ECT. These changes occurred in parallel with the alleviation of depressive symptoms in TRD patients.

The mechanism of ECT has been suggested to be in part related to dopaminergic neurons and BDNF (Okamoto et al. 2008). They found that serum BDNF levels were increased in ECT responders while levels in non-responders were not changed. Moreover, higher serum levels of BDNF were also found after ECT in TRD patients (Bocchio-Chiavetto et al. 2006) and in MDD patients (Marano et al. 2007). By contrast, another study reported no change in serum BDNF levels due to ECT (Gronli et al. 2007). Animal studies have shown that electroconvulsive shocks (ECS) produce an increase in BDNF mRNA (Nibuya et al. 1995) and BDNF protein (Altar et al. 2004) in different rat brain areas. Transcriptional regulation of several genes is induced by ECS, including BDNF the transcription of which is upregulated (Conti et al. 2007). Accordingly, in recent animal studies it has been suggested that the therapeutic action of ECT is associated with neurogenesis, synaptogenesis and synaptic plasticity (Perera et al. 2007, Huang and Chen 2008, Chen et al. 2009). In ECS treated animals the actions of ECS have been suggested to be mediated by neurotrophic growth factors and angiogenic systems (Newton et al. 2003). The authors suggested that ECSs inducing growth factors provide neurotrophic, neurogenic and neuroprotective effects.

After a single ECT stimulus an acute increase in the plasma thyroid-stimulating hormone (Esel et al. 2002), ACTH (Whalley et al. 1987, Kronfol et al. 1991), prolactin (Lisanby et al. 1998), cortisol (Kronfol et al. 1991) and vasopressin (Weinger et al. 1991) have been reported. ACTH, prolactin and cortisol levels were decreased after repeated ECT (Kronfol et al. 1991).

It has been suggested that the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) is involved in the neurobiology of depression. GABA has been suggested also to be related to the mechanism of the anticonvulsant and antidepressant actions of ECT (Sackeim et al. 1983). Serum GABA level has been reported to be lower in MDD patients than in healthy controls and ECT causes its normalization. (Esel et al. 2008). Sanacora et al. (2003) reported a significant increase in occipital cortex GABA concentrations after a course of ECT. Response to ECT has been associated with increased vascular perfusion and GABAergic neurotransmission in the right temporal and bilateral parietal cortices (Mervaala et al. 2001). Devanad et al. (1995) reported free plasma GABA reduction acutely after ECT. In the small sample of Palmio et al. (2005) a significant decrease in plasma GABA level was also found two hours after a single ECT session.

It is proposed that the mechanism of action is related to the anticonvulsive properties of ECT (Sackeim 1999). During the course of the ECT there is an increase in seizure threshold and decrease in seizure duration (Coffey et al. 1995, Kales et al. 1997). It has been shown that the seizure threshold varies as much as 35-fold (Boylan et al. 2000). The efficacy of ECT has been related to the change in seizure threshold over the ECT course (Sackeim et al. 1987b, Sackeim 1999). However, in a recent study by Fink et al. (2008) the seizure threshold increased in only 21 % of patients at remission with bilateral ECT. The authors concluded that this finding did not support the anticonvulsive hypothesis about ECT.

Short-term increase of slow-wave activity (delta and theta) in electroencephalography (EEG) produced by ECT in prefrontal cortex has been suggested to be linked to the efficacy of ECT (Sackeim et al. 1996, Heikman et al. 2001). Sackeim et al. (1996) suggested that interictally increased delta power in the prefrontal regions was associated with response to ECT. Greater ictal power and delta coherence and postictal suppression in EEG have been suggested to correlate with good outcome (Perera et al. 2004). Accordingly, Azuma et al. (2007) reported that postictal suppression predicted therapeutic outcome in ECT, which has also been proposed earlier by Sackeim et al. (1999). In magnetoencephalographic (MEG) recordings the increase of the theta activity in the left frontal cortex was associated with the response to ECT treatment (Heikman et al. 2001).

#### *1.2.3.2 Indications*

ECT has a shorter latency of onset of response than ADs and has been considered as a treatment of choice in severe MDD especially when accompanied by psychotic features, catatonic stupor, severe suicidality or denying of nutrition (APA 2001, Petrides et al. 2001, Husain et al. 2004, Patel et al. 2006). If a rapid response is needed, ECT should be considered as a first line treatment method in MDD. One indication for ECT is also a prior good response to this treatment or the patients' own request (APA 2001). Older people are likely to have ECT treatment in MDD more often than the middle-aged (Thomson et al. 1994, Prudic et al. 2001, Huuhka

2005, Rapoport et al. 2006). They also seem to benefit from this treatment most (O'Connor et al. 2001). This may be related to a lower rate of comorbid axis II psychopathology in this age group (APA 2001). TRD is also a common indication for ECT, although resistance to ADs may predict a poorer response to ECT (APA 2001). The effectiveness of ECT in TRD has not been studied in randomized placebo-controlled clinical trials but it is evident that it is also effective in this indication (Suomen psykiatriyhdistys 2004). It has been suggested that ECT should be used more often than as a treatment of last resort (APA 2001, Husain et al. 2004).

ECT is an effective treatment in severe mania (Mukherjee et al. 1994, Barekatin et al. 2008, Mohan et al. 2009) in patients who have not responded to pharmacological treatments (APA 2001). Schizophrenic patients with acute onset of psychosis and shorter episode duration are more likely to benefit from ECT than patients with persistent unremitting symptoms (APA 2001). There is some evidence that ECT combined with antipsychotic drugs provide acute benefit to medication-resistant schizophrenic patients (Kupchik et al. 2000, Suzuki et al. 2004, Tharyan and Adams 2005).

#### *1.2.3.3 Contraindications*

There are no absolute contraindications for ECT; risks and benefits should be evaluated individually (APA 2001). Recent myocardial infarction, unstable angina pectoris, decompensated congestive heart failure and severe valvular disease increase the risks in ECT. Other conditions with elevated risk to ECT are increased intracranial pressure, aneurysm, recent cerebral infarction, severe pulmonary conditions or patient's rated at level 4 to 5 on the scale by American Society of Anesthesiologists (ASA 1963).

#### *1.2.3.4 Predictors of efficacy*

There are some clinical factors which may predict the response to ECT. These include characteristics of patients and technique of ECT (discussed later). TRD patients are associated with lower rates of remission after acute ECT (Dombrowski et al. 2005). Thus the high response rate to ECT in MDD patients in general (80-90 %) is lowered in TRD patients to 50 % -60% (Devanand et al. 1991, Prudic et al. 1996). However, in some earlier studies it has also been reported that AD medication resistance does not affect short term response to ECT (Pluijms et al. 2002, Kho et al. 2003, Rasmussen et al. 2007, Heijnen et al. 2008). Psychotic depression may also be a predictor of good response to ECT (Petrides et al. 2001, Birkenhäger et al. 2003). Older patients are more likely than younger ones to benefit from this treatment (O'Connor et al. 2001). It has also been suggested that psychomotor retardation predicts a good response (Hickie et al. 1996). Longer episode duration has been associated with poorer outcome (Prudic et al. 2004). Despite some previous claims (Carney et al. 1965), melancholic features in MDD

did not predict better acute response to ECT (Fink et al. 2007). Poorer response to ECT is also associated with comorbid somatic or psychiatric conditions (Sareen et al. 2000, DeBattista and Mueller 2001, Heikman et al. 2002, Feske et al. 2004, Prudic et al. 2004).

#### *1.2.3.5 Clinical practice*

The method of ECT is standardized and well established and the course of ECT is safe with modern monitoring techniques and well trained staff. Evaluation before ECT treatment should be done for all patients to identify the severity of MDD, previous episodes and treatment strategies and also the severity of medical risks and course of previous anesthetics. The medical examination should be focused on the neurological, cardiovascular and pulmonary systems. Depressive symptoms should be evaluated and monitored with some of the depression ratings scales e.g. MARDS, HRSD and general cognitive status using e.g. Mini-Mental State Examination Scale (MMSE). ADs and antipsychotics are nowadays usually continued during ECT (APA 2001, Greenberg and Kellner 2005).

Methohexital or propofol are used as anesthetics and succinylcholine as a relaxant for ECT (APA 2001). Oxygenation using positive pressure ventilation is maintained during anesthesia until adequate spontaneous respiration resumes (APA 2001).

Electrode placement and stimulus dose affect the efficacy and adverse effects of ECT. Some practitioners use only bilateral (bifrontotemporal) ECT and some right unilateral. If treatment is started with right unilateral ECT and the response is inadequate switching to bilateral ECT may be beneficial. Conversely, if cognitive adverse effects are a problem, bilateral ECT could be changed to right unilateral (APA 2001). Bifrontal electrode placement is less used but it has been suggested to have fewer cognitive adverse effects than bilateral (Bakewell et al. 2004). Its efficacy compared to bilateral ECT is still disputed (Bailine et al. 2000, Bakewell et al. 2004). Bilateral ECT is considered a “gold standard” for its efficacy (Greenberg and Kellner 2005). The stimulus dose should be adjusted to the seizure threshold. Particularly in right unilateral ECT the empirical stimulus titration has been suggested to be the only accurate method to determine the dosage (Sackeim et al. 1987a, Boylan et al. 2000). The other strategy for stimulus dosing is the preselected stimulus dose method based on the age of the patient to which the dose is adjusted (Petrides and Fink 1996, Swartz and Abrams 1996).

Seizure duration should be monitored during each treatment. The modern ECT devices deliver brief pulse stimulation and are equipped with EEG, electrocardiogram (ECG) and electromyography (EMG). In addition the physiological monitoring during the ECT includes blood pressure and pulse oximetry. A generalized epileptic seizure has been traditionally considered necessary for a therapeutic response. Seizure duration of at least 20 seconds for the

motor response and/or 25 seconds for the ictal EEG response is considered adequate (Beyer et al. 1998). The common frequency of treatment is three times a week. If ECT results in severe cognitive adverse effects, a frequency of twice a week should be considered (Shapira et al. 2000, APA 2001).

#### *1.2.3.6 Adverse effects*

ECT is a safe treatment. Mortality is estimated to be one death per 80,000 treatments (APA 2001). Nuttall et al. (2004) performed a retrospective review of 2,279 patients given 17,394 ECT treatments in Minnesota during the period 1988 – 2001. There were no deaths during or immediately after ECT.

The most common adverse effects of ECT treatment are related to memory impairment and cognitive dysfunction (Calev et al. 1991b, Sackeim et al. 2000). Memory disturbances consist of both anterograde and retrograde amnesia. Bilateral ECT causes more severe and persistent retrograde amnesia than right unilateral ECT (Lisanby et al. 2000, Sackeim et al. 2007). Advanced age, lower premorbid intellectual function, and female gender have also been associated with greater cognitive defects after ECT (Sackeim et al. 2007). Patients with poor response are also likely to report memory impairment after a treatment course (Prudic et al. 2000). Lekwauwa et al. (2006) suggested that smaller hippocampal volume is associated with ECT induced memory adverse effects. However, it has been reported that ECT may even improve cognitive functioning when depression is relieved (Bosboom and Deijen 2006, Tielkes et al. 2008). Anterograde memory defect usually disappears in a few days or weeks after completion of the ECT course (APA 2001). Fujita et al. (2006) reported that anterograde memory improved after one week. Retrograde amnesia is the most troublesome cognitive adverse effect of ECT. It affects more public than personal events and it is greater for recent events before ECT (Lisanby et al. 2000). Long seizure duration and high stimulus dosage relative to seizure threshold may exacerbate the cognitive adverse effects (Sackeim et al. 1987a, Calev et al. 1991b, Sackeim et al. 1993, McCall et al. 2000). In objective measures retrograde memory loss has been found to disappear 6 months posttreatment (Fraser et al. 2008). Disorientation in the period immediately after ECT is a common occurrence (Calev 1991a, APA 2001), severe delirium is rare (APA 2001).

Cardiovascular complications are more likely to occur in patients with pre-existent cardiac diseases (Zielinski et al. 1993). Transient and benign arrhythmias are common in patients with pre-existing arrhythmias (Huuhka et al. 2003, McCully et al. 2003). Headache is quite a common adverse effect, likewise muscle soreness. Treatment-emergent mania and prolonged apnea are relatively uncommon (APA 2001).



## 1.2.4 Other treatment methods of Major Depressive Disorder

### 1.2.4.1 *Transcranial magnetic stimulation*

In transcranial magnetic stimulation (TMS) a rapidly alternating current passes through a small coil placed over the scalp and generates a magnetic field that induces an electric field in the underlying areas of the brain, ionic currents are generated and neuronal depolarization occurs. During the treatment session the patient is awake, coil location is currently determined by identifying the motor cortex and then moving the coil 5 cm rostrally to approximate the location of the dorsolateral prefrontal cortex. Treatments may last 45 min, and may be given daily. In general, patients tolerate repetitive TMS (rTMS) well and are able to resume their daily activities immediately after treatment. Headache may be an adverse effect and some seizures have been reported. Patients with MDD who have not responded to one adequate AD trial in the current episode are suggested to have better response to TMS treatment than those who have already failed 2–4 AD trials (Lisanby et al. 2008). TMS is in any case not as effective as ECT (Knapp et al. 2008). TMS can be considered an adjuvant therapy for younger MDD patients, MDD patients who are not resistant to ADs and have no psychotic features. The clinical benefits of this treatment are marginal and there is still some uncertainty about the optimal stimulation parameters and clinical practice (Mitchell et al. 2006).

### 1.2.4.2 *Vagus nerve stimulation*

In vagus nerve stimulation (VNS) a pacemaker-like device is implanted surgically in the left chest wall and delivers an electrical signal through an implanted lead that is wrapped around the left cervical vagus nerve. Pulses are delivered to the vagus nerve, typically for 30 s every 5 min, 24 h a day or until turned off. Intermittent stimulation of the vagus nerve provides bilateral activation of the brain circuits. The vagus nerve sends sensory information from the periphery to the brain, including the locus ceruleus, the raphe nuclei, and the nucleus tractus solitarius. The most common adverse effects of VNS are voice alteration and hoarseness during the period of active stimulation. Of patients who had not responded to two or three adequate AD trials 50 % responded to VNS (Sackeim et al. 2001c). However, this response rate dropped with four or more inefficacious AD trials. Response is thus associated with the degree of resistance to ADs which is also true of response to ECT (Prudic et al. 1990, Prudic et al. 1996, Sackeim et al. 2000). Efficacy of VNS may increase over time (Rush et al. 2000, Nahas et al. 2005, Schlaepfer et al. 2008) and adherence to this treatment is at least guaranteed.

#### 1.2.4.3 *Deep brain stimulation*

In deep brain stimulation (DBS) a battery-powered pulse generator is implanted near the clavicle similar to pacemakers or VNS devices. One or two leads (unilateral or bilateral) are tunneled from the device(s) under the scalp along the skull. Neuroimaging and brain stimulation recording during the implantation procedure helps the exact placement of the lead in the targeted brain area. Inferior thalamic peduncle DBS was found to be effective in TRD (one patient) (Jimenez et al. 2005), as well as internal globus pallidus DBS (one patient) (Kosel et al. 2007). DBS to the nucleus accumbens relieved the symptoms of TRD patients (Schlaepfer et al. 2007). DBS to the subcallosal cingulate gyrus resulted in a response of 35 % and remission of 10 % one month after surgery in TRD patients (Lozano et al. 2008). After six months the response rate was 60% and the remission rate 35%. These percentages were maintained in 12-month follow-up. DBS of the subgenual cingulum region can also help patients with TRD (Mayberg et al. 2005). Four out of six patients achieved response or remission at the end of 6 months without changes in concurrent medications. DBS of the ventral anterior internal capsule/ventral striatum can also help TRD patients (Malone et al. 2009). Infection of the implant site, skin erosion, subcutaneous seroma, intracerebral hematoma and extension cable discomfort have been reported as adverse effects. Some suicides have been reported in DBS in movement disorders (Burkhard et al. 2004). Cognitively this treatment is supposed to be safe (McNeely et al. 2008). This treatment method, however, is still being researched and is used in ultimate cases of TRD.

#### 1.2.5 Neuroimaging studies associated with different treatment methods in Major Depressive Disorder

In neuroimaging studies after response or remission with different treatments for MDD alterations have also been found in the metabolism and in the activation of different brain regions.

##### 1.2.5.1 *Psychotherapies*

Martin et al. (2001) found right posterior cingulate and right basal ganglia activation in single photon emission computed tomography (SPECT) in MDD patients who had interpersonal psychotherapy (IPT) sessions for six weeks. Decreased metabolism in right middle frontal gyrus including DLPFC and ventrolateral prefrontal cortex (VLPFC) and in left anterior cingulate gyrus and increased metabolism in left temporal lobe and anterior insula were found in PET with IPT treated MDD patients (Brody et al. 2001).

Hippocampal and dorsal cingulate increased metabolism was detected with PET after response to CBT (Goldapple et al. 2004). Decreased metabolism was found in the ventral prefrontal cortex, DLPFC, VLPFC, superior and inferior medial frontal regions, posterior cingulate, inferior parietal, and inferior temporal cortex. In another study, response to CBT was associated with decreased metabolism in the posterior cingulate and in the thalamus, increased metabolism was found in the left inferior temporal cortex, the anterior portion of the subgenual cingulate/ventromedial frontal cortex and the right occipital-temporal cortex (Kennedy et al. 2007).

#### 1.2.5.2 *Antidepressants*

Remission of MDD with ADs was associated with a regional decrease in glucose metabolism found in a PET study on left prefrontal and anterior temporal cortices, left anterior cingulate cortex and bilateral thalamus, putamen and cerebellum (Holthoff et al. 2004). Fluoxetine effects on regional brain glucose metabolism detected with PET were subcortical and limbic decreases (in subgenual cingulate, hippocampus, insula, and pallidum) and cortical as well as brain stem increases (prefrontal, parietal, anterior and posterior cingulum) (Mayberg et al. 2000). Adaptive changes occurred from week 1 to week 6 and failure in these adaptations may affect treatment response. Paroxetine treatment induced increases in metabolism in DLPFC, inferior parietal, inferior temporal, brainstem and cerebellum regions studied with PET (Goldapple et al. 2004). Decreases in this study were found in ventral prefrontal cortex, hippocampus, ventral subgenual cingulate and insula regions. In another study after paroxetine treatment, metabolism was decreased in the middle frontal gyrus including the VLPFC and DLPFC and left ventral anterior cingulate gyrus and increased metabolism was found in left temporal lobe and right insula in a PET study (Brody et al. 2001). Paroxetine was associated with increases in metabolic activity in DLPFC, VLPFC and ventral prefrontal areas, dorsal medial prefrontal, anterior cingulate and inferior parietal regions (Kennedy et al. 2001). Increases were mostly on the left side. Moreover, decreases in glucose metabolism were reported in left anterior and posterior insular regions, right hippocampal and parahippocampal regions. Response to venlafaxine was associated with PET detected brain glucose metabolism increases in the posterior cingulate and decreases in the left inferior temporal cortex, right nucleus accumbens and a posterior part of the subgenual cingulate (Kennedy et al. 2007). Venlafaxine treatment increased right basal ganglia blood flow detected with SPECT (Martin et al. 2001). A relationship with SERT occupancy in SPECT in paroxetine treated MDD patients and serotonin transporter gene promoter polymorphism (5-HTTLPR) II genotype has been reported (Ruhe et al. 2009). This higher occupancy was associated with better clinical improvement.

In the comparison of brain metabolic changes with PET in MDD, patients treated with IPT or paroxetine metabolic changes were comparable (Brody et al. 2001). Brain metabolic abnormalities at baseline compared to healthy controls seemed to

normalize with both treatments. In another comparison of brain metabolic changes associated with response to either CBT or paroxetine the sites of changes were quite similar but the directions were mostly opposite (Goldapple et al. 2004). Similar to both treatments were decreases in metabolism in ventral prefrontal cortex. The study by Kennedy et al. (2007) compared the effects of venlafaxine and CBT. Decreased metabolism in orbitofrontal cortex, left medial prefrontal cortex and increased metabolism in right occipital temporal cortex were similar in both treatments in responders. Venlafaxine treatment was associated with increases in posterior cingulum while decreases with CBT. In left inferior temporal cortex venlafaxine caused decreases and CBT increases. Unique to each were decreases in right thalamus with CBT and decreases in right posterior subgenual cingulum with venlafaxine.

### 1.2.5.3 *Electroconvulsive therapy*

Contradictory results have been reported on the cerebral metabolism and blood flow after and during ECT, while some studies have reported decreased and others increased metabolism. This may relate to the time point of measure and differences in ECT technique. The therapeutic action of ECT has been suggested to be related to the reduction in glucose metabolism after ECT measured with PET regularly in bilateral anterior and posterior frontal areas (Schmidt et al. 2008). Accordingly, reduced glucose metabolism Nobler et al. (2001) found bilateral ECT to reduce metabolism in MDD patients in the frontal, prefrontal and parietal cortexes after a few days of treatment. Segawa et al. (2006) reported a reduction in regional cerebral blood flow in the left medial prefrontal area and the left limbic regions after ECT. The greater the improvement was the greater was the reduction. Henry et al. (2001) found decrease in metabolism in the right parietal lobe, right anterior and left posterior frontal lobes. This correlated with the response to ECT. In the same study relative increases of metabolism were found in the right basal ganglia, occipital lobe and brainstem possibly associated with dopaminergic innervations (Henry et al. 2001). However, an anterior hypofrontality was detected in the SPECT of MDD patients at baseline compared to controls (Navarro et al. 2004). After 12 months of follow-up AD and ECT treatments this hypofrontality disappeared. In the PET study by McCormick et al. (2007) the antidepressive effect of ECT was associated with increased metabolism in the left subgenual anterior cingulum and hippocampus. In addition, during bifrontal ECT the cerebral blood flow increased in the prefrontal and anterior cingulate cortex and during bitemporal ECT it increased in lateral frontal cortex and anterior temporal lobes (Blumenfeld et al. 2003). The cerebral blood flow was also found to be increased in basal ganglia, brain-stem, diencephalon, amygdala, vermis and the frontal, temporal and parietal cortices during bitemporal ECT, whereas soon after ECT decreases were found in the anterior cingulate and medial frontal cortex (Takano et al. 2007).

#### 1.2.5.4 *Transcranial magnetic stimulation*

Treatment with TMS changes regional cerebral blood flow in peri-insular cortex examined with SPECT and this may correlate to the treatment effect (Mottaghy et al. 2002). If the cerebral blood flow is high in this area before TMS the response to it may be positive. TMS also reversed left-right asymmetry in regional cerebral blood flow. High frequency (20 Hz) rTMS is associated with increases in regional cerebral blood flow in the prefrontal cortex, cingulate gyrus, left amygdala, bilateral insula, bilateral thalamus, bilateral hippocampus and other limbic structures (Speer et al. 2000). However, low frequency (1 Hz) rTMS is associated with decreases in the right prefrontal cortex, left amygdala, left medial temporal cortex and left basal ganglia studied with PET.

#### 1.2.5.5 *Vagus nerve stimulation*

In a SPECT study it was shown that VNS induced changes in regional cerebral blood flow resembling those seen with ADs (Zobel et al. 2005). In a PET study VNS increases of cerebral blood flow were found in the bilateral orbitofrontal cortex, bilateral anterior cingulate cortex and right superior and medial frontal cortex (Conway et al. 2006). Decreases were found in the bilateral temporal cortex and right parietal area.

#### 1.2.5.6 *Deep brain stimulation*

DBS of the nucleus accumbens relieved the symptoms of patients with treatment resistant depression and significantly increased metabolism in the nucleus accumbens, amygdala, and DLPFC and DMPFC and decreased metabolism in the ventral and ventrolateral medial prefrontal cortex after one week of stimulation imaged with PET (Schlaepfer et al. 2007). DBS of the subgenual cingulate region increased the cerebral blood flow in prefrontal cortex and also reversed the pretreatment abnormally elevated subgenual cingulate blood flow (Mayberg et al. 2005). DBS to the subcallosal cingulate gyrus produced metabolism decreases in orbital, medial frontal cortex and insula and increases in lateral prefrontal cortex, parietal, anterior midcingulate and posterior cingulate areas (Lozano et al. 2008).

## 1.2.6 Treatment of subtypes of Major Depressive Disorder

Some specific aspects of treatment methods concerning the subtypes of MDD in this series of studies are reviewed here.

### 1.2.6.1 *Treatment of treatment resistant Major Depressive Disorder*

For patients who do not benefit from the initial adequate AD trial the next-step strategies are switching within and between classes of ADs, augmentation or combination treatments and also adding or switching to psychotherapy. There are no established data on which of these strategies is better. In STAR\*D study bupropion, sertraline and venlafaxine were found to have quite similar outcomes, tolerability and adverse events as second step alternatives (Rush et al. 2006c). The remission rate after switching from inefficient citalopram was around 25 % in each alternative drug switched to. Bupropion and buspirone augmentation with SSRI resulted in around 30 % remission rate after an unsuccessful trial with SSRI alone, bupropion was better tolerated (Trivedi et al. 2006a). After two unsuccessful trials with ADs, augmentation of tri-iodothyronine with ongoing AD resulted in around 25 % remission rate and around 16 % rate was reported with lithium augmentation but the difference in remission rates was not significant (Nierenberg et al. 2006). After three unsuccessful treatment trials with ADs a switch to tranylcypromine (irreversible MAOI, not available in Finland) resulted in a remission rate of about 7 % and venlafaxine together with mirtazapine in about 14 % remission rate. The latter combination was better tolerated and easier to use, but the difference in remission rates was not significant (McGrath et al. 2006). Olanzapine and fluoxetine in combination may also be effective in some cases of TRD (Thase et al. 2007a).

ECT is one of the eligible strategies for TRD (APA 2001, UK ECT Review Group 2003). It is reported that 50-60% of AD resistant patients respond to ECT and achieve remission (Devanand et al. 1991, Prudic et al. 1996, Khalid et al. 2008). DBS should also be considered in ultimate TRD cases (Jimenez et al. 2005, Mayberg et al. 2005, Kennedy and Giacobbe 2007, Kosel et al. 2007, Schlaepfer et al. 2007, Lozano et al. 2008, Malone et al. 2009).

### 1.2.6.2 *Treatment of psychotic Major Depressive Disorder*

Adding an antipsychotic to AD in the treatment of psychotic depression is recommended because the outcome with AD alone is poor. Only around 20 % of these patients respond to AD alone. TCAs may be more effective than newer ADs in psychotic depression (Wijkstra et al. 2006). Response rates in psychotic depression were 64 % for combined olanzapine and fluoxetine treatment compared to 35 % for

olanzapine alone and 28 % for placebo (Rothschild et al. 2004). Patients with psychotic depression had poorer response to combined AD and psychological treatment in a 6-month follow-up study than nonpsychotic patients (Gaudiano et al. 2005).

ECT is a highly effective treatment in psychotic depression, 95% remission rate has been reported and remission also occurs earlier in psychotic depression than in nonpsychotic depression (Petrides et al. 2001). Treatment response is thus suggested to be better in psychotic than in nonpsychotic depression (Petrides et al. 2001, Birkenhäger et al. 2003). Birkenhäger et al. (2003) found that the relapse rate at 4 and 12 months after the response to ECT was lower in psychotic than in nonpsychotic patients. In a 2-year follow-up study in elderly psychotic MDD patients who had remitted with ECT and nortriptyline, the combination of these was better in continuation/maintenance than nortriptyline alone (Navarro et al. 2008). Because psychotic depression is associated with factors that can be life-threatening, ECT should be considered as a first-line treatment method for such patients (Maixner and Taylor 2008).

#### *1.2.6.3 Treatment of late-onset Major Depressive Disorder*

The treatment methods in late-onset depression are the same as those with MDD in general. However, hospital admission should be considered earlier than with younger patients at least if there is a lack of social support (Joska and Stein 2008). In elderly patients lower dosage of AD at initiation should be considered, although the response may be slower than in younger patients. TCAs have adverse effects especially harmful to elderly patients and it is obvious that newer ADs are better tolerated. ECT should also be considered because its response may be even better in elderly than in middle-aged patients (O'Connor et al. 2001). ECT is generally considered to be safe for elderly patients (Mulsant et al. 1991, Manly et al. 2000).

#### **1.2.7 Genetics of the treatment response in Major Depressive Disorder**

In recent years more genetic studies on treatment response in MDD have been published. Most of these have focused on the genetic factors associated with AD drug treatments based on monoamine theory. Other treatment methods are studied rarely in this context. There is a great deal of negative and contradictory data, and only some associations are presented here. Every polymorphism associated with the neurotransmitters, receptors or the signal transduction molecules in brain monoamine circuits can affect the response to different treatment methods. These studies are seeking an effective treatment method for individual patients based on their genetic profiles.

### 1.2.7.1 *Psychotherapies*

So far there is no published data on the association between genetic polymorphisms and treatment response to any psychotherapies in mood disorders. Psychotherapies are suggested to affect the receptor density in the brain but genetic variations associated with these have not yet been reported.

### 1.2.7.2 *Antidepressants*

5-HTTLPR polymorphism has been studied extensively because SERT is the major target of most antidepressants. Better response to fluvoxamine (100-300 mg) was found in 5-HTTLPR polymorphism l allele carriers (Caucasian MDD inpatients, n=53) assessed with Hamilton Rating Scale for Depression (HRSD, Hamilton 1960, Hamilton 1967) compared to ss genotype (p=0.0012) (Smeraldi et al. 1998). Likewise Caucasian inpatients diagnosed for MDD (n=58) carrying l allele showed a better and faster treatment response to paroxetine (40 mg for four weeks) assessed with HRSD compared to ss genotype (p=0.0001) (Zanardi et al. 2000). Faster treatment response to sertraline (50-100 mg) was also found in a sample of 106 Caucasian outpatients with ll genotype (compared to s allele carriers, p=0.01) (Durham et al. 2004). With Caucasian MDD outpatients (n=131) citalopram treatment (20-40 mg) resulted in nonremission in 12 week follow-up mostly with ss genotype carriers (p=0.006, OR=3.23) (Arias et al. 2003). Inpatients with MDD and bipolar depression (n=88) carrying ss genotype had poorer response in six weeks of treatment with fluvoxamine (100-300 mg) (p=0.029) (Zanardi et al. 2001). In a study by Pollock et al. (2000) s allele carriers showed a slower response to paroxetine (20-30 mg) (p=0.027) but there was no difference in number of responders compared to ll genotype (n=51 MDD out-, and inpatients, scale HRSD). Caucasian inpatients (n=220), diagnosed for MDD (n=127) and bipolar depression (n=93) were treated with fluvoxamine (300 mg) and paroxetine (20-40 mg) for six weeks (Serretti et al. 2004b). Patients with ss genotype had poorer response compared to l allele carriers (p=0.034). In a study by Bozina et al. (2008) s allele carriers of 130 Caucasian MDD patients treated with paroxetine (20 mg for six weeks) showed poorer response (assessed with HRSD) compared to ll genotype (p=0.0004). The ss genotype was associated with poorer response to fluoxetine and nortriptyline treatment in MDD patients older than 25 years (Joyce et al. 2003). Less favourable effect of SSRIs in Caucasian patients carrying ss genotype compared to l allele carriers was found in a meta-analysis (Smits et al. 2004). Serretti et al. (2007) found that patients carrying l allele showed significantly better response compared to ss genotype (p=0.004) in a sample of 106 Caucasian MDD patients assessed with HRSD and treated with SSRIs for six weeks. Smits et al. (2008) found a less favourable response to SSRI treatment in women and younger (<44 years old) MDD patients associated with s allele.

In addition in Asian patients the results have been contradictory, suggesting better treatment response even with s allele and poorer outcome with ll genotype.



Better response to fluoxetine (20-50 mg) and paroxetine (20-60 mg) in six weeks was found with 120 Asian MDD and bipolar depression patients carrying ss genotype compared to l allele carriers ( $p=0.0074$ ) (Kim et al. 2000). In Asian MDD patients ( $n=66$ ) s allele frequency was higher in responders than in nonresponders ( $p=0.01$ ) (Yoshida et al. 2002a). Likewise, ss genotype carriers had better response to SSRIs in 136 Asian MDD patients compared to l allele carriers ( $p=0.03$ ) (Kim et al. 2006). However, better response with ll genotype versus s allele carriers has also been reported in Asians. Fluoxetine (20-60 mg) treated MDD patients ( $n=121$ , Asian) carrying ll genotype showed a better response assessed with HRSD ( $p=0.013$ ) compared to s allele carriers (Yu et al. 2002). Asian 224 MDD patients treated with fluoxetine had better treatment response assessed with HRSD when carrying ll genotype compared to s allele carriers ( $p<0.001$ ) (Hong et al. 2006). A similar finding was suggested by Kato et al. (2006) in a sample of 100 Asian MDD patients treated with paroxetine (20-40 mg) and fluvoxamine (50-150 mg) for four weeks, l allele carriers were more often responders compared to ss genotype ( $p=0.025$ ).

Another SERT polymorphism, SERTin2 has also been studied. Patients carrying ss genotype had better treatment response to paroxetine treatment compared to ls and ll genotype ( $p=0.035$ ) (Bozina et al. 2008). Conversely, Kim et al. (2000, 2006) found in two different Korean MDD samples that ll genotype carriers had better treatment response to SSRIs compared to s allele carriers ( $n=120$  and  $136$ ,  $p<0.0001$  and  $p=0.003$  respectively).

5-HT1A polymorphism C1019G (rs6965) has been shown to be associated with treatment response to ADs, GG genotype carrying MDD patients ( $n=137$ , Asian) had better response than C allele carriers ( $p=<0.0001$ ) (Kato et al. 2008). Some previous reports have also suggested similar findings in MDD (Arias et al. 2005, Hong et al. 2006, Yu et al. 2006) and bipolar depression (Serretti et al. 2004a). In addition, a poorer treatment response to SSRIs was found in Caucasian melancholic MDD patients ( $n=98$ ) carrying the C1019G polymorphism CC genotype compared to G allele carriers ( $p=0.02$ ) (Baune et al. 2008). 5-HT1A polymorphism rs10042486 CC genotype and rs1364043 TT were also associated with AD treatment response (Kato et al. 2008). 5-HT2A encoding gene second intron SNP rs7997012 AA genotype was associated with better treatment outcome in MDD patients treated with citalopram at least six weeks than GG genotype in a large sample of 1,380 STAR\*D patients ( $p<0.0001$ ), although treatment was less effective among black patients who also had A allele six times less frequently than white patients (McMahon et al. 2006). The 5-HT2A -1438A/G GG genotype was associated with a good response to SSRIs (Kato et al. 2006). The 5-HT3A 178C/T TT genotype was also associated with treatment response to SSRI.

TPH1 polymorphism A218C AA genotype carriers with MDD and bipolar depression ( $n=217$ , Caucasian) had slower response to fluvoxamine treatment (300 mg for six weeks) than C allele carriers ( $p=0.001$ ) (Serretti et al. 2001b). The same polymorphism A allele carriers with MDD and bipolar depression ( $n=121$ ,

Caucasian origin) showed a poorer response to paroxetine treatment (20-40 mg for four weeks) than those with CC genotype ( $p=0.005$ ) (Serretti et al. 2001a). A trend in the same direction was found in a different sample ( $n=221$  inpatients) diagnosed for MDD and bipolar depression with this same polymorphism (Serretti et al. 2004b). AA genotype suggested poorer response to SSRI treatment in six weeks compared to C allele carriers ( $p=0.09$ ). In addition, carrying the A allele of this same polymorphism predicted poorer remission rate in citalopram treatment (10-20 mg for eight weeks) than CC genotype ( $p=0.017$ ) in MDD patients ( $n=105$ , Asian) (Ham et al. 2007). Some other TPH2 polymorphisms (rs2171363, rs10897346 and rs1487278) have also been associated with antidepressant treatment response in two different studies (Tzvetkov et al. 2008, Tsai et al. 2009b).

Serum BDNF levels are elevated following treatment with ADs (Shimizu et al. 2003, Aydemir et al. 2006, Yoshimura et al. 2007, Huang et al. 2008, Piccinni et al. 2008, Sen et al. 2008), although different ADs may have different effects on BDNF levels (Matrisciano et al. 2009). Choi et al. (2006) found that the Val66Met polymorphism of BDNF was associated with efficacy of citalopram (10-60 mg for eight weeks) in Asian MDD patients ( $n=83$ ). Met-allele carriers were more often responders than non-responders ( $p=0.003$ ,  $OR=4.375$ ). Tsai et al. (2003) found a trend for improved fluoxetine (20-60 mg) treatment response in a four-week trial in Asian MDD patients ( $n=110$ ) carrying BDNF Val66Met polymorphism Val/Met genotype in comparison to both homozygous patients ( $p=0.086$ ). In addition, heterozygote MDD patients of Asian origin ( $n=134$ ) using either milnacipran (50-100 mg) or fluvoxamine (50-200 mg) for six weeks had better treatment response compared to homozygote patients ( $p=0.029$ ) (Yoshida et al. 2007). The AD used did not influence the result.

The COMT polymorphisms' association with treatment response to different ADs is contradictory. Most studies report of COMT Val108/158Met (rs4680) polymorphism. In a study by Tsai et al. (2009a) a poorer response to fluoxetine (20-40 mg for eight weeks) was found in Chinese MDD patients ( $n=344$ ) with COMT Val158Met Val/Val genotype compared to Met allele carriers ( $p=0.02$ ). On the other hand MDD patients ( $n=102$ , Caucasian) carrying Val108/158Met polymorphism Met/Met genotype had slower and poorer response to mirtazapine treatment (45 mg for six weeks) than patients carrying Val allele ( $p=0.015$ ), but no association was found with paroxetine (20-40 mg) treatment (Szegedi et al. 2005). Arias et al. (2006) found Met/Met genotype to be associated with increased risk for nonremission in Caucasian inpatients with MDD ( $n=139$ ) after 6-8 weeks' ( $p=0.006$ ) treatment with citalopram (20-40 mg) but this effect was not any more found after 12 weeks treatment. On the contrary, the Met/Met genotype of the same COMT polymorphism was associated with faster therapeutic effect of milnacipran (100 mg for six weeks) in Japanese MDD patients ( $n=81$ ,  $p=0.011$  for Val/Met and  $p=0.011$  for Val/Val) (Yoshida et al. 2008). COMT Val108/158Met polymorphism was also associated with response to paroxetine treatment (mean dose 31.64 mg) in Caucasian MDD patients ( $n=55$ ) (Benedetti et al. 2009). Val/Val homozygotes had worse HRSD scores than Met/Met homozygotes after four weeks trial ( $p=0.0317$ ).

Furthermore, COMT Val158Met Val/Val genotype carriers had worse AD treatment response compared to heterozygote Val/Met genotype carriers ( $p < 0.0001$ ) in Caucasian MDD and bipolar depression ( $n = 256$ ) (Baune et al. 2008). Another COMT SNP rs165599 GG genotype was found to be associated with response and remission in MDD patients treated with duloxetine (Perlis et al. 2009).

GNB3 C825T polymorphism TT genotype carriers in a Caucasian population of 76 MDD patients have been reported to show a better response to AD treatment (four weeks) than C allele carriers ( $p = 0.012$ ) (Zill et al. 2000). In a sample of 490 Caucasian patients (bipolar depression  $n = 200$ , MDD  $n = 290$ ) treated either with fluvoxamine (300 mg) or with paroxetine (40 mg) TT genotype carriers showed better treatment response compared to other genotypes ( $p = 0.009$ ) (Serretti et al. 2003b). T allele was associated with better response to antidepressant treatment in Asian MDD patients ( $n = 106$ ,  $p = 0.035$ ) (Lee et al. 2004). However, young (under 25-year-old) Caucasian MDD patients ( $n = 169$ ) showed a T allele association with poorer response to nortriptyline (Joyce et al. 2003). Moreover, in Caucasian MDD patients ( $n = 166$ ) TT genotype carrying resulted in worse treatment outcome both with initial AD ( $p = 0.04$ ) and with second switch therapy ( $p = 0.03$ , OR 0.26) (Wilkie et al. 2007).

Female MDD patients homozygous for the T allele of MAOA T941G polymorphism responded better and faster to mirtazapine treatment than female patients with G allele (Tadić et al. 2007).

MDD patients carrying the C allele of the alpha-2-adrenergic receptor gene (ADRA2A) polymorphism C-1297G fared better improvement than GG genotype carriers in milnacipran treatment (Wakeno et al. 2008).

The gene coding dopamine transporter (DAT1) VNTR polymorphism 9-repeat allele predisposed to poorer or nonresponse to AD therapy in MDD patients compared to 10/10 genotype carriers (Kirchheiner et al. 2007). Smaller number of rapid responders was found in 9/9 genotype carriers than in 10-repeat allele carriers.

Response to nortriptyline was associated with the NET G1287A polymorphism. GG genotype had a better response compared to pooled GA and AA genotypes in Chinese MDD patients (Kim et al. 2006).

Associations of some polymorphisms with treatment response to ADs are presented in Table 2.

Table 2. Gene polymorphisms associated with treatment response to ADs

<b>Gene</b>	<b>Variant</b>	<b>Association</b>	<b>Study reference</b>	<b>N Ethnicity Scale</b>	<b>P (OR)</b>	
<b>SERT</b>	5-HTTLPR	l allele or ll genotype faster response to sertraline	Durham et al. 2004	206, Caucasian, HRSD	0.01	
		ll genotype and l allele better response to fluvoxamine	Smeraldi et al. 1998	53, Caucasian, HRSD	0.0012	
		l allele carriers better response to SSRIs	Serretti et al. 2007	106, Caucasian, HRSD	0.004	
		ss genotype poorer response to citalopram	Arias et al. 2003	131 Caucasian, HRSD	0.006 (3.23)	
		ss genotype poorer response to fluvoxamine	Zanardi et al. 2001	88, Caucasian, HRSD	0.029	
		ss genotype poorer response to SSRIs	Serretti et al. 2004b	200, Caucasian, HRSD	0.034	
		s allele poorer response to paroxetine	Bozina et al. 2008	130, Caucasian, HRSD	0.0004	
		s allele slower response to paroxetine	Pollock et al. 2000	51, Caucasian, HRSD	0.027	
		ss genotype better response to fluoxetine or paroxetine	Kim et al. 2000	120, Asian, HRSD	0.0074	
		ll genotype better response to fluoxetine	Yu et al. 2002	121, Asian, HRSD	0.013	
		ss genotype better response to fluoxetine or sertaline	Kim et al. 2006	136, Asian, HRSD	0.03	
		s allele frequency was higher in responders than in nonresponders	Yoshida et al. 2002a	66, Asian, MADRS	0.01	
		ll genotype better response to fluoxetine	Hong et al. 2006	224 Asian, HRSD	<0.001	
		SERTin2	ss genotype better response to paroxetine	Bozina et al. 2008	130, Caucasian, HRSD	0.035
		ll genotype better response to SSRIs	Kim et al. 2000	120, Asian, HRSD	< 0.0001	
	ll genotype carriers a better response to SSRIs	Kim et al. 2006	136, Asian, HRSD	0.003		

<b>Gene</b>	<b>Variant</b>	<b>Association</b>	<b>Study reference</b>	<b>N Ethnicity Scale</b>	<b>P (OR)</b>
<b>BDNF</b>	Val66Met	Met allele better response to citalopram	Choi et al. 2006	83, Asian, HRSD	0.003 (4.38)
		Val/Met heterozygote better response to ADs	Tsai et al. 2003	110, Asian, HRSD	0.086 (trend)
		Val/Met heterozygote better response to ADs	Yoshida et al. 2007	134, Asian, MADRS	0.029
<b>5-HT1A</b>	C1019G	GG genotype better response to Ads	Kato et al. 2008	137, Asian, HRSD	<0.0001
		CC genotype poorer response to different ADs	Baune et al. 2008	98, Caucasian, HRSD, CGI	0.02
<b>5-HT2A</b>	rs 7997012	AA genotype better response to citalopram	McMahon et al. 2006	1,380, white and black, QIDS-C <sub>16</sub>	<0.0001
<b>TPH1</b>	A218C	AA genotype carriers slower response to fluvoxamine	Serretti et al. 2001b	217, Caucasian, HRSD	0.001
		A allele poorer response to paroxetine	Serretti et al. 2001a	121, Caucasian, HRSD	0.005
		AA genotype poorer response to SSRI	Serretti et al. 2004b	221, Caucasian, HRSD	0.09 (trend)
<b>GNB3</b>	C825T	TT genotype better response to AD treatment	Zill et al. 2000	76, Caucasian, HRSD	0.012
		TT genotype better response to fluvoxamine and paroxetine	Serretti et al. 2003b	490, Caucasian, HRSD	0.009
		T allele better response to AD treatment	Lee et al. 2004	106, Asian, HRSD	0.035
		TT genotype worse outcome with ADs	Wilkie et al. 2007	166, Caucasian, HRSD	initial AD 0.04 second switch therapy 0.03 (0.26)

<b>Gene</b>	<b>Variant</b>	<b>Association</b>	<b>Study reference</b>	<b>N Ethnicity Scale</b>	<b>P (OR)</b>
<b>COMT</b>	Val158Met	Val/Val genotype poorer response to fluoxetine	Tsai et al. 2009a	344, Asian, HRSD	0.02
		Met/Met genotype slower and poorer response to mirtazapine	Szegedi et al. 2005.	102, Caucasian, HRSD	0.015
		Met/Met genotype increased risk for nonremission with citalopram in 6-8 weeks	Arias et al. 2006	139, Caucasian, HRSD	0.006
		Met/Met genotype associated with faster therapeutic effect of milnacipran treatment	Yoshida et al. 2008	81, Asian, MADRS	0.011
		Val/Val genotype worse response to paroxetine	Benedetti et al. 2009	55, Caucasian, HRSD	0.0317
		Val/Val genotype worse response to ADs	Baune et al. 2008	256, Caucasian, HRSD	<0.0001

### 1.2.7.3 *Electroconvulsive therapy*

Genetic studies on treatment response to ECT are rare. In a recent study 5-HTTLPR l/s polymorphism was not found to be associated with the outcome of ECT (Rasmussen et al. 2009). COMT Val158Met polymorphism Val allele was associated with better treatment response to ECT (Domschke et al. 2009). Apolipoprotein E4 allele carriers were more likely to respond to ECT treatment (Fisman et al. 2001) but this finding was not replicated later on (Huuhka et al. 2005). In a recent study by Stewart et al. (2009) angiotensin I converting enzyme polymorphism was not found to be associated with treatment response to ECT.

## 1.2.8 Outcome

MDD is a chronic illness with a high lifetime risk of recurrence up to 85% (Mueller et al. 1999). In two to five-year follow-up the recurrence rate was 46-74 % (Piccinelli and Wilkinson 1994). The duration of an MDE is reported to be around 3 to 4 months (Spijker et al. 2002, Kessler et al. 2003). The recovery rate after one year was about 64% within a year of the index episode about 25% had a recurrence while 15 % were still depressed (Piccinelli and Wilkinson 1994). The more AD treatment steps are required the fewer patients benefit at every step (Rush et al. 2006b). However, more than 80 % were reported to have recovered after two years (Coryell et al. 1987). The most serious complication of MDD is suicide. In 2007 there were altogether 995 suicides in Finland and of these 817 were among those of working age (Tilastokeskus 2008). Preventing chronicity and recurrence of MDD is the main aim of the treatment (APA 2000b, Suomen Psykiatriyhdistys 2004).

Response to the treatment of MDD is defined as clinically significant degree of depressive symptom alleviation following treatment initiation, usually  $\geq 50\%$  reduction in pretreatment symptom severity (Rush et al. 2006a) assessed e.g. by the Montgomery and Åsberg Depression Rating Scale (MADRS, Montgomery and Åsberg 1979) or HRSD. Response is heavily dependent on the initial pretreatment symptom severity and its ascertainment requires the systematic assessment of symptoms before and after the treatment course. A clinically significant reduction in symptoms may lead to a wide variety of scores and the most severely depressed responding patients may still suffer from numerous depressive symptoms and have high scores even on post-treatment depression scales.

Remission is defined by the absence of the signs and symptoms of the illness for two months (APA 2000a). The patient also resumes the daily functioning that was habitual before the onset of symptoms. Remission is assessed by the clinician using rating scales such as MADRS and HRSD. In HRSD a score of  $\leq 7$  (Nierenberg and Wright 1999) and in MADRS a score between 5 and 12 has been suggested as a threshold of remission (Zimmerman et al. 2004a, Zimmerman et al. 2004b,

Zimmerman et al. 2004c, Rush et al. 2006a). In the Handbook of Psychiatric Measures, remission is set as a score of 7 on MADRS (Yonkers and Samson 2008). In partial remission symptoms of Major Depressive Episode (MDE) are present but full criteria are not met, or the period without symptoms of MDE has lasted less than two months (APA 2000a).

A relapse is the return of an MDE during the remission state. Recurrence is a return of MDE after at least two consecutive months of partial or full remission. Recovery occurs when the state of remission has lasted over 4 months. Patients with residual symptoms have a greater risk of relapse and recurrence compared to symptom-free patients (Rush et al. 2006a). MDD tends to recur (Keller 2003). 77.5% of patients in an Australian follow-up study of 5 years had a chronic or relapsing course (Wilson et al. 2003), a 40-50 % relapse rate has been reported by Spijker et al. (2002) in a follow-up of 2 years, and the risk of chronicity was considerable (20 %). Psychotic symptoms in MDD predict more severe depression and tendency to recurrence (Kessing 2003). In a one-year follow-up of elderly patients with MDD treated with AD or ECT the relapse rate was high (41 %) in both groups (Huuhka et al. 2004). Relapse rates in elderly depression have been reported to be higher than in the middle-aged (Mitchell and Subramaniam 2005).



## 2. Aims of the study

The general aim of this study was to investigate whether genetic factors, single nucleotide polymorphisms (SNPs) are associated with TRD or with treatment response to ECT. The SNPs were selected focusing on serotonergic and dopaminergic neurotransmission as well as neurogenesis.

The specific aims of this study were:

1. To study the association of BDNF polymorphisms G196A and C270T and the response to ECT in TRD patients. (I)
2. To study whether TRD associated with the interaction between 5-HT1A C1019G and BDNF G196A genotypes. (II)
3. To study the role of the interaction of TPH1 A218C and GNB3 C825T polymorphisms in TRD and in treatment response to ECT. (III)
4. To study the association of RGS4 polymorphism with TRD and with treatment response to ECT. (IV)
5. To study the effect of COMT polymorphism Val158Met in treatment response to ECT. (V)
6. To study the effect of DRD2 C957T polymorphism on TRD and in treatment response to ECT as well as the interaction of DRD2 C957T and COMT Val158Met. (VI)

## 3. Materials and methods

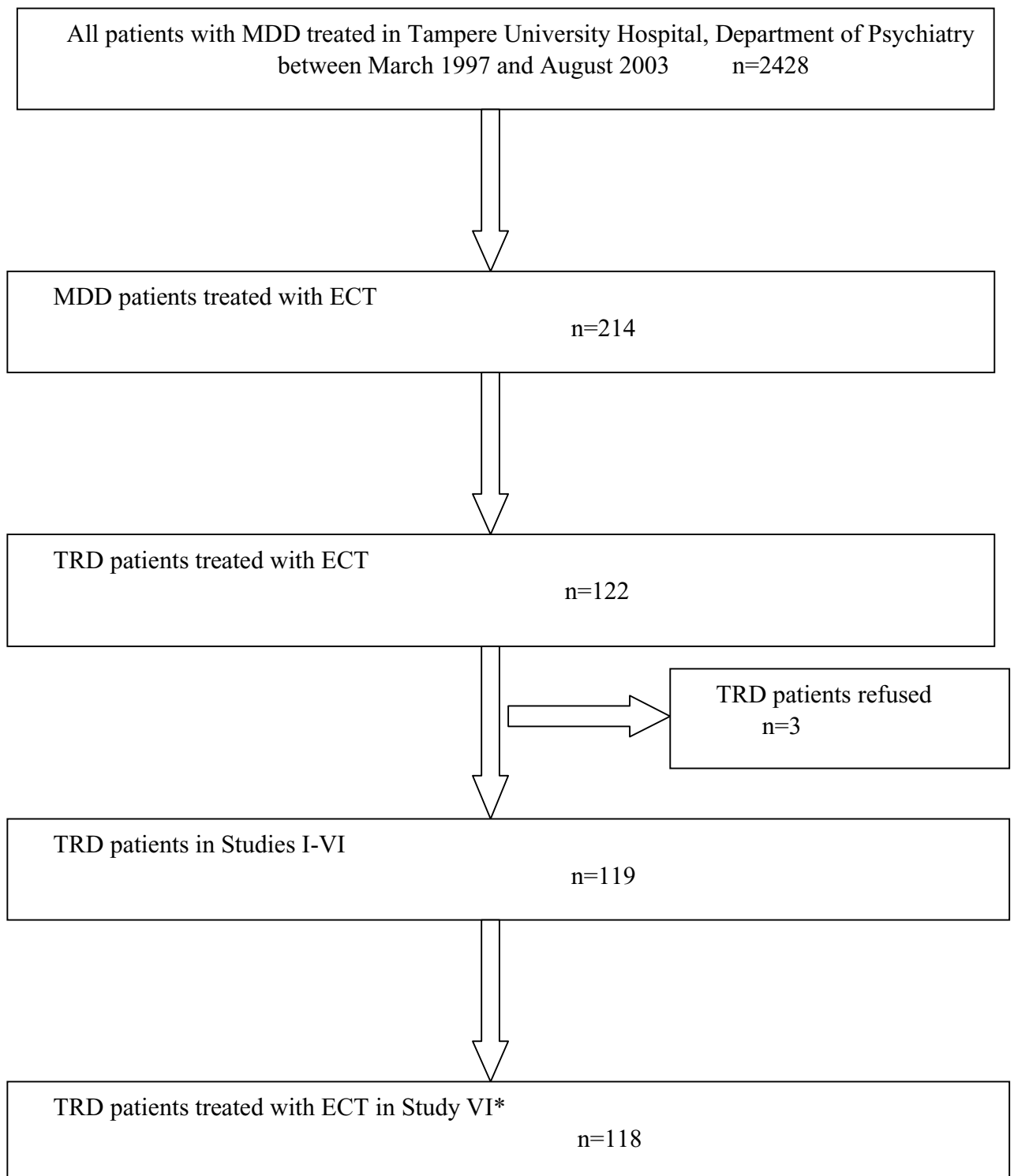
### 3.1 Patients and controls

#### 3.1.1 Patients

The study group consisted of 119 Finnish Caucasian patients suffering from TRD, consecutively admitted for ECT to the Department of Psychiatry, Tampere University Hospital. The patients (n=122) meeting the eligibility criteria were invited to participate in the study. Three of them declined and in Study VI one of them was excluded because the genotyping of DRD2 failed (n=118). The majority of the patients with TRD who were treated with ECT in this geographical region between March 1997 and August 2003 were recruited for this study (Figure 1.).

All the patients fulfilled the diagnostic criteria of DSM-IV for MDD (APA 1994), and 51 of them had psychotic symptoms. The diagnosis was made on a clinical basis by an experienced psychiatrist. All the patients were referred for ECT because of TRD. Treatment resistance to ADs was defined as two or more unsuccessful trials with antidepressants of different groups at an adequate dose for at least four weeks. Patients with neurological disorders, dementia, schizophrenia, bipolar disorder and alcohol or other substance abuse were excluded from the study. Of these patients 27.7 % were suffering from first episode of MDD and ECT was used after  $0.8 \pm 1.5$  years of suffering from TRD. The study group finally consisted of 65 women and 54 men (n=119), (in Study VI 64 women and 54 men, n=118). The mean age of the patients was  $57.7 \pm 14.0$  years, range 22-84 years. Blood samples were taken at the time of entry into the study and coded. The patients received their ongoing medication during the ECT course. Sociodemographic and medical data are given in Table 3. All except 12 patients had some psychotropic medication, (details in Table 3). Fifty-five patients had additional non-psychotropic medication. All the patients gave written informed consent. The study was approved by the Tampere University Hospital Ethics Committee.

Figure 1. Flow chart of the selection of the patients



\* 1 patient missing due to technical error

Table 3. Sociodemographic and medical data of the patients

	All Patients n=119	Males n=54	Females n=65	Responders n=45	Partial responders n=42	Non-responders n=32
Gender (M/ F)	54/65			22/23	14/28	18/14
Age, mean $\pm$ SD	57.7 $\pm$ 14.0	55.4 $\pm$ 12.5	59.5 $\pm$ 14.9	58.3 $\pm$ 12.1	58.7 $\pm$ 17.4	55.3 $\pm$ 11.4
Psychotic/ non-psychotic (n)	51/68	21/33	30/35	19/26	18/24	14/18
MADRS						
baseline, MADRS0, mean $\pm$ SD	32.5 $\pm$ 8.2	33.3 $\pm$ 8.7	31.9 $\pm$ 7.7	31.7 $\pm$ 9.0	32.6 $\pm$ 7.8	33.5 $\pm$ 7.7
after treatment, MADRS1, mean $\pm$ SD	11.3 $\pm$ 8.8	12.3 $\pm$ 9.5	10.4 $\pm$ 8.1	3.2 $\pm$ 2.4	10.9 $\pm$ 2.3	23.1 $\pm$ 6.4
Number of electroconvulsive treatments, mean $\pm$ SD	9.5 $\pm$ 1.7	9.7 $\pm$ 1.8	9.3 $\pm$ 1.6	9.3 $\pm$ 1.7	9.5 $\pm$ 1.6	9.7 $\pm$ 1.9
Age of onset	50.2 $\pm$ 15.3	49.4 $\pm$ 14.9	50.9 $\pm$ 15.7	49.8 $\pm$ 16.4	51.4 $\pm$ 16.9	49.3 $\pm$ 11.5
Early-onset/Late-onset depression ( $\leq$ 45 vs. $>$ 45 years) (n)	51/68	25/29	26/39	20/25	16/26	15/17
First episode/recurrent depression (n)	33/86	12/53	21/33	15/30	11/31	7/25
Proportion of patients using concomitant drugs %						
No psychotropic medication	9.2	3.4	5.9	4.4	9.5	15.6
Antidepressants	73.1	70.4	75.4	75.6	73.8	68.8
Antipsychotics	66.4	70.4	63.1	71.1	69.0	56.3
Anxiolytics or hypnotics	67.2	59.3	73.8	80.0	66.7	50.0
Non-psychotropic drugs	46.2	50.8	40.7	51.1	40.5	46.9

### 3.1.2 Controls

The controls were 392 (179 women and 213 men) (II), 398 (182 women and 216 men) (III), 384 (174 women and 210 men) (IV), 392 (V), 383 (VI) healthy blood donors from the Finnish Red Cross Blood Transfusion Service in Tampere, Finland. The mean age of the controls was  $44.4 \pm 11.1$  years, range 19-65 years. Only age and gender were available. The blood donors complete a written health statement, including information on neurological and mental health at every blood donating session. Blood donors are interviewed by a nurse before each donation and asked about medication, allergies, heart diseases, infectious diseases and other chronic diseases. Blood donors are not paid in Finland. All the patients and controls were Finnish citizens of Caucasian origin.

### 3.1.3 Selection of polymorphisms

The selection of the polymorphisms studied was based on the assumption that the serotonin- or dopamine-linked or neurotrophic and signal transduction associated polymorphisms may affect treatment resistance in MDD and treatment response to ECT.

## 3.2 Methods

### 3.2.1 Clinical assessment of patients

All the patients were evaluated before and after ECT treatment. Patients' psychiatric and medical histories were assessed, medical examination was carried out focusing particularly on neurological, cardiovascular and pulmonary systems. MADRS was used to assess the severity of depression (before ECT, MADRS0, after ECT, MADRS1) and the clinical change (MADRS0-MADRS1). Five experienced psychiatrists assessed the MADRS. All personnel were blind to the genetic data. Age at first onset of MDD was defined using information from the medical records and the patient's interview. Cognition was assessed in 78 patients using the Mini-Mental State Examination Scale (MMSE) (Folstein et al. 1975).

### 3.2.2 Procedure in electroconvulsive therapy

All the patients were treated at the ECT treatment unit in the Psychiatric Clinic of Tampere University Hospital. The ECT team included a psychiatrist, an anesthesiologist, a treatment nurse and a recovery area nurse.

ECT was administered three times a week with a brief pulse constant current device (Thymatron DGx, Somatics, Inc., Lake Bluff, IL, USA). The initial stimulus dosage was adjusted with the age method for 30 patients (Swartz and Abrams 1996). The seizure threshold was determined for 89 patients by administering successive stimuli of increasing intensity until generalized seizure was induced. Anesthesia was induced with methohexital and muscle relaxation with succinylcholine. The initial dose was 1 mg/kg of methohexital and 0.5 mg/kg of succinylcholine. The patients were ventilated with 100 % oxygen until resumption of spontaneous respiration. Physiological monitoring included pulse oximetry, blood pressure, ECG, one channel EEG and EMG.

The criterion for adequate generalized seizure duration was at least 20 seconds of motor response and 25 seconds of EEG seizure activity. If needed, at subsequent treatments during the course of ECT, the dosage was increased to maintain adequate seizure duration. All the patients were treated with standard bilateral (bifrontotemporal) ECT, the number of treatments ranged between seven and 15,  $9.4 \pm 1.79$  (mean  $\pm$  SD). The total number of treatments administered was determined by clinical judgment. ECT was continued until patients were symptom-free or had received at least eight treatments without any further improvement being noted during the past two treatments.

### 3.2.3 Genotyping of the polymorphisms

Genomic DNA was extracted from peripheral blood leukocytes using QIAamp®DNA Blood Minikit and automated biorobot M48 according to manufacturer's instructions (Qiagen, Hilden, Germany). DNA samples were genotyped using the 5' nuclease polymerase chain reaction (PCR) assays with TaqMan MGB probes (Livak 1999). The 5' ends of the allele-specific probes are fluorescently labeled with either FAM or VIC reporter dyes (corresponding to the two alleles). At the 3' end, there is a nonfluorescent quencher and a MGB molecule which binds to the minor groove of the DNA helix and thus improves hybridization-based assays by stabilizing the MGB-probe/template complex. For the genotyping of DRD2 C957T (rs6277), TPH1 A779C (rs 1799913), and GNB3 C825T (rs6489738) pre-designed assays (C\_11339240\_10, C\_2645661\_10, C\_2184734\_10) were available from Applied Biosystems. If no pre-designed assay was available the nucleotide sequences of primers and probes used in the PCR were deduced from published sequences deposited in the GenBank and Celera databases and custom assays were designed by Applied Biosystems' design service (Foster City, CA, USA). Custom assays were used in the genotyping of TPH1 A218C (rs1800532), COMT Val158Met (rs4680), BDNF C270T (rs56164415), BDNF G196A (rs6265), 5-HT1A C1019G (rs6295), RGS4 (rs951436).

PCR reaction containing genomic DNA, 1xUniversal PCR Master Mix, 900 nM of each primer and 200 nM of each probe was performed in 96-well plates using the TaqMan Universal Thermal Cycling Protocol in a total volume of 25  $\mu$ l. After PCR,

end-point fluorescence intensity was measured by the ABI Prism 7900HT Sequence Detection System (Applied Biosystems) and allelic discrimination performed resulting in clear identification of different genotypes for polymorphisms. The performance of the assays was monitored by analyzing random duplicate samples and by including negative controls (no DNA template in the reaction) in the analysis. Genotyping was always performed without knowledge of the clinical data.

### 3.2.4 Statistical methods

#### 3.2.4.1 *Association between treatment resistant Major Depressive Disorder and studied polymorphisms*

Pearson's chi-square ( $\chi^2$ ) test was used to compare genotypes and allele frequencies between the different study groups (II, III, IV, VI). OR was calculated with 95% confidence interval (CI) (II, III). Multiple logistic regression was used to calculate gene-gene interaction in relation to the studied trait (II, III).

#### 3.2.4.2 *Association with response to electroconvulsive therapy*

For statistical analyses the remission after ECT was defined in different ways.

1. MADRS endpoint score less than 8 was considered as remission and more than 15 points as non-response. By this criterion, patients with MADRS score between 8 and 15 were excluded from the efficacy analyses due to partial response (I-VI).
2. By using one cutpoint, MADRS score less than 8 = remission, MADRS score 8 or more = partial or non-response (I in subgroups, IV).
3. In post hoc analysis using MADRS score 10 as one cutpoint respectively (III).

The age of 45 years was used as a cut-off between early and late onset depression, as in some earlier studies (Fisman et al. 2001, Krishnan et al. 1996, Zubenko et al. 1996) (I).

Analysis of variance was used to compare the means of MADRS0 and MADRS1 scores in remitter and non-responder groups likewise in subgroups of psychotic and late-onset depression (I, V, VI). The OR was calculated with 95% CI using binary logistic regression analysis (III, V, VI). For analyzing the association between MADRS1 score and genotype, general linear model was used with MADRS1 score as a dependent variable (IV). In this analysis, patient's age, gender, age at onset, psychotic or non-psychotic depression, first episode or recurrent depression, the number of ECT treatments and MADRS baseline score were used as covariates (IV). The analysis of covariance with genotype as a fixed factor and age, gender,

age at onset, psychotic/non-psychotic depression, first episode/recurrent depression, the number of ECT and MADRS baseline score as covariates was used to analyze the association with genotype and the endpoint MADRS score (IV).

T-test (VI) and analysis of variance (V, VI) were used to analyze differences between genotypes in MADRS0 and in MADRS1 respectively.

The limit of statistical significance was set at 0.05. Data analysis was carried out using SPSS/Win software (Version 14.0, SPSS Inc., Chicago, IL, USA).



## 4. Results

### 4.1 Association between treatment resistant Major Depressive Disorder and studied polymorphisms

The BDNF polymorphisms G196A and C270T were not associated with TRD compared to controls (I, unpublished data). 5-HT1A C1019G polymorphism alone was not associated with TRD (II). However, the combination of 5-HT1A GG genotype and BDNF G196A GA+AA genotypes was associated with TRD compared to controls. (OR=3.18; 95% CI 1.32-7.68; p=0.007) (II).

TPH1 A218C polymorphism CC genotype was more frequent in patients than in controls (compared to pooled other genotypes) (OR=1.96; 95% CI 1.28-3.01; p=0.002) (III). In males CC genotype was also more frequent in patients compared to controls (OR 2.33; 95% CI 1.27-4.28; p=0.006) but not in females. In the whole patient population GNB3 C825T polymorphism was not associated with TRD, but female patients had CC genotype vs. pooled CT+TT genotypes less frequently compared to controls (OR=0.52; 95% CI 0.29-0.92; p=0.023). However, there was an opposite trend in males (OR=1.68; 95% CI 0.90-3.12; p=0.101). TPH1 and GNB3 pooled genotypes (CC vs. CA+AA for TPH1 and CC vs. CT+TT for GNB3) had a significant interaction for the risk of being a patient (p=0.013). TPH1 CC genotype and GNB3 CT+TT genotypes together were associated with increased risk of being a patient in females (OR=4.22; 95% CI 1.80-9.86; p=0.0004). In males the risk of being a patient was associated with a combination of TPH1 CC genotype and GNB3 CC genotype (OR=2.34; 95% CI 1.18-4.58; p=0.013) (III).

RGS4 rs 951436 genotypes were not associated with TRD compared to controls (IV). COMT Val158Met and DRD2 C957T polymorphisms alone or their combined effect was not associated with TRD (V, VI). However, the patients with the combination of DRD2 TT genotype and COMT Val/Met+Met/Met genotypes assumed to be the HIGH dopamine activity group had higher MADRS0 scores (37.2±7.7) than the patients with the combination of DRD2 CC+CT genotypes together with COMT Val/Val genotype assumed to be the LOW dopamine activity group (MADRS0 31.0±8.6) or the remaining combinations (REST group) (MADRS0 31.2±7.7) (p=0.004) (VI).

The associations of the studied polymorphisms with TRD are presented in Table 4.

Table 4. Association of the studied polymorphisms with TRD.

Article	Polymorphism	Whole group	Males	Females
I	BDNF G196A	- *	- *	- *
I	BDNF C270T	- *	- *	- *
II	5HT1A C1019G	-	-*	-*
II	BDNF G196A+5HT1A C1019G	+ (p=0.007 OR=3.18) GA+AA together with GG	+*(p=0.005 OR=6.50)	-*
III	TPH1 A218C	+ (p=0.002 OR=1.96) CC	+ (p=0.006 OR=2.33) CC	-
III	GNB3 C825T	-	(+) (p=0.101 OR=1.68) CC more frequent in pat.	+ (p=0.023 OR=0.52) CC less frequent in pat.
III	TPH1 A218C+GNB3 C825T	-*	+ (p=0.013 OR=2.34) CC together with CC	+ (p=0.0004 OR=4.22) CC together with CT+TT
IV	RGS4	-	-*	-*
V	COMT Val158Met	-	-*	-*
VI	DRD2 C957T	-	-*	-*
VI	COMT Val158Met+ DRD2 C957T	-	-	-

+ Significant    - Nonsignificant    (+) Trend

\*Unpublished data

## 4.2 Association with response to electroconvulsive therapy

In the whole study group the MADRS0 scores decreased from  $32.5 \pm 8.2$  to  $11.3 \pm 8.8$  (MADRS1). Out of 119 patients 45 were considered to have achieved remission (MADRS1 < 8) and 32 were nonresponders (MADRS1 > 15).

BDNF polymorphisms G196A and C270T were not associated with the treatment response to ECT (I). However, the CC genotype of BDNF C270T polymorphism was more frequent in patients with remission in subgroups of late-onset and psychotic depression ( $p=0.038$ , OR=7.27 and  $p=0.028$ , OR=5.32, respectively), using cut-point less than 8 for remission (I). The 5-HT1A C1019G polymorphism as well as the interaction of these 5-HT1A and BDNF polymorphisms was not associated with treatment response to ECT (II, unpublished data).

Both TPH1 A218C polymorphism and GNB3 C825T polymorphism alone were not associated with treatment response to ECT (III). In addition, the interaction of these polymorphisms in relation to ECT response was not significant. In the post hoc (cut-point MADRS 10 for remission) analysis there was no association with treatment response and TPH1 genotypes alone (CC vs. pooled CA+AA). GNB3 genotypes alone (CC vs. pooled CT+TT) likewise were not associated with treatment response. However, the interaction of these polymorphisms (TPH1 CC vs. pooled CA+AA and GNB3 CC vs. pooled CT+TT) for treatment response was significant in female patients ( $p=0.044$ ). The combination of TPH1 CC and GNB3 CT+TT genotypes was associated with better treatment response in female patients (OR=5.24; 95% CI 1.30-21.10;  $p=0.013$ ). In the whole study group and in males the interaction was not significant (III).

RGS4 rs 951436 polymorphism was not associated with the treatment response to ECT (IV). COMT polymorphism Val158Met Val/Val (high enzyme activity resulting in low dopamine) genotype carriers were more often in remission than nonresponders (OR=4.37; 95% CI 1.14-16.78;  $p=0.023$ ) (V). DRD2 C957T polymorphism alone was not associated with treatment response to ECT (VI, unpublished data). Patients with assumed LOW dopamine activity were more often in remission than nonresponders compared to the patients with the assumed HIGH dopamine activity (OR=11.0; 95% CI 1.7-70.0;  $p=0.011$ ) (VI). When the remaining genotype combinations of the DRD2 and COMT polymorphisms (REST group) were compared to the HIGH dopamine activity group, OR was not significant (VI). MADRS0 scores were not associated with COMT Val158Met polymorphism (V). MADRS1 scores were significantly lower in patients with Val/Val genotype compared to patients with the two other COMT genotypes ( $7.40 \pm 5.99$  vs.

12.32±9.15; p=0.012) (V). DRD2 C957T polymorphism TT genotype carriers had higher MADRS0 scores compared to the CT and CC genotypes (36.4±7.4 vs. 31.1±8.1; p=0.002) (VI). TT genotype carriers also had higher MADRS1 scores than the other two genotypes (14.1±10.2 vs. 10.4±8.1; p=0.046) (VI).

The associations of the polymorphisms studied with treatment response to ECT are presented in Table 5.

Table 5. Association of the studied polymorphisms with treatment response to ECT.

Article	Polymorphism	Whole group	Males	Females	Late-onset	Psychotic
I	BDNF G196A	-	-	-	-	-
I	BDNF C270T	-	-	-	+ <sup>#</sup> (p=0.038 *OR=7.27) CC	+ <sup>#</sup> (p=0.028 *OR=5.32) CC
II	5HT1A C1019G	-*	-*	-*		
II	BDNF G196A+5HT1A C1019G	-*	-*	-*		
III	TPH1 A218C	-	-*	-*		
III	GNB3 C825T	-	-*	-*		
III	TPH1 A218C+GNB3 C825T	-	-	+ <sup>α</sup> (p=0.013 OR=5.24) CC together with CT+TT		
IV	RGS4	-	-*	-*		
V	COMT Val158Met	+ Val/Val (p=0.023 OR 4.37)	+*Val/Val (p=0.011 OR=8.40)	-*		
VI	DRD2 C957T	-*	-*	-*		
VI	COMT Val158Met+ DRD2 C957T	+ LOW (p=0.011 OR 11.0)	+*LOW (p=0.034 OR=9.0)	-*		

+ Significant    - Nonsignificant    \*Unpublished data

# < 8 vs. ≥ 8    α < 10 vs. ≥ 10

LOW = DRD2 CC+CT together with COMT Val/Val

## 5. Discussion

### 5.1 General aspects

The populations in genetic studies of depression treatment response are generally heterogeneous concerning ethnicity, diagnosis, severity and outcome measures. The classification of TRD also still varies. The present population was unique and represented the most severe end of MDD treated with ECT. The most important finding in this study could be an association of COMT Val158Met polymorphism with treatment response to ECT. Later on, Domscke et al. (2009) replicated this finding.

The majority of genetic studies in this field concern ADs. However, studies on ECT response are rare. The mechanism of action of ECT may differ from that of ADs concerning neurotransmitters, receptors, transporters and regulation of genes. Thus different genetic polymorphisms may contribute more to the variation in ECT treatment response than in AD response. Despite numerous studies focusing on genetic polymorphisms associated with mood disorders, most results remain contradictory and clinically inadequate.

The present series of studies is the first one in the genetic research of ECT treatment response. These results, even if still speculative, may focus the research in future. In light of the present findings dopamine related genetic polymorphisms may be involved in treatment response of ECT.

### 5.2 Association between treatment resistant Major Depressive Disorder and studied polymorphisms

The BDNF polymorphisms G196A (Val66Met) and C270T studied were not associated with TRD. This finding is thus in line with earlier negative findings concerning MDD and BDNF G196A (Hong et al. 2003, Tsai et al. 2003, Oswald et al. 2005), although some positive findings have also been reported. The Met (A) allele has been suggested to be associated with MDD in males (Verhagen et al. 2008) and elderly patients (Hwang et al. 2006). Met (A) allele has also been suggested to predispose to suicidal behavior in MDD and also to be associated with higher stress hormone response in MDD patients than in Val (G) allele carriers (Schüle et al. 2006). A relation between Met (A) allele and both anxiety and

depression has also been reported (Jiang et al. 2005). The association of C270T polymorphism with MDD has not been studied earlier. However, both these polymorphisms are functional, G196A (Val66Met) A (Met) allele being associated with decreased activity-dependent secretion of BDNF protein in hippocampus (Egan et al. 2003). C127T polymorphism T allele is connected to lower production of BDNF protein (Kanemoto et al. 2003). Thus, at least the findings about the possible role of A allele of G196A in the pathogenesis of MDD may be reasonable. The role of another BDNF polymorphism C270T has not previously been studied in MDD patients and the present data produced a negative finding.

However, when the BDNF G196A polymorphism was combined with the 5-HT1A C1019G polymorphism in the present study an association was found with TRD. A allele of G196A and GG genotype of C1019G were found to indicate over three times higher association with TRD compared to the remaining combinations of these polymorphisms between patients and controls. 5-HT1A polymorphism GG genotype alone has previously been found to be associated with MDD and increased risk of suicide (Lemonde et al. 2003, Parsey et al. 2006). However, in the present study no association with TRD was found concerning the 5-HT1A polymorphism alone. The combination of these polymorphisms has not been studied earlier with regard to MDD. In animal models of depression BDNF and serotonin systems may both make an important contribution to the pathophysiology and treatment of depression (Gardier 2009). Both BDNF and serotonin regulate synaptic plasticity and neuronal survival. Serotonin stimulates the expression of BDNF, and BDNF enhances the growth and survival of serotonin neurons (Mattson et al. 2004). Serotonin and the selective 5-HT1A receptor agonist are able to produce an increased expression of BDNF mRNA (Galter and Unsicker 2000). The association of mechanisms with the risk of MDD seems reasonable when BDNF G196A A allele results in decreased secretion of BDNF (Egan et al. 2003) and 5-HT1A C1019G G allele derepresses 5-HT1A receptors leading to decrease serotonin neurotransmission (Lemonde et al. 2003).

TPH1 A218C polymorphism was associated with TRD in the present study. CC genotype was more common in patients than in controls in the whole population and in males but not in females. This finding is in line with previous findings suggesting that the A allele is associated with milder symptomatology of MDD (Mann et al. 1997, Serretti et al. 2001c). However, several other studies have found no association between this TPH1 polymorphism and depression (Furlong et al. 1998b, Frish et al. 1999, Kunugi et al. 1999, Cusin et al. 2001, Serretti et al. 2002). Other studies have subsequently suggested that the A allele is a risk allele for suicide attempts (Bellivier et al. 2004, Galfalvy et al. 2009) and MDD (Gizatulin et al. 2006). The role of TPH1 polymorphism in MDD is therefore still disputed.

In the present study the CC genotype of GNB3 C825T polymorphism was less frequent in TRD patients in females. In males an opposite trend was found, CC genotype being more frequent in TRD patients. Previously it has been reported that T allele carriers of this polymorphism are at risk of depression (Zill et al. 2000,

Bondy et al. 2002, Exton et al. 2003, Lee et al. 2004). Thus, the present study found the association to be in line with previous findings only in female TRD patients. The gender differences have not previously been reported in the association with this polymorphism and MDD. MDD affects females twice as often as males, and there is some evidence of gender differences in the binding potentials of the major serotonin receptors (Arango et al. 1995, Biver et al. 1996, Parsey et al. 2002).

According to the present findings an interaction of TPH1 and GNB3 polymorphisms may be associated with TRD. In males the combination of TPH1 A218C polymorphism CC genotype together with GNB3 C825T CC genotype associated with TRD. In females the combination of TPH1 CC genotype with GNB3 CT and TT genotypes associated with TRD. No similar findings have been reported so far but if replicated, at least in females this combination could associate with the course of MDD.

The RGS4 allele frequencies did not differ between the present patients and controls. RGS4 has not been studied earlier in MDD, however, it may be a risk factor for bipolar disorder (Kato 2007). T allele has been found to increase the risk of schizophrenia (Mirnics et al. 2001, Prasad et al. 2005, Talkowski et al. 2006) but according to the present findings it was not associated with TRD.

The COMT polymorphism Val158Met examined in the present study did not differ between patients and healthy controls. Earlier findings suggested that the Met allele associated with the onset of mood disorder after stressful life-events (Mandelli et al. 2007), higher endocrine and reported stress responses (Jabbi et al. 2007) and emotional dysregulation of affective responses (Drabant et al. 2006). On the contrary, Val/Val genotype is suggested to be associated with early onset MDD (Massat et al. 2005) and subsequently Val allele with greater severity of TRD (Domschke et al. 2009) and with a risk of affective disorder in general (Funke et al. 2005). In the present study not even an association with the severity of depression (higher MADRS0 scores) was found. The pathophysiology of TRD may thus not be fundamentally associated with the genetic variation in dopamine levels in the brain, although in the light of the monoamine hypothesis it would be logical to hypothesize that patients with less dopamine in certain brain areas, e.g. in prefrontal cortex, may be more prone to depression and more severely depressed than otherwise (Lambert et al. 2000). Thus, the role of dopamine in the pathophysiology of MDD is strongly implicated but still open (Dunlop and Nemeroff 2007, Montgomery 2008). Maybe the genetic risk of depression is associated merely with other factors than dopamine alone, like with serotonin linked polymorphisms.

No association of MDD with DRD2 gene has been reported in earlier studies (Furlong et al. 1998a, Kõks et al. 2006) but DRD2 polymorphism (rs 1800497) influenced the effect of stressful life-events on depressive symptoms (Elovainio et al. 2007). DRD2 C195T polymorphism and MDD has not earlier been studied but this polymorphism was suggested to be associated with the risk of schizophrenia (Hänninen et al. 2006). In the present study no association with DRD2 C195T

polymorphism and TRD was found. The C allele of this polymorphism is related to lower dopaminergic activity, striatal D2 binding is lowest with CC genotype (Hirvonen et al. 2004). TT genotype (with higher assumed dopaminergic activity, respectively) was associated with higher MADRS0 scores compared to C allele carriers in the present study. This may indicate an association between the severity of MDD and the C195T polymorphism.

The combination of COMT Val158Met and DRD2 C195T polymorphisms was not associated with TRD in this patient population but in this combination an association was found with more severe depression in baseline (MADRS0) in TRD patients. Higher MADRS0 scores were found in patients with HIGH dopamine activity (DRD2 TT genotype together with COMT Val/Met and Met/Met genotypes) compared to other genotype combinations. Domschke et al. (2009), however, found this COMT polymorphism (alone) Val allele to be associated with more severe TRD. This combination of polymorphisms has not been studied earlier in relation to the risk of MDD, and it may be an interesting target in the future.

### 5.3 Association with response to electroconvulsive therapy

It has been suggested that ECT is the most effective treatment option in MDD (APA 2001). Patients with TRD are often referred for ECT and they also achieve reasonable response to this treatment.

Neither of BDNF polymorphisms studied here, G196A and C270T, were associated with treatment response to ECT in the whole patient population. In subgroups of psychotic and late-onset depression, however, an association with remission and BDNF C270T CC genotype was found. Serum BDNF levels have been found to be increased after ECT in MDD and TRD patients (Bocchio-Chiavetto et al. 2006, Marano et al. 2007, Okamoto et al. 2008) and this increase has been suggested to relate to ECT treatment response (Okamoto et al. 2008). In animal studies ECS has also been reported to increase BDNF mRNA (Nibuya et al. 1995) and BDNF protein (Altar et al. 2004) in different brain areas in rats. It has been suggested that the therapeutic action of ECT is partly associated with neurogenesis, synaptogenesis and synaptic plasticity (Perera et al. 2007, Huang and Chen 2008, Chen et al. 2009) where BDNF is involved. The BDNF Val66Met (G196A) polymorphism Met (A) allele has been found to be associated with the reduction in the volume of the hippocampus (Bueller et al. 2006) and Met (A) allele is also associated with decreased activity-dependent secretion of BDNF in the hippocampus (Egan et al. 2003). Hippocampal volume reduction has been suggested to be reversed by ADs (Duman and Monteggia 2006) and the formation and stabilization of the synaptic connectivity was improved (Saarelainen et al. 2003, Castren 2004). BDNF Val66Met (G196A) polymorphism Met-allele carriers had better treatment response to citalopram (Choi et al. 2006), Val/Met heterozygote



MDD patients were likely to have better treatment response to fluoxetine treatment compared to carriers of each homozygote (Tsai et al. 2003) which was also replicated after different AD treatments (Yoshida et al. 2007). According to these experimental and clinical findings, ECT may have an impact on BDNF and its response may vary according to the gene polymorphisms involved. No association, however, was found with ECT treatment and the polymorphisms studied in the present whole TRD population. Concerning the BDNF C270T polymorphism findings in subgroups, caution should be exercised. This polymorphism has not been studied earlier with regard to the treatment response in MDD patients. Although T allele is connected to lower production of BDNF (Kanemoto et al. 2003) and thus may be associated with MDD or its treatment response, the association is still rather theoretical. In the present population in subgroups of psychotic and late-onset depression an association was found between remission and CC genotype of BDNF C270T polymorphism. This finding, however, remains highly speculative due to absence of earlier data.

Neither 5-HT1A C1019G polymorphism alone nor BDNF G196A polymorphism together with 5-HT1A polymorphism were associated with treatment response in the present patient group. In earlier studies 5-HT1A C1019G polymorphism GG genotype or G allele have been associated with better treatment response to ADs compared to C allele carrying MDD patients (Arias et al. 2005, Hong et al. 2006, Yu et al. 2006, Kato et al. 2008) and same finding was also detected with bipolar depression (Serretti et al. 2004a). The finding of Baune et al. (2008) suggesting in melancholic subtype of depression a poorer treatment response to SSRIs with CC genotype was also in line with our finding. This polymorphism has not been previously studied with regard to TRD patients and ECT treatment response and the present population may thus differ from other study populations. Moreover, the relationship to treatment response in MDD has not been studied earlier concerning this combination of BDNF and 5-HT1A polymorphisms even though they could be hypothesized to have some interactions. BDNF converging in the level of neurogenesis, neuronal survival and synaptic plasticity and 5-HT1A crucially influencing serotonin input may theoretically have additive or potentiative effects on each other (Galter and Unsicker 2000, Mattson et al. 2004, Gardier et al. 2009).

TPH1 A218C polymorphism was not associated with the treatment response to ECT in the present patient population and neither were GNB3 C825T polymorphism alone or the combination of these. TPH1 polymorphism A218C AA genotype or A allele has been found to be associated with poorer treatment response to ADs in MDD in several earlier studies compared to C allele containing genotypes (Serretti et al. 2001a, Serretti et al. 2001b, Serretti et al. 2004b, Ham et al. 2007). GNB3 C825T polymorphism TT genotype or T allele have been reported to be associated with better treatment response to ADs than other genotypes (Zill et al. 2000, Serretti et al. 2003b, Lee et al. 2004) but contradictory findings have also been reported (Joyce et al. 2003, Wilkie et al. 2007). The combination of these TPH1 and GNB3 polymorphisms has not been studied earlier. In post hoc analysis (using a cut-point < 10 in MADRS as one cut-point for remission) an association between better

treatment response and the combination of TPH1 CC and GNB3 CT+TT genotypes was found in female patients. This finding concerning the interaction of these genotypes is relatively in line with most of the studies reviewed (although both previously were studied only alone). However, no reports of gender differences relating to these polymorphisms and treatment response to ADs have previously been published. In general, there may be gender differences in treatment response to various antidepressive options (Kornstein and Schneider 2001, Yonkers and Brawman-Mintzer 2002, Cohen 2003, Sloan and Kornstein 2003, Bloch et al. 2005).

The treatment response was not associated with RGS4 polymorphism in the present patient population. RGS proteins are a family over 20 subtypes shaping G protein coupled receptor signaling (De Vries et al. 2000). RGS4 is associated with serotonin signaling on prefrontal cortex in rats (Gu et al. 2007). RGS4 T allele has been suggested to be connected with decreased neuro-cognitive functioning in healthy subjects (Buckholz et al. 2007). In animal models of ECT G protein coupled receptor signaling cascades have been studied. Alterations have been found after chronic ECS among others in 5-HT1A (Stockmeier et al. 1992) and 5HT2A (Butler et al. 1993) receptors. ECS has also been suggested to regulate gene expression of neurotrophic signaling pathways in the hippocampus of rats (Altar et al. 2004, Sun et al. 2005). In rats RGS4 mRNA levels in brain have been shown to be altered by ECS at transcriptional level (Gold et al. 2002). Subtype, time and region specific alterations were found. G protein coupled receptor signaling cascades may thus be involved in the antidepressant action of ECS and can be regulated by RGS activity. However, in humans RGS4 genotype may not be a crucial factor in the mechanism of the antidepressive action of ECT.

The present COMT Val158Met polymorphism Val/Val genotype carriers were more often in remission after ECT treatment than Met allele carriers. This leads to the hypothesis that lower pretreatment levels of dopamine in the prefrontal cortex may be associated with better results in ECT treatment. This finding was also replicated in a very recent study (Domschke et al. 2009). ECT increases dopaminergic activity in animal models (Yoshida et al. 1998, Andrade et al. 2002, Strome et al. 2007) and also decreases the auto-receptor functions also in presynaptic dopaminergic neurons resulting in increased release of dopamine (Ishihara and Sasa 1999). These suggestions may lead to the assumption that patients who have less pretreatment dopaminergic activity in prefrontal cortex may achieve better response from ECT treatment than those with moderate to high dopaminergic activity, respectively. The findings of the efficacy of ECT in Parkinson's disease also support this assumption (Fall et al. 1995, Moellentine et al. 1998, Kennedy et al. 2003). Moreover, an increase in CSF dopamine metabolites after ECT has been found, reflecting increased dopamine neurotransmission (Fall et al. 1995). By contrast, Markianos et al. (2002b) suggested that ECT has only a minor effect on dopaminergic responses. The studies on AD treatment response and COMT Val158Met are also contradictory. COMT Val allele carriers have been reported to respond better to mirtazapine treatment in MDD compared to Met allele carriers (Szegedi et al. 2005) as well as to SSRI treatment (Arias et al. 2006).

However, in other studies on treatment response with ADs Val allele has been suggested to be associated with poorer outcome in MDD compared to Met allele (Baune et al. 2008, Yoshida et al. 2008, Benedetti et al. 2009, Tsai et al. 2009a). Although different treatment methods like ADs and ECT may have something in common, the principal mechanisms may be very different. The present patients receiving ECT continued their previous AD treatment, which makes the conclusions more complicated. However, both the ECT studies available (the present and the study by Domschke et al. 2009) concerning COMT Val158Met polymorphism in TRD have similar results.

TT genotype of DRD2 C957T indicates high striatal dopamine activity and binding potential (Hirvonen et al. 2004) and C allele conversely low activity. In the present TRD patients, however, DRD2 C957T polymorphism was not alone associated with treatment response to ECT. On the contrary, COMT Val158Met polymorphism high activity Val allele (resulting low dopamine levels) was associated alone with better treatment response. When these two polymorphisms were combined this effect actually strengthened. According to the “inverted U” model there is a narrow range in dopamine activity to be optimal (Mattay et al. 2003). In patients with the LOW baseline dopamine activity, the ECT-induced extra dopamine load may result in better dopamine levels and thus a better treatment response. Accordingly, in patients with the HIGH baseline dopamine activity, ECT induced dopamine excess does not provide any extra benefit. It is possible that this is the reason why MADRS scores did not diminish as expected in this latter group. Moreover, the patients with the HIGH dopamine pretreatment activity also had more severe TRD than the other patients. This is not in line with some earlier suggestions (Lambert et al. 2000, Rocc et al. 2002, Dunlop and Nemeroff 2007) but may be also be explained by the “inverted U” model of optimal dopamine activity.

## 5.4 Study population and methods

### 5.4.1 Patients

The patients in the present study were diagnosed with MDD and subsequently TRD. The definition of TRD varies in the literature and in clinical practice (Berlim and Turecki 2007). In the present study the definition used was: “two or more unsuccessful trials with antidepressants of different groups at an adequate dose for at least four weeks”. Based on a clinical assessment patients were treated with ECT. About 43 % of the patients had psychotic symptoms and 57 % had late-onset MDD. All the patients fulfilled the actual inclusion criteria and agreed to participate and gave their written informed consent. Only three patients declined. All the patients in this study subjected to careful clinical assessment, likewise the severity of MDD. General evaluation of outcome for all the study population was performed according to the recommendations of APA (2001).

## 5.4.2 Controls

The controls were healthy middle-aged blood donors on whose age and gender information was available. Before donation they were asked to complete a questionnaire about their health and medication. It cannot be excluded that some of the controls may have been suffering from depression or were at risk of or had a family history of MDD.

## 5.4.3 Selection of polymorphisms

The BDNF gene G196A (Val66Met) polymorphism was chosen for this study because it is a functional polymorphism (Egan et al. 2003) affecting the BDNF protein levels and its connection to the pathophysiology of depression has been studied earlier (Tsai et al. 2003, Jiang et al. 2005, Oswald et al. 2005, Schumacher et al. 2005, Hwang et al. 2006). Stress has been suggested to lower the levels of BDNF in brain regions associated with depression and ADs to have an elevating effect of BDNF levels (Duman et al. 2006). The expression of BDNF is thus modified by antidepressant treatment (Saarelainen et al. 2003). The poorer acting A (Met) allele could be hypothesized to be associated with the risk of depression by reducing the secretion of BDNF in the hippocampus and ADs mediate their therapeutic effect partly by increasing levels of BDNF in the hippocampus (Duman and Monteggia 2006). The C270T polymorphism of BDNF gene T allele is connected to lower production of BDNF (Kanemoto et al. 2003). In light of the neurotrophic hypothesis of depression these BDNF functional polymorphisms were chosen for this study (Duman and Monteggia 2006).

Depression and treatment response to ADs could be associated with signaling through 5-HT<sub>1A</sub> receptor as well as neurogenesis in hippocampus (Santarelli et al. 2003, Castren 2004). Earlier studies have suggested that 5-HT<sub>1A</sub> C1019G polymorphism of the 5-HT<sub>1A</sub> gene promoter region GG genotype or G allele is associated with MDD and also poorer response to ADs (Hong et al. 2006, Parsey et al. 2006). This SNP was therefore a target of this study and also the interaction of BDNF gene polymorphism G196A (Val66Met) and 5-HT<sub>1A</sub> polymorphism C1019G was also hypothesized to interact with the risk of TRD.

TPH is the rate-limiting enzyme in the biosynthesis of serotonin so it is a suitable candidate gene for the study of depression. TPH1 gene polymorphism A218C A allele was associated with decreased serotonin synthesis (Jonsson et al. 1997). This polymorphism has been reported to be associated with depression so that the A allele may protect males against MDD (Serretti et al. 2001c) or may be associated with milder symptomatology of MDD (Mann et al. 1997). However, several other studies have found no association between depression and TPH1 polymorphisms (Furlong et al. 1998b, Frisch et al. 1999, Kunugi et al. 1999, Cusin et al. 2001, Serretti et al. 2002, Serretti et al. 2004b). In earlier studies the A allele or AA

genotype of this polymorphism has been associated with poorer response to SSRIs (Serretti et al. 2001a, Serretti et al. 2001b, Serretti et al. 2004b), in other studies no such association was found (Yoshida et al. 2002b, Peters et al. 2004).

In European populations an association with GNB3 C825T polymorphism and MDD has been found (Zill et al. 2000, Bondy et al. 2002, Exton et al. 2003) but not in Asian studies (Lin et al. 2001, Kunugi et al. 2002). GNB3 C825T polymorphism has been found to be associated with treatment response to SSRIs and other antidepressants in earlier studies (Zill et al. 2000, Joyce et al. 2003, Serretti et al. 2003b, Lee et al. 2004). These earlier findings contributed to the choice of this polymorphism to this study.

RGS4 has been found to increase the risk of schizophrenia (Talkowski et al. 2006). This may be due to imbalance in the dopamine system of the brain (Mirnics et al. 2001). RGS4 alters schizophrenia-associated hypofrontality in the prefrontal area (Prasad et al. 2005), which can lead to neuro-developmental hypofunction in reduced number or function of synapses (Mirnics et al. 2001). Both of these mechanisms may also be associated with abnormal stress response, which may be present in the onset of mood disorders (Tafet and Smolovish 2004). However, the functional changes in the prefrontal cortex of patients with schizophrenia are not comparable to the functional changes of those with a mood disorder (Mirnics et al. 2001). RGS4 gene polymorphism (rs951436) T allele is also connected to decreased neuro-cognitive functioning through altered connectivity in neural network, resulting in delayed reaction time, and hypoactivity during working memory tasks in healthy subjects (Buckholz et al. 2007). Regardless of the diagnosis, it may be assumed in light of these findings that RGS4 polymorphism may affect the vulnerability mechanism of severe mental disorders in general, including MDD.

The association of dopamine with treatment response in ECT has previously been studied (Markianos et al. 2002a). In animal models an increasing amount of dopamine has been detected in the CNS after ECS (McGarvey et al. 1993, Yoshida et al. 1997). In addition, dopamine is suggested to be linked to the pathophysiology of depression (Papacostas 2006, Dunlop and Nemeroff 2007, Montgomery 2008). COMT enzyme in the prefrontal cortex is the major enzyme metabolizing dopamine. The COMT gene contains a functional polymorphism Val158Met, which Met (low enzyme activity) allele homozygosity leads to three- to fourfold reduction in enzymatic activity compared to Val (high enzyme activity) allele homozygosity (Lachman et al. 1996). Thus it is suggested that patients with Met/Met genotype have higher levels of dopamine in prefrontal cortex (Chen et al. 2004). Met allele has been reported to be associated with emotional dysregulation of affective responses (Drabant et al. 2006) and also higher endocrine and reported stress responses (Jabbi et al. 2007). High-activity COMT Val allele has been reported to be associated with early onset MDD (Massat et al. 2005). However, other studies found no association with this polymorphism and the risk of MDD (Kunugi et al. 1997, Frisch et al. 1999, Serretti et al. 2003a, Serretti et al. 2006). MDD patients with Val allele were suggested to respond better to treatment with mirtazapine

(Szegeedi et al. 2005). Met allele carriers were suggested to have higher risk for nonremission with SSRI treatment (Arias et al. 2006). However, no association was found between treatment response to paroxetine and this COMT polymorphism (Szegeedi et al. 2005). Because this polymorphism is studied with regard to MDD and also to treatment response in MDD, it was a suitable candidate for this study.

DRD2 gene polymorphism C957T (rs6277) affects mRNA folding, stability and translation and changed dopamine induced up-regulation of DRD2 expression (Duan et al. 2003). This functional polymorphism is thus associated with striatal DRD2 receptor availability, subjects carrying T allele had markedly higher striatal D2 receptor availability and thus higher DRD2 receptor binding potential (Hirvonen et al. 2004). It has been associated with the risk of schizophrenia (Hänninen et al. 2006). Lower dopaminergic activity is suggested also to be associated with MDD and drugs acting on the dopamine system have been studied in MDD (Dunlop and Nemeroff 2007). In addition, TRD patients showed lower levels of both brain norepinephrine and dopamine (Lambert et al. 2000). As a consequence of these findings this polymorphism was selected and its interaction with COMT Val158Met polymorphism was also hypothesized to be of interest associating with the levels of dopamine in the brain.

#### 5.4.4 Procedure in electroconvulsive therapy

Bilateral ECT was chosen because this method is considered to be the most effective strategy even though it may induce more cognitive adverse effects more than right unilateral ECT (Greenberg and Kellner 2005). The age method was used in 30 patients and stimulus titration in 89 patients. The stimulus titration method has been recommended because it is the most precise method for the individual selection of ECT stimulus dose relative to the seizure threshold (Sackeim et al. 1987a, Heikman et al. 1999, APA 2001). The value of the stimulus titration method has also been criticized (Petrides and Fink 1996, Abrams 2002) and the optimal dosage level may still be unknown (Fink et al. 2001, Abrams 2002). The pre-selected stimulus dose adjusted by patient's age in decades or half age from chronological age used as a percent of the charge for the Thymatron ECT device is also recommended (Petrides and Fink 1996, Swartz and Abrams 1996). However, the age method is a relatively inaccurate method possibly resulting in too low or considerably suprathreshold stimulus dose. The stimulus titration method on the other hand requires multiple stimulations and may increase the risk of cardiovascular adverse effects (Petrides and Fink 1996, Burd and Kettl 1998). In the present study the method was chosen individually, with the age method preferred for elderly patients or for patients with cardiac diseases.

### 5.4.5 Ratings

The MADRS scale is easy to administer and the sensitivity and specificity have been found to be good (Yonkers and Samson 2000, Yonkers and Samson 2008). It has been approved as a reliable and valid instrument to evaluate the severity and the change of depressive symptoms (Davidson et al. 1986). In the present studies, the definition of remission to ECT has been MADRS score less than 8. Nonresponse was defined as more than 15. This strict definition was used in order to achieve two clinically and perhaps biologically clearly different groups for response. The patients with partial response according these criteria, (MADRS score between 8 and 15), were thus excluded from the primary analyses. In the analyses of subgroups such as late-onset and psychotic depression only one cut-point (MADRS score less than or = 8) was used in order to avoid missing cases. In one post hoc analysis a cut-point less than 10 for remission was used because this cut-point has also been used elsewhere (Fisman et al. 2001). The change in MADRS scores between MADRS0 and MADRS1 was not used as a main outcome measure because it would not accurately differentiate the remitters from the non-responders.

### 5.4.6 Genotyping of the polymorphisms

The 5' nuclease assay has been successfully used to discriminate alleles that differ by a single base substitution (Livak 1999). TaqMan assays allow fast and sensitive genotyping and are especially suitable for studies including large numbers of participants (Koch et al. 2002). A standard method of analysis has been developed that enables automated genotype determination. Applications of this assay have included typing a number of polymorphisms e.g. in pharmacogenetic and human drug metabolism studies (Livak 1999).

## 5.5 Limitations and strengths of the study

The relatively small patient population is a main limitation of the present study and thus may lead to lack of power in statistical analyses. Especially in the subgroups, the number of patients was rather small. The study design also leads to small patient population when the partial responders were excluded from the efficacy analyses, but the aim was to create clinically and thus possibly biologically two clearly different groups to compare. Analyses in subgroups resulted in multiple testing. Due to the small sample size no Bonferroni corrections were performed to avoid type II error.

A further limitation is that also that the diagnosis was made only on a clinical basis and no formal, standardized diagnostic interview was used. In defining TRD, a four-week trial of AD may be considered too short, maybe 6 to 8 weeks would have

been more reliable. Only one rating scale for severity and change of MDD symptoms was used and using e.g. a self-rating scale in addition would have given some extra information on outcome. For practical reasons remission was assessed based on only one cut-point, the outcome was not confirmed during a time course.

No comparison was made between these TRD patients and the rest of the patients excluded from this study but treated with ECT. This information may have been interesting and weakens the generalizability of the results to ECT treated patient groups other than TRD.

A limitation of this study is also that the patients were medicated during the ECT trial. Although this is a general procedure, it makes it difficult to assess the ECT response alone. The mechanism of the antidepressive effect of ECT and antidepressants may be complementary, and the effect of AD treatment cannot entirely be ruled out in such a study setting.

The controls were not examined. They were healthy blood donors but may also have been suffering from or have been at a risk for depression; they were also somewhat younger than the patients.

The relatively small number of SNPs was also a limitation in the present study. Nowadays comprehensive SNP genotyping in the candidate genes or genome-wide analysis would be state-of-the-art.

The main strengths of the present study are that the study setting was naturalistic, the ethnic background of the patients and controls was homogeneous and the severity and change of MDD was carefully evaluated. Patients were referred for ECT according to clinical criteria and TRD was also diagnosed according to the specified criteria. The present series of studies is the first one in the genetic research of ECT treatment response.

## 5.6 Conclusions and future implications

The genetic risk factors of MDD have been widely studied. Some candidate genes and polymorphisms have been found and in light of recent literature it could be hypothesized that the risk is mostly associated with serotonin-linked polymorphisms. The data about dopamine-linked polymorphisms is somewhat contradictory. Genetic studies seem to agree with the monoamine hypothesis of depression, indicating some relationship with the functioning of the specific neurotransmitter systems and MDD. Subsequently the functioning of the hippocampus along with the neurotrophic systems is suggested to be an important factor in developing MDD.



According to the present results serotonin-linked polymorphisms TPH1 A218C and GNB3 C825T may be associated with the risk of TRD. This effect was also seen when these polymorphisms were combined although it was different with males than with females. This may be due to the different gender specific risk factors of TRD. When 5-HT1A C1019G polymorphism was combined with BDNF G196A polymorphism an association was found with the risk of TRD even if there were no associations with these BDNF and 5-HT1A polymorphisms alone. There may be some synergistic action between these mechanisms, perhaps at the level of neuronal plasticity and neurogenesis. However, the dopamine-linked polymorphisms studied which may affect the levels or activity of dopamine in the CNS, were not associated with the risk of TRD.

The response to ECT seemed to be associated with studied dopamine-linked polymorphisms. COMT Val158Met polymorphism genotype (Val/Val) which results in lower dopamine activity in prefrontal cortex (high enzyme activity respectively) may contribute to the better response to ECT. This effect was even strengthened when combined with DRD2 C957T polymorphism genotype (CC+CT) also resulting in lower dopamine binding potential and activity in prefrontal cortex. ECT has been known to affect dopamine levels in the brain and it seems reasonable that TRD patients who have less pretreatment dopamine activity in prefrontal cortex may benefit most from this treatment. The entire mechanisms of action of ECT treatment are still unknown. It is the most effective treatment method for TRD patients but not all patients benefit. The responding patients may have decreased baseline prefrontal activity of dopamine. Some neurotrophic factors may also be involved.

The number genetic studies associated with ECT is scanty and the results remain speculative. In the future genome-wide analyses are needed with larger homogenous patient groups and reliable diagnostics. The clinical use of these genetic polymorphisms as markers for MDD or TRD in general and markers for treatment response in particular is still far away. However, it is important to elucidate the genetic basis of MDD and its treatment response in order in future to have an opportunity to choose individually the most effective treatment method.

## 6. Summary

The main findings of the study were:

I BDNF polymorphisms G196A and C270T were not associated with TRD. C270T polymorphism was associated with better treatment response in subgroups of psychotic and late-onset depression.

II 5-HT1A C1019G polymorphism was not associated with TRD but together with BDNF G196A polymorphism an association with TRD was found. These polymorphisms were not associated with treatment response to ECT either alone or combined.

III TPH1 A218C polymorphism was associated with TRD, as was the case with GNB3 C825T polymorphism. There seemed to be a gender specific manner in these associations; together these polymorphisms were associated with TRD differently in both genders. The combination of these polymorphisms was also associated with a chance to benefit from ECT treatment in females. Even this effect was gender specific.

IV RGS4 gene polymorphism was not associated with TRD or treatment response to ECT

V COMT Val158Met polymorphism was not associated with TRD. An association with treatment response to ECT was found.

VI DRD2 C957T polymorphism was not associated with TRD. It was not associated alone with the treatment response to ECT either. When combined with the COMT polymorphism an association with the treatment response was found.

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## ORIGINAL PAPER

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**Brain-derived neurotrophic factor (BDNF) polymorphisms G196A and C270T are not associated with response to electroconvulsive therapy in major depressive disorder**

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**Abstract** The aim of the present study was to examine an association of brain-derived neurotrophic factor (BDNF) polymorphisms G196A and C270T and the response to electroconvulsive therapy (ECT) in major depressive disorder (MDD). The study group consisted of 119 patients consecutively admitted for ECT in the Department of Psychiatry, Tampere University Hospital. All patients fulfilled the diagnostic criteria of DSM-IV for MDD. ECT was administered three times a week with a brief pulse constant current device. The Montgomery and Åsberg Depression Rating Scale (MADRS) was used as an outcome measure of depression. Genotyping was performed using fluorescent allele-specific TaqMan probes. No association between either G196A or C270T and the response to ECT was found in the whole population. There were no significant differences in responses between men and women or between psychotic and non-psychotic patients. However, within subgroups such as in psychotic and in late-onset depression CC genotype of C270T may predict good response. BDNF may not be associated with response to ECT in general, but some association in subgroups may exist.

**Key words** BDNF · polymorphism · major depressive disorder · electroconvulsive therapy

**Introduction**

Major depressive disorder (MDD) is a debilitating mental illness causing major social and economic burden. It is a heterogeneous disease, involving genetic and environmental factors [1]. Electroconvulsive therapy (ECT) is the most effective treatment method in MDD [2], although its mechanism of action is still largely unknown.

Brain-derived neurotrophic factor (BDNF) is a small dimeric protein and is a member of the nerve growth factor family. The human BDNF gene is mapped in chromosome 11p13 [3]. The action of BDNF is mediated mainly by its receptor, protein tyrosine kinase B (TrkB). BDNF has been found to promote neuronal survival, differentiation and neuroprotection [4]. It has an important role in activity-dependent synaptic plasticity. This has been shown in BDNF knock-out mice, which exhibit a reduction in hippocampal long-term potentiation. This can be reversed by adenovirus induced overexpression of BDNF [5, 6]. Stress and depression have been found to lower the BDNF levels in the CNS in animal models [7, 8] but increased levels of BDNF have also been found [9]. BDNF also produces an antidepressant-like effect when injected into rat brain [10, 11]. These findings support the suggestion that BDNF plays a role in depression [12].

There are some reports of volume and cell loss in the hippocampus of patients with mood disorders [13, 14]. It has been reported that repeated electroconvulsive shocks and long-term antidepressant medication increasing the BDNF mRNA in rat hippocampus and so increasing the proliferation and survival of neurons [15–19]. Chronic antidepressant medication increases

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BDNF expression in human CNS and thus may normalize the hippocampal levels of BDNF [20, 21].

G196A (val66met) polymorphism in the coding region of the BDNF gene is a functional polymorphism (met allele is associated with decreased activity-dependent secretion of BDNF in hippocampus) [22]. The BDNF polymorphism G196A is associated with susceptibility to bipolar disorder (BD) in some but not in all studies [23–27]. This may imply ethnic variation. Two recent studies [28, 29] found no association between this polymorphism and MDD or panic disorder [30].

To our knowledge, there are no reports of any association between depression and the BDNF polymorphism C270T. This polymorphism has not been found to be associated with BD [24].

The aim of the present study was to examine the association between BDNF polymorphisms G196A and C270T and the response to ECT in major depression.

## Materials and methods

### Subjects

The study group consisted of 119 patients (65 women and 54 men, age  $57.7 \pm 14.0$  [mean  $\pm$  SD]) consecutively admitted for ECT to the Department of Psychiatry, Tampere University Hospital. Patients ( $n = 122$ ) who met the eligibility criteria were invited to participate in the study, and three of them declined. All the patients fulfilled the diagnostic criteria of DSM-IV for MDD [31], and 51 of them had psychotic symptoms. Patients with neurological disorders, dementia, schizophrenia, BD and alcohol or other substance abuse were excluded from the study. Blood samples were taken at the time of entry into the study and coded. The participants gave written informed consent. The study was approved by the Tampere University Hospital Ethics Committee.

### Treatment and clinical evaluation

ECT was administered three times a week with a brief pulse constant current device (Thymatron DGx, Somatics, Inc., Lake Bluff, IL, USA). The initial stimulus dosage was adjusted with the age method for 30 patients [32]. The seizure threshold was determined for 89 patients by administering successive stimuli of increasing intensity until generalized seizure was induced. Anesthesia was induced with methohexital and muscle relaxation with succinylcholine. The initial dose was 1 mg/kg of methohexital and 0.5 mg/kg of succinylcholine. The patients were ventilated with 100% oxygen until resumption of spontaneous respiration. Physiological monitoring included pulse oximetry, blood pressure, electrocardiogram (ECG), one channel electroencephalogram (EEG) and electromyography (EMG). The criterion for adequate generalized seizure duration was at least 20 s of motor response and 25 s of EEG seizure activity. If needed, at subsequent treatments during the course of ECT, the dosage was increased to maintain adequate seizure duration. All patients were treated with standard bilateral (bifrontotemporal) ECT, the number of treatments ranged between 7 and 15,  $9.4 \pm 1.79$  (mean  $\pm$  SD). The total number of treatments administered was determined by clinical judgment. ECT was continued until patients were symptom-free or had received at least eight treatments without any further improvement being observed during the last two treatments. The patients received their ongoing medication during the ECT. All except 12 patients had some psy-

chotropic medications (87 had antidepressants, 79 had antipsychotics and 80 had small doses of anxiolytics or hypnotics). Altogether, 55 of the patients had additional non-psychotropic medications during the treatment.

The Montgomery and Åsberg Depression Rating Scale (MADRS) [33] was used to assess the severity of depression and the clinical change. Cognition was assessed in 78 patients using the Mini-Mental State Examination Scale (MMSE) [34].

### Genotyping of the BDNF polymorphisms

Purification of genomic DNA from whole blood and buffy-coats was performed using standard methods. DNA samples were genotyped by employing the 5' nuclease assay and fluorescent allele-specific TaqMan MGB probes [35] using the ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA). The nucleotide sequences of primers and probes used in the PCR were deduced from published sequences deposited in the GenBank and Celera databases and synthesized in conjugation with Applied Biosystems. PCR reaction containing genomic DNA,  $1 \times$  Universal PCR Master Mix, 900 nM of each primer and 200 nM of each probe was performed in 96-well plates using the standard protocol in a total volume of 25  $\mu$ l. End-point fluorescence was measured and genotype calling carried out by the allelic discrimination analysis module after PCR resulting in clear identification of three genotypes for both BDNF polymorphisms.

### Statistical analysis

The distributions of the categorical variables between different study groups were compared by the Fisher exact test or  $\chi^2$  test. One-way analysis of variance was used in determining differences in clinical variables between BDNF genotype groups. The statistical analysis was carried out using SPSS/Win (Version SPSS 11.0.1, Inc, Chicago, IL). A *P*-value of less than 0.05 was considered significant.

In order to obtain meaningful subgroups for statistical analysis, the polymorphisms were pooled. G196A was pooled to GG versus GA + AA genotypes and C270T were pooled to CC versus CT + TT genotypes. These polymorphisms were also pooled to homozygotes versus heterozygotes.

For statistical analysis, those scoring less than 8 in MADRS were considered responders and those scoring more than 15 were considered non-responders [36]. The patients with MADRS score between 8 and 15 were excluded because of borderline response. Moreover, the subgroups (such as psychotic and late-onset depression) were analyzed separately, where less than and  $\geq 8$  was applied as a cut-off point for response because of the small number of patients. The age of 45 years was used as a cut-off between early and late onset depression, as in some earlier studies [37–39]. The patients were dichotomized by change in MMSE during ECT (equal or improved versus impaired).

## Results

In the whole group, the pre-ECT values of the MADRS score decreased from  $32.5 \pm 8.2$  to  $11.3 \pm 8.8$  after ECT (mean  $\pm$  SD,  $P < 0.0001$ ). Out of a total of 119 patients 45 were considered responders (MADRS  $< 8$ ) and 32 non-responders (MADRS  $> 15$ ). The demographic and clinical characteristics of responders and non-responders are presented in Table 1.

No associations with G196A or C270T BDNF pooled polymorphisms and the response to ECT were found ( $P = 0.763$ ,  $P = 0.104$ , respectively). Moreover, no difference was found between homozygotic and

**Table 1** Demographic and clinical characteristics of responders and non-responders to ECT

	Responders (n = 45)	Non-responders (n = 32)
Sex (M/F)	22/23	18/14
Age, mean (SD)	58.3 (12.1)	55.3 (11.4)
Age at onset of depression, mean (SD)	58.3 (12.1)	55.3 (11.4)
Psychotic/non-psychotic	19/26	14/18
<b>MADRS</b>		
Baseline, mean (SD)	31.7 (9.0)	33.5 (7.7)
After treatment, mean (SD)	3.2 (2.4)	23.1 (6.4)
<b>MMSE</b>		
Baseline, mean (SD)	27.8 (2.3)	25.5 (4.9)
After treatment, mean (SD)	27.6 (2.5)	26.0 (4.3)
Number of ECT treatments, mean (SD)	9.3 (1.7)	9.7 (1.9)

MADRS = Montgomery and Åsberg Depression Rating Scale

MMSE = Mini-Mental State Examination

heterozygotic patients. In addition, no significant differences in these polymorphisms concerning the ECT response were found among men, women, psychotic or non-psychotic patients. Neither of the polymorphisms was associated with the MMSE score change caused by ECT. Distributions of these genotypes are given in Table 2.

In the patients suffering from psychotic depression ( $n = 51$ ) as well as in those with late-onset depression ( $n = 68$ ), CC genotype of BDNF C270T polymorphism was more frequent in those with good response ( $P = 0.028$ ,  $P = 0.038$ , respectively). In the other subgroups, however, no significant differences in the genotypes were found between responders and non-responders. Distributions of these genotypes in subgroups are given in Table 3.

## Discussion

In the present study, an association of BDNF polymorphisms G196A and C270T and response to ECT in MDD was investigated. These polymorphisms were chosen because G196A is a functional polymorphism and its connection to the pathophysiology of depression has been studied earlier [28, 29] and even C270T allele is connected to lower production of BDNF [40]. To our knowledge, there are no previous studies examining the BDNF polymorphisms and the response to ECT. It is suggested that BDNF has antidepressant-like effects and may be associated with

the pathogenesis of major depression [15, 41, 42]. The role of BDNF in the pathogenesis of depression and in antidepressant response in MDD is still disputed.

According to the present study, neither of these BDNF polymorphisms is directly associated with treatment response to ECT. Even though no difference between the patients with response and non-response was found in C270T polymorphism of BDNF in the whole population, the CC genotype was associated with good response in subgroups with psychotic features as well as late-onset depression. In such small subgroups, however, the findings should be approached with caution. This polymorphism has not been studied earlier concerning MDD.

Tsai et al. [29] found a trend for improved therapeutic response to fluoxetine in G196A heterozygotic patients in comparison to homozygotic patients. In the present study, no such association was found. However, the antidepressant action of ECT may differ from the antidepressive action of antidepressant medication. Although both antidepressants and ECT have been suggested to increase the levels of BDNF in hippocampus, this antidepressant action is not fully understood and the antidepressants and ECT may differ essentially from each other.

The present findings may thus support the suggestion of Tsai and others [29] that at least BDNF G196A polymorphism plays no major role in the pathogenesis of MDD. Since BDNF G196A polymorphism has been suggested to influence BDNF secretion, an association between poorer acting met allele and depression could be hypothesized. This, however, was not found in the present severely depressed population.

In the present study, the definition of the response to ECT was the same as that used in the earlier study of the same patient sample [43]. Response was defined as less than 8 in MADRS and non-response as more than 15. This strict definition was used to differentiate two clearly separate response groups, although MADRS 8 or less in fact means remission. Forty-two patients with borderline response were thus excluded from primary analyses. However, when analysing the subgroups only one cut-off point (less than and  $\geq 8$  in MADRS) for response was used in order to avoid missing cases because these populations were relatively small (Table 3). In the present study, all the patients were severely depressed and were admitted consecutively for ECT treatment. Even if this group

**Table 2** Distribution of genotypes of the BDNF polymorphisms G196A and C270T

	G196A			C270T		
	GG	GA	AA	CC	CT	TT
Patients ( $n = 119$ )	82 (68.9%)	35 (29.4%)	2 (1.7%)	101 (84.9%)	17 (14.3%)	1 (0.8%)
Responders ( $n = 45$ )	31 (68.9%)	14 (31.1%)		42 (93.3%)	3 (6.7%)	
Non-responders ( $n = 32$ )	21 (65.6%)	11 (34.4%)		26 (81.3%)	6 (18.8%)	



**Table 3** Distribution of genotypes of the subgroups

	G196A			C270T		
	GG	GA	AA	CC	CT	TT
Psychotic depression (n = 51)	34 (66.7%)	17 (33.3%)		44 (86.3%)	6 (11.8%)	1 (2.0%)
Non-psychotic depression (n = 68)	48 (70.6%)	18 (26.5%)	2 (2.9%)	57 (83.8%)	11 (16.2%)	
Late-onset depression (n = 68)	43 (63.2%)	23 (33.8%)	2 (2.9%)	57 (83.8%)	11 (16.2%)	
Psychotic (n = 34)	22 (64.7%)	12 (35.3%)	28 (82.4%)	6 (17.6%)		
Non-psychotic (n = 34)	21 (61.8%)	11 (32.4%)	2 (5.9%)	29 (85.3%)	5 (14.7%)	
Early-onset depression (n = 51)	39 (76.5%)	12 (23.5%)		44 (86.3%)	6 (11.8%)	1 (2.0%)
Psychotic (n = 17)	12 (70.6%)	5 (29.4%)		16 (94.1%)		1 (5.9%)
Non-psychotic (n = 34)	27 (79.4%)	7 (20.6%)		28 (82.4%)	6 (17.6%)	

was originally very well selected the associations were found only in the subgroups.

Neither of the studied BDNF polymorphisms was associated with the change of MMSE. This rating, however, may not be sensitive enough to detect minor cognitive alterations caused by ECT.

In conclusion, neither of the BDNF polymorphisms studied (G196A, C270T) may be associated with response to ECT in MDD. Within subgroups such as in psychotic and in late-onset depression, CC genotype of C270T may predict good response. These results cannot be explained in the light of earlier studies, so they remain highly speculative.

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## RGS4 polymorphism and response to electroconvulsive therapy in major depressive disorder

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### ABSTRACT

We studied the association between RGS4 (rs951436) polymorphism and treatment response in electroconvulsive therapy (ECT) as well as risk of treatment-resistant depression. The study sample consisted of 119 patients with major depressive disorder (MDD) and 384 healthy control subjects. RGS4 polymorphism was not associated with treatment response in ECT or risk of MDD. According to the present data, the impact of RGS4 genotype is not decisive in major depressive disorder. The results provide preliminary data on the impact of RGS4 polymorphism in treatment response in ECT.

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The regulators of G protein signalling (RGS) proteins are a diverse protein family, modulating the activity of G proteins [6]. RGS proteins accelerate the hydrolysis of GTP from G $\alpha$ , thereby driving the G protein complex into its inactive, GDP-bound state [3,11]. RGS proteins terminate the G protein stimulated signals. RGS4 is highly abundantly expressed in neocortex, striatum, parahippocampus and hippocampus [8,13].

Electroconvulsive therapy (ECT) is the most effective treatment for patients with severe depression [25]. However, the mechanism of action of ECT is not known. Among many other factors, the regulators of G protein signalling proteins are associated with the action of ECT. In animal models of ECT, electroconvulsive shocks (ECS) induced subtype-, time-, and region-specific alteration of RGS proteins [7]. ECS have been shown to regulate gene expression of distinct neurotrophic signalling pathways particularly in the hippocampus of rats [1,20].

Several lines of evidence link RGS4 in depression and ECT. RGS4 modulate several G protein coupled receptors suggested to have major role in the treatment of depression: serotonin, dopamine and metabotropic glutamate receptors [10,5]. RGS4 is also associated

with serotonin signalling in prefrontal cortex [9]. In addition, RGS4 expression is modulated by dopamine receptors [24]. Acute and chronic electroconvulsive seizures regulate RGS4 mRNA levels in hippocampus and prefrontal cortex. However, the exact mechanism of this regulation is still unknown [7]. RGS4 mRNA is also regulated by COMT enzyme activity in the brain [14].

The expression of RGS4 protein has been found to be diminished in the cortical regions of subjects with schizophrenia [4,17]. SNP4 of RGS4 (rs951436) has been shown to be associated with altered prefrontal volumes in patients with schizophrenia, and to a lesser degree with healthy control subjects [19]. RGS4 may also be associated with bipolar disorder [12]. On the other hand, Mirnics et al. [17] found no altered RGS4 expression in the prefrontal cortex in subjects with major depressive disorder. Buckholtz et al. [5] found in their study using functional MRI in healthy subjects that RGS4 A (T) allele (rs951436) was associated with hypofunctionality in working memory tasks and delayed information processing, reflecting decreased grey matter connectivity.

The aim of the present study was to investigate whether there is an association with a single nucleotide polymorphism at RGS4 gene (T>G, rs951436) and the response to electroconvulsive therapy in patients with treatment-resistant depression. The association between RGS4 polymorphism and the risk of treatment-resistant depression was also studied.

The study group consisted of 119 patients (65 women and 54 men) consecutively admitted for ECT to the Department of Psychi-

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atry, Tampere University Hospital. The patients ( $n = 122$ ) who met these eligibility criteria were invited to participate in the study, and three of them declined. The patients fulfilled the diagnostic criteria of DSM-IV for major depressive disorder (MDD) [2], and 51 of them had psychotic symptoms. Patients with neurological disorders, dementia, schizophrenia, bipolar disorder and alcohol or other substance abuse were excluded from the study. The mean age of the patients was 57.7 years (S.D.  $\pm 14.0$ , range 22–84 years). Blood samples were taken at the time of entry into the study and coded. The controls were 384 (210 males and 174 females) healthy blood donors. The mean age of the controls was 44.4 years (S.D.  $\pm 11.1$ , range 19–65 years). The participants gave written informed consent. The study was approved by the Tampere University Hospital Ethics Committee.

ECT was administered three times a week with a brief pulse constant current device (Thymatron DGx, Somatics, Inc., Lake Bluff, IL, USA). The initial stimulus dosage was adjusted with the age method for 30 patients [21]. The seizure threshold was determined for 89 patients by administering successive stimuli of increasing intensity until generalized seizure was induced. Anaesthesia was induced with methohexital and muscle relaxation with succinylcholine. The initial dose was 1 mg/kg of methohexital and 0.5 mg/kg of succinylcholine. The patients were ventilated with 100% oxygen until resumption of spontaneous respiration. Physiological monitoring included pulse oximetry, blood pressure, electrocardiogram (ECG), one channel electroencephalogram (EEG) and electromyography (EMG). The criterion for adequate generalized seizure duration was at least 20 s of motor response and 25 s of EEG seizure activity. If needed, at subsequent treatments during the course of ECT, the dosage was increased to maintain adequate seizure duration. All the patients were treated with standard bilateral (bifrontotemporal) ECT, the number of treatments ranged between 7 and 15,  $9.4 \pm 1.79$  (mean  $\pm$  S.D.). The total number of treatments administered was determined by clinical judgment. ECT was continued until patients were symptom-free or had received at least eight treatments without any further improvement being noted during the past two treatments. The patients received their ongoing medication during the ECT. All except 12 patients had some psychotropic medications (87 had antidepressants, 79 had antipsychotics and 80 had small doses of anxiolytics or hypnotics). Altogether 55 of the patients had additional non-psychotropic medications during the treatment.

The Montgomery and Åsberg Depression Rating Scale (MADRS) [18] was used to assess the severity of depression and the clinical change. Age at first onset of major depression was defined using information from the medical records and the patient's interview. The ratings were done by five experienced psychiatrists.

Rs951436 is located  $\sim 5.5$  kb apart from the 5'UTR of the open reading frame of the gene *RGS4* on chromosome 1q23.3. Genomic DNA was extracted from peripheral blood using a commercially available kit (Qiagen Inc., Hilden, Germany). DNA samples were genotyped by employing the 5' nuclease assay in combination with fluorogenic TaqMan MGB probes; [15] using the ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA,

USA). The nucleotide sequences of primers and allele-specific wild-type and variant probes, labelled with the reporter dyes FAM or VIC, were deduced from sequences deposited in the GenBank database and synthesized in conjugation with Applied Biosystems using the Custom TaqMan® SNP Genotyping Assay. PCR reaction containing genomic DNA,  $1 \times$  Universal PCR Master Mix, 900 nM of each primer and 200 nM of each probe was performed in 96-well plates using the standard protocol in a total volume of 25  $\mu$ l. The endpoint reading of the fluorescence generated from each probe during the PCR amplification was measured by the ABI Prism 7900HT Sequence Detection System (Applied Biosystems) using the allelic discrimination analysis module resulting in clear identification of three genotypes.

The possible transcription factor binding sites in SNP sequence were analysed using TRANSFAC® database [16]. Match™ program did not find any transcription factor binding site at this position of *RGS4* gene promoter region when tested with both possible alleles.

For statistical analyses the ECT response was defined in two ways. First, MADRS endpoint score less than 8 was considered as remission and more than 15 points as non-response. With this criterion, the patients with MADRS score between 8 and 15 were excluded from the analysis due to partial response. The whole sample was also analysed by using one cutpoint (MADRS score less than 8 = remission, MADRS score 8 or more = partial or non-response). Pearson's chi-square test was used to compare genotype and allele frequencies between the study groups. For analyzing the association between MADRS endpoint score and *RGS4* genotype, GLM model was used with MADRS endpoint score as the dependent variable. In this analysis, patient's age, gender, age of onset, psychotic or non-psychotic depression, first episode or recurrent depression, the number of ECT and MADRS baseline score were used as covariates. The limit of statistical significance was set at 0.05. Due to the small sample size no Bonferroni corrections were performed to avoid type II error. Data analysis was carried out using SPSS/Win (Version 14.0, SPSS Inc., Chicago, IL) software.

There were no differences in *RGS4* genotype distributions between patient and control groups ( $p = 0.19$ , chi-square). *RGS4* genotype and allele distributions for patient and control groups are presented in Table 1.

In analyses there was found no difference in *RGS4* genotype distributions between patients with remission ( $n = 45$ ) and non-responders ( $n = 32$ ) ( $p = 0.36$ ), or patients with remission and the rest of the sample ( $n = 74$ ), ( $p = 0.25$ ). Moreover, patients with remission were not more frequently T allele carriers (TT + TG compared to GG genotype) than those with no response ( $p = 0.79$ ) or the rest of the sample ( $p = 0.85$ ). In corresponding analyses with allele frequencies, no differences were likewise not found between patients with remission and non-responders ( $p = 0.33$ ) or the rest of the sample ( $p = 0.29$ ) (Table 2). The ANCOVA model with *RGS4* genotype as a fixed factor and age, gender, age at onset, psychotic/non-psychotic depression, first episode/recurrent depression, the number of ECT and MADRS baseline score as covariates did not predict the endpoint MADRS score on a significant level ( $p = 0.26$ , partial Eta squared 0.10, power 0.6).

**Table 1**  
RGS4 genotype and allele distributions between patients and controls

	GG	TG	TT	Total	G-allele	T-allele	Total
Patients	28 (23.5%)	57 (47.9%)	34 (28.6%)	119 (100%)	113 (47.5%)	125 (52.5%)	238 (100%)
Men	10 (18.5%)	27 (50.0%)	17 (31.5%)	54 (100%)	47 (43.5%)	61 (56.5%)	108 (100%)
Women	18 (27.7%)	30 (46.2%)	17 (26.2%)	65 (100%)	66 (50.8%)	64 (49.2%)	130 (100%)
Controls	108 (28.1%)	187 (48.7%)	89 (23.2%)	384 (100%)	403 (52.5%)	365 (47.5%)	768 (100%)
Men	58 (27.6%)	97 (46.2%)	55 (26.2%)	210 (100%)	213 (50.7%)	207 (49.3%)	420 (100%)
Women	50 (28.7%)	90 (51.7%)	34 (19.5%)	174 (100%)	190 (54.6%)	158 (45.4%)	348 (100%)

Statistics:  $p = 0.19$  between genotypes of patients and controls (chi-square test).

**Table 2**  
Characteristics of responders, partial responders and non-responders

	Remission (n = 45)	Partial responders (n = 42)	Non-responders (n = 32)
Gender (M/F)	22/23	14/28	18/14
Age, mean (S.D.)	58.3 (12.1)	58.7 (17.4)	55.3 (11.4)
Psychotic/non-psychotic	19/26	18/24	14/18
MADRS			
Baseline, mean (S.D.)	31.7 (9.0)	32.6 (7.8)	33.5 (7.7)
After treatment, mean (S.D.)	3.2 (2.4)	10.9 (2.3)	23.1 (6.4)
Number of electroconvulsive treatments, mean (S.D.)	9.3 (1.7)	9.5 (1.6)	9.7 (1.9)
RGS4 genotypes (GG/TG/TT) <sup>1,2</sup>	11/25/9	10/18/14	7/14/11
RGS4 G allele (n) <sup>3</sup>	47	38	28
RGS4 T allele (n) <sup>3</sup>	43	46	36

Statistics: 1. According to *RGS4* genotype:  $p = 0.36$  between remission and non-responders,  $p = 0.25$  between patients with remission and the rest of the sample. 2. According to *RGS4* T-allele carrying status (TT + TG vs. GG genotype):  $p = 0.79$  between remission and non-responders,  $p = 0.85$  between patients with remission and the rest of the sample. 3. According to *RGS4* alleles:  $p = 0.33$  between remission and non-responders,  $p = 0.29$  between patients with remission and the rest of the sample (chi-square test).

This study explored the role of a single nucleotide polymorphism of *RGS4* gene (rs951436) in treatment-resistant major depression and in the treatment response of electroconvulsive therapy. This particular SNP has been found to be important in the pathophysiology of schizophrenia, and there are three important mechanisms of evidence known so far. First, the SNP has been found to increase the risk of this disorder [23]. This may reflect the increased risk for imbalance in the dopamine system of brain [17]. Second, the *RGS4* (rs951436) genotype alters schizophrenia-associated hypofrontality in the prefrontal area [19], which is likely to reflect the neuro-developmental hypofunction through reduced number or function of synapse [17]. Both of these vulnerability mechanisms may be associated with abnormal stress response, which is in most cases also present during onset of mood disorders [22]. *RGS4* genotype may also be a risk factor for bipolar disorder [12]. On the other hand, the functional changes in the prefrontal cortex of patients with schizophrenia have not been comparable to those with a mood disorder [17]. The third mechanism for *RGS4* to be involved in schizophrenia has been tested among healthy subjects, and these results prove conclusively that *RGS4* (rs951436) A (T)-allele is connected with decreased neuro-cognitive functioning through altered connectivity in neural network, resulting in delayed reaction time, and hypoactivity during working memory tasks [5]. Regardless of the diagnostic category, it is likely that these findings form an important framework for a vulnerability mechanism in any severe mental disorder, including major depression. (Note that because of a different genotyping method the A allele in the study by Buckholz et al. [5] is equated with T allele in the present report.)

If the *RGS4* SNP studied had a similar impact on MDD as on schizophrenia, allelic variation should have been found between the patient and control groups and specifically overrepresentation of T-allele in the patient population. The study population was selected according to clinical treatment practice, among those not responding to antidepressive drugs and presenting with the most severe form of MDD. However, the *RGS4* allele frequencies in the present study did not differ between patients and controls. Despite the large control sample it is possible that there is a type II error, and therefore further attempts to replicate the study are necessary. It is unlikely that individuals with an actual MDD would have been selected in the control group due to the screening practice in the blood donor services. The risk of lifetime MDD in the control group can thus be considered greatest at the level of an average population. On the basis of earlier reports we assumed the SNP studied to be in Hardy–Weinberg equilibrium [26].

There was no association within the patient group between *RGS4* genotype and treatment response to ECT in both comparisons between patient groups. In rat models, *RGS4* mRNA levels

in brain have been shown to be regulated by electroconvulsive seizures at transcriptional level, however, the exact mechanism is still unknown [7]. According to this model, the G protein-coupled receptor (GPCR) signalling cascades are involved in the antidepressant action of ECT, which may also function as a regulatory mechanism controlling RGS activity. The *RGS4* genotype may not have an impact on the course of MDD, including response to ECT, or at least this is not the crucial mechanism for the antidepressive effect with ECT in humans. To eliminate the many confounding factors present in clinical setting, we performed a covariate analysis, which did not change the negative results.

The results provide preliminary data on the impact of *RGS4* polymorphism in treatment response in ECT. Because of the limitations mentioned above, the results need to be repeated in an independent sample. The role of *RGS4* (rs951436) in MDD may not be a risk or course-affecting antecedent contrary to schizophrenia.

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# Catechol-O-methyltransferase (COMT) polymorphisms predict treatment response in electroconvulsive therapy

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Several lines of evidence suggest that catechol-O-methyltransferase (COMT) may be associated with treatment response in depression. We conducted a study on 119 patients with treatment-refractory depression admitted consecutively for electroconvulsive therapy (ECT). The COMT high/high genotype leads to a higher enzyme activity and thus lowers dopaminergic activity in the prefrontal cortex. In the present sample, those homozygous to high-active allele of COMT responded significantly more frequently to ECT. *The Pharmacogenomics Journal* advance online publication, 14 August 2007; doi:10.1038/sj.tpj.6500468

**Keywords:** COMT; polymorphism; major depression; electroconvulsive therapy; ECT

## Introduction

Electroconvulsive therapy (ECT) is the most effective treatment in major depression.<sup>1,2</sup> However, the mechanism of the action of ECT is unknown. Several lines of evidence suggest that dopaminergic actions in the prefrontal cortex may be involved.<sup>2–8</sup>

Catechol-O-methyltransferase (COMT) is a major enzyme in dopamine metabolism in the prefrontal cortex. A common functional polymorphism at codon 158 in the gene coding for COMT (COMT Val158Met) results in substantial effects, with Met (low-activity) allele homozygosity leading to a three- to fourfold reduction in enzymatic activity compared with the Val (high-activity) allele homozygosity.<sup>9</sup>

Given that ECT has been shown to affect the dopaminergic functions of the prefrontal cortex, *COMT* is a suitable candidate gene for a pharmacogenetic study. As *COMT* significantly affects the dopaminergic activity in prefrontal cortex, we hypothesized that *COMT* polymorphism may be associated with treatment response in ECT.

## Results

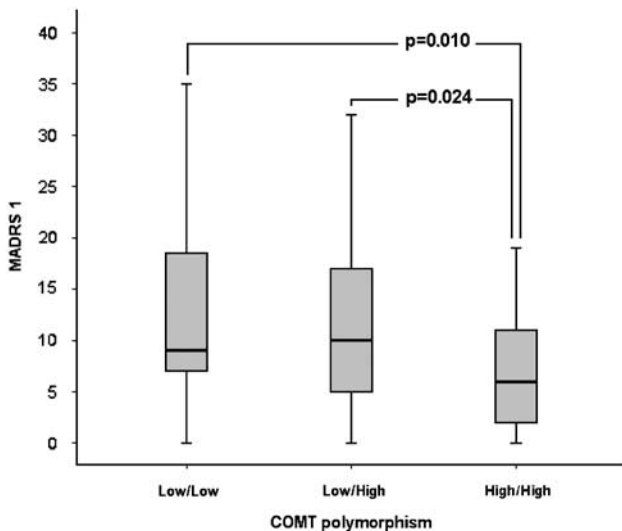
Patients who had <8 scores in Montgomery and Åsberg Depression Rating Scale (MADRS) 1 were considered responders ( $n=45$ ). Scores >15 in MADRS1 indicated nonresponse ( $n=32$ ). *COMT* high/high genotype carriers were more often responders than nonresponders: odds ratio (OR) = 4.366 (95% confidence interval (CI) 1.137–16.770;  $P=0.023$ ) (Table 1).

Before ECT treatment, MADRS0 scores were not associated with *COMT* polymorphisms ( $P>0.22$ , analysis of variance (ANOVA)) (Table 1).

**Table 1** Distribution of the *COMT* gene polymorphisms in responders ( $MADRS1 < 8$ ) and nonresponders ( $MADRS1 > 15$ ) ( $n = 77$ )

	COMT polymorphism			Total
	Low/low (%)	Low/high (%)	High/high (%)	
MADRS1 > 15	9 (28.13)	20 (62.50)	3 (9.38)	32
MADRS1 < 8	8 (17.78)	23 (51.11)	14 (31.11)	45
Total	17 (22.08)	43 (55.84)	17 (22.08)	77

Abbreviations: COMT, catechol-O-methyltransferase; MADRS, Montgomery and Åsberg Depression Rating Scale.

**Figure 1** Patients carrying catechol-O-methyltransferase (*COMT*) high/high ( $n = 25$ ) genotype have significantly lower scores than patients with low/high ( $n = 66$ ) and low/low ( $n = 28$ ) genotype in Montgomery and Åsberg Depression Rating Scale ( $MADRS1$ ) after ECT treatment.

After ECT treatment,  $MADRS1$  was significantly lower in patients with the *COMT* high/high genotype compared to patients with the two other *COMT* genotypes ( $7.40 \pm 5.99$  vs  $12.32 \pm 9.15$ ;  $P = 0.012$ , ANOVA) (Table 1, Figure 1).

The allele and genotype frequencies from the study population were in Hardy-Weinberg equilibrium.

The effect of initial  $MADRS$  scores ( $MADRS0$ ) on treatment response was studied using univariate analysis of variance.  $MADRS0$  as a covariate did not affect treatment response ( $P = 0.328$ ). Age, age of onset, sex and the number of ECT treatment did not affect the treatment response ( $P = 0.170$ , 0.489, 0.524 and 0.148, respectively).

The concomitant uses of antipsychotics, antidepressants and anxiolytics or hypnotics are shown in Table 2.

In power analysis the sample size needed for 30% unit difference between responders and nonresponders was 72 ( $\alpha = 0.05$ ,  $\beta = 0.2$ , power for uncorrected  $\chi^2 = 0.6982$ ). This indicates that the sample size of the present study is adequate.

## Discussion

The present study is the first to address the impact of *COMT* polymorphism on treatment response in ECT. We report that the *COMT* high/high genotype was associated with better treatment response to ECT than were low allele-containing genotypes. As this functional polymorphism of the *COMT* gene is associated with dopaminergic activity, we can assume that lower dopamine levels in the prefrontal cortex are associated with substantially better results in ECT.

Our results suggest that *COMT* high/high genotype carriers are more often responders to ECT than other genotype carriers. High/high genotype is also associated with considerably lower posttreatment  $MADRS$  scores. As there is practically no earlier data on an association between

**Table 2** Demographic data of the patients

N	COMT polymorphism			P-value
	Low/low 28	Low/high 66	High/high 25	
MADRS before ECT ( $MADRS0$ ) (mean $\pm$ s.d.)	31.00 $\pm$ 7.89	33.68 $\pm$ 8.37	31.16 $\pm$ 7.86	0.227
MADRS after ECT ( $MADRS1$ ) (mean $\pm$ s.d.)	13.54 $\pm$ 9.97	11.80 $\pm$ 8.81	7.40 $\pm$ 5.99	0.030
Number of ECT treatments (mean $\pm$ s.d.)	9.29 $\pm$ 1.63	9.56 $\pm$ 1.70	9.52 $\pm$ 1.76	0.769
Age (years) (mean $\pm$ s.d.)	56.36 $\pm$ 14.64	58.70 $\pm$ 13.45	56.36 $\pm$ 14.92	0.666
Age at onset (years) (mean $\pm$ s.d.)	49.75 $\pm$ 16.69	50.27 $\pm$ 15.24	50.64 $\pm$ 14.50	0.978
Proportion of male patients	39.29%	50.00%	40.00%	0.527
Proportion of psychotics patients	35.71%	43.94%	48.00%	0.642
Proportion of recurrent depression	71.43%	72.73%	72.00%	0.991
Proportion of stimulus titration method	64.29%	60.94%	70.83%	0.689
Proportion of concomitant antipsychotics	57.14%	69.70%	68.00%	0.490
Proportion of concomitant antidepressants	71.43%	72.73%	76.00%	0.927
Proportion of concomitant anxiolytics or hypnotics	67.86%	62.12%	80.00%	0.268

Abbreviations: COMT, catechol-O-methyltransferase; ECT, electroconvulsive therapy; MADRS, Montgomery and Åsberg Depression Rating Scale; s.d., standard deviation.

COMT and ECT, interpreting our results is difficult. There is a possibility that those patients with depression having less dopaminergic activity in prefrontal cortex (due to high-active COMT enzyme) may respond better to ECT, which in turn increases dopaminergic activity.<sup>2,10</sup> However, studies of this topic are far from conclusive and further studies are needed to confirm these results.

Our result is in line with Szegedi *et al.*<sup>11</sup> who reported that COMT high (Val)-allele carriers responded better to treatment with mirtazapine in major depression. In addition, Arias *et al.*<sup>12</sup> likewise reported that low/low (Met/Met) genotype was associated with increased risk of nonremission in the clinical response to selective serotonin reuptake inhibitors in depressive patients. Thus, it is possible that these three different kinds of treatments have some characteristics in common. However, Szegedi *et al.*<sup>11</sup> did not find any association between treatment response to paroxetine and COMT polymorphism. Thus, one may suggest that COMT polymorphism is associated with treatment response in major depression only in some cases. However, many more studies are needed before we can conclude which components these different kinds of treatments of depression have in common.

It has been suggested that the effect of the COMT polymorphism in treatment response in depression may be associated with the capability of the COMT enzyme to regulate norepinephrine levels in the brain.<sup>11</sup> However, studies of the role of norepinephrine in ECT have been inconsistent.<sup>10</sup> Moreover, lack of COMT enzyme has not been associated with significant changes in norepinephrine levels in frontal cortex.<sup>13</sup> Thus, the role of norepinephrine may be limited in this context.

Only few studies on the pharmacogenetics of ECT have been published, and with partially conflicting results.<sup>14–17</sup> Thus, comparison of results with those of the present study is not possible.

The relatively small patient sample is the main limitation of the study. However, we were able to create a naturalistic study and recruit almost all the patients with treatment-resistant major depression who were treated with ECT in this geographical region over a period exceeding 8 years.

Another limitation of the study is the concomitant use of psychotropic drugs during the ECT treatment. However, there are no differences in the use of psychotropic drugs between different COMT polymorphisms (Table 2). This may diminish the effect of drug use on the results of the present study. However, because of insufficient data, we cannot rule out the effect of the concomitant drug use to the results of the present study.

The third limitation is the lack of precise data of the patients' earlier episodes of depression and treatments.

## Materials and methods

### Patients and controls

A total of 122 patients with *Diagnostic and Statistical Manual of Mental Disorders*, fourth edition major depressive disorder

(MDD) were treated with ECT in the Department of Psychiatry, Tampere University Hospital. Only 3 patients refused and the study group finally consisted of 119 patients (65 women and 54 men). The high recruitment rate is based on several factors as general positive attitude to medical studies in Finland, regular staff in the ECT unit and the contribution of one author (MH).

The patients were consecutively admitted for ECT because of treatment-resistant MDD. Treatment resistance to antidepressants was defined as two or more unsuccessful trials with antidepressants at an adequate dose for at least 4 weeks. Patients with neurological disorders, dementia, schizophrenia, bipolar disorder and alcohol or other substance abuse were excluded. The mean age of the patients was  $57.7 \pm 14.0$  years. As much as 27.73% of the patients had the first episode of depression. However, they were treated with ECT only after  $0.82 \pm 1.53$  years (range 0–39 years) of suffering from treatment-resistant MDD. There was no difference between MADRS1 in patients who had age-based or stimulus titration method. MADRS1 were  $11.05 \pm 9.12$  for age-based method and  $11.61 \pm 8.76$  for stimulus titration method ( $P = 0.745$ , ANOVA). The patients' demographic data are presented in Table 2. The blood was drawn when a patient entered the study. All patients gave written informed consent. The study was approved by the Tampere University Hospital Ethics Committee.

### Treatment and clinical evaluation

ECT was administered three times a week with a brief pulse constant current device (Thymatron DGx, Somatics Inc., Lake Bluff, IL, USA). The initial stimulus dosage was adjusted with the age method for 30 patients. The seizure threshold was determined for 89 patients by administering successive stimuli of increasing intensity until generalized seizure was induced. Anesthesia was induced with methohexital and muscle relaxation with succinylcholine. The initial dose was 1 mg/kg of methohexital and 0.5 mg/kg of succinylcholine. The patients were ventilated with 100% oxygen until resumption of spontaneous respiration. Physiological monitoring included pulse oximetry, blood pressure, electrocardiogram, one-channel electroencephalogram (EEG) and electromyography. The criterion for adequate generalized seizure duration was at least 20 s of motor response and 25 s of EEG seizure activity. If needed, at subsequent treatments during the course of ECT, the dosage was adjusted upward to maintain adequate seizure duration. Electrical dosages were 100% above the seizure threshold. All the patients were treated with standard bilateral (bifrontotemporal) ECT, the number of treatments ranged between 7 and 15, or  $9.4 \pm 1.79$  (mean  $\pm$  s.d.). The total number of treatments administered was determined by clinical judgment. ECT was continued until patients were free of symptoms or had received at least eight treatments without any further improvement being noted during the past two treatments. The patients received their ongoing medication during the ECT. All except 12 patients had some psychotropic medications (87 had antidepressants, 79 had antipsychotics and 80 had small doses of anxiolytics or hypnotics). Altogether, 55

of the patients had additional no-psychotropic medications during the treatment.

When the study design was determined, there was insufficient research to determine which treatment frequency would be more efficient, two or three times a week. Now we know that they seem to be equally effective.<sup>1</sup>

The severity of depression in all patients was assessed by the MADRS before (MADRS0) and after (MADRS1) ECT treatment course. Five experienced psychiatrists assessed the MADRS.

#### DNA extraction and COMT Val108Met genotyping

Genomic DNA was extracted from peripheral blood leukocytes using commercially available method (Qiagen Inc., Hilden, Germany) and DNA samples were then genotyped by employing the 5' exonuclease assay.<sup>18</sup> Primers and allele-specific probes were designed by Applied Biosystems' design service (Foster City, CA, USA). Probes were fluorescently labeled with either FAM (A allele) or VIC (G allele) reported dyes at the 5' end. At the 3' end, there was a nonfluorescent quencher. PCR reaction mixture consisted of genomic DNA, 1 × Universal PCR Master Mix, 900 nM of each primer and 200 nM of each probe in a total reaction volume of 25 µl. Amplification was performed in 96-well plates using the TaqMan Universal Thermal Cycling Protocol. After PCR, end-point fluorescence intensity was measured by the ABI Prism 7900HT Sequence Detection System (Applied Biosystems) and allelic discrimination performed resulting in clear identification of three genotypes for the COMT Val108Met polymorphism.

#### Statistical analysis

Pearson's  $\chi^2$ -test was used to compare genotypes and allele frequencies between the study groups. The OR was calculated with 95% CI. ANOVA was used to compare means of MADRS1 scores. Univariate analysis of variance was used in covariate analysis. The limit of statistical significance was set at 0.05. Data analysis was carried out using SPSS/Win software (Version 14.0, SPSS Inc., Chicago, IL, USA). Power analysis was performed by PS Power and Sample Size Calculations, Version 2.1.30.

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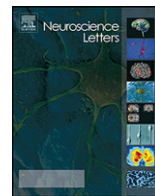
#### Duality of interest

We declare that we have no duality or conflict of interest.

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## Dopamine 2 receptor C957T and catechol-o-methyltransferase Val158Met polymorphisms are associated with treatment response in electroconvulsive therapy

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### ABSTRACT

Alterations in dopamine levels and dopamine receptors in brain are suggested to be associated with treatment response in electroconvulsive therapy (ECT). Dopamine 2 receptor gene (DRD2) polymorphism C957T (rs6277) and catechol-o-methyltransferase (COMT) polymorphism Val158Met (rs4680) interaction was studied in 118 patients suffering from major depressive disorder (MDD) treated with ECT and 383 healthy controls. It was found that the combination of COMT Met allele and DRD2 T allele predicted more severe depression in those already affected but did not predict the risk of depression when compared to normal population. The genotype modified the response to ECT. The patients with TT genotype of D2 receptor gene C957T polymorphism combined with COMT gene polymorphism Met/Met genotype did not achieve remission as often as those with CC genotype of DRD2 C957T combined with COMT Val/Val genotype. Thus the interaction of these polymorphisms may be associated with response to ECT.

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Several independent studies suggest that alterations in dopamine levels and dopamine receptors in the brain are associated with treatment response in electroconvulsive therapy (ECT) [17,23,24]. In addition, a number of studies have highlighted the role of dopamine in the pathophysiology of depression [11,28,30]. In animal models of ECT an increasing amount of dopamine has been detected in the CNS [27,37]. We therefore focused on two functional gene polymorphisms related to dopaminergic activity in our genetic study dealing with response in ECT and the risk of treatment-resistant depression: dopamine 2 receptor (DRD2) gene C957T (rs6277) and catechol-o-methyltransferase (COMT) gene Val158Met (rs4680) polymorphisms.

COMT enzyme in the prefrontal cortex is the major enzyme metabolizing dopamine. The COMT gene contains a functional polymorphism Val158Met. Val allele has higher enzymatic activ-

ity than Met allele, thus Met/Met genotype carriers have higher levels of dopamine in prefrontal cortex [8,20]. The COMT polymorphism has also been associated with treatment response to several antidepressants. Patients with COMT Met/Met genotype may have a slower treatment response to citalopram and mirtazapine. This effect was not found with fluvoxamine or paroxetine treatment [6,36]. In the study by Szegedi et al. [36] the response to mirtazapine treatment was also poorer in patients with COMT Met/Met genotype compared to other genotypes. On the other hand, Baune et al. [7] recently reported that COMT Val/Val genotype predisposed to non-response to treatment with different antidepressants. The recent study by our study group, however, suggested a significant association between COMT polymorphism and treatment response in ECT [5]. Patients who had one or two high-activity Val alleles responded better in ECT. Thus lower dopaminergic activity in prefrontal cortex may be associated with treatment response in ECT.

Duan et al. [10] found in the DRD2 gene a new polymorphism with effects on mRNA folding, stability and translations as well as changed dopamine induced up-regulation of DRD2 expression. This polymorphism is also associated with striatal DRD2 receptor

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availability in vivo [13] and the risk of schizophrenia [16]. It has also been suggested that lower dopaminergic activity may increase the risk of depression [11]. In addition, treatment-resistant patients with unipolar depression showed lower levels of brain norepinephrine and dopamine [21].

COMT Val158Met has not been directly associated with the risk of depression [12,19,32,33]. However, COMT Val158Met has been reported to be associated with anxiety and emotional dysregulation [9]. A recent study by Jabbi et al. [18] showed that patients homozygotes on Met allele had higher stress response and suggested that this genotype may predispose to major depressive disorder (MDD). Massat et al. [25] reported that high-activity COMT Val allele was more frequent in early onset patients with major depressive disorder.

There are so far no reports of an association of DRD2 C957T polymorphism and depression or its treatment response. However, theoretically C allele with lower dopaminergic activity may be associated with both of these.

The main aim of the present study was to explore the effect of DRD2 polymorphism and the interacting effect of DRD2 C957T and COMT Val158Met polymorphisms on treatment response in ECT and risk of depression. We hypothesized that the combination of C allele in DRD2 C957T and Val allele in COMT Val158Met leading to lower dopaminergic activity may modify the treatment response in ECT as well as predispose to MDD.

The study group consisted of 122 Finnish Caucasian patients (64 women and 54 men) consecutively admitted for ECT to the Department of Psychiatry, Tampere University Hospital. The patients ( $n = 122$ ) who met these eligibility criteria were invited to participate in the study. Three of them declined and one of them was excluded because the genotyping of D2 failed. The patients fulfilled the diagnostic criteria of DSM-IV for MDD [1], 51 with psychotic depression. All the patients were treatment-resistant to at least two antidepressants (ADs) of different groups at an adequate dose in a trial of at least 4 weeks. Patients with neurological disorders, dementia, schizophrenia, bipolar disorder and alcohol or other substance abuse were excluded from the study. The mean age of the patients was 57.7 years ( $SD \pm 14.0$ , range 22–84 years). Blood samples were taken at the time of entry into the study and coded. The controls were 383 Finnish Caucasian (392 in COMT genotyping) (209 males and 174 females) healthy blood donors. The mean age of the controls was 44.4 years ( $SD \pm 11.1$ , range 19–65 years). The age difference between patients and controls was significant ( $p < 0.001$ ). The participants gave written informed consent. The study was approved by the Tampere University Hospital Ethics Committee.

ECT was administered three times a week with a brief pulse constant current device (Thymatron DGx, Somatics, Inc., Lake Bluff, IL, USA). The initial stimulus dosage was adjusted with the age method for 30 patients [35]. The seizure threshold was determined for 89 patients by administering successive stimuli of increasing intensity until generalized seizure was induced. Anesthesia was induced with methohexital and muscle relaxation with succinylcholine. The initial dose was 1 mg/kg of methohexital and 0.5 mg/kg of succinylcholine. The patients were ventilated with 100% oxygen until resumption of spontaneous respiration. Physiological monitoring included pulse oximetry, blood pressure, electrocardiogram (ECG), one channel electroencephalogram (EEG) and electromyography (EMG). The criterion for adequate generalized seizure duration was at least 20 s of motor response and 25 s of EEG seizure activity. If needed, at subsequent treatments during the course of ECT, the dosage was increased to maintain adequate seizure duration. All the patients were treated with standard bilateral (bifrontotemporal) ECT, the number of treatments ranged between seven and 15,  $9.4 \pm 1.79$  (mean  $\pm$  SD). The total number of treatments adminis-

**Table 1**

Concomitant psychotropic medication of the patients.

	n = 118	%
No medication	12	10.2
Antidepressants	5	4.2
Antipsychotics	4	3.4
Anxiolytics or hypnotics	3	2.5
Antidepressants + antipsychotics	18	15.3
Antidepressants + anxiolytics or hypnotics	20	16.9
Antidepressants + antipsychotics + anxiolytics or hypnotics	43	36.4
Antipsychotics + anxiolytics or hypnotics	13	11.0

tered was determined by clinical judgment. ECT was continued until patients were symptom-free or had received at least eight treatments without any further improvement being noted during the past two treatments.

The patients received their ongoing medication during the ECT. The details of their psychotropic medications are given in Table 1. The most commonly used antidepressants were: citalopram, venlafaxine, mirtazapine; antipsychotics: risperidone, olanzapine and levomepromazine; anxiolytics/hypnotics: oxazepam, lorazepam, temazepam. Altogether 55 of the patients had additional non-psychotropic medication during the treatment. It was decided to continue the psychiatric drug treatment in order to prevent an early relapse and perhaps to obtain the synergistic therapeutic action [2]. The dosages of concomitant anxiolytics and/or hypnotics were kept as small as possible. The Montgomery and Åsberg Depression Rating Scale (MADRS) [29] was used to assess the severity of depression and the clinical change. The ratings were done by five experienced psychiatrists.

Pearson's Chi-square ( $\chi^2$ ) test was used to compare genotypes and allele frequencies between the different study groups.

T-test was used to compare the difference of MADRS0 (baseline, before ECT) and MADRS1 (after ECT) scores in TT homozygotes of DRD2 gene C957T polymorphism with C allele carriers and analysis of variance (ANOVA) when comparing COMT polymorphisms. Age, gender and AD treatment adjusted analyses were also done.

The ECT response was defined in two ways. First, MADRS end-point score less than 8 was considered as remission and more than 15 points as non-response. In order to achieve two clearly biologically and clinically different groups, patients with MADRS score between 8 and 15 were excluded from the efficacy analysis due to partial response. This same definition has also been used in other reports on this same patient population [5,14,15]. Odds ratios were calculated with 95% confidence interval using binary logistic regression analysis. The limit of statistical significance was set at 0.05. Data analysis was carried out using SPSS/Win software (Version 14.0, SPSS Inc., Chicago, IL, USA).

Genomic DNA was extracted from peripheral blood leukocytes using QIAamp<sup>®</sup> DNA Blood Minikit and automated biorobot M48 extraction (Qiagen, Hilden, Germany). D2 receptor C957T (rs6277) genotyping was performed by using Taqman<sup>®</sup> SNP Genotyping Assay C\_11339240\_10. Primers and allele specific probes used in genotyping the COMT V158M (rs4680) with a Custom Taqman<sup>®</sup> SNP Genotyping Assay were designed by Applied Biosystems' design service (Applied Biosystems, Foster City, CA, USA). Genotyping was performed using the ABI Prism 7900HT Sequence Detection System according to manufacturer's instructions (Applied Biosystems, Foster City, CA, USA). A description of the 5' nuclease assay method can be found in Livak [22]. Random duplicates were run in parallel with unknown DNA samples.

The distribution of the DRD2 gene and COMT gene polymorphisms did not differ between the patients and the controls (Table 2). Moreover, the pooled populations of these polymorphisms did not differ either. Thus these polymorphisms were

**Table 2**

Distribution of DRD2 and COMT gene polymorphisms and the MADRS scores in the study population.

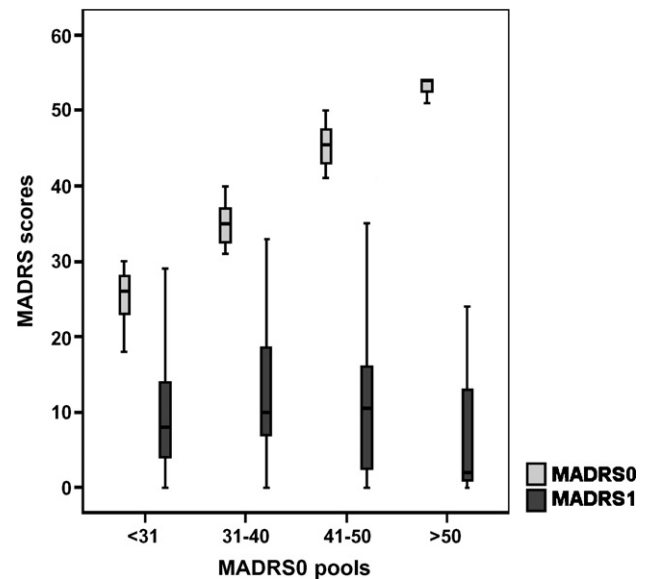
	DRD2			COMT		
	TT	CT	CC	Met/Met	Val/Met	Val/Val
Controls	104 (27.2%)	175 (45.7%)	104 (27.2%)	106 (27.0%)	205 (52.3%)	81 (20.7%)
Patients	30 (25.4%)	59 (50.0%)	29 (24.6%)	28 (23.5%)	66 (55.5%)	25 (21.0%)
MADRS0	36.4	31.0	31.4	31.0	33.7	31.1
MADRS1	14.1	10.7	9.8	13.5	11.8	7.4
MADRS > 15	11 (34.4%)	15 (46.9%)	6 (18.8%)	9 (28.1%)	20 (62.5%)	3 (9.4%)
MADRS < 8	8 (18.2%)	24 (54.5%)	12 (27.3%)	8 (17.8%)	23 (51.1%)	14 (31.1%)

DRD2: Dopamine 2 receptor gene; COMT: Catechol-o-methyltransferase; MADRS0: Baseline, score before ECT; MADRS1: Score after ECT.

not associated in the risk of treatment-resistant depression in the present sample. Both polymorphisms studied were in Hardy–Weinberg equilibrium.

In patients DRD2 polymorphisms were associated with MADRS0 (scores before treatment) and MADRS1 (scores after treatment). TT homozygotes of DRD2 gene C957T polymorphism had higher scores before treatment (MADRS0  $36.4 \pm 7.4$ ) than the CT and CC genotypes (C allele carriers) (MADRS0  $31.1 \pm 8.1$ ),  $p = 0.002$ . TT homozygotes also had higher scores after treatment (MADRS1  $14.1 \pm 10.2$ ) than C allele carriers (MADRS1  $10.4 \pm 8.1$ ),  $p = 0.046$  (*t*-test). COMT polymorphisms were not associated with MADRS0 but they were associated with MADRS1,  $p = 0.012$  [5] (Table 2). Age, gender and AD treatment were not associated with the treatment response. In the whole patient population the severely depressed patients achieved the greatest reduction in MADRS scores after ECT,  $p < 0.0001$  (ANOVA) (Fig. 1). Based on the assumption that dopamine activity is associated with treatment response, three groups were formed: HIGH dopamine activity group [DRD2(TT)+COMT(Val/Met+Met/Met)], LOW dopamine activity group [DRD2(CC+CT)+COMT(Val/Val)] and the REST gene polymorphisms. The patients with HIGH activity were at baseline more severely depressed (MADRS0 =  $37.2 \pm 7.7$ ) than the patients with LOW activity (MADRS0 =  $31.0 \pm 8.6$ ) or REST genotypes (MADRS0 =  $31.2 \pm 7.7$ ) ( $p = 0.004$ ) (Table 3). The combination of DRD2 + COMT polymorphisms were associated with MADRS scores after treatment ( $p = 0.007$ , ANOVA, Table 3). The result remained the same adjusted by age, gender and AD treatment. Patients with different dopamine activity (HIGH, LOW, REST) were differentially distributed according to their remitter vs. non-responder status ( $p = 0.023$ , Table 3). Patients in the LOW dopamine activity group were after ECT significantly more often in remission (MADRS1 < 8) than non-responders (MADRS1 > 15) compared to that in the HIGH activity group (OR = 11.0, 95% CI 1.7–70.0;  $p = 0.011$ ). When the REST group was compared to the HIGH group, OR was not significant (OR = 2.8, 95% CI 0.8–9.5;  $p = 0.097$ ) (Table 3). These results remained when adjusted by age, gender and AD treatment.

In the present study the gene polymorphisms examined did not differ between patients and healthy controls, so it is unlikely that they were associated with the biological pathophysiology of severe depression. The combination of COMT Met allele and DRD2



**Fig. 1.** MADRS end score in the whole population pooled in ten point range. Response to ECT in whole patient population.

T allele (HIGH dopamine activity) predicted more severe depression in those who were already affected but it did not predict the risk of depression when compared to normal population.

In light of the monoamine hypothesis it would be logical to hypothesize that patients who have less dopamine in certain brain areas may be more severely depressed than otherwise [31]. The obvious discrepancy between this theory and the present results may lead us to hypothesize that the severity of depression in most patients is associated with other factors than dopamine alone. Indeed, in two recent studies concerning the present population, the risk of depression was associated with serotonin linked polymorphisms of TPH1 and GNB3 as well as of 5HT1A and BDNF [3,4].

It has been suggested that ECT is the most effective treatment method in major depression. Patients with treatment-resistant severe depression are usually referred for ECT and they also achieve

**Table 3**

Distribution of the pooled polymorphisms of DRD2 and COMT and the MADRS scores in these subgroups.

	HIGH	REST	LOW	<i>p</i> -value HIGH vs. LOW
Controls <i>n</i> = 383	80 (20.9%)	224 (58.5%)	79 (20.6%)	
Patients <i>n</i> = 118	25 (21.2%)	73 (61.9%)	20 (16.7%)	
MADRS0 (mean ± SD)	$37.2 \pm 7.7$	$31.2 \pm 7.7$	$31.0 \pm 8.6$	
MADRS1 (mean ± SD)	$15.6 \pm 10.1$	$10.9 \pm 8.5$	$7.6 \pm 5.7$	
MADRS change 0–1 (mean ± SD)	$21.6 \pm 12.6$	$20.3 \pm 11.6$	$23.4 \pm 9.0$	
MADRS1 > 15	10 (40.0%)	20 (27.4%)	2 (10.0%)	0.024
MADRS1 < 8	5 (20.0%)	28 (38.4%)	11 (55.0%)	0.015

HIGH: High dopamine activity, DRD2(TT)+COMT(Val/Met+Met/Met); REST: Other genotypes; LOW: Low dopamine activity, DRD2(CC+CT)+COMT(Val/Val); MADRS0: Baseline, score before ECT; MADRS1: Score after ECT.

the best response to this treatment. This was also true in the present whole sample when the outcome was considered without the genotype. However, the genotype modified the response to ECT and it was not as expected in patients with TT genotype of DRD2 gene C957T polymorphism combined with COMT gene Val158Met polymorphism Met/Met genotype. Those patients with HIGH dopamine activity and severe depression did not achieve remission as often as those with LOW dopamine activity. However, this was only seen when clinically clearly different subgroups (remitters vs. non-responders) were considered. When three groups of different dopamine activity were compared with regard to their direct change in MADRS (MADRS0-MADRS1) in the post hoc analyses, no significant differences between the groups were found (Table 3). Age, gender and AD treatment did not contribute to the treatment results in the whole population or in subgroups. In a recent study on the present patient population an association was also found between lower dopamine levels in CNS (COMT 158 Val/Val) and better response to ECT [5]. This phenomenon actually seems to increase when this COMT genotype is combined with DRD2 C allele. According to the “inverted U” model there is a narrow range in dopamine activity to be optimal [26]. In patients with LOW dopamine activity, the antidepressant or ECT-induced extra dopamine load may result in better dopamine levels according to the inverted U curve and thus in a better treatment response. Thus in patients with HIGH baseline dopamine activity, ECT-induced dopamine excess does not provide any extra benefit. Therefore MADRS scores did not diminish as expected in this severely depressed group. Patients who are depressed but have intact dopamine activity may have insufficiency in some other neurotransmitter systems.

The main limitation of this study was the relatively small patient population leading to small cell sizes in the analyses of subgroups. A major limitation was that the CNS dopaminergic activities were not measured in this study and the hypothesis of an association between lower dopaminergic activity and depression was thus based on the literature. Another limitation is the concomitant use of psychotropic drugs during ECT. Thus, the effect of concomitant medication on the present results cannot be entirely ruled out. However, almost all the patients with treatment resistant major depression who were treated with ECT in this geographical region over a period exceeding 8 years were recruited for this study. A further limitation is that the cases were older than the controls. It was impossible to match the cases and controls by age because the controls were volunteer middle-aged blood donors.

It is obvious that there is a genetically determined difference in dopamine activity. According to the present results dopamine may not as such be associated with the risk of depression. However, it may modify the treatment response so that patients with LOW dopamine activity may have a better response to ECT. This finding is very preliminary and far from biomarker predicting the treatment response.

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