

PHARMACOGENETICS OF ANTIDEPRESSANTS

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Summary

Antidepressants are the first line for the short and long term treatment of mood disorders. Recently, some gene variants have been associated with antidepressant efficacy. This is a first step toward a more detailed molecular comprehension of the pathophysiology, an individualized therapy and, in the long run, toward a targeted gene therapy. In detail the functional polymorphism in the upstream regulatory region of the serotonin transporter gene (5-HTTLPR), the A218C gene variant on the tryptophan hydroxylase gene (TPH), the 102TC variant in the 5HT2A receptor, the G-protein beta3-subunit (Gbeta3) C825T gene variant and the Circadian Locomotor Output Cycles Kaput (CLOCK) gene variants were independently associated with short term SSRIs antidepressant efficacy. The effects of 5-HTTLPR and TPH polymorphisms were more pronounced in subjects not taking pindolol. Marginal associations were reported for ADRB1, ACE I/D, BDNF and IL-1beta. DRD2, DRD4, Mao-A, SERT-STin2, 5HT6, NOS gene variants were not associated with outcome.

Key Words: Pharmacogenetics – Antidepressant response – Major depression – SSRI

Declaration of interest: none

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Introduction

The pharmacological treatment of mood disorders reduced their morbidity and improved mental health for millions of individuals worldwide, favouring a considerable reduction of the direct and indirect costs caused by these common pathologies. The main supporters of these results are antidepressant drugs (AD), available since the first fifties, which improved well-being and increased the chance of a good long term outcome. During the last decade selective serotonin reuptake inhibitors (SSRIs) have revolutionized the treatment of depression (Grunze et al. 2002). These drugs show high efficacy, and relatively few adverse reactions compared with the previously used tricyclic antidepressants, even if their mechanism of action is not entirely understood yet. From the late eighties, also other not-pharmacological treatments, such as sleep deprivation (SD) have proved their efficacy, both as the only and as augmentation therapy, in addition to antidepressants.

Unfortunately, not all subjects benefit from treatments: a percentage of 30-40% of the patients do not show a complete response to treatment (Geddes et al. 2000). Such difficulties could be avoided if it would be possible to determine more quickly the most suitable drug for each subject. Efficient clinical predictors are not available: genetic factors are thought to play a substantial (but complex) role in antidepressant response

(Berrettini 1998, Orsini 1987, Pare and Mack 1971, Sederer 1986).

Polymorphisms in drug target genes, changing protein amino-acid sequence, or protein expression level (if the polymorphism is located in a gene promoter) could influence its transcriptional control and thus drug responsiveness.

Genes coding for proteins somewhat involved in monoaminergic pathways and other possible targets of antidepressant action could present functional polymorphisms, altering their constitutive activity. Pharmacogenetics, which investigates the influence of genetic features on pharmacological response, gained increasing attention and holds great promises for clinical psychiatry. This success is mainly due to its great potential in optimising psychopharmacology, given the lack of biologically based treatment guidelines (Dettling et al. 2001, Pickar and Rubinow 2001, Roses 2000, Segman et al. 1999). A major obstacle to the advance of pharmacogenetics is the difficulty in finding candidate polymorphisms. They are in fact very common, therefore millions of them must be identified and analysed to determine their involvement (if any) in drug response. Further complicating the process is our limited knowledge of which genes are involved with each drug response. Since many genes are likely to influence responses, obtaining the big picture on the impact of gene variations is highly time-consuming and complicated. For this reason, candidate genes should be chosen ac-

cording to their possible implications with pharmacological action and pathogenic mechanisms. Furthermore, the complexity of genetic influences is the result of the combination of various single gene mutations, as each of them, taken individually is unlikely to cause the variability observed in treatment response.

The foremost theory for explaining the biological basis of antidepressant action is their ability to improve monoaminergic transmission. Antidepressant drugs are therefore classified according to their properties in improving monoaminergic transmission in the different biogenic monoamines systems: serotonin, (5HT) noradrenaline (NA), dopamine (DA).

Pharmacogenetic studies in mood disorder were performed only during recent years (for a review see Serretti et al. 2002). Polymorphisms at some genes have been studied to date, to test their association with antidepressant response. The present paper intended to offer a brief overview on the various pharmacogenetic studies published to date, which analyse the commonest treatments for depression: antidepressants and sleep deprivation (figure 1).

than twice that of the short form of the SERT gene promoter. These two functional polymorphisms (Stin2 and SERTPR) were investigated to date.

Our group tested the hypothesis that SERTPR could be related to the response of fluvoxamine and/or augmentation with pindolol. One hundred and two inpatients with delusional major depression were analysed; they were randomly assigned to treatment with fluvoxamine and either placebo or pindolol for 6 weeks. Depression severity was assessed weekly with HAMD, and allelic variations were detected by a PCR-based method. Both homozygous for the long variant (l/l) and heterozygous (l/s) showed a better response to fluvoxamine than homozygous for the short allelic variant (s/s) in the fluvoxamine plus placebo group, while in the fluvoxamine plus pindolol group these differences were not observed (Smeraldi et al. 1998). We then replicated this finding on a sample of 155 inpatients treated in the same standardized method of the previous study, and doing the same assessment of the antidepressant outcome. Also in this study SERTPR s* allele was associated with a poor response to fluvoxamine treatment, and the diagnosis, the presence of psychotic features,

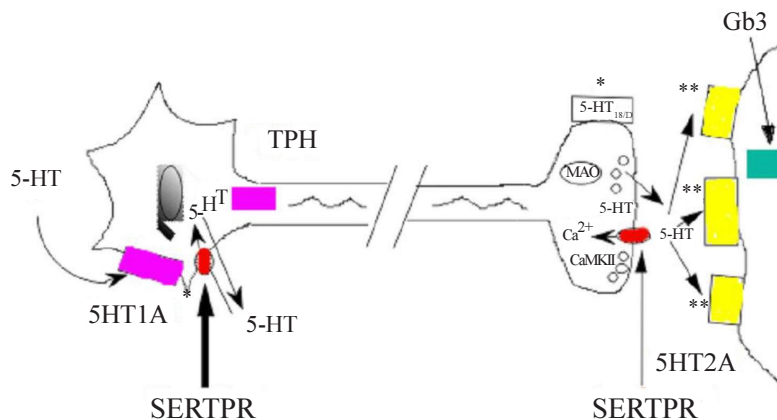


Figure 1. In the figure we graphically displayed the gene variants that have been associated with response to SSRI in depression

Pharmacogenetics of antidepressants

A lot of polymorphisms investigated in pharmacogenetic studies are located in genes coding for proteins belonging to the catecholamine systems (serotonergic, noradrenergic, dopaminergic).

The most widely investigated were those of the brain 5-HT transporter (SERT). SERT takes back 5-HT into the pre-synaptic neurons, which thus recycle it into the neurotransmitter pool.

The brain 5HT transporter (SERT) appears to be a principal site of action of many antidepressants and may mediate behavioural and toxic effects of cocaine and amphetamines.

Ramamoorthy et al. (Ramamoorthy et al. 1993) identified and cloned a single gene encoding the human

SERT, localised to chromosome 17q11.1-q12. The gene spans 31 kb and consists of 14 exons (Lesch et al. 1994). Ogilvie et al. (Ogilvie et al. 1996) identified a variable number tandem repeat (VNTR) polymorphisms (Stin2). Heils et al. (Heils et al. 1996) reported a polymorphism in the transcriptional control region upstream of the SERT coding sequence (SERTPR). Initial experiments demonstrated that the long and short variants of this SERT gene-linked polymorphic region have different transcriptional efficiencies. The polymorphism is located approximately 1 kb upstream of the transcription initiation site and is composed of 16 repeat elements. It consists of a 44-bp insertion or deletion involving repeat elements 6 to 8. Lesch et al. (Lesch et al. 1996) called it 5-HTTLPR and studying lymphoblastoid cell lines found that the basal activity of the long variant was more

and the severity of depressive symptomatology did not influence the association (Zanardi et al. 2001). In a third study we investigated the persistence of the finding for another antidepressant treatment, analysing the association of alleles at SERTPR polymorphism with paroxetine treatment and evidencing an analogue finding (Zanardi et al. 2000). the presence of the *l variant of the SERTPR was associated with a more favorable and faster response (Zanardi et al. 2000), and the finding was independently replicated by Pollock et al. (Pollock et al. 2000) in a sample of 95 elderly patients receiving paroxetine or nortriptyline in a standardized treatment. Patients were treated for up to 12 weeks and assessed weekly with clinical ratings and measurements of plasma drug concentrations; they found a significantly more rapid mean reductions from baseline in HAMD for patients with the l/l genotype, despite equivalent paroxetine concentrations.

Similar findings were obtained using citalopram in a Spanish sample (Arias et al. 2003); s/s genotype was associated with non-remission at 12th week ($p=0.013$), and homozygous for the s allele presented three times more the risk for non reaching remission of depressive episode after citalopram treatment. In an American elderly sample, l/l subjects showed a significant increase in response at first weeks of treatment with sertraline, as assessed by the CGI-I scale, with no significant difference between genotypes in a placebo group (Durham et al. 2004).

Recently, in a study on 51 patients with major depression treated with fluoxetine (1.25 mg to 40 mg per day) also platelet SERT kinetics before treatment was measured, in order to investigate the possibility that one genotype could express a lower-dose fluoxetine response. After a 1-week placebo lead-in, the 24 item Hamilton depression was administered, and repeated after 6, 12, and 18 weeks of treatment. A repeated-measures analysis of variance of 24-item Hamilton depression change through baseline was performed, to test a genotype effect on outcome. Genotype, as well as affinity constant and dose show a significant effect on outcome. SERTPR seemed to show both a placebo response effect, and a drug dose response effect: in fact, the l allele group was more responsive to placebo, as well as more responsive to drug dose than the s allele group. Of course, the small size of the sample does not allow any definite conclusion (Rausch et al. 2002).

In the Asian population contrasting results were obtained; Kim et al. found an association in the opposite direction, by comparing 120 patients and 252 healthy controls; in their sample homozygous subjects for the s allele showed a better response both to fluvoxamine and to paroxetine. (Kim et al. 2000). A subsequent study confirmed this result: one analysed 66 Japanese patients with major depressive disorder in a 6-week study with fluvoxamine; the short allele frequency was significantly higher in responsive individuals than in non responsive ones (Yoshida et al. 2002a). Conversely, the second study on 121 Chinese patients diagnosed with major depression revealed that patients with the l/l genotype had a significantly better response to fluoxetine, as evaluated on the basis of total, core, psychic-anxiety and somatic-anxiety HAMD score percentage change, Surprisingly confirming those performed on Caucasian population (Yu et al. 2002). Finally, Ito et al. studied the

association between SERTPR and response to fluvoxamine prescribed up to 200 mg/day for 6 weeks in 66 patients with major depressive disorder, and found no significant association either for s or for l variant (Ito et al. 2002). More recently, Yoshida replicated the negative findings about SERTPR (Yoshida et al. 2004), in a study investigating also other polymorphisms; the authors found association between the T allele of the NET T-182C and a better antidepressant response, and between the A/A genotype of the NET G1287A and a slower onset of response. No association was found besides SERTPR, for SERT-STin2 and 5HT2A G-1438A polymorphism. In Asian population the results are thus very conflicting, most probably the small sample sizes; moreover, different ethnicity and different definition of responders do not allow to draw a definite conclusion on the role of the SERTPR polymorphism.

Recently, a new polymorphism (an A to G SNP) was observed in the *l variant of the SERTPR gene: the *G variant of the *l allele is less expressed than the *A variant, in immortalized cell lines, more similarly to the expression of the s allele (Goldman et al. 2004). Probably, this finding could explain some of the differences between the various studies on SERTPR. Beyond the biological aspects, also some clinical aspects ought to be considered: disease features are not always the same in all patients, and clinical improvement should be considered not only as a whole but also in each of its single components.

To our knowledge, three studies investigating the association between a genetic polymorphism and switch were performed: the one by Mundo et al. (Mundo et al. 2001), reported an association between the short (*s) variant of the functional polymorphism located in the upstream regulatory region of the serotonin transporter gene (SERTPR) and antidepressant-induced mania. This finding was not confirmed by our data (Artioli et al. 2002, Serretti et al. 2004), and also a recently published retrospective study found no association between this SERTPR variant and switch, either using a broad or a restrictive definition (Rousseva et al. 2003).

Other analysed polymorphisms belong to the synthesizing and catabolising pathways of the monoamines. One of them, the human tryptophan hydroxylase gene (TPH), has been cloned (Boularand et al. 1990), is located on chromosome 11p14-15.3 (Craig et al. 1991). and codes for the rate-limiting enzyme of serotonin biosynthesis;

The best-studied TPH variants are the two bi-allelic polymorphisms, on position 218 (A218C) and 779 (A779C) of intron 7, in strong disequilibrium (Nielsen et al. 1997). The polymorphism A218C is located in a potential GATA transcription factor-binding site, so that it may influence gene expression, and consequently the antidepressant response. The rarer TPH*a of A218C allele showed in fact to be associated to a decreased serotonin synthesis (Jonsson et al. 1997). Our group published two papers, which demonstrated the influence of this polymorphism in SSRIs response. The first study involved a sample of 217 subjects affected by major depression and bipolar disorder, with or without psychotic features, treated with fluvoxamine 300 mg and either placebo or pindolol in a double blind design for 6 weeks, assessing the severity of depressive symptoms weekly with the Hamilton Rating Scale for Depression.

TPH allelic variants were determined in each subject by using a PCR-based technique. No significant finding was observed in the overall sample as well as in the pindolol group, while TPH*A/A was associated with a slower response to fluvoxamine treatment in subjects not taking pindolol ($P = 0.001$) (Serretti et al. 2001c). For the second study, a sample of 121 inpatients affected by a major depressive episode was recruited; each patient was treated with paroxetine 20-40 mg with either placebo or pindolol in a double blind design for 4 weeks. Again, the authors found that A/A and A/C genotypes were associated with a poorer response to paroxetine without pindolol, when compared to TPH* C/C ($p = 0.005$); this difference was not present in the pindolol augmented group. Other variables, such as sex, diagnosis, presence of psychotic features, severity of depressive symptomatology at baseline and paroxetine plasma level, were not associated with the outcome (Serretti et al. 2001b). Even if two different SSRIs (fluvoxamine and paroxetine) were used, the A218C polymorphism (probably together with other genetic or environmental factors) seemed to be involved in modulating antidepressant response.

Yoshida et al. reported a contrasting result (Yoshida et al. 2002b) on a sample of 54 Japanese subjects affected by major depressive disorder and treated with fluvoxamine. However, it must be observed that the sample was smaller than the previous one and belonging to a different ethnicity, which shows, probably, a different distribution of the variants of this polymorphism. The same Japanese group also published a paper reporting lack of association between this and other polymorphisms with the development of fluvoxamine-induced nausea (Takahashi 2002).

Recently, a second gene encoding for TPH was found in vertebrates, called TPH2 (Walther 2003). TPH2 is predominantly expressed in the brain stem, while the classical TPH gene (now called TPH1) is expressed in the gut, pineal gland, spleen and thymus. These findings lead to a new concept of the serotonin system, with the presence of two serotonin systems in vertebrates, independently regulated and with distinct functions (Walther and Bader 2003). At the light of these recent findings, pharmacogenetic studies on TPH1 should be reconsidered; it could seem surprising that a polymorphism located on TPH1 could influence antidepressant response. Positive findings in association study could be explained with the presence of an involvement of the "peripheral" serotonin system in pharmacological antidepressant response. We may also hypothesize the presence of a genetic variant in strong linkage disequilibrium with this gene, which could be involved in antidepressant response, or other unknown peripheral interactions.

The catabolism of catecholamines is catalysed by catechol-o-methyltransferase (COMT) and monoamine oxidase A (MAOA). These enzymes, being involved in the elimination of biogenic amines from the synaptic cleft, could also be involved in the different individual response to ADs, as potentially impaired variants could be more constitutionally active in amine catabolism, contrasting the action of ADs. In addition, MAOA is the specific target of the first synthesised ADs, MAO inhibitors (MAOI). Monoamine oxidase A (MAO-A) which catalyses also 5HT catabolism (Berry et al. 1994)

and Catechol-O-methyltransferase (COMT) catalyse the elimination of biogenic monoamines from the synaptic cleft.

MAOA is also supposed to influence the mechanism of action of SSRIs through interaction with the 5-HT transporter. Its gene is located on chromosome Xp11-23 (Sabol et al. 1998).

A polymorphism located 1.2 kb upstream of the MAO-A coding sequences has been shown to affect the transcription of the MAO-A gene promoter. This mutation consists of a 30 bp repeat in 3, 3.5, 4, or 5 copies. The polymorphism was shown to affect transcriptional activity of the MAOA gene promoter (Denney et al. 1999, Sabol et al. 1998). Muller et al. (Muller et al. 2000) investigated the possible association between a polymorphism called MAOA-VNTR (located 1.2 kb upstream of the MAO-A coding sequence) and antidepressant response to moclobemide on a sample of 64 major depressive patients (15 males and 49 females), with negative results. Yoshida et al., by studying MAOA-VNTR polymorphism together with TPH A218C on 54 Japanese depressives, did not find any association with fluvoxamine treatment (Yoshida et al. 2002b). We replicated this negative result on a sample of 443 inpatients (248 were affected by major depression and 195 by bipolar disorders) treated with 300 mg fluvoxamine and 136 with 20-40 mg paroxetine for 6 weeks. The severity of depressive symptoms was assessed weekly with the HAMD, and allele variants were determined by a PCR-based technique (Cusin et al. 2002).

Other possible candidate genes for pharmacogenetic studies were found among genes coding for catecholamine receptors. Serotonin (5HT) is found in neuronal cell bodies clustered specifically in the brainstem. The axons of these cells, however, innervate almost every area of the central nervous system. Beside its fundamental role in mood tone, 5HT is also involved in eating, sleeping, sexual behaviour, the circadian cycle, and other neuroendocrine functions. In addition, the serotonergic pathway is the main target of SSRIs, the most widely used antidepressant compounds.

There are multiple subtypes of 5HT receptors, with varying affinity. They are categorised into seven main classes (5HT1-7).

For example, Serotonin 1A (5HT1A) receptors are located both at a postsynaptic and at a presynaptic level; in the first case, they mediate the action of serotonin on cortical and limbic neurons and are thought to play an important role in the pathogenesis of depressive symptomatology, in the second case, they act as serotonergic auto-receptors on serotonergic neurons in the raphe nuclei and prevent the release of serotonin by a negative feedback (Dubovsky and Thomas 1995, Kapur and Remington 1996). 5HT1A presynaptic receptors exert a self-inhibitory function and when they are stimulated by 5HT with the result of a decrease in neuronal firing and 5HT synthesis and release. 5HT1A receptor gene was mapped on the long arm of chromosome five (5q11.2-13) and it appears to be intronless (Kobilka et al. 1987). It contains an uninterrupted long open reading frame encoding a G protein-coupled receptor, that acts primarily via inhibition of adenylate cyclase. A functional new variant in the promoter region of the gene was recently reported (Wu and Comings 1999). This

polymorphism consists of a G to C substitution and is located at position 92928 bp (GDB: AC008965) of the human 5HT1A gene. It is inside a palindromic region of 26, which bounds a single repressor, the so-called Nuclear DEAF-1-related (NUDR) protein (Lemondé et al. 2003). This variant was demonstrated to be involved in modulating the rate of transcription of 5HT1A gene. When the G-allele is incorporated, it prevents the binding of this putative repressor to DNA, leading, in this way, to an increase of 5HT1A auto-receptors and a reduction of serotonergic neurotransmission (Stahl 1994). This C(-1019)G polymorphism of the 5-HT1A promoter was associated with a number of psychiatric disorders including major depression, suicide and anxiety related traits (Lemondé et al. 2003, Rothe et al. 2004, Strobel et al. 2003). Lemondé et al. found a trend association ($p=0.0497$) between a G to C substitution at position -1019 in the promoter region of 5-HT1A and antidepressant response to citalopram (Lemondé et al. 2004). More recently, we investigated the same polymorphism according to fluvoxamine response in a sample of 252 mood disorders subjects (151 were major depressed and 111 bipolars). 5-HT1A appeared to be associated with AD response in BP subjects only ($p=0.036$) (Serretti et al. In press-a).

5HT2A gene was mapped on chromosome 13q14-q21 (Sparkes et al. 1991); it consists of 3 exons separated by 2 introns and spans over 20 kb. When the 5HT2A receptor is stimulated by 5HT, it causes the production of second messengers, modulating phosphatidylinositol production and intracellular Ca^{2+} flux. The activation of 5HT2A receptors of medial prefrontal cortex and anterior cingulate cortex is thought to mediate the hallucinogenic properties of LSD, whereas in amygdala 5HT2A receptor activation is a component of the response. The 5HT2A receptors may mediate some of the antidepressant effects seen in experimental animal models of depression (Skrebuhova et al. 1999). Nefazodone exerted its antidepressant effects partially through 5HT2A receptor antagonism (Hemrick-Luecke et al. 1994).

A polymorphism in the promoter region was identified (A-1438G) which creates an HpaII restriction site (Spurlock et al. 1998) and associated to clozapine response (Arranz et al. 1998). A silent polymorphism T102C was identified by Warren (Warren et al. 1993) and was associated to individual responses to risperidone (Lane et al. 2002) and clozapine (Arranz et al. 1995).

Our group showed a marginal association between T102C polymorphism and antidepressant response in 443 subjects affected by major depression and bipolar disorder, the same sample of MAOA study (248 major and 195 bipolar depressed patients, with or without psychotic features, see above) (Cusin et al. 2002). Also Minov et al. (Minov et al. 2001) have previously examined the same polymorphism together with another one (His452Try) and SERTPR, in a sample of 173 patients and 121 healthy controls. They observed a different treatment response (calculated as a decrease in HAMD 17 and CGI item 1) in C allele carriers after antidepressant treatment. Actually, the paper investigated the association between T102C polymorphism and major depression, and tested the association with antidepressant treatment response in a sub-sample of patients; the treat-

ment were non standardized and the sub-sample size was exiguous.

The former polymorphism was also investigated for involvement in side effects in an 8-week, double-blind, randomized pharmacogenetic study comparing paroxetine and mirtazapine in 246 elderly patients with major depression. The presence and the severity of paroxetine-induced side effects were strongly associated with C/C genotype (Murphy et al. 2003).

Another polymorphism in the promoter region of the gene (A-1438G) was investigated according to fluvoxamine response in a Japanese sample (66 patients with major depression disorder) treated with fluvoxamine; Fifty-four patients only completed the study, and the genotype distribution. No significant differences were detected between the variants and the time-course of the Montgomery-Asberg Depression Rating Scale scores (Sato et al. 2002).

More recently, Peters et al. focused their investigation on seven candidate genes in the serotonergic pathway on a sample of 96 subjects with unipolar major depression treated with fluoxetine, analysing the response after 12 weeks. Patients were genotyped at 110 SNPs and four repeat polymorphisms located in seven candidate genes (HTR1A, HTR2A, HTR2C, MAOA, SLC6A4, TPH1, and TPH2). Three SNPs in the TPH1 gene and one SNP in the SLC6A4 gene show significant single-locus association when response to fluoxetine is compared to nonresponse ($P=0.02-0.04$). Then a specificity of response was analysed, by first comparing specific response vs nonspecific and nonresponse, and then comparing specific response to nonspecific response. In the first comparison three SNPs in the TPH2 gene ($P=0.02-0.04$) were positively associated, while one SNP in the HTR2A gene ($P=0.02$) was negatively associated, while in the second comparison significant negative associations in three SNPs in the HTR2A gene ($P=0.001-0.03$) and two SNPs in the MAOA gene ($P=0.03-0.05$) were found. Significant haplotype associations were found in all but the HTR1A and HTR2C genes. Moreover, the authors observe that a number of the less frequent alleles of many of the SNP markers were associated with the nonresponse and nonspecific phenotypes (Peters et al. 2003, Peters et al. 2004).

Another investigated gene was 5-HT6 receptor gene; Kohen et al. (Kohen et al. 1996) cloned and characterized its 440-amino acid polypeptide and mapped its gene to human chromosome 1p36-p35, not far from the location of the 5HT1D alpha receptor. They showed that the receptor is expressed in several human brain regions, most prominently in the caudate nucleus. Study of genomic clones revealed that the open reading frame is interrupted by 2 introns in positions corresponding to the third cytoplasmic loop and the third extracellular loop, respectively. In vivo microdialysis studies showed that a selective 5HT6 antagonist produced an increase in extracellular glutamate levels in the frontal cortex. This receptor is G protein coupled and stimulates adenylyl cyclase. Wu et al investigated the involvement in antidepressant response of a silent thymidine to cytosine polymorphism within the first exon of 5-HT6 receptor genes (T267C) (Kohen et al. 1996) on 34 subjects affected by Major Depressive Disorder. They were completing a 4-week treatment with various antidepres-

sants, administered at not fixed doses, and were assessed with the HAMD before antidepressant treatment and at the end of the trial; no association was found. However, the use different antidepressants and the smallness of the sample did not allow a definite conclusion (Wu et al. 2001).

Disturbances of dopamine transmission have also been implicated in antidepressant response.

Antidepressant treatments showed to enhance dopaminergic neurotransmission by increasing the behavioural sensitivity to the stimulation of dopamine receptors in the mesolimbic dopamine system, and such super sensitivity might underlie the antidepressant therapeutic effect (D'Aquila et al. 2000).

At least five different dopamine receptors were identified: D1, D2, D3, D4, D5/D1b. Dopamine Receptor D2 (DRD2) was mapped on 11q23 (Grandy et al. 1989), and seems to exert a central role in the neuromodulation of appetitive behaviours (Balk et al. 1995, Grandy et al. 1989).

Itokawa et al. (Itokawa et al. 1993) reported a putatively functional polymorphism causing a structural change from Serine to Cysteine at codon 311 of DRD2 (S311C). The signal transducing action of the DRD2 receptor following ligand binding is to inhibit cAMP synthesis and the Cys311 variant is less effective in inhibiting it.

DRD4 showed considerable homology to DRD2, and its activation was shown to inhibit presynaptically glutamatergic neurotransmission (Price and Pittman 2001).

DRD4 was mapped on chromosome 11p15.5; it is one of the most variable known human genes. Most of this diversity is the result of length and single-nucleotide polymorphism (SNP) variation in a 48-bp VNTR in exon 3 (Van Tol et al. 1992), which encodes the third intracellular loop of this dopamine receptor. Variant alleles containing 2 (2R) to 11 (11R) repeats are found, with the resulting proteins having 32 to 176 amino acids at this position. The frequency of these alleles varies widely, the 4-fold repeat (D4.4) being most frequent.

We investigated the possible association of DRD2 Ser311Cys and DRD4 exon3 VNTR polymorphisms and antidepressant response on a sample of 364 inpatients affected by a major depressive disorder, treated with fluvoxamine 300 mg/day (266) or paroxetine 20-40 mg/day (98), finding no positive results. The severity of depressive symptoms was assessed weekly with the Hamilton Rating Scale for Depression. Possible stratification factors, such as sex, diagnosis, presence of psychotic features, depressive symptoms at baseline, paroxetine and fluvoxamine plasma levels, and pindolol augmentation did not significantly influence the negative results (Serretti et al. 2001a).

Regarding noradrenergic pathway, norepinephrine transporter (NET) was recently investigated, together with SERTPR, SERT-STin2 and 5HT2A G-1438A in Ninety-six Japanese patients with major depressive disorder, treated with milnacipran, 50-100 mg/day, for 6 weeks. Severity of depression was assessed with the Montgomery-Asberg Depression Rating Scale at baseline and at 1, 2, 4, and 6 weeks of treatment. Only eighty patients completed the study. The presence of the T allele of the NET T-182C polymorphism was associated with a superior antidepressant response,

whereas the A/A genotype of the NET G1287A polymorphism was associated with a slower onset of therapeutic response. In contrast, no influence of 5-HTT polymorphisms on the antidepressant response to milnacipran was detected (Yoshida et al. 2004).

According to receptors, Beta-adrenergic receptors, which are only postsynaptic, stimulate the formation of cAMP after the linkage of the agonists to the receptor. ADRB1 serve as important regulators of central nervous system (CNS)-mediated behaviour and several neural functions, including mood, memory, neuroendocrine control, and stimulation of autonomic function. ADRB1 5' flanking region contains AP-2 consensus elements which can interact with trans-activating elements (Kirigiti et al. 2000). Studies on the action of antidepressants suggest that they may play a critical role in affective disorders and their treatment; centrally active beta-1 and beta-2 adrenergic agonists produce antidepressant-like effects in several behavioural tests, suggesting that these receptors may be involved in the mediation of the effects of antidepressant drugs (Crissman et al. 2001). ADRB1 was mapped on 10q24-q26 (Yang-Feng et al. 1990). A functional polymorphism (G1165C) was recently identified, leading to an amino acid variation (Gly389Arg). This variant of the human ADRB1 gene results in alterations of receptor-Gs protein interaction, with functional consequences on signal transduction (Mason et al. 1999).

This polymorphism was investigated for a possible association with drug response in a sample of 259 patients compared to 206 healthy controls, finding an association between CC genotype and a better response to various antidepressant treatments (Zill et al. 2003). In fact, tendency for a better and even faster response to various antidepressant treatments was found, determined by the HAMD and CGI score ($P = 0.05$). Even if after correction for multiple testing (Bonferroni) these results did not remain significant, these findings suggest that the presence of the C allele might be an indicator for antidepressant treatment response.

Abnormal signal transduction pathways are possibly involved in response to treatment with antidepressants. G-proteins are key elements of these pathways, so they were investigated in pharmacogenetics. Beta subunit could be subdivided into three subtypes: 1, 2 and 3. The majority of the studies focused on G-beta 3 subunit (GNB3); G beta3 subunit (GNB3) gene was mapped on locus 12p13, and spans 7.5 kb and is composed of 11 exons and 10 introns. Its promoter lacks a TATA box but harbours GC-rich regions. A polymorphism of a G-protein beta3 subunit (C825T) has been shown to be associated with increased signal transduction and ion transport activity (Siffert et al. 1995); GNB3 825T variant is associated with the occurrence of the splice variant Gbeta3s, which, despite a deletion of 41 amino acids, is functionally active in reconstituted systems. Although the polymorphism did not affect the amino acid sequence of the beta-3 subunit, the T allele was associated with deletion of nucleotides 498-620 of exon 9; this was found to be an example of alternative splicing caused by a nucleotide change outside the splice donor and acceptor sites.

Zill et al. by analysing a sample composed by 88 depressive patients (10 bipolar disorder, 78 major

depressives) 68 schizophrenic patients and 111 healthy controls, found a significant association between TT genotype of the C825T polymorphism and a good response to various AD treatments (Zill et al. 2000). More recently, our group confirmed these results in a sample of 490 subjects, bipolars and major depressives, treated with SSRIs fluvoxamine and paroxetine., by finding a significant association ($p=0.009$) between Gbeta3 T/T genotype and good response to antidepressant treatment, independently from demographic and clinical variables (Serretti et al. 2003b). Recently, these results were further replicated by Lee et al. in a Korean sample of 106 MDD patients and 133 healthy controls. Hypertensive subjects were excluded from the study because association between GNB3 variants and hypertension has been reported in previous studies. The T-allele carriers showed higher scores than those with the CC genotype in the baseline total and in some subcategories of the Hamilton Depression Rating Scale ($P<0.05$). They also found a statistically significant association between T-allele carriers and antidepressant treatment response ($P<0.05$). These are to our knowledge the first studies investigating association between a polymorphism in a molecule involved in intracellular signal transduction pathway and AD action.

The dysbindin gene (dystrobrevin-binding-protein 1, DTNBP1), which was recently reported to be associated with schizophrenia is widely expressed in the human brain and binds to the dystrophin-associated protein complex (DPC) which appears to be involved in signal transduction pathways. Zill et al. investigated five SNPs in the dysbindin gene in a sample of 293 patients and 220 healthy controls. Neither single SNP, nor haplotypes resulted associated to antidepressant response (Zill et al. 2004).

Other genes were the objects of pharmacogenetic investigation of antidepressant response: for example nitric oxide (NO). It is probably involved in the processes of learning and memory, as it influences synaptic plasticity in the striatum and elsewhere.

The enzyme NO synthase (NOS) produces NO starting from precursor L-arginine. It includes at least three distinct isoforms - neuronal (NOS1), endothelial, and inducible NOS. The neuronal but so far not the endothelial NO Synthase isoform (NOS3) was detected in some striatal interneurons with a large axonal arborisation. On the other hand, in the hippocampus, NOS1 is localized to GABAergic interneurons, whereas endothelial NOS is found in pyramidal neurons. The NOS 1 gene was mapped on chromosome 12q24.2-q24-31. In a population-based association study A NOS1 polymorphism (C276T) was investigated for association with fluoxetine response in 114 major depressed patients treated with fluoxetine, with negative results (Yu et al. 2003b).

Also angiotensin I converting enzyme (ACE), or kininase II, was the object of psychopharmacologic investigation. Angiotensin I-converting enzyme, or kininase II, is a dipeptidyl carboxypeptidase that plays an important role in blood pressure regulation and electrolyte balance. The ACE gene encodes 2 isozymes (Ramaraj et al. 1998). Ehlers et al. (Ehlers et al. 1989) determined the cDNA sequence for human testicular ACE, identical, from residue 37 to its C terminus, to the second half or C-terminal domain of the endothelial ACE

sequence. The inferred protein sequence consists of a 732-residue preprotein including a 31-residue signal peptide. Jeunemaitre et al. (Jeunemaitre et al. 1992) mapped ACE to 17q22-q24, and it was shown that 50% of the interindividual variability of plasma ACE concentration is determined by an insertion (I)/deletion (D) polymorphism located in intron 16 of the ACE gene and known as the ACE I/D polymorphism (Rigat et al. 1990), with the D allele showing dominance rather than codominance relative to the I allele (Jeffery et al. 1999).

Baghai et al. analysing an insertion (I)/deletion (D) polymorphism located in intron 16 on 99 Caucasians unrelated patients with MD and 99 age- and sex-matched healthy controls from the general population, found that D- allele carriers treated with different antidepressant treatments (tricyclic anti-depressants, mirtazapine, SSRIs, and others) showed significantly better response and had significantly shorter hospitalisation periods ($p=0.02$). After 4 weeks of treatment, D-allele carriers showed significantly lower HAM-D17 scores, remitted more often and had a significantly shorter duration of hospitalisation. Also, the number of treatment alterations during hospitalisation was significantly higher in I/I-genotypes (Baghai et al. 2001). On the other hand, a recent study showed conflicting results in a sample of 58 affective patients treated with venlafaxine slow-release 75mg or fluoxetine 20 mg (Hong et al. 2002).

Interleukin-1 (IL-1) is a proinflammatory cytokine, mainly produced by blood monocytes, which mediate the acute phase response. The interleukin-1 (IL-1) complex consists of 3 linked genes (IL-1 Alpha, IL-1 Beta and IL-1 receptor antagonist) mapping to chromosome 2q13-14 (Patterson et al. 1993). All three molecules bind to IL-1 receptors. Auron et al. (Auron et al. 1984) isolated human IL-1 cDNA, while Webb et al. (Auron et al. 1985) assigned the IL-1 gene to chromosome 2q13-q21. IL-1 Beta was assigned to the end of 18q (Le Beau and Rowley 1986).

In IL-1 Beta gene, assigned to the end of the chromosome, four SNPs were reported: two in the promoter region -31C/T and -511C/T one in exon 5 (3954C/) and another one (5810A/G) in intron 4 (Di Giovine et al. 1992, Guasch et al. 1996, Pociot et al. 1992). The -31 SNP is in strong linkage disequilibrium with the -511 SNP (El-Omar et al. 2000). Yu and his group studied the association of the -511C/T polymorphism and fluoxetine response in 119 major depressed receiving a 4-week fluoxetine treatment, and found a trend to a less severe depressive symptomatology and a more favourable fluoxetine response in homozygous subjects for the -511T allele (Yu et al. 2003a).

In the latest years researchers have focused their attention to the biological clock genes, according to the hypothesis that the pathogenesis of mood disorders could be linked to alteration in the circadian rhythm. We investigated the possible role of CLOCK gene polymorphism in the regulation of diurnal mood fluctuations during a major depressive episode. The sample consisted of 101 patients affected by bipolar disorder type I, during a depressive episode without psychotic features, free of psychotropic medications. Perceived mood levels were assessed three times a day with self-administered visual analogue scales. Genotype groups showed no significant difference in diurnal mood fluctuations. When stratifying the sample by including only patients with

an adequate period of observation (duration of illness higher than 5 years, $n = 69$), we post-hoc observed a significantly higher recurrence rate in homozygotes for the C variant, which was almost double than that of the other genotype groups. This preliminary observation leads to hypothesize a role for the CLOCK gene polymorphism in the regulation of long-term illness recurrence in bipolar disorder. Given the post-hoc nature of the finding, replication in independent samples is necessary to confirm it (Benedetti et al. 2003b). Then, we investigated the possible effect of the 311T/C CLOCK gene polymorphism on sleep disorders in a sample of 620 patients affected by major depressive disorder (MDD) and bipolar disorder (BP), detecting a significantly higher recurrence of initial ($P = 0.0001$), middle ($P = 0.0009$), and early ($P = 0.0008$) insomnia in homozygous for the C variant and a similar trend concerning decreased need of sleep in BP ($P = 0.0074$). Other demographic and clinical features were found not related with CLOCK polymorphisms. This preliminary observation leads to hypothesize a possible involvement of the CLOCK gene polymorphism in the sleep dysregulations in MDD and BP (Serretti et al. 2003a). In a subsequent paper we analysed the association between the antidepressant outcome and CLOCK genes. Our findings assessed that a significantly higher insomnia rate was present throughout the trial in homozygous for the C variant ($p=0.026$). Other demographic and clinical features were found not related with CLOCK polymorphisms. The sample was composed by one hundred and seventy-eight inpatients treated with fluvoxamine 300 mg/day ($n=147$) or paroxetine 20-40 mg/day ($n=31$) and either placebo or pindolol in a double blind design for 6 weeks. The severity of depressive symptoms was weekly assessed with the Hamilton Rating Scale for Depression. Overall these findings may suggest that CLOCK genotype influences the time course of insomnia during antidepressant treatment. This, together with previous findings on this polymorphism, could lead to a further dissection of the complexity of Mood Disorders (Serretti et al. In press-b).

In conclusion, our researches stated the complexity of the involvement both of SERTPR and of CLOCK genes in a plethora of pharmacological effects and numerous features of affective disorders: in fact, also SERTPR was shown to be involved both in pharmacological response (see above), and in illness time course (Cusin et al. 2001), as well as in switches from depression to mania, even with contrasting results (see above).

Pharmacological treatment is not the only therapeutic chance for depression. Other methods have recently demonstrated their therapeutic efficacy, especially as augmenting strategies.

Sleep deprivation, for example, is a rapid and effective antidepressant treatment, but long-lasting benefit is only observed in a small part of the subjects, pharmacogenetic studies could be therefore useful for explaining this observation.

We investigated the influence of dopamine receptor D4 exon 3 (DRD4) variants on TSD antidepressant efficacy on one hundred and twenty-four depressed inpatients affected by bipolar disorder (DSM-IV). DRD4 variants were not associated with TSD outcome. Consideration of possible stratification effects such as gen-

der, age at onset and duration of illness did not reveal any association either (Serretti et al. 1999). Other studies on DRD3 and DRD2 were performed, all with negative results (Benedetti et al. 2003c, Schumann et al. 2001).

SERTPR long variant was associated with a good response, an effect similar to that observed for Selective serotonin reuptake inhibitors (Benedetti et al. 1999).

Our group also tested the hypothesis that allelic variation of SERTPR could influence the response to the combination of light therapy and TSD on Twenty-two bipolar depressed inpatients. Light therapy sustained the effect of TSD, by preventing relapse after recovery sleep, and the effect was more marked in homozygous for the long variant of SERTPR (Benedetti et al. 2003a).

Also GSK3beta showed association with sleep deprivation outcome. We studied the effect of this polymorphism on the acute response to total sleep deprivation of 60 depressed bipolar type I inpatients. CC homozygous showed a later onset of bipolar illness, and better acute effects of TSD treatment on perceived mood (as rated on VAS). Overall, these observations suggest a protective role for this genotype in respect to bipolar illness (Benedetti et al. 2004).

Perspectives

The frequently reported statement that it will soon be possible to individualize and tailor the choice and dosing of pharmacotherapy, not only for depressive illness, is an idealistic view at present, and further investigation is required. Considering the major clinical and economic importance of individual drug response, this field of research deserves major attention, not only by specialists but also by primary care physicians, health officials and the health industry.

The introduction of new technologies, such as DNA microarray (DNA chip) analysis, represents a further chance to implement and make the use of genetic information available in everyday clinical practice. In fact, it enables a quick genome-wide scanning, using the high-density single nucleotide polymorphisms map. This procedure shows however substantial limitations. Although microarray-related studies can monitor global changes in gene expression, these studies are limited by access and cost; moreover, a simultaneous analysis of thousands of genes, many of which are non functional or not related with the trait under analysis, leads to a risk of false positive findings.

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