Article highlights.

- Genetic polymorphisms represent the most promising and easily accessible biological marker of antidepressant response so far.
- The complexity of the genetic component of antidepressant response gives rise to the issue of methodological appropriateness of pharmacogenetic studies.
- Despite the limitations of candidate gene studies and genome-wide association studies (GWAS), several genetic regions provided encouraging results and overlapping between candidate studies and GWAS results exist.
- Technological and methodological innovations are expected to provide higher covering of genetic variants across the whole genome and particularly to increase the capability of capturing their interactions.
- Pharmacoeconomic studies investigating the cost-utility of genotype-guided antidepressant treatment are increasing in number and suggested favorable cost-utility profile of genotyping.

This box summarizes key points contained in the article.

individuals [3] gave rise to the hypothesis of a genetic contribution. Recent data confirmed the relevant contribution of common genetic variations to antidepressant efficacy, since they were estimated to explain 42% of individual differences [5] without including rare variants. Despite these encouraging results and several pharmacogenetic studies providing positive findings, no group of polymorphisms has been consistently demonstrated to explain a relevant proportion of the variance in antidepressant response [3], preventing the development and diffusion of genotype-based treatments.

The complex polygenic modulation of antidepressant response set the problem of which is the most appropriate approach to disentangle it and put into question if methodological issues may be the main reason behind unsatisfactory outcomes of antidepressant pharmacogenetics. Anyway, genetic polymorphisms still remain the most promising and more easily applicable biological marker of antidepressant response in clinical practice. Further, the need of any reliable biological marker to guide antidepressant treatment results is of primary relevance in order to reduce the heavy burden of depressive and anxiety disorders.

Thus, the present article will review pharmacogenetic knowledge focusing on the most commonly used antidepressant class, that is, SSRIs. Starting from the above-mentioned methodological issues, possible strategies to better integrate the available knowledge deriving from candidate gene studies and genome-wide association studies (GWAS) are suggested.

2. Areas covered

Candidate gene studies and GWAS focused on SSRI pharmacogenetics were collected and discussed. MEDLINE database was searched (articles published till January 2014) using any combination of the following terms: pharmacogenetics, gene, polymorphism, variation, genetic, candidate, genome-wide, GWAS, pathway, antidepressant, SSRI, efficacy, response, side effect, suicide, major depression, mood disorder, anxiety disorder and individual gene names according to the official nomenclature.

GWAS signals localized in promising genetic regions derived from candidate gene studies were analyzed using Ricopili [6] - a tool for visualizing regions of interest in selected GWAS data sets available via the Broad Institute. Further, genes within a range of (0.1 cm + 300 kb) from single nucleotide polymorphisms (SNPs) with $p < 0.0001$ in a previous GWAS meta-analysis [7] (only results obtained in the subsample treated with SSRIs) were analyzed through the GeneMANIA plug-in [8] in Cytoscape [9] in order to identify potential interactions among them.

3. Candidate gene studies

Candidate gene studies are focused on a limited number of genes that are selected on the base of evidence from molecular, cellular, animal or human studies that suggest a relevant biological function under the perspective of antidepressant action.

3.1 Established candidates

In this section, candidate genes confirmed by almost five studies are discussed, independently from the specific polymorphism(s) associated with phenotype. Indeed, the basic functional unities of genome can be identified in genes rather than individual polymorphisms. A summary of this group of candidate genes is provided in **Table 1** for serotonergic genes and **Table 2** for non-serotonergic genes.

3.1.1 5-HT transporter and 5-HT receptors

The monoaminergic system represents a 'historic' piece of research in the field of SSRI pharmacogenetics. Indeed, SSRIs have been engineered starting from the hypothesis that a central deficit in monoamines and particularly 5-HT is pivotal in the pathogenesis of depression and anxiety disorders [3]. Clearly, this represents an oversimplification, but central 5-HT and norepinephrine (NE) are thought to have a key role in the regulation of anxiety, irritability, loneliness and guilt [10]. On the other hand, loss of pleasure, loss of interest, fatigue and loss of energy - each of which contributes to the loss of drive and motivation - are mainly associated with dysregulation of dopamine (DA) and NE neurotransmission [11].

The serotonin transporter (SERT) gene (SLC6A4) has been the most investigated in the field of SSRI pharmacogenetics, since it represents the main target of this class of antidepressants. A 44 bp insertion/deletion serotonin-transporter-linked polymorphic region (5-HTTLPR) and the rs25531 A/G have been particularly studied, since their functional effect on transcription. Briefly, the former variant can carry the

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Gene	Polymorphism	Diagnosis	Drug(s)	Outcome	Result	Ref.
SLC6A4	5-HTTLPR	MDD+BP DSM IV	Fluvoxamine	w6 response	L or L/L ↑ outcome	[190]
		MDD+BP DSM IV	Paroxetine	w4 remission		[191]
		MDD+BP DSM IV	Fluvoxamine ± pindolol	w6 remission		[192]
		MDD DSM IV	Paroxetine	w2 response		[193]
		MDD DSM IV	Citalopram	w4 response		[194]
		MDD DSM IV	Sertraline	w2, w4, w6, w8 response		[195]
		MDD+BP DSM IV	Fluvoxamine or paroxetine ± lithium	w6 remission		[196]
		MDD DSM IV	Paroxetine	w6 response		[40]
		MDD DSM IV	Paroxetine	w12 response		[197]
		MDD DSM IV/ ICD10	Escitalopram	w12 response		[39]
		MDE DSM IV	Paroxetine, fluoxetine, escitalopram, citalopram, sertraline	w4 response		[17]
		MDD DSM IV	Citalopram, fluoxetine, paroxetine	w3, w6 response and remission		[198]
		MDD DSM IV	Fluoxetine	w6, w12, w18 response		[199]
		MDD DSM IV	Fluoxetine	w4 response and remission		[200]
		MDD DSM IV	Paroxetine and fluvoxamine	w2, w4, w6 response		[74]
		MDD DSM IV	Paroxetine	w12 response		[193]
		MDD in the elderly DSM IV	Citalopram	w14 response and remission		[22]
		MDD in the elderly DSM IV	Escitalopram	w12 remission		[23]
		MDD and/or anxiety disorders in children/ adolescents	Citalopram	w8 response		[87]
		PD DSM IV	Paroxetine	w12 response	L allele ↑ outcome in females	[18]
		PTSD DSM IV	Sertraline	w4, w12 response	L/L ↑ outcome	[201]
		MDD	Paroxetine	w1, w2, w3, w4, w6, w8 response and adverse events	LL ↑ outcome and ↓ adverse events	[21]
		MDD DSM IV	Fluoxetine	Adverse events	S ↑ adverse events	[26]
		MDD DSM IV	Escitalopram	Sexual dysfunction	Negative results	[29]
		MDD DSM IV	Fluvoxamine	w1, w2, w4, w6 response	S or S/S ↑ outcome	[202]
		MDD DSM IV	Sertraline	w6 response		[203]

↑: Higher side effects; ↓: Lower side effects; 5-HTTLPR: Serotonin-transporter-linked polymorphic region; BP: Bipolar disorder; DSM: Diagnostic and Statistical Manual of Mental Disorders; GAD: Generalized anxiety disorder; MD: Major depression; MDD: Major depressive disorder; MDE: Major depressive episode; OCD: Obsessive-compulsive disorder; PD: Panic disorder; PTSD: Post-traumatic stress disorder; SNP: Single nucleotide polymorphism; SSRI: Serotonin reuptake inhibitor.

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Gene	Polymorphism	Diagnosis	Drug(s)	Outcome	Result	Ref.
		PD DSM IV	Paroxetine	w2 response		[204]
		MDD DSM IV	Fluoxetine, sertraline	w2, w4, w6 response		[14]
		MDD DSM IV	Escitalopram	w8 response		[205]
		OCD DSM IV	Fluvoxamine	w12 response and % improvement	S ↑ reduction in compulsion scores	[206]
		MDD DSM IV	Escitalopram	w8 response	Negative results	[207]
		PD DSM IV	Paroxetine	w2 response and side effects		[208]
		PD DSM IV	Paroxetine	w10 response		[209]
	5-HTTLPR and STin2	MDD in the elderly DSM IV	Fluoxetine	w4 response	L/L ↑ outcome	[41]
		MDD DSM IV	Fluoxetine, paroxetine, citalopram	w6 response	L/L and 12/12 ↑ outcome	[45]
		MDD DSM III	Fluoxetine, paroxetine	w6 response		[44]
		MDD DSM IV	Fluoxetine, sertraline	w6 response and remission	S and 12/12 ↑ outcome	[43]
		MD ICD-10	Several serotonergic antidepressants	Adverse events	S/S or S ↑ adverse events	[27]
		MD DSM IV	SSRIs	Adverse events		[28]
		MDD DSM IV	Paroxetine	w6 response	L/L and 9-10/9-10 ↑ outcome	[46]
		MD	Sertraline	Response	Negative results	[40]
		MDD DSM IV	Fluvoxamine	Drug-induced nausea		[49]
		MDD ICD-10	Paroxetine	Response, remission and adverse reactions	S/S ↑ outcome	[50]
	5-HTTLPR, rs25531, STin2	MDD DSM IV/ICD-10	Escitalopram	w12 response and remission	L ↑ outcome	[39]
		MDD DSM IV	Citalopram	w12 remission	L/L and 9/12 ↑ outcome; S-A-12 haplotype ↓ outcome	[47]
	5-HTTLPR and rs25531	MDD ICD-10	SSRIs	w5 response and remission	↑ LA outcome	[35]
		GAD in the elderly DSM IV	Escitalopram	w12 response		[77]
		MD	Fluoxetine	w12 remission		[36]
		MD	Paroxetine, fluvoxamine	w2, w4, w6 response	↑ LA outcome to fluvoxamine	[15]
		MDD DSM IV	Citalopram	w12 response and remission, adverse effects	S or L_G ↑ adverse effects	[24]
		MDD DSM IV	Escitalopram	w12 response and % improvement, side effects	S ↑ adverse effects	[25]
	STin2	MDD DSM IV	Fluoxetine, sertraline	w6 response and remission	S and 9/12 ↑ outcome	[43]
		MDD	Fluvoxamine	w6 response	Negative results	[42]

↑: Higher side effects; ↓: Lower side effects; 5-HTTLPR: Serotonin-transporter-linked polymorphic region; BP: Bipolar disorder; DSM: Diagnostic and Statistical Manual of Mental Disorders; GAD: Generalized anxiety disorder; MD: Major depression; MDD: Major depressive disorder; MDE: Major depressive episode; OCD: Obsessive-compulsive disorder; PD: Panic disorder; PTSD: Post-traumatic stress disorder; SNP: Single nucleotide polymorphism; SSRI: Serotonin reuptake inhibitor.

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Gene	Polymorphism	Diagnosis	Drug(s)	Outcome	Result	Ref.
HTR1A	rs6295	PD DSM IV	Sertraline, paroxetine	w6 response	C ↑ outcome	[210]
		MD DSM IV	Fluvoxamine	w6 response	G ↓ outcome	[52]
		MDD DSM IV	Citalopram	w12 response		[53]
		MD DSM IV	Fluoxetine	w4 response		[54]
		MDD DSM IV	Fluoxetine	w4 response	G ↑ outcome	[41]
		Depressive disorders	SSRIs	w4 response	Negative results	[56]
		MDD DSM IV	SSRIs	w6 response and remission	Negative results	[57]
	10 SNPs among which rs6295, rs6294, rs1800044	MDD DSM IV	Fluoxetine	w12 response		[55]
	rs1800042	Depressive disorders DSM IV	Fluvoxamine	w12 response and remission	A ↑ outcome	[59]
	rs1800042, rs6295	MD DSM IV	Fluoxetine	w4 response	rs6295C/C ↑ outcome in females	[54]
HTR2A	rs7997012	MDD DSM IV	Citalopram	w12 response and remission	A ↑ outcome	[62]
		MDD DSM IV	Citalopram	w12 response and remission		[63]
	rs7997012, rs9316233, rs2224721	MDD DSM IV	Escitalopram	w12 response and remission	rs9316233 G and rs2224721 A ↑ outcome	[64]
	rs6313, rs6314	MDD ICD-10	Paroxetine	Response, remission and adverse reactions	rs6314 C/T ↑ outcome; rs6313 C/C ↑ adverse events	[50]
	rs6311, rs6313, rs7997012, rs1928040	MDD DSM IV	Fluvoxamine, paroxetine or sertraline	w8 response and remission	rs6311-rs6313-rs1928040 G-C-T ↑ outcome	[65]
	17 SNPs among which rs6314, rs1923882, rs3125	MDD DSM IV	Fluoxetine	w12 response	rs6314 A, rs1923882 T, rs3125 G ↑ outcome	[55]
	rs6311	OCD DSM IV	Paroxetine	w12 response	G/G ↑ outcome	[211]
	rs6313, rs6306	MD DSM IV	Fluvoxamine, paroxetine	w6 response	rs6306 TT trend of ↓ outcome	[68]
	rs6311	MDD DSM IV	Citalopram	w4 response and w12 remission	Negative findings	[70]
	rs6311	MDD DSM IV	Fluvoxamine	w6 response	Negative findings	[69]
	rs6313	MDD DSM IV	Fluoxetine	w4 response	Negative findings	[41]
	rs6313, rs6311, rs7997012, rs6313,	MDD DSM IV	SSRIs	w6 response and remission	Negative results	[57]
	rs6311	MD DSM IV	Paroxetine	Adverse events	CC ↑side effects and ↑ discontinuation	[71]

↑: Higher side effects; ↓: Lower side effects; 5-HTTLPR: Serotonin-transporter-linked polymorphic region; BP: Bipolar disorder; DSM: Diagnostic and Statistical Manual of Mental Disorders; GAD: Generalized anxiety disorder; MD: Major depression; MDD: Major depressive disorder; MDE: Major depressive episode; OCD: Obsessive-compulsive disorder; PD: Panic disorder; PTSD: Post-traumatic stress disorder; SNP: Single nucleotide polymorphism; SSRI: Serotonin reuptake inhibitor.

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Gene	Polymorphism	Diagnosis	Drug(s)	Outcome	Result	Ref.
		Depressive disorders	SSRIs	Sexual dysfunction	GG ↑ sexual dysfunction	[72]
		Depressive or anxiety disorders DSM IV	Paroxetine	Drug-induced nausea	Negative findings	[76]
	rs6313	Mixed DSM IV diagnosis	Serotonergic antidepressants	Serotonin toxicity in overdose	Negative findings	[78]
	rs7997012	MD	SSRIs	Response and adverse events	AA ↑ side effects	[34]
TPH1	rs1800532	MD DSM IV	Fluvoxamine ± pindolol	w6 response	AA or A ↓ outcome in subjects not taking pindolol	[80]
		MD DSM IV	Paroxetine ± pindolol	w4 response		[79]
		MDD DSM IV	Citalopram	w8 remission	A ↓ outcome	[81]
		MDD DSM IV	Citalopram	w4 response and w12 remission	A ↓ outcome in psychotic and melancholic MDD	[70]
		MDD DSM IV	Fluvoxamine	w6 response	Negative results	[82]
		MDD DSM IV	Fluoxetine	w4 response		[41]
		MD DSM IV	SSRIs	w6 response and adverse effects		[83]
		MDD DSM IV	SSRIs	w6 response and remission		[57]
	rs1800532	MDD DSM IV	Fluvoxamine	Drug-induced nausea		[49]
	19 SNPs among which rs1800532, -7180T/G, -5806T/G	MDD DSM IV	Fluoxetine	w12 response	-7180 C and -5806 T ↑ outcome	[55]
TPH2	14 SNPs among which rs1843809, rs1386492, rs1487276	MDD DSM IV	Fluoxetine	w12 response	rs1843809 T, rs1386492 A and rs1487276 C ↑ outcome	[55]
	rs10897346, rs1487278	MD DSM IV	SSRIs	w3 response	rs10897346 C ↓ outcome	[85]
	rs2171363	MDD	Fluoxetine, citalopram	w8 response	C/T ↑ outcome	[86]
	rs4570625	Depressive and anxiety disorders	Citalopram	w8 response	G ↑ outcome and interaction with 5-HTTLPR	[87]

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16-repeat long (L) allele, which is associated with a twice basal SERT expression compared to the 14-repeat short (S) allele, while the rs25531 G variant in conjunction with the L allele (L_G) may result in a reduced expression of SLC6A4, equivalent to that conferred by the S allele [3]. Cumulative evidence from reviews and meta-analyses mainly suggests that the L allele of 5-HTTLPR is a predictor of better SSRI response in Caucasian populations, while in Asian populations the same allele may be associated with poorer SSRI outcome [3,12]. Recently, this hypothesis that arose from pharmacogenetics has found support in functional neuroimaging. Indeed, in Chinese Han subjects, in contrast to Caucasians, the L allele was demonstrated to confer vulnerability to anxiety and depression and to weak top-down emotional control between the prefrontal cortex (PFC) and amygdala [13], which may confer higher risk of poor SSRI response. A molecular explanation of the stratification effect deriving from ethnicity is provided by the observation of different functional effect of the S allele between Caucasians and Asians. In detail, Asian S/S subjects show higher SERT function expressed as rate of platelet 5-HT

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Gene	Polymorphism	Diagnosis	Drug(s)	Outcome	Result	Ref.
COMT	rs4680 (Val108/158Met)	MDD DSM IV	Paroxetine	w4 response	A (Met) ↑ outcome	[88]
		MDD DSM IV	Fluvoxamine	w6 response		[89]
		MDD DSM IV	Fluoxetine	w4, w8 response	A (Met) ↑ outcome in males	[90]
		MD	Fluoxetine	w12 remission	A (Met) ↑ outcome	[36]
		MDD DSM IV	Citalopram	w4 response, w12 remission	A/A ↓ outcome	[92]
		MDD DSM IV	Paroxetine	w6 response	Negative results	[93]
		MD DSM IV	SSRIs	w6 response and remission		[94]
		MDD DSM IV	Fluoxetine	w4 response		[31]
	23 SNPs among which rs13306278 and rs9332381	MDD DSM IV	Citalopram	w12 response and remission	rs13306278 C and rs9332381 G ↑ outcome in White non-Hispanic	[95]
BDNF	rs6265 (Val66Met)	MDD DSM IV	Fluoxetine	w4 response	Val/Met trend of ↑ outcome	[101]
		MDD DSM IV	Fluvoxamine	w2, w4, w6 response	Val/Met ↑ outcome	[102]
		MDD DSM IV	Fluoxetine	w6 response and remission; side effects	Val/Met ↑ outcome; Met ↓ insomnia and sexual dysfunction	[103]
		MDD DSM IV	Citalopram	w8 response and remission	Met ↑ outcome	[104]
		MDD in the elderly DSM IV	Escitalopram	w12 remission		[105]
	Several SNPs among which rs6265	MDD in the elderly DSM IV	Paroxetine	w8 response	Met ↓ outcome	[106]
	Several SNPs, among which rs10835210 and rs6265	MDD DSM IV	Escitalopram	w12 response and remission	rs10835210 associated with outcome	[64]
	rs6265	MDD DSM IV	Citalopram	w12 response and remission	Negative results	[62]
	18 SNPs among which rs2049046 and rs6265	MD DSM IV	SSRIs	w5 response	rs2049046 T ↑ outcome	[111]
	rs6265, rs7124442, rs7103411	MD DSM IV	SSRIs	w6 response	rs7124442 TT ↓ outcome	[110]
ABCB1	rs1045642, rs2032582, rs1128503	MD DSM IV	Paroxetine	w6 response	rs1045642 T and C-G-T haplotype ↓ outcome	[115]
	rs1045642 rs2032582	MDD DSM IV	Citalopram	w4 response	rs2032582 G ↑ outcome	[116]
	95 SNPs	MD DSM IV	P-gp substrates among which paroxetine and citalopram	w4, w5 and w6 response and remission	rs2032583 C and rs2235015 T ↑ outcome	[117]
	15 SNPs	MD DSM IV	Paroxetine	w8 response and remission	rs2032583 C and rs2235040 A ↑ outcome	[118]
	81 SNPs	MDD DSM IV	Fluoxetine	w8 response and remission	Haplotypic association with remission	[119]

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Gene	Polymorphism	Diagnosis	Drug(s)	Outcome	Result	Ref.
	rs6946119, rs28401781, rs4148739, rs3747802	MDD DSM IV	SSRIs	w6 response	rs28401781 A and rs4148739 G ↑ outcome	[120]
	20 SNPs	MDD DSM IV	Escitalopram	w8 response and remission	rs1882478-rs2235048-rs2235047-rs1045642-rs6949448 T-T-T-C-C ↓ remission	[121]
	rs61615398, rs2032582, rs1045642	Depression ICD-10	Paroxetine	w2, w4 response	Negative results, but rs61615398 interacted with CYP2D6 genotypes	[122]
	rs1045642, rs2032582	MD DSM IV	Paroxetine	w6 response	Negative results	[123]
	rs1128503, rs2032582, rs1045642	MDD DSM IV	Citalopram	w12 response and remission; adverse events		[124]
	rs2032582 and rs1128503	MDD DSM IV	Escitalopram	w8 remission	↑ Dose needed to remit for C rs1045642	[125]
	rs1128503, rs2032582, rs1045642, rs2032583, rs2235040, rs2235015	MDD DSM IV	SSRIs	Adverse effects	rs2032583 C and rs2235040 A ↑ adverse events	[127]
	rs2032582	Eating disorder	Paroxetine	Drug-induced sexual dysfunction	rs2032582 A ↑ sexual dysfunction	[128]
	rs1045642, rs2032582, rs1128503	Several diagnosis	SSRIs	Switching and discontinuation	T-T-T haplotype ↑ risk of discontinuation	[129]

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uptake, while the contrary has been reported in Caucasians [14]. Therefore, ethnic background should be taken into account in gene-related studies. It should be nevertheless underlined that results regarding 5-HTTLPR and SSRI efficacy are not univocal, and ethnic stratification is only a possible explanation of the conflicting results. Given that contradictory results were found in the same ethnic group treated with the same class of antidepressants in some cases, another possible confounding factor is represented by peculiarity of the pharmacodynamic profile shown by different SSRIs. In detail, the level of selectivity and affinity of each SSRI molecule for the SERT may influence pharmacogenetic associations. This hypothesis is supported by previous studies that found a selective impact of 5-HTTLPR and rs25531 on paroxetine response compared to fluvoxamine response [15]. Other hypothesized stratification factors are gender and age. Indeed, the deleterious effect of the S allele may be particularly evident in females, as suggested in both major depressive disorder (MDD) [16,17] and panic disorder [18]. These findings may be explained by the demonstration that rates of 5-HT synthesis, as well as 5-hydroxy-indoleacetic acid levels in the cerebrospinal fluid, are sexually dimorphic in humans and tryptophan depletion may induce depressive symptomatology only in women [19]. The activity of the 5-HT system (as well as other neurotransmitter systems) is also influenced by aging [20], and age-related alterations of the 5-HT system occur at multiple levels, such as the density of 5-HT neurons in the raphe nuclei, the metabolism and level of 5-HT in the central nervous stystem (CNS), the expression of the SERT and 5-HT receptors [20]. Pharmacogenetic studies performed in elderly populations supported higher risk of SSRI-induced adverse events [21] and worse SSRI response [22,23] in S carriers. Further, microstructural white matter abnormalities in frontolimbic networks were demonstrated in elderly depressed S carriers [23]. Thus, if the elderly and women are groups with some higher biological liability of the serotonergic system, the S allele may be more detrimental in these patients compared to young people and males.

5-HTTLPR S allele has been repeatedly associated to SSRI-induced side effects [21,24-28], with the exception of sexual dysfunction [29]. Similarly to the therapeutic effect, the association between the polymorphism and side effects was selective to Caucasians [3]. The S allele may be also a risk factor for antidepressant-induced mania according to a meta-analysis, which anyway underlined the high heterogeneity among studies and the confounding effect deriving from concomitant mood stabilizer treatment in some of them [30].

Studies focused on the tri-allelic 5-HTTLPR/rs25531 variant showed several negative findings [25,31-33], despite some of them confirmed that LA may predict better outcome [15,34-36].

Despite the quite common description of 5-HTTLPR as a tri-allelic locus (L_G, L_A, and S), the allelic variation in the SLC6A4 upstream regulatory region is more complex. Indeed,

10 additional sequence variants were identified, suggesting that the alleles reported as S and L are divided into four and six kinds of allelic variants, respectively, that show significant different distribution between Caucasian and Asian populations [37]. Rare variants for the L allele (16A, 16D and 16F) and two others for the S allele (14A and 14B) were examined in depressed patients to improve predictability [38]. Overall, the presence of these rare polymorphisms added 0.6% to the previous 7% with no difference in response rate by the presence of a different S allele. Subjects with the excess of 16F L allele (A of the A/G polymorphism together with a T instead of a C in the sixth nucleotide of the sixth repeat) may have more treatment resistant depression.

Another functional variant of SLC6A4 has been particularly investigated given its functional effect, that is, a 17 bp VNTR within intron 2 (STin2). 9, 10 or 12 copies of a 16–17 bp repeat can be found and may influence gene transcription with a synergistic effect with 5-HTTLPR. Particularly, the 12 repeat variant was shown to cause higher gene expression *in vitro* and *in vivo* [3]. This polymorphism was repeatedly investigated, but results were mainly negative [16,39-42], while positive results suggested better response in L allele carriers in Asian samples [43-45], including elderly patients [43], and better response in S allele carriers in Caucasians [46,47]. No apparent stratification effect deriving from the antidepressant class used was retrieved. Meta-analytic results further suggested an opposite effect of the variant in Caucasians versus Asians, but results remained contradictory with evidence of high heterogeneity among studies [48]. Similarly, no association was found between STin2 and SSRI-induced side effects [27,28,49,50], as confirmed by a meta-analysis [48].

Given that additive effects of multiple genes are likely to contribute to pharmacogenomic associations, recent studies were interested in identifying molecules that interact with SERT and modulate its function, such as ITGB3 – an integrin subunit that is required for SERT activity and modulates SERT [51] – and microRNAs (miRNAs), which regulate SERT expression. Interestingly, the reduction of SERT expression obtained by miRNA regulates serotonergic neurotransmission more rapidly than pharmacological blockade of SERT [3], suggesting a strategy for developing fast-acting antidepressants.

Among 5-HT receptors, the most established candidate genes for involvement in SSRI efficacy are HTR1A and HTR2A. HTR1A has been considered a good candidate since several antidepressants desensitize this inhibitory autoreceptor, consistently to the antidepressant effect of pindolol (an HTR1A blocker). rs6295 (1019C/G), in the upstream regulatory region of HTR1A, has received particular attention since the G allele causes an upregulation of the gene [3] and may contrast the therapeutic effect of antidepressants. Some pharmacogenetic studies confirmed the hypothesis reporting worse fluvoxamine [52], citalopram [53] and fluoxetine [54] response in G allele carriers, while one finding in the opposite direction [41] regarded an Asian sample treated with fluoxetine. Interestingly, the study by Yu *et al.* suggested that rs6295 CC genotype was a female-specific factor for the prediction of a beneficial outcome [54]. On the other hand, several negative studies exist [55-57], as well as a recent meta-analysis that also performed subanalyses stratified by ethnic origin and treatment (SSRIs and non-SSRIs) [58]. Another variant that was hypothesized to modulate SSRI efficacy is HTR1A rs1800042, since it is in linkage disequilibrium (LD) with rs6295. Nevertheless, findings are scarce and contradictory [54,59]. Despite the contradictory and inconclusive findings reported for rs6295 and rs1800042, several studies demonstrated that HTR1A knockout mice and HTR1A receptor block by small interference RNA are characterized by increased 5-HT release in the PFC and robust antidepressant-like effects [60]. It is reasonable to hypothesize that in previous pharmacogenetic studies the focus on only one/two polymorphisms within HTR1A may have limited the understanding of the role of the gene. Recent findings suggested the involvement of other HTR1A SNPs in SSRI efficacy, as well as the interaction between HTR1A and HTR1B [53]. HTR1B is another subtype of 5-HT inhibitory autoreceptor. Insufficient evidence regarding the role of the gene in SSRI pharmacogenetics is available, but an interesting modulating effect of HTR1B rs130058 on the risk of suicidal ideation during treatment was suggested to depend from the antidepressant class (SSRIs vs non-SSRIs [61]).

HTR2A codes for a G protein-coupled receptor that shows mainly excitatory activity and was demonstrated to be involved in depression and antidepressant mechanisms of action. Behavioral studies demonstrate that 5-HT2A receptor antagonists elicit antidepressant-like activities and potentiate the antidepressant-like effect of fluoxetine in rodents [3]. Several HTR2A variants have been studied in the field of SSRI pharmacogenetics, despite the possible functional impact of these variants is still unknown. rs7997012 was identified as a modulator of antidepressant efficacy that seems selective to SSRIs in a large sample [62,63]. In another study, other HTR2A SNPs (rs9316233 and rs2224721) but not rs7997012 were among the best predictors of escitalopram response [64]. Another positive finding regarded rs6314 and paroxetine treatment [50]. The poor replication of results may be linked to the complex (multilocus) nature of the genetic factors involved, as confirmed by several studies suggesting interactions among different HTR2A polymorphisms and other genes. Indeed, some HTR2A haplotypes may predict SSRI efficacy [55,65] and an interaction between HTR2A rs6313 (in LD with rs6311) and glutamate receptor, ionotropic, kainate 4 (GRIK4) (that codes for glutamate receptor, ionotropic, kainate 4) rs1954787 was reported to affect citalopram efficacy [66]. HTR2A x GRIK4 interaction does not seem surprising, since the serotonergic-glutamatergic molecular connections at multiple levels, thus the joined study of both systems has been suggested to provide a more informative perspective than the study of each individual system [67]. Under this perspective, the sequence of negative findings – including a meta-analysis [58] – provided by studies focused on only few HTR2A variants [41,57,68-70] may be at

least partly explained. HTR2A may also modulate SSRI-induced side effects, particularly rs6311/rs6313 variants may affect the risk of paroxetine discontinuation [71], sexual dysfunction [72], fluvoxamine- and paroxetine-induced gastrointestinal side effects [73,74], and preliminary evidence suggested the involvement of rs7997012 in side effect risk [34]. On the other hand, some studies did not confirm the association between rs6311 and fluvoxamine- or paroxetine-induced nausea [75,76]. As HTR2A receptor plays a role in the modulation of cognition, rs6311 may also be associated with reductions in attention during SSRI treatment in elderly people [77]. Meta-analytic results confirmed that rs6311 GG and rs6313 CC genotypes may be risk factors for side effects development, particularly when considering only SSRI-treated subjects [48]. The effect of rs6313 on adverse events seems to be limited to side effects and not to influence 5-HT toxicity after drug overdose [78].

Other subtypes of serotonergic receptors were less studied in the pharmacogenetic field, but interestingly some of them may be involved especially in the modulation of side effects risk. Particularly, HTR2C rs6644093 may be a selective predictor of serotonergic antidepressant-induced adverse reactions, while HTR3A and HTR3B were associated with paroxetine- and fluvoxamine-induced gastrointestinal side effects [3].

3.1.2 5-HT and other monoamines metabolism

The levels of monoamines in the CNS are largely dependent from the activity of the enzymes involved in their synthesis and breakdown. Tryptophan hydroxylase (TPH) catalyzes the rate-limiting step in 5-HT biosynthesis while catechol-O-methyl transferase (COMT) and monoamine oxidase (MAO) are involved in the degradation of monoamines. MAOA and MAOB genes are not discussed in this paragraph, since SSRI pharmacogenetic studies mainly provided negative findings, while evidence suggesting a possible association between MAOA/MAOB variants mainly regarded non-SSRI antidepressants [3].

Despite TPH2 is the TPH isoform predominantly expressed in the CNS, TPH1 and TPH2 are expressed at similar levels in some brain areas that are relevant to depressive and anxiety disorders pathogenesis (e.g., frontal cortex, thalamus, hippocampus and amygdala [3]). TPH1 rs1800532 (A218C) A allele is associated with decreased 5-HT synthesis, and according to the monoaminergic theory of depression it may determine predisposition to anxious/depressive symptoms and worse antidepressant efficacy [3]. Pharmacogenetic studies that show agreement with the molecular and imaging findings mainly used SSRI treatments [70,79-81], suggesting a selective effect on this class of antidepressants. Studies that did not report any association were mainly performed on Asian samples [41,82,83] but not only [55,57]. A possible selective effect of rs1800532 on response in major depression with psychotic and melancholic features was reported [70]. Despite clinical features may remain a confounding factor, a recent meta-analysis did not find any effect of the polymorphism on SSRI efficacy neither in Caucasians nor in Asians [58].

rs1800532 did not demonstrate any evidence of involvement in the modulation of SSRI-induced side effects [49,83], an effect (nonspecific for SSRIs) on the risk of body weight gain apart [84].

Other polymorphisms within TPH1 were only marginally investigated, but associations between -7180T/G, -5806T/G and fluoxetine response were reported [55], suggesting that a better covering of TPH1 variability may be useful to clarify the role of the gene.

Results regarding TPH2 are more heterogeneous in terms of investigated variants. The most promising polymorphisms are rs1843809, rs1386492, rs1487276 [55], rs10897346, 1487278 [85] and rs2171363 [86]. In children and adolescents with depressive and anxiety disorders, response to citalopram was influenced by TPH2 G703T (rs4570625), and showed an interaction with SLC6A4. Indeed patients with the combination of TPH2 -703G and 5-HTTLPR L alleles were the most likely to respond [87]. The hypothesis of an additive effect on antidepressant response exerted by TPH2 and SLC6A4 is plausible since the key role of both genes in the modulation of 5-HT neurotransmission.

COMT is a highly polymorphic gene, but the greatest part of studies were focused on the functional rs4680 (Val108/158Met). The Val/Val homozygote catabolizes DA at up four times the rate of Met/Met homozygote, with a possible impact on bioavailability of DA in the frontal cortex and clinical response to SSRIs [11]. Consistently with the monoaminergic theory, six pharmacogenetic studies reported the Met variant as the favorable allele for SSRI response [36,88-91], with an allele dose effect (better outcome in Met/Met carriers and intermediate outcome in Met/Val carriers). One of these studies [90] suggested a possible gender-dimorphic effect of rs4680, since Val/Val poorer antidepressant response was observed only in males. To the best of our knowledge, only one study demonstrated an opposite effect of the polymorphism on SSRI efficacy [92], while some others showed no evidence of association [31,93,94], as well as a meta-analysis [58]. All the cited studies were unfortunately performed on samples of relatively small size, while in a larger sample rs13306278 was the only COMT polymorphism that impacted on symptom remission; this variant was associated with altered ability to bind nuclear proteins [95].

3.1.3 Growth factors and cellular signaling

The neurotrophic hypothesis of MDD was formulated after the observation that hippocampus atrophy following stress was reversed by antidepressants in parallel to an increase in the expression of neurotrophic factors, in particular brain-derived neurotrophic factor (BDNF) [96]. The neurotrophic hypothesis is not independent from the monoaminergic one, since 5-HT depletion negatively modulates long-term potentiation (LTP), suggesting that 5-HT facilitates LTP induction [97].

The BDNF rs6265 (196G/A or Val66Met) polymorphism was particularly investigated, since it has been reported to affect intracellular trafficking and activity-dependent secretion of BDNF. The Met/Met mice show decreased basal levels of BDNF in the hippocampus and do not show any BDNF increase during fluoxetine treatment. Further, Met/Met mice have impaired survival of newly born cells in the dentate gyrus [98]. In humans, the Met allele was associated with poorer episodic memory and abnormal hippocampal activation [99]. On the other hand, animal models suggested that too high BDNF levels may have a detrimental effect on mood and especially anxiety [100]. The positive heterosis effect supported by this study found confirmation in Asian samples of depressed patients treated with SSRIs [101-103], including a recent meta-analysis [58]. Conversely, other studies reported a more favorable outcome in Met allele carriers treated with the most SSRIs (citalopram or escitalopram) [104,105], also in quite large Caucasian samples [62,64]. Regarding geriatric depression, two studies obtained opposite findings (better [105] and worse [106] response in Met carriers, treatments were escitalopram and paroxetine, respectively). Excluding studies performed in particular populations (e.g., geriatric depression), studies that reported a positive heterosis effect were performed in Asian samples treated with fluoxetine or fluvoxamine [101-103] while negative studies were performed in Caucasian samples treated with escitalopram or citalopram. The level of selectivity for the SERT may modulate the impact of the BDNF gene on the efficacy of each specific SSRI drug. This hypothesis is supported from the demonstration of higher increase in BDNF gene expression induced by citalopram combined with electroconvulsive therapy (ECT) than citalopram alone, even without any difference in treatment outcome [107]. ECT is hypothesized to exert its antidepressant activity through a modulation of 5-HT, NE and DA neurotransmission in different brain areas [108]. Thus, the antidepressant profile of modulation of different monoamines may modify the effect of BDNF variants on treatment outcome. Age is another modulating factor, as discussed for 5-HTTLPR, given that modifications in neurotrophic factor level normally occur with aging. Consistently, neural plasticity is severely affected in aging, as demonstrated by changes in hippocampal morphology and impairment in LTP [109]. As reported above, pharmacogenetic studies on geriatric depressive samples showed contradictory results that may be related to still poorly understood modifications of BDNF production/function in the elderly.

Besides ethnicity, SSRI drug type and age, clinical type of depression was suggested as a possible modulating factor of BDNF effect on treatment response. Indeed, rs6265 Met/Met (i.e., AA) and rs7103411 TT were found to be predictors of worse response selectively in melancholic depression, as well as rs7124442 TT predicted worse outcome particularly in anxious depression [110]. These results however were not specific for SSRI antidepressants. On the other hand, an association between BDNF rs2049046 and response was reported to be selective to SSRIs compared to other antidepressant classes, without influence from mood stabilizer co-medication [111]. Second messengers are pivotal for the transmission and amplification of signals that originate through the binding of molecules such as growth factors, neurotransmitters and hormones with the respective receptors. Guanine nucleotide-binding protein (G protein), β polypeptide 3 (coded by the GNB3 gene), is involved in the generation of second messengers in response to a number of signals, with a possible modulating effect on a number of cellular responses. GNB3 rs5443 (C825T) T allele was associated with the occurrence of a splice variant that appears to have reduced biological activity [3] and was hypothesized to affect antidepressant efficacy. Despite the interesting functional impact of the SNP, rs5443 is not discussed in detail in this context because only one pharmacogenetic study found that the T allele predicted better SSRIs response, while several negative findings exist [3]. The study performed on the largest sample [112] found an association between rs5443 TT genotype and better response to nortriptyline but not escitalopram, suggesting an antidepressant class-specific effect. A recent meta-analysis confirmed that the effect of this SNP is probably not specific to SSRI antidepressants [58].

3.1.4 *SSRI transport and metabolism*

P-glycoprotein (P-gp) (coded by the ABCB1 gene) and the CYP superfamily represent the most investigated among genes involved in antidepressant pharmacokinetics.

P-gp is an ATP-dependent drug efflux pump for xenobiotic compounds that limit uptake and accumulation of some lipophilic drugs into key organs such as the brain. Among P-gp substrates, several SSRIs are included (citalopram, fluoxetine, fluvoxamine, paroxetine, sertraline [113]). Recently, also escitalopram was demonstrated to be a substrate of P-gp. In rats, the administration of a P-gp inhibitor enhanced the brain distribution of escitalopram by 70 – 80%, increased 5-HT turnover inhibition in the PFC, and antidepressant-like effects of escitalopram [114], suggesting that P-gp inhibitors may be useful for antidepressant augmentation in treatment-resistant depression.

Some ABCB1 SNPs, that is, rs2032582 and rs1045642, were demonstrated to alter P-gp expression and/or function [3], pointing out a possible modulation effect on antidepressant efficacy. This hypothesis was confirmed by pharmacogenetic studies on paroxetine [115] and citalopram [116]. Two further SNPs (rs2032583 and rs2235040) were associated with paroxetine efficacy by two independent studies [117,118], and one ABCB1 haplotype has been selectively associated with fluoxetine response [119]. A wider covering of ABCB1 genetic variability suggested that rs28401781 and rs4148739 may also modulate SSRI response [120], as well as intron 26-27 regions [121]. On the other hand, the association between rs2032582-rs1045642 and paroxetine [122,123] or citalopram [124] response was not confirmed by other studies. Given that several different polymorphisms within the gene were related to SSRI efficacy

and negative studies were focused on only 2-3 SNPs, the hypothesis of an involvement of ABCB1 seems reasonable while the exact regions that are implicated were not definitively identified. Despite this degree of uncertainty, the association between rs2032582 and SSRI efficacy found encouraging results at meta-analytic level [58] and rs1045642 (in LD with rs2032582) showed an impact on escitalopram dose needed for remission in MDD [125]. Further, the clinical application of ABCB1 genotyping has been recently tested in patients treated with P-gp substrates (not only SSRIs), providing the evidence that in rs2032583 and rs2235015 major homozygotes an increase in dose was associated with a shorter duration of hospital stay, whereas other treatment strategies did not improve outcome [126]. This encouraging finding may also be related to ABCB1 impact on SSRI-induced side effects [127,128] and risk of treatment discontinuation, despite this association is still debated [129].

While P-gp is pivotal in regulating the access of lipophilic drugs into the brain, CYP superfamily has a major role in their oxidation and reduction, thus regulating drug plasma levels. The main isoforms involved in SSRI metabolism are CYP2D6 (fluoxetine, fluvoxanime, paroxetine), CYP2C19 (fluoxetine, fluvoxanime, escitalopram), CYP2C9 (fluoxetine, fluvoxanime), CYP3A4 (escitalopram), while sertraline is equally metabolized by CYP2D6, CYP2C19, CYP2C9 and CYP1A2 [130]. The genes coding for these isoforms are highly polymorphic, and alleles may show normal, partially or totally defective activity, defining some theoretical metabolizing groups. CYP genotypes and related metabolizing status have been demonstrated to affect antidepressant pharmacokinetics. In detail, the most confirmed evidence is the effect of CYP2D6 polymorphisms on fluoxetine and paroxetine pharmacokinetics, and CYP2C19 polymorphisms on citalopram and escitalopram pharmacokinetics [3]. Theoretical dose adjustments have been calculated on the base of these findings [131], despite no clear evidence of correlation between SSRI plasma levels and response has been demonstrated [130]. Pharmacogenetic studies mainly did not find any association between CYP polymorphisms and SSRI response [3]. Consequently, the genotyping of CYP2D6 and CYP2C19 (e.g., AmpliChipTM test) has not been recommended so far since the lack of validated prescription guidelines. Cost-effectiveness trials that include pretreatment genotyping of CYP2D6, CYP2C19, CYP2C9 and CYP1A2 polymorphisms are providing first results. Preliminary findings suggested that genotyping can identify subjects at risk of high health-care utilization, greater number of medical absence days and greater disability claims [132].

3.2 Less replicated candidate genes

In this section, candidate genes replicated by less than five studies but showing particular relevance under the perspective of SSRI mechanisms of action are discussed. The focus is pointed again on genes rather than individual polymorphisms, similarly to paragraph 3.1.

3.2.1 *Glutamatergic system*

Besides the monoaminergic theory has been central in the last decades, the contribution of dysfunction in the glutamatergic system has been recently underlined. Indeed, monoamine depletion paradigms failed to find a final common pathway for antidepressant efficacy [133]. The glutamatergic theory posits that the glutamate may shape the risk of depression, influencing the neuronal fate (neurotoxicity) or the unfolding of new neuronal nets (neuroplasticity) [3]. Compounds with glutamatergic activity have demonstrated antidepressant properties, for example molecules that downregulated the glycine B site and the NMDA receptor [133]. Further, also SSRI antidepressant action may be partially mediated through the modulation of the glutamatergic system. Indeed, repetitive administration of fluoxetine induced maturation of telencephalic dendritic spines that is associated with the presence of a higher proportion of GluA2- and GluN2A-containing glutamate receptors [134].

Despite the encouraging findings provided by preclinical studies, SSRI pharmacogenetics still provided few studies focused on glutamatergic genes. GRIK4 was identified as candidate gene as the reduced anxiety and antidepressant-like phenotype of the KO mice [135]. While a preliminary study on the first two-third of the STAR*D (Sequenced Treatment Alternatives for Depression) cohort provided negative findings for all glutamate NMDA and kainate receptor genes [62], a following study on the whole STAR*D sample identified GRIK4 rs1954787 G allele as a predictor of citalopram efficacy [66]. The result was independently replicated in a sample treated with SSRIs or SNRIs [136], and the possible functional effect of the SNP on gene expression makes the finding even more encouraging [137]. On the other hand, negative findings regarding GRIK4 gene were obtained in samples treated with antidepressants of mixed classes and in some cases combinations with mood stabilizers and/or antipsychotics [3]. Besides GRIK4, glutamate receptor, metabotropic 7 (GRM7) represents a promising candidate gene for involvement in early (second week) SSRI response [67], given the observation that glutamatergic-based antidepressants are faster in their action than their monoaminergic counterparts. Other glutamatergic genes were reported to be possible predictors of citalopram-induced sexual dysfunction (GRIK2, GRIA1, GRIA3 and GRIN3A) [138] and treatment-emergent suicidal ideation (GRIA3, GRIK2) [139], but these findings still remain without replication.

3.2.2 *Neuropeptides*

In recent years, a prominent role of various neuropeptides in anxiety and mood disorders emerged. Given the typical neurovegetative symptoms of MDD (sleep-wake cycle disruption, gastrointestinal symptoms, arousal alterations), several peptides that are involved in the regulation of circadian rhythms have been recently investigated in depressed subjects. Disruptions in the diurnal rhythms of the release of

melatonin, vasoactive intestinal polypeptide (VIP), adrenocorticotropic hormone (ACTH), IGF-1 and growth hormone were described in MDD, and they are combined with disruption in the expression of peptides involved in the regulation of the biological clock (PERIOD1, PERIOD2, CRY1, BMAL1, NPAS2 and GSK-3β). Several of these disruptions (i.e., PER1, CRY1, melatonin, VIP, ACTH and IGF-1) were found to persist after escitalopram treatment, suggesting that these neurobiological alterations may contribute to the risk of MDD recurrence or incomplete remission [140].

Besides peptides involved in circadian rhythms regulation, neuropeptide Y (NPY), leptin and galanin recently gained attention since their role in anxiety, mood, feeding and cognition [141,142]. These neuropeptides also exert modulating influence on the monoaminergic system and the hypothalamic-pituitary-adrenal (HPA) axis [143] and have antidepressant-like effects in animal models [144,145]. In depressed patients, a robust suppression of cerebrospinal fluid NPY levels has been found [146] and treatment with citalopram led to an increase in NPY cerebrospinal fluid concentrations [147], providing further support to the relevance of the gene in antidepressant mechanisms of action. In particular, rs16147 has been considered a good candidate SNP, since it is located in the promoter of NPY and has been shown to alter gene expression [148]. Preliminary evidence suggests that the less active rs16147 C allele conferred slow and worse response to antidepressant treatment, even if not only SSRI antidepressants were used in this study [149]. The effect of rs16147 on response was observed particularly in anxious depression and was combined with stronger bilateral amygdala activation in response to threatening faces in an allele-dose fashion. Further studies should focus on NPY gene to clarify its contribution to SSRI efficacy.

Despite preclinical and first clinical data are also encouraging for galanin (e.g., galanin has been reported to have an antidepressant effect in patients with depression under standard antidepressant treatment [150]), few pharmacogenetic findings still exist. rs948854 in the preprogalanin gene (i.e., the gene coding for the proteic precursor of galanin) was demonstrated to play a role in anxiety and depressive vegetative symptoms, as well in antidepressant response, with a gender-specific effect that is related to the role of estrogens in the regulation of HPA-axis [151]. The SNP was associated with higher HPA-axis activity before treatment in a consistent way. Even in this case, the study was not focused on SSRIs but mixed antidepressant classes were used, thus further studies are needed to provide clearer statements.

As reported above, leptin has biological functions that go beyond the modulation of appetite and energy balance. Modifications of leptin levels in the course of antidepressant treatment suggest a link between antidepressant action, resolution of psychopathology and regulation of leptin release in both rodents and humans [152,153]. In depressed patients, nominal associations of several polymorphisms in the upstream vicinity of rs10487506 were associated with antidepressant response across different samples, among which the STAR*D. Further, unfavorable treatment outcome was accompanied with decreased leptin mRNA and leptin serum levels, but without selectivity of these last findings for SSRI antidepressants [142].

Endogenous opioid peptides have also been implicated in the modulation of anxiety [154] and depression [155]. Interestingly, women with MDD who did not respond to antidepressant treatment demonstrated attenuated μ-opioid receptor binding potential when compared with women with MDD who did respond to medication, as well as depressive state itself was associated with a defect in the endogenous opioid neurotransmission [155]. A pharmacogenetic study suggested that rs540825 in the OPRM1 gene (coding for the μ-opioid receptor) may be a modulator of citalopram response in MDD [156]. The polymorphism is a nonsynonymous SNP in the final exon of the OPRM1 gene that results in a histidine to glutamine change in the intracellular domain of the receptor. In the same study, the rs1799971 – which results in an asparagine to aspartic acid amino acid substitution and produces a threefold increase in binding to β-endorphin and 10% reduction of protein level – provided negative findings, whereas it was associated with non-SSRI antidepressant response [157].

3.2.3 *Ion channels*

Mouse models have recently provided compelling evidence implicating some neuronal ion channels in antidepressant mechanisms of action. In detail, hyperpolarization-activated cyclic nucleotide-gated (HCN) 1 gene – coding for a potassium/sodium channel – and particularly KCNK2 – a neuronal potassium channel – have been proposed as promising candidates. TREK1 is inhibited by therapeutic doses of SSRIs, and mice lacking the KCNK2 gene are resistant to depression-like behavior, leading to speculation that KCNK2 inhibition may contribute to the therapeutic effects of SSRIs [158]. In humans, genetic variations in KCNK2 were associated with anhedonic symptoms of depression [159]. In depressed patients treated with citalopram, KCNK2 gene was associated with treatment response, as well as with response to different antidepressant therapies in treatment-resistant subjects [160]. Nevertheless, pharmacogenetic replication of these results is still lacking.

HCN1 gene has not been studied yet by pharmacogenetic studies in humans, but animal models suggested it is involved in the antidepressant effect. HCN channels underlie the hyperpolarization-activated current, a functionally important current in the nervous system [161]. HCN localization in the CNS is most striking in dendrites of hippocampal and cortical pyramidal neurons – both of which are relevant to depression – and spatially enriched hyperpolarization-activated current controls local dendritic processes such as Ca2+ spikes and synaptic plasticity [162]. Mutant mice lacking HCN1 demonstrated antidepressant-like behaviors [163]; following experiments showed that the selective HCN1 knockdown in the dorsal hippocampal CA1 region was enough to produce antidepressant- and anxiolytic-like behaviors, in association with an upregulation of BDNF signaling pathways [164]. The reported

findings highly suggest the usefulness of investigating this gene in clinical pharmacogenetic studies.

3.2.4 *Inflammation and immune system*

Inflammation is hypothesized to play a subtle role in the pathophysiology of MDD, as demonstrated by abnormal peripheral inflammatory biomarkers and HPA-axis functioning in these subjects [3]. In line with these findings, treatment outcome of MDD is influenced by the antidepressant-induced modulation of cytokines, which occurs through a direct or indirect action on intracellular cAMP, 5-HT metabolism and HPA-axis activity [165].

Corticotropin releasing hormone (CRH) receptors 1 and 2 (CRHR1 and CRHR2) hold a primary role within the HPA-axis since they are the mediators of the effects of glucocorticoids in the CNS. Interestingly, CRHR1 antagonists have demonstrated antidepressant-like effects in animals [166]. The CRHR1 rs242941 G/G genotype and one haplotype block including two SNPs in LD with rs242941 (rs1876828 and rs242939) were associated with SSRI response [167], particularly in anxious depression [168]. Nevertheless, the result was not confirmed by a further study [169], neither in a numerous sample. The latter study suggested the role of another CRHR1 SNP (i.e., rs12942300, that is 40 Kbp from rs242941). Studies focused on the CRHR2 gene suggested the involvement of rs2270007 [169] and rs2267716-rs255105 [170] in citalopram response.

Besides CRH receptors, the glucocorticoid receptor (GR), (i.e., coded by the NR3C1 gene) represents the target of glucocorticoid hormones and is expressed in almost every cell in the body. GR regulates the transcription rate of genes controlling the development, metabolism and immune response through the binding with specific chromatin sequences. In the absence of hormone, GR resides in the cytosol complexed with a variety of proteins including FK506-binding protein 52 (FKBP5) that regulates GR sensitivity [3]. Pharmacogenetic studies suggested several NR3C1 polymorphisms as putative modulators of SSRI efficacy (rs852977, rs10482633 and rs10052957 [64]), but the effect does not appear specific for SSRI antidepressants, while negative results were provided for BcII [171]. Studies focused on FKBP5 firstly suggested that the gene does not play a major role in SSRI response [3], while recent studies on more numerous cohorts suggested that rs4713916 may modulate remission to citalopram [172] and rs352428 may affect SSRI response [173]. Unfortunately, among pharmacogenetic studies that investigated NR3C1 and FKBP5 only a minor part was focused on SSRI antidepressants, but they mainly investigated the efficacy of heterogeneous pharmacological treatments [3]. An effect of FKBP5 was anyway hypothesized on the risk of suicidal ideation during SSRI treatment [61,174] and may be related to the high HPA-axis dysfunctions associated with suicidal behavior.

Interesting but preliminary results were reported for CRH-binding protein gene that encodes for a plasma protein involved in the inactivation of CRH. This gene is hypothesized to act as a selective modulator of citalopram efficacy in African-American and Hispanic populations, but not in Caucasians [170].

Given that the brain is not a sanctuary for the immune system as hypothesized in the past, inflammatory cytokines represent putative modulators of neurotransmitter metabolism, neuroendocrine function and neural plasticity [3]. The IL-1β gene is considered a good candidate for involvement in the modulation of SSRI response, since its multiple and reciprocal interactions with the monoaminergic, cholinergic and GABAergic systems in the CNS [11]. Further, pretreatment IL-1β expression is higher in antidepressant nonresponders and is significantly decreased after treatment [175]. Pharmacogenetic studies further support the role of IL-1β gene in the modulation of antidepressant efficacy. In detail, the promoter SNP rs16944 has been selectively associated with SSRI response by some studies [176,177].

4. GWAS and methods based on genome-wide data

In the 2000s, GWAS were proposed as innovative approach to study human complex traits and rapidly gained approval and enthusiasm. Alongside a series of advantages - mainly the genotyping of hundreds of thousands of polymorphisms within the whole genome without any a priori hypothesis - some limitations of GWAS emerged. In detail, i) the available platforms are able to provide only a relative narrow genomic covering (e.g., less of 50% in the STAR*D); ii) current GWAS technology does not allow the reliable genotyping of rare variants (< 1% of the population); iii) the risk of the so-called flip-flop phenomenon [178], since the functional effect of the genotyped variants is largely unknown and polymorphisms apparently associated with outcomes may be only in LD with variants truly associated; iv) the difficulty in balancing the risk of false-positive and false-negative findings [179]. Other limitations that are specific of pharmacogenetics in psychiatry are represented by: i) difficulty in collecting a sufficiently powered sample size since the consistent administration of a drug for a period of several weeks is required; ii) the lack of objective measures of efficacy (e.g., laboratory tests) and tendency to high placebo effect that anyway remains undetermined in previous GWAS since they lacked a placebo arm. Two GWAS were performed on cohorts with a substantial part that was treated with SSRI monotherapy, that is, the STAR*D [180] and the Genome-based Therapeutic Drugs for Depression (GENDEP) [181]. Among the top STAR*D findings, the RAR-related orphan receptor A gene shows the highest biological rationale for association with antidepressant response, since it is involved in the regulation of the circadian rhythm [182]. In the GENDEP study instead, the best findings were localized within two intergenic regions, but copy number variants that may influence the expression of genes at longer distances through changes of the chromatin structure have been identified in both regions [181]. Results of both GWAS have been extensively

reviewed elsewhere [3], while suicidal ideation during antidepressant treatment deserves here some attention given the clinical relevance of this dramatic adverse event. In the GENDEP study, two suggestive signals were found within Kv channel-interacting protein 4 (KCNIP4) and near elongation protein 3 homolog (ELP3) in subjects treated with escitalopram [183], while the papilin, proteoglycan-like sulfated glycoprotein and IL28RA (IFN, lambda receptor 1) genes were predictors of the phenotype in the STAR*D [184]. Papilin is a component of the extracellular matrix whose relevance remains unknown in relation to suicide and antidepressant treatment, while KCNIP4 and ELP3 are involved in several neuronal functions but their possible role in suicide is still unclear. On the other hand, IL28RA calls the attention to the known inflammatory mechanisms that are associated with depression and suicide, as well as for the FKBP5 gene (see paragraph 3.2.4).

Given the hypothesis that sample sizes of previous individual GWAS could be insufficient to provide adequate statistical power, recently GWAS meta- and mega-analysis have been performed. In particular, a meta-analysis was focused on STAR*D and GENDEP subsample treated with escitalopram [7], providing as top finding an intergenic region on chromosome 5 with no evidence of transcription so far. As reported above, regulatory regions may modulate gene expression also at longer distances, thus this finding should not be overlooked.

Instead of further discussing findings of previous GWAS, the reporting of GWAS results in genetic regions identified by the candidate gene approach represents an interesting perspective to study the replication of findings across the two methodologies. Data set used corresponds to results reported by a previous GWAS meta-analysis [7]. Among genes considered as established candidates in this review (paragraph 3.1), SLC6A4 and HTR2A regions show interesting signals (**Figure 1**). Among less replicated candidate genes (paragraph 3.2), GWAS signal overlapping was more evident in CRHR2, GRIK4, GRM7, KCNK2, NPY and OPRM1 genetic regions (Supplementary Figure 1).

4.1 Molecular pathways involved in SSRI efficacy

Recently, pathway analysis has been proposed in order to balance the limitations of GWAS hypothesis free approach. The basic principle of the method is the analysis of variants within genes involved in the same biological pathway. Pathway analysis may yield more insights into disease biology because it overcomes the genetic heterogeneity bias (e.g., due to population stratification, differential rates of genotyping error between the groups under analysis), and setting the variants within the same pathway as the unit of analysis may increase power to detect associations and replicate findings [185]. As in the previous paragraph, a cumulative evidence on individual candidate genes derived from GWAS data was reported, in this paragraph molecular pathways involving genes within a range of (0.1 cm + 300 kb) from SNPs with $p < 0.0001$ in a previous GWAS meta-analysis [7] were reported (**Table 3**). The pathways involved were mainly related to cell proliferation and apoptosis (including neuronal apoptosis) and immune system modulation. Both these processes have been extensively implicated in the mechanisms of antidepressant action accordingly to the inflammation [165] and neurotrophic [96] hypotheses of depression. Other identified pathways converged into corticosteroid and lipid metabolism, which are interestingly connected to HPA-axis dysfunction and neurosteroids role in stress and depression. Indeed, neurosteroids are potent and effective neuromodulators that are synthesized from cholesterol in the brain. Neurosteroids and their synthetic derivatives influence the function of multiple signaling pathways including GABA and glutamate receptors [186]. The other identified pathways are involved in protein metabolism or assembly, DNA transcription, and second messengers. These processes show clearly less specificity for antidepressant mechanisms of action. Anyway, a list of the genes included in each of the identified pathways was reported in **Table 3** in order to provide some suggestions about putative candidate genes.

5. Conclusion

Previous pharmacogenetic studies often provided findings that are difficult to summarize and interpret. This difficulty partly derived from heterogeneity in clinical features, type of treatment, trial design, genotyped polymorphisms and sample ethnicity, all of which reduce the comparability among studies. Taking into account these factors of stratification, the involvement of SLC6A4, HTR1A, HTR2A, BDNF and ABCB1 genes in SSRI response seems plausible (**Tables 1** and **2**). Among innovative candidates, CRHR2, GRIK4, GRM7, KCNK2, NPY and OPRM1 are promising genes taking into account both candidate studies and genome-wide findings.

The rapid progress in technology and reduction in genotyping costs provide the opportunity to clarify the role of these genes and other genes potentially involved. The increasing amount of GWAS data paves the way to large mega-analysis (e.g., [187]), and sequencing technologies are spreading in genetic research. The possibility to extend both sample size and covering of genetic variability represents pivotal issues to improve the knowledge in the field, since they were among the main limitations of previous studies. Further, the need of shifting from the study of individual polymorphisms to genes and molecular pathways represents an equal importance issue. Indeed, polymorphisms are not independent from each other and the same can be stated for genes. The study of gene x environment interactions and epigenetic modulation of gene expression have encountered parallel development, together with genome-wide transcriptomics. Epigenetics (modulation of gene expression dependent from non-sequence modifications, e.g., DNA methylation) and transcriptomics (RNA sequencing) represent levels of regulation that are complementary to genetics and can fill the gaps in biological mechanisms of SSRI action that are left after genetic analysis. Progress in technology provides the

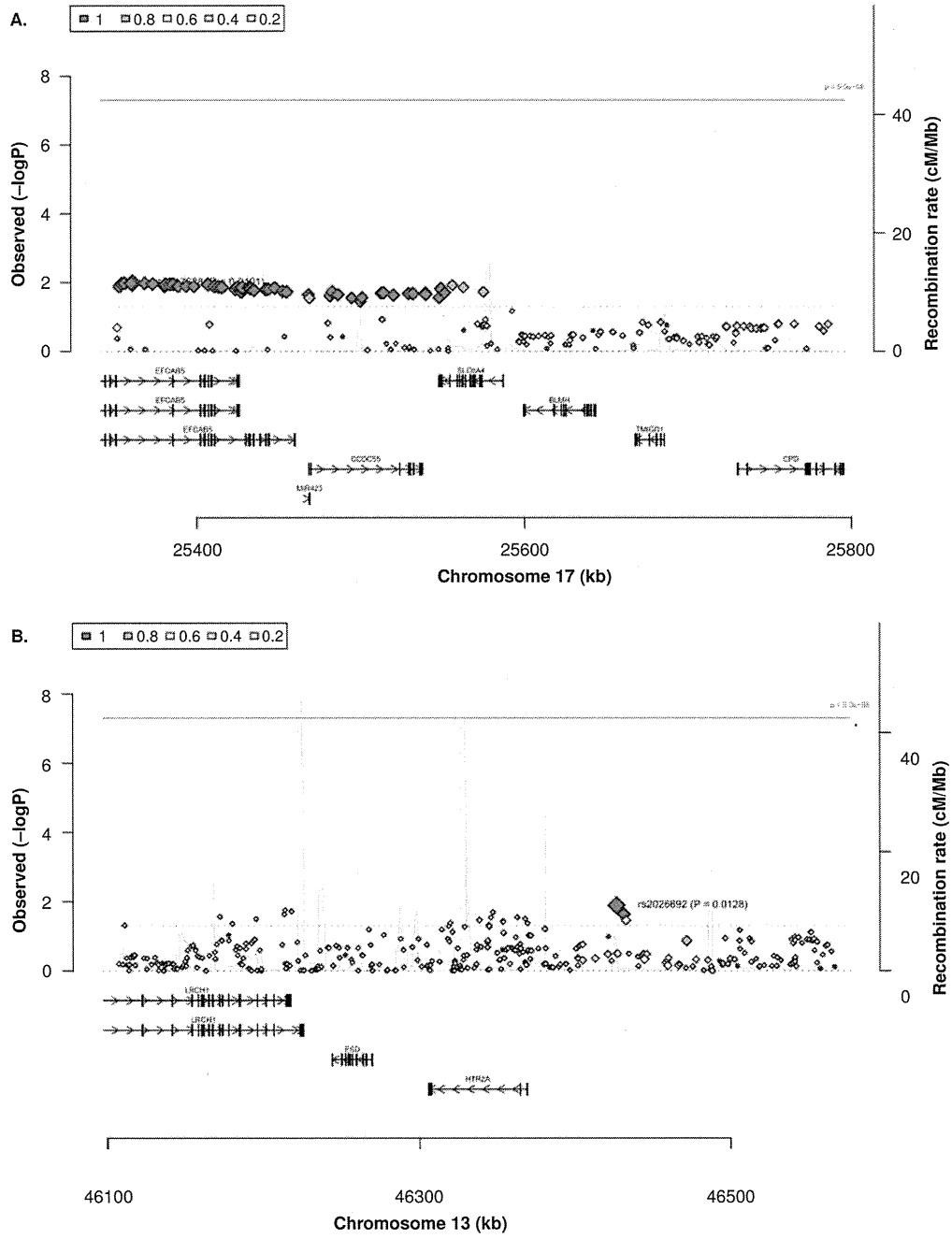

Figure 1. Results reported by a previous genome-wide association studies meta-analysis [7] in SLC6A4 (A) and HTR2A (B) genetic regions are reported. Plots were obtained using Ricopili [6]. Colors in the legend indicate the level of linkage disequilibrium among the identified SNPs.

Table 3. Molecular pathways involved in serotonin reuptake inhibitor efficacy (results obtained by including genes within a range of [0.1 cm + 300 kb] from SNPs with p < 0.0001 in a previous meta-analysis of genome-wide studies [7]).

Biological process	Pathway	Genes	q-value
Cell proliferation, cell damage and apoptosis	p53 binding	USP7, TP53, SMARCA4, SIRT1, SETD7, EHMT2, DAXX	6.91e-05
	Induction of apoptosis by extracellular signals	VAV2, TNF, SSTR3, MAP3K5, DAXX, BAX, BAK1	0.0053
	Cellular component disassembly	SOD1, SMARCA4, RPS11, RPL7A, RPL7, RPL18, PMAIP1, BAX	0.0063
	Induction of apoptosis by intracellular signals	BAK1, BAX, MAP3K5, PMAIP1, SIRT1, TP53	0.0074
	Positive regulation of cysteine-type endopeptidase activity involved in apoptotic process	TP53, TNF, SIRT1, PMAIP1, MAP3K5, BAX	0.0081
	Positive regulation of neuron apoptosis	TP53, PCSK9, BAX	0.0178
	Positive regulation of cellular component organization	TP53, TNF, SMAD2, PMAIP1, PCSK9, HCK, DNMT1, BAX, ARF6	0.0203
	Regulation of cellular response to stress	SIRT1, PMAIP1, MAP3K7, MAP4K5, MAP3K5, DAXX, CEBPG	0.0275
	Apoptotic signaling pathway	TNF, PMAIP1, MCL1, BAX	0.0275
	Nuclear body	TP53, TFIP11, SIRT1, HSPA1A, GFI1, DAXX	0.0275
	Cellular response to radiation	TP53, SIRT1, RUVBL2, BAK1	0.0309
	Cellular response to starvation	TP53, SIRT1, PMAIP1, PCSK9	0.0323
	Regulation of cellular component biogenesis	TNF, RAF1, PMAIP1, HSPA1A, HCK, BAX, ARF6	0.0401
	Macromolecular complex disassembly	TNF, SMARCA4, RPS11, RPL7A, RPL7, RPL18	0.0428
	Cellular response to biotic stimulus	TP53, TNF, HCK, GFI1	0.0441
Immune system	Cytokine-mediated signaling pathway	TYK2, TNF, PIN1, NUP210, IFNAR2, IFNAR1, HLA-DMB, HLA-DMA, HCK, GFI1, CEBPA, CDC37, ARIH1	7.20e-05
	Regulation of defense response	TYK2, TNF, MEF2A, MAP3K7, IFNAR2, IFNAR1, HCK,GFI1, CHUK, CDC37	0.0080
	Regulation of innate immune response	TYK2, MEF2A, MAP3K7, IFNAR2, IFNAR1, HCK, GFI1, CHUK, CDC37	0.0018
	Regulation of type I IFN-mediated signaling pathway	TYK2, IFNAR2, IFNAR1, CDC37	0.0150
	Leukocyte apoptosis	TP53, SIRT1, CTSL1, BAX	0.0176
	Regulation of response to cytokine stimulus	TYK2, IFNAR2, IFNAR1, GFI1, CDC37	0.0275
	Immune response-activating signal transduction	MEF2A, MAP3K7, HLA-DMB, HLA-DMA, HCK, GFI1, CHUK	0.0408
	Positive regulation of immune response	SIRT1, MEF2A, MAP3K7, HLA-DMB, HLA-DMA, HCK, GFI1, CHUK	0.0428
Protein assembly	Protein heterodimerization activity	TP53, MEF2A, MCL1, GTF2A2, GTF2A1, EXT2, CEBPG, BAX, BAK1	0.0018
	Regulation of protein complex assembly	TNF, PMAIP1, HCK, BAX, ARF6	0.0177
Protein and peptide metabolism	Peptidyl-lysine modification	TAF10, SUPT7L, SIRT1, SETD7, RUVBL2, RUVBL1, MAP3K7, EHMT2	0.0018
	Positive regulation of peptidase activity	ADRM1, BAX, MAP3K5, PMAIP1, SIRT1, TNF, TP53	0.0018
	Positive regulation of endopeptidase activity*	TP53, TNF, SIRT1, PMAIP1, MAP3K5, BAX, ADRM1	0.0018
	Positive regulation of cysteine-type endopeptidase activity*	TP53, TNF, SIRT1, PMAIP1, MAP3K5, BAX	0.0081
	Ubiquitin protein ligase binding*	USP7, TP53, SMAD2, HSPA1A, DAXX, ARIH1	0.0082
	Activation of protein kinase activity	DAXX, MAP3K5, MAP3K7, MAP4K5, PICK1, RAF1, SOD1, TNF	0.0082
	Small conjugating protein ligase binding	USP7, TP53, SMAD2, HSPA1A, DAXX, ARIH1	0.0082
	Unfolded protein binding	RUVBL2, PFDN6, HSPA2, HSPA1A, CDC37	0.0086
	Peptidyl-lysine acetylation	TAF10, SUPT7L, SIRT1, RUVBL2, RUVBL1, MAP3K7	0.0125
	Positive regulation of protein serine/threonine kinase activity	TNF, SOD1, SIRT1, MAP4K5, MAP3K7, MAP3K5, DAXX	0.0150
	Protein acetylation		0.0178

*These molecular pathways are involved also in apoptosis.

Table 3. Molecular pathways involved in serotonin reuptake inhibitor efficacy (results obtained by including genes within a range of [0.1 cm + 300 kb] from SNPs with p < 0.0001 in a previous meta-analysis of genome-wide studies [7]) (continued).

Biological process	Pathway	Genes	q-value
		TAF10, SUPT7L, SIRT1, RUVBL2, RUVBL1, MAP3K7	
	Protein acylation	TAF10, SUPT7L, SIRT1, RUVBL2, RUVBL1, MAP3K7	0.0205
DNA transcription	Histone modification	TP53, TAF10, SUPT7L, SIRT1, SETD7, RUVBL2, RUVBL1, MAP3K7, EHMT2, DNMT1	0.0018
	Covalent chromatin modification	TP53, TAF10, SUPT7L, SIRT1, SETD7, RUVBL2, RUVBL1, MAP3K7, EHMT2, DNMT1	0.0018
	Regulation of sequence-specific DNA binding transcription factor activity	USP7, TNF, SMARCA4, SIRT1, MAP3K7, HCK, GTF2A2, GFI1, CHUK, CEBPG	0.0058
	mRNA catabolic process	RPS11, RPL7A, RPL7, RPL18, LSM3, LSM2, HSPA1A, EXOSC5	0.0078
	Nuclear chromatin	TP53, SMARCA4, SIRT1, RUVBL2, RUVBL1, MEF2A	0.0143
	Nuclear-transcribed mRNA catabolic process	RPS11, RPL7A, RPL7, RPL18, LSM3, LSM2, EXOSC5	0.0185
	Histone acetyltransferase complex	TAF10, SUPT7L, RUVBL2, RUVBL1, MAP3K7	0.0203
	Positive regulation of sequence-specific DNA binding transcription factor activity	TNF, SMARCA4, MAP3K7, GTF2A2, CHUK, CEBPG	0.0402
Energetic metabolism	Regulation of mitochondrial membrane potential	SOD1, PMAIP1, BAX, BAK1	0.0018
	Mitochondrial outer membrane	RAF1, PMAIP1, MCL1, BAX, BAK1	0.0143
Corticosteroid and lipid metabolism	Regulation of steroid biosynthetic process	TNF, SOD1, SIRT1, GFI1	0.0084
	Regulation of lipid biosynthetic process	TNF, SOD1, SIRT1, LDLR, GFI1	0.0152
	Regulation of steroid metabolic process	TNF, SOD1, SIRT1, GFI1	0.0224
	Cholesterol homeostasis	SIRT1, PCSK9, LIPG, LDLR	0.0323
	Sterol homeostasis	SIRT1, PCSK9, LIPG, LDLR	0.0323
Second messengers	Activation of MAPK activity	TNF, SOD1, MAP4K5, MAP3K7, MAP3K5, DAXX	0.0085
	Positive regulation of JUN kinase activity	MAP4K5, MAP3K7, MAP3K5, DAXX	0.0239

*These molecular pathways are involved also in apoptosis.

opportunity to extend the study of biological predictors of SSRI response from genetics to genomics and more in general to 'omics,' giving a preferential way to enter into biological mechanisms that are still partly unknown. The identification of molecular targets for innovative antidepressant drugs would be a natural consequence, offering the opportunity of significant changes in the treatment and prognosis of depression.

6. Expert opinion

Despite the efforts aimed to the identification of genes involved in SSRI efficacy, clinical applications of antidepressant pharmacogenetics are still in the first experimental phase.

Given previous pharmacogenetic results concerning 5-HTTLPR, models of cost-effectiveness of antidepressant treatment guided by 5-HTTLPR genotype have been investigated. Incorporating into the model the joint effect of 5-HTTLPR on antidepressant response and tolerability, cost-effectiveness acceptability showed a > 80% probability of being under the commonly accepted threshold (three times Gross Domestic Product [GDP] according to the WHO) [188]. The hypothesis of favorable cost-effectiveness ratio of treatment guided by 5-HTTLPR versus treatment as usual was confirmed by a further simulation using data from 27 European states stratified by GDP. This study demonstrated that cost-effectiveness was favorable in > 90% in high-income countries (Euro A) [189]. Given these encouraging results, recent studies began to investigate the cost-effectiveness of genotyping in real-world clinical practice. A retrospective blinded study evaluated health-care utilization measures for patients with depressive or anxiety disorder in relation to an interpretive pharmacogenomic test that was based on DNA variations in CYP genes (CYP2D6, CYP2C19, CYP2C9 and CYP1A2), SLC6A4 and HTR2A. Subjects that were classified as 'at risk' according to the gene-based interpretive report and drug regimen had 69% more total health-care visits, 67% more general medical visits, greater than threefold more medical absence days and greater than fourfold more disability than subjects categorized as having no risk or intermediate risk [132]. Present technology allows to provide a gene-based interpretive report in two working days, a time that is compatible with routine clinical practice. Nevertheless, a number of kits are currently commercially available, but sufficient stability across products is still lacking (i.e., different genetic markers are included in the available kits) and their prediction value is still not clinically overwhelming. Besides the high heterogeneity across previous pharmacogenetic studies,

another reason of this lack of consistence is that previous evidence in literature largely regards individual polymorphisms, mRNA or proteins, without any integration of information derived by complementary levels of regulation. Indeed, protein level and functionality are influenced by genetic variants but also non-sequence variants (i.e., epigenetics) and RNA editing and degradation. Further, variability in protein transport and metabolism can affect drug pharmacodynamics and pharmacokinetics. Given the multiple levels of biological regulation, recent studies are moving from individual polymorphisms and individual genes to whole genotyping of multiple regions, epigenetics, RNA sequencing or candidate approach, study of the level of selected proteins in serum or lymphocytes. The extension from individual genes/molecules to molecular systems moved the focus on networks, which probably represents the most suitable strategy to identify interrelated groups of genes that are critically involved in antidepressant action.

In conclusion, the lacking of any genotyping test approved by guidelines for use in routine clinical practice may become the past. As a matter of fact, resources to overcome the limits of previous pharmacogenetic studies are currently more and more available at both technological and theoretical level.

Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties. No funding or any financial interest is related to the present paper. Financial disclosure is referred to general (past or current) assignments.

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