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Novel strategies for pharmacogenetic and metabolomic analysis of antidepressant treatment response in depression

Kenneth Chappell

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Novel strategies for pharmacogenetic and metabolomic analysis of antidepressant treatment response in depression

Des nouvelles stratégies de l'analyse pharmacogénétique et métabolomique de la réponse aux traitements antidépresseurs dans la dépression

Thèse de doctorat de l'université Paris-Saclay

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CESP (Université Paris-Saclay, UVSQ, Inserm) et
UMR-S 1180 (Université Paris-Saclay, Inserm)
sous la direction de **Céline VERSTUYFT**, Professeur des Universités – Praticien hospitalier
et de **Jérôme BOULIGAND**, Professeur des Universités – Praticien hospitalier

Thèse soutenue à Paris-Saclay, le 11 décembre 2023, par

Kenneth CHAPPELL

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Titre : Des nouvelles stratégies pour l'analyse pharmacogénétique et métabolomique de la réponse aux traitements antidépresseurs dans la dépression

Mots clés : Dépression, Antidépresseurs, Pharmacogénétique, Métabolomique, Bioinformatique

Introduction Le trouble dépressif caractérisé (TDC) est un fardeau mondial dont le traitement par des antidépresseurs (ATD) est modestement efficace. Les prédicteurs de la réponse aux antidépresseurs peuvent contribuer à améliorer les résultats thérapeutiques. Des centaines d'associations biologiques sont observées au niveau génétique et métabolomique, mais de nouvelles méthodes, outils et stratégies continuent d'évoluer, ouvrant de nouvelles voies d'analyse. L'objectif de cette thèse est d'exploiter de nouvelles stratégies pour explorer l'association de la variation génétique au sein des gènes candidats avec les résultats cliniques et les concentrations de métabolites après le traitement ATD.

Méthodes Ce travail est basé sur l'analyse des données de METADAP, une cohorte prospective, multicentrique et naturaliste de 624 patients âgés de 18 à 65 ans souffrant d'un épisode dépressif caractérisé (EDC) dans le contexte de TDC et nécessitant un nouveau traitement ATD. Les patients ont subi une évaluation clinique et biologique à l'inclusion et 1, 3 et 6 mois après le début du traitement par ATD. Les données analysées comprenaient des mesures cliniques, des données génétiques provenant d'un panel de gènes séquencés à haut débit ou d'un génotypage TaqMan et des concentrations de sérotonine (5-HT) et d'acide 5-hydroxyindoléacétique (5-HIAA).

Résultats 1) le polymorphisme génétique *ERICH3* rs11580409(A>C) a été associé à une amélioration clinique, mais pas aux niveaux de 5-HT, après un traitement ATD chez des patients déprimés ; 2) l'allèle A du polymorphisme génétique *MAOA* rs979605(A>G) a été associé à une plus mauvaise amélioration clinique chez les femmes que chez les hommes, tandis que le polymorphisme génétique *MAOB* rs1799836(T>C) a été associé au rapport 5-HIAA/5-HT plasmatique ; 3) l'accumulation de variations génétiques rares d'*ARRB1* et deux polymorphismes génétiques d'*ARRB1* ayant des conséquences fonctionnelles potentielles, rs553664(G>A) et rs536852(A>G), ont été associés à des résultats cliniques plus défavorables ; 4) dans un panel de gènes candidats, la variation génétique de *SLC1A1* a été la plus associée à des mesures cliniques ; l'allèle C du polymorphisme génétique *SLC1A1* rs301435(T>C) a été associé à une amélioration clinique plus défavorable.

Conclusion Ce travail s'est appuyé sur de nouvelles stratégies pour révéler plusieurs associations entre les variations génétiques candidates et les résultats cliniques à la suite d'un traitement ATD. Ces résultats suggèrent plusieurs associations dépendantes du contexte. Cependant, la plupart d'entre elles restent des associations nécessitant une réplification indépendante et une validation fonctionnelle dans le contexte du traitement ATD de TDC.

Title: Novel strategies for pharmacogenetic and metabolomic analysis of antidepressant treatment response in depression

Keywords: Depression, Antidepressants, Pharmacogenetics, Metabolomics, Bioinformatics

Introduction Major Depressive Disorder (MDD) is a global burden whose treatment with antidepressant drugs (ATDs) is modestly effective. Predictors of ATD response may help improve therapeutic outcomes. Hundreds of biological associations are observed at both the genetic and metabolomic level, but novel methods, tools, and strategies continue to evolve, opening new avenues of analysis. The objective of this thesis is to leverage novel strategies to explore the association of genetic variation within candidate genes with clinical outcomes and metabolite concentrations following ATD treatment.

Methods This work is based on the analysis of data from METADAP, a prospective, multicentric, and naturalistic cohort of 624 patients between 18 and 65 years of age suffering from a major depressive episode (MDE) in the context of MDD and requiring a new ATD treatment. Patients underwent clinical and biological evaluation at inclusion and 1, 3, and 6 months after beginning ATD treatment. Analyzed data included clinical measures, genetic data from a high-throughput sequencing gene panel or TaqMan genotyping, and serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) concentrations.

Results 1) the *ERICH3* rs11580409(A>C) genetic polymorphism was associated with clinical improvement, but not 5-HT levels, following ATD treatment in depressed patients; 2) the A allele of the *MAOA* rs979605(A>G) genetic polymorphism was associated with worse clinical improvement in females compared to males, while the *MAOB* rs1799836(T>C) genetic polymorphism was associated with the plasma 5-HIAA/5-HT ratio; 3) rare *ARRB1* genetic variation accumulation and two *ARRB1* genetic polymorphisms with potential functional consequences, rs553664(G>A) and rs536852(A>G), were associated with worse clinical outcomes; 4) across a candidate gene panel, *SLC1A1* genetic variation was most associated with clinical measures; the C allele of the *SLC1A1* rs301435(T>C) genetic polymorphism was associated with worse clinical improvement.

Conclusion This work leveraged novel strategies to reveal several associations between candidate genetic variation and clinical outcomes following ATD treatment. Several context-dependent associations are suggested by these findings. However, most remain associations requiring independent replication and functional validation in the context of ATD treatment for MDD.

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Abbreviations

5-HIAA	5-hydroxyindoleacetic acid
5-HT	serotonin
AMPA	α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ATD	antidepressant drug
BDNF	brain derived neurotrophic factor
CPIC	Clinical Pharmacogenetics Implementation Consortium
CYP	cytochrome P450
DSM	Diagnostic and Statistical Manual of Mental Disorders
ECT	electroconvulsive therapy
eQTL	expression quantitative trait loci
G2PSR	Genome-to-Phenome Sparse Regression
GC	gas chromatography
GPCR	G protein-coupled receptor
GWAS	genome-wide association study
HPA	hypothalamic–pituitary–adrenal
HTS	high-throughput sequencing
LC	liquid chromatography
LCECA	liquid chromatography electrochemical arrays
LD	linkage disequilibrium
MAF	minor allele frequency
MAO	monoamine oxidase
MDD	Major Depressive Disorder
MDE	major depressive episode
MS	mass spectrometry
NMR	nuclear magnetic resonance
NMDA	N-methyl-D-aspartate
OCD	obsessive compulsive disorder
PD	pharmacodynamics
PGx	pharmacogenetics/pharmacogenomics
PGRN-AMPS	Pharmacogenomics Research Network–Antidepressant Medication Pharmacogenomics Study
PK	pharmacokinetics
PMx	pharmacometabolomics

PRS polygenic risk score
rTMS repetitive transcranial magnetic stimulation
SKAT Sequence Kernel Association Test
SNRI serotonin-norepinephrine reuptake inhibitor
SNP single nucleotide polymorphism
SNV single nucleotide variation
SSRI selective serotonin reuptake inhibitor
rs reference SNP
STAR*D Sequenced Treatment Alternatives to Relieve Depression
TCA tricyclic antidepressant
TF transcription factor
UTR untranslated region

Preface

Major Depressive Disorder is a psychiatric disorder of global and high financial burden, with debilitating consequences for patients. Unfortunately, its treatment with pharmacological therapies including antidepressant drugs remains modestly effective. The identification of biological factors that can predict whether an individual will respond to antidepressant treatment may help improve therapeutic outcomes. Hundreds of biological associations with clinical outcomes following antidepressant treatment spanning myriad biological pathways are known, including those related to genetic variation. Owing to advancements in methods, tools, and analytical strategies, as well as the accessibility of data, novel approaches for genetic investigation in association with the response to antidepressant treatment are possible.

The objective of this thesis project was to explore the association of genetic variation within candidate genes with clinical outcomes and metabolite concentrations following antidepressant drug treatment in depressed individuals using novel strategies. These genetic targets included: (1) the *ERICH3* rs11580409(A>C) genetic polymorphism that was previously associated with variable serotonin levels and clinical response in several cohorts of depressed individuals; (2) genetic variation within the X chromosome-linked *MAOA* and *MAOB*, encoding monoamine oxidase, which metabolizes monoamine neurotransmitters, the targets of first-line antidepressant treatments; (3) genetic variation—both rare and common with potential functional consequences on gene expression—within *ARRB1*, encoding a protein that modulates receptor activity; (4) genetic variation within all candidate genes of a high-throughput sequencing gene panel.

Synthèse de la Thèse

1. Introduction

Le trouble dépressif caractérisé (TDC) est un fardeau mondial dont le traitement par les antidépresseurs (ATD) est modestement efficace. Les indicateurs de la réponse aux traitements ATD peuvent contribuer à l'amélioration des résultats thérapeutiques et comprennent des facteurs biologiques dont ceux liés à la pharmacocinétique—le devenir du médicament dans l'organisme—et le pharmacodynamie—l'action du médicament sur l'organisme. Le mécanisme d'action des traitements ATD reste mal connu, mais leur influence sur les concentrations synaptiques des neurotransmetteurs monoamines dont sérotonine (5-HT) entraîne une augmentation de la neurotransmission monoaminergique et, par conséquent, des processus liés à la neuroplasticité. Des facteurs au niveau de la synapse et liés à la neurotransmission monoaminergique sont donc d'intérêt.

Des associations génétiques à ce niveau sont connues, dont des gènes encodant des métaboliseurs des neurotransmetteurs et des protéines liées à la signalisation des récepteurs. Cependant, ces associations génétiques sont rarement répliquées, n'impliquent généralement que des polymorphismes fréquents et n'expliquent pas leurs conséquences biologiques potentielles. Des nouvelles méthodes, outils et stratégies continuent d'évoluer et s'ouvrent aux nouvelles voies d'analyse pour ces facteurs génétiques. L'objectif de cette thèse est d'exploiter de nouvelles stratégies pour explorer l'association de la variation génétique au sein des gènes candidats liés plutôt à la neurotransmission monoaminergique avec les résultats cliniques et les concentrations de métabolites à la suite d'un traitement ATD chez des patients souffrants d'un épisode dépressif caractérisé (EDC) dans le contexte de TDC.

2. Patients, Matériels et Méthodes

Ce travail se base sur l'analyse des données de METADAP, une cohorte prospective, multicentrique et naturaliste de 624 patients âgés de 18 à 65 ans souffrants d'un EDC dans le contexte de TDC, nécessitant un nouveau traitement ATD et ayant un score de ≥ 18 sur l'échelle de dépression de Hamilton à 17-items (HDRS). Les évaluations cliniques ont été faites à l'inclusion et 1, 3 et 6 mois après le début du traitement ATD. Les facteurs cliniques analysés comprenaient le HDRS, les scores des échelles de l'impression clinique globale (CGI), la réponse (définie par un changement du score HDRS $\geq 50\%$) et la rémission (définie par un score HDRS ≤ 7). La 5-HT et l'acide 5-hydroxyindoleacétique (5-HIAA), le métabolite principal de la 5-HT, ont été dosés du plasma sanguin par la chromatographie en phase liquide à très haute performance. Les données génétiques sont issues soit de la technologie

TaqMan soit du séquençage à haut débit selon un panel de 72 gènes candidats. Les informations de cette dernière méthode ont été stockées dans des fichiers de Variant Call Format (VCF). Les VCFs ont été téléchargés sur R pour le prétraitement et l'analyse. Les analyses ont été réalisées principalement en utilisant des modèles à effets mixtes. La variation génétique rare a été analysée en utilisant le package GMMAT. L'ensemble des polymorphismes fréquents issus de HTS a été analysé en utilisant Genome-to-Phenome Sparse Regression (G2PSR).

3. Résultats

Premièrement, on a analysé le polymorphisme génétique rs11580409(A>C) du gène *ERICH3*, un gène associé aux taux de la 5-HT et un polymorphisme associé à la réponse aux traitements ATDs. Chez des patients déprimés de METADAP, le polymorphisme rs11580409 a été associé à une amélioration clinique après un traitement ATD. Les homozygotes CC avaient un score HDRS plus bas et un taux de réponse plus élevé par rapport aux porteurs de l'allèle A après 6 mois de traitement. Cependant, on n'a pas répliqué l'association de ce polymorphisme aux taux de la 5-HT.

Deuxièmement, on a regardé la variation génétique au sein de *MAOA* et de *MAOB*, encodant la monoamine oxydase (MAO). Cinq polymorphismes de *MAOA* et un de *MAOB* ont été identifiés par le HTS. Le déséquilibre de liaison était élevé entre les 5 polymorphismes de *MAOA*. On a donc analysé le polymorphisme rs979605(A>G) de *MAOA* comme proxy de ces 5 et le polymorphisme rs1799836(T>C) de *MAOB*. Une interaction entre le polymorphisme rs979605 et le sexe a été associée au score HDRS. Les femmes homozygotes AA avaient un score HDRS significativement plus élevé par rapport aux hommes portant l'allèle A après 6 mois de traitement. Le polymorphisme rs1799836 de *MAOB* a été associé au ratio plasmatique de 5-HIAA/5-HT.

Troisièmement, on a regardé la variation génétique d'*ARRB1* encodant beta-arrestine 1, une protéine multifonctionnelle au niveau des récepteurs. Plus de 900 polymorphismes ont été identifiés, dont 643 rares et 9 fréquents et annotés d'avoir une haute probabilité de perturber la liaison d'un facteur de transcription (TF) ou d'influencer l'expression génique selon leur score de RegulomeDB (i.e., <3). Les 643 polymorphismes rares ont été associés au score HDRS et à la rémission dans des tests de *burden*, suggérant que la majorité de la variation rare avait une influence dans la même direction. Une accumulation de variants rares était bien associé à une moins bonne amélioration après un traitement ATD dans des modèles des effets mixtes. Deux polymorphismes fréquents et annotés, rs553664(G>A) et rs536852(A>G), ont été associés au score HDRS et à la rémission. Le polymorphisme

rs536852 n'était pas fortement corrélé aux autres polymorphismes et a été annoté de potentiellement perturber la liaison de TFs comme FOS et JUN.

En dernier, on a analysé l'ensemble des polymorphismes fréquents du panel de 72 gènes candidats en utilisant la méthode G2PSR pour agréger les polymorphismes selon leurs contextes géniques. Puisque *ARRB1* et *ARRB2* ont été séquencés entièrement par rapport aux autres gènes du panel (des régions exoniques uniquement) 3 analyses ont été réalisées : l'une avec tous les polymorphismes ; l'une où *ARRB1* a été divisé en 8 sous-groupes ; l'une où *ARRB1* et *ARRB2* ont été limités aux régions exoniques. Le gène *SLCIA1* a été identifié dans chaque analyse. Les 7 polymorphismes de *SLCIA1* ont été analysés en utilisant des modèles à effets mixtes. Parmi eux, le polymorphisme rs301435(T>C) a été associé à plusieurs mesures dont le score HDRS et la rémission. Dans chaque cas, l'allèle C a été associé à une moins bonne amélioration clinique après un traitement ATD.

4. Discussion & Conclusion

Ce travail s'est appuyé sur l'utilisation des nouvelles stratégies pour analyser l'association des polymorphismes des gènes candidats monoaminergiques à la réponse aux traitements ATDs chez des patients souffrants d'un EDC dans le contexte de TDC.

L'association du polymorphisme rs11580409(A>C) d'*ERICH3* aux mesures d'amélioration après un traitement ATD chez des patients de METADAP rejoint et enrichie d'autres résultats décrits dans la littérature. *ERICH3* a été récemment identifié dans le contexte de la réponse aux ATDs qui soulignent aussi l'image incomplète du mécanisme d'action des ATDs et les facteurs qui influencent la variabilité observée de la réponse.

L'allèle A du polymorphisme rs979605(A>G) de *MAOA* était associé à une moins bonne amélioration chez les femmes mais à une meilleure amélioration chez les hommes, qui suggère une association qui dépend du sexe. Différents facteurs peuvent influencer l'expression et/ou l'activité de MAO, dont les hormones sexuelles, qui peuvent aider à expliquer cette observation.

L'analyse de la variation génétique d'*ARRB1* souligne l'usage de RegulomeDB pour la priorisation des polymorphismes ainsi que l'importance de la variation génétique rare dans le contexte de l'amélioration après un traitement ATD. En effet, la variation rare est très peu analysée dans ce contexte. Malgré l'association des polymorphismes fréquents et annotés, ces associations doivent être répliquées dans des cohortes indépendantes et leurs conséquences potentielles doivent être investiguées dans des analyses fonctionnelles.

Nos résultats soulignent l'association de la variation génétique liée à la neurotransmission monoaminergique à l'amélioration à la suite d'un traitement ATD, mais

désigne également celle du système glutamatergique. Ce système continue à gagner d'intérêt surtout grâce aux résultats cliniques liés au traitement par kétamine, un antagoniste de récepteur NMDA de glutamate disposant d'une action antidépresseur rapide.

Ce projet bénéficie de sa taille d'échantillon, son design, son homogénéité diagnostique et sa durée de suivi. Il est limité par le taux de perte de vue, l'analyse des taux de métabolites périphériques qui diffèrent probablement de ceux du cerveau et l'influence simultanée des différents facteurs sur l'amélioration clinique dont le placebo, les facteurs environnementaux et d'autres thérapies.

Plusieurs associations génétiques ont été identifiées dans le contexte de l'amélioration aux traitements antidépresseurs pour le TDC en utilisant des nouvelles stratégies, mais elles nécessitant la répllication dans des cohortes indépendantes et, pour la majorité des résultats, la validation par des analyses fonctionnelles.

I. Introduction

1.1. Major Depressive Disorder

1.1.1. Background

Major Depressive Disorder (MDD) is the leading contributor to global disability. Over 300 million individuals worldwide (~4.4% of the global population) suffer from MDD (World Health Organization, 2017). Lifetime prevalence suggests that 20% of all individuals meet the criteria for a major depressive episode (MDE) during their lifetimes. Compared to general populations, MDD is associated with an increased risk for overall mortality, suicide, and the development of other health conditions (Chesney et al., 2014; Penninx et al., 2013). Females across all age ranges suffer more frequently from MDD (World Health Organization, 2017) and experience longer and more severe depressive episodes compared to males (LeGates et al., 2019). The economic burden of MDD is substantial. Among US adults with MDD, economic burden rose from 236.6 billion USD in 2010 to 326.2 billion USD in 2018, of which 60% was attributable to increased costs (Greenberg et al., 2021).

According to the Diagnostic and Statistical Manual of Mental Disorders (DSM) (American Psychiatric Association, 2022), MDD is characterized by at least two weeks of experiencing five or more of the following, including, necessarily, a depressed mood or a loss of interest or pleasure in most regular activities (i.e., anhedonia) and (1) decreased or increased sleep, (2) significant changes in weight and/or appetite, (3) impaired psychomotor status (e.g., slowed thought or reduced physical activity), (4) fatigue or loss of energy, (5) feelings of worthlessness or excessive guilt, (6) diminished ability to think or concentrate, or indecisiveness, and (7) recurrent thoughts of death or suicidal ideation, or suicidal behavior.

An MDE related to MDD can be further specified by several subcategorizations, including severity (e.g., mild or severe), anxious distress (e.g., increased reporting of suicidal thoughts), and presentation with different features [e.g., mixed, melancholic (e.g., anhedonia), atypical, psychotic (e.g., hallucinations), and catatonic (e.g., decreased motor activity)] (Otte et al., 2016). Its numerous and varied clinical presentations (Zimmerman et al., 2015) suggest that many diverse and heterogeneous populations fall under the diagnostic of MDD (Goldberg, 2011).

Many methods exist to measure and quantify depressive symptoms and severity in clinical practice and research, including self- and clinician-administered interviews and scales [for several examples, see **Table 1** (page 2)] (Baer and Blais, 2010; Busner and Targum, 2007). The 17-item Hamilton Depression Rating Scale (HDRS), for example, poses questions related to mood, anxiety, diet, and somatic symptoms (Hamilton, 1960). Scores for

Table 1: Depression rating scales

Scale	Abbreviation	Administration	Cut-offs (depression)	Strengths	Limitations
Beck Depression Inventory	BDI	Self-rated	1–10: normal 11–16: mild disturbance 17–20: borderline 21–30: moderate 31–40: severe >40: extreme	<ul style="list-style-type: none"> • Self-rating saves time and does not require trained professionals 	<ul style="list-style-type: none"> • Validity is highly variable
Clinical Global Impressions-Severity and Improvement	CGI-S and CGI-I	Clinician-rated	1: not at all ill 2: borderline mentally ill 3: mildly ill 4: moderately ill 5: markedly ill 6: severely ill 7: among the most extremely ill	<ul style="list-style-type: none"> • Correlates well with other established scales (e.g., HDRS) • Single-question format is quick 	<ul style="list-style-type: none"> • General for use in other psychiatric disorders • Poor inter-rater reliability due to dependence on clinical experience and judgment
Hamilton Depression Rating Scale (17-items)	HDRS	Clinician-rated	0–6: not present 7–17: mild 18–24: moderate >24: severe	<ul style="list-style-type: none"> • Widely used in clinical trials • High internal consistency, inter-rater reliability, and validity 	<ul style="list-style-type: none"> • Does not include all MDD symptom domains • Uneven weighting for different symptom domains
Montgomery and Åsberg Depression Rating Scale	MADRS	Clinician-rated	<10: remission >30: severe	<ul style="list-style-type: none"> • High internal consistency and validity • Sensitive to changes resulting from antidepressant treatment 	<ul style="list-style-type: none"> • Focused more on psychological rather than somatic symptoms • Reliability may vary between professionals
Quick Inventory of Depressive Symptomatology	QIDS	Clinician (C) and self-rated (SR) versions	<6: not present 6–10: mild 11–15: moderate 16–20: severe >21: very severe	<ul style="list-style-type: none"> • High internal consistency, inter-rater reliability, and validity • Includes more symptom domains according to DSM definition 	<ul style="list-style-type: none"> • Clinician-rated is time-consuming and requires trained professionals

DSM: Diagnostic and Statistical Manual of Mental Disorders; **MDD:** Major Depressive Disorder

stratification by severity are often employed by these scales (e.g., a score of 18–24 on the HDRS suggests moderate depression) (Baer and Blais, 2010). Along with clinical judgement, these scales can be used to measure and assess improvement during treatment. Other treatment-related outcomes derived from these scales are also commonly used in research, including response (e.g., an improved depressive symptom score $\geq 50\%$ compared to baseline) and remission [e.g., a depressive symptom score below a certain threshold (e.g., < 8 on the HDRS)] (Rush et al., 2006; Trivedi et al., 2006).

1.1.2. Treatments for Major Depressive Disorder

Several therapies for the treatment of MDD are used today, including psychotherapies, somatic therapies, and pharmacological therapies [for a non-exhaustive list, see **Table 2** (page 4)]. Although each is effective in the treatment of MDEs, antidepressant drug (ATD) pharmacotherapy remains the first-line recommended treatment (Malhi and Mann, 2018). Most prescribed ATDs fall into two main classes, selective serotonin reuptake inhibitors (SSRI) and serotonin-norepinephrine reuptake inhibitors (SNRI), though tricyclic antidepressants (TCA), monoamine oxidase inhibitors (MAOI), and other “atypical” ATDs with different pharmacological targets are also available and may be prescribed under different clinical circumstances (Malhi and Mann, 2018; Otte et al., 2016).

Dozens of ATDs with similar efficacy are commercially available in France today. Although this offers a variety of options, it ultimately lends itself to a “trial-and-error” approach in the treatment of MDD (Nemeroff, 2020). This becomes more burdensome given that ATDs share two discouraging characteristics: (1) a typically weeks-long delay to any measurable benefit; (2) modest efficacy, with comparable response rates around 50% (Malhi and Mann, 2018; Otte et al., 2016). Indeed, following 4–12 weeks of ATD treatment, rates of response (47%–69%) and remission (27%–51%) remain modest across cohorts of depressed individuals [see **Table 3** (page 6)]. These rates were equally modest in the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) cohort—the largest cohort of ATD-treated depressed patients (Laje et al., 2009)—in which one-half responded and one-third remitted following 14 weeks of citalopram treatment (Trivedi et al., 2006). Moreover, remission rates fall with successive treatments, while relapse rates rise (Rush et al., 2004).

Table 2: Types of therapies for the treatment of MDD

Therapy	Abbrv	Target	Strengths	Limitations	References
<i>Psychotherapy</i>					
Cognitive behavioral therapy	CBT	Aims to replace distorted, negative thoughts with realistic, positive ones	<ul style="list-style-type: none"> • Comparable efficacy compared to pharmacological therapies • Improved efficacy when combined with pharmacological therapies 	<ul style="list-style-type: none"> • Limited use due to time constraints, lack of resources, and cost 	Karyotaki et al., 2016; Otte et al., 2016; Sverre et al., 2023; Weitz et al., 2015
Mindfulness-based therapy	MBT	Aims to non-judgmentally experience and accept negative thoughts			
<i>Somatic therapy</i>					
Electroconvulsive therapy	ECT	Generally used on patients with TRD	<ul style="list-style-type: none"> • High remission rates (60%–95%) • Successful in treating severe cases (TRD, psychotic MDE) • Safer compared to ECT 	<ul style="list-style-type: none"> • High relapse rate (~80%) • Cognitive side effects • Less effective than ECT • Treatment duration does not differ greatly from first-line pharmacological therapies 	Haddad et al., 2015
Repetitive transcranial magnetic stimulation	rTMS				
Vagus nerve stimulation	VNS				
<i>Pharmacological therapy</i>					
Ketamine	KET	Antagonist of the NMDA glutamate receptor	<ul style="list-style-type: none"> • Rapid (~24 hours) and long-lasting (1–2 weeks) antidepressant action 	<ul style="list-style-type: none"> • Improvement not sustained with KET alone • Findings remain preliminary • Adverse side effects (e.g., cognitive decline, dissociation) 	Haddad et al., 2015; LeGates et al., 2019; Lener et al., 2017; Sanacora et al., 2012
Monoamine oxidase inhibitor	MAOI	Blocks the metabolism of monoamine neurotransmitters by MAO			
Tricyclic antidepressant	TCA	Blocks the reuptake of 5-HT and norepinephrine	<ul style="list-style-type: none"> • More effective in treating MDE compared to SSRIs and SNRIs • Improved selectivity and tolerability 	<ul style="list-style-type: none"> • Adverse side effects (e.g., cardiac effects) 	Carvalho et al., 2016; Cipriani et al., 2018; Malhi and Mann, 2018; Undurraga and Baldessarini, 2017
Serotonin-norepinephrine reuptake inhibitor	SNRI	Blocks the reuptake of 5-HT and norepinephrine			
Selective serotonin reuptake inhibitor	SSRI	Blocks the reuptake of 5-HT		<ul style="list-style-type: none"> • Modest efficacy • Weeks-long delay in therapeutic response • Side effects remain common especially with long-term use 	

5-HT: serotonin; MAO: monoamine oxidase; MDE: major depressive episode; NMDA: N-methyl-D-aspartate

1.1.3. Drug response and factors of variability

Although response to ATD therapies depends on adequate dosing, treatment duration, and adherence to the prescribed drug (Haddad et al., 2015), it can be influenced by other factors. Pharmacology, the study of drug action and response, seeks to examine and better understand the factors underlying the interindividual variability in drug response and toxicity. This often requires prior knowledge, most importantly about the individual, to determine the “right” pharmacological therapy (e.g., the correct drug, dose, and frequency) (Vallance and Smart, 2006).

Although this concept of precision medicine is a promising idea, various factors can influence drug response, including drug characteristics (e.g., dosage and drug-drug interactions), environmental factors (e.g., diet), and one’s own biology (Turner et al., 2015). As such, biological factors that predict drug response (i.e., biomarkers) are of interest when searching for the “right” pharmacological treatment. These factors are often related to drug pharmacokinetics (PK) and pharmacodynamics (PD), or, respectively, how the body affects the drug—including its absorption, distribution, metabolism, and excretion—and how the drug affects the body (Turner et al., 2015).

ATDs differ greatly with respect to their PK (Hiemke and Härtter, 2000), which plays into drug selection and dosing since active metabolites must maintain specific concentrations to have a therapeutic effect (Wyska, 2019). Various biological factors can influence ATD concentrations, including members of the cytochrome P450 (CYP) superfamily. Indeed, interindividual variability in ATD concentrations, which depends on hepatic transport and CYP-mediated metabolism, is associated with CYP activity (Wyska, 2019). Genetic variation in CYP-encoding genes can reduce, enhance, or abolish CYP activity and/or gene expression (Tracy et al., 2016) and variation specifically within *CYP2D6* and *CYP2C19* is associated with SSRI concentrations (Ji et al., 2014; Jukić et al., 2018). The Clinical Pharmacogenetics Implementation Consortium (CPIC) also recommends clinical guidelines to help guide ATD dosing and selection based on *CYP2D6* and *CYP2C19* genetic variation that is expertly validated to influence the efficacy and tolerability of ATDs (Bousman et al., 2023; Hicks et al., 2017).

Like the cause(s) of depression, the PD of ATDs remains less well understood. ATD treatment outcomes have been explored and associated with factors related to hypothalamic–pituitary–adrenal (HPA) axis dysregulation, altered glutamatergic signaling, neurodegeneration, immunological status, and more (Kamran et al., 2022; Leboyer et al., 2016). Though important, ATDs were not designed to directly target these factors, but rather

Table 3: Cohorts of depressed patients

Cohort	N	F/M ratio	Ethnicity	Inclusion	Treatment(s)	F/u (weeks)	Assessment	Response/remission definition	Response rate	Remission rate	Strengths	Limitations	Reference
GENDEP	811	1.74	European	<ul style="list-style-type: none"> • Aged >18 years • Moderate MDE (ICD-10 or DSM-IV) 	citalopram, nortriptyline	12	MADRS, HDRS, BDI	<ul style="list-style-type: none"> • ≥50% reduction in HDRS score • HDRS score ≤7 • HDRS score <17 after ≤2 adequate consecutive ATD trials during the last MDE 	57%	41%	<ul style="list-style-type: none"> • Ancestry determined up to 2 generations (population stratification) 	<ul style="list-style-type: none"> • Use of nortriptyline (generalizability) 	Uher et al., 2009b
GSRD	702	2.64	European	<ul style="list-style-type: none"> • MDD (DSM-IV) • ≥1 adequate ATD treatment¹ during current MDE 	naturalistic n.r.	8	HDRS	<ul style="list-style-type: none"> • n.r. 	51%	n.r.	<ul style="list-style-type: none"> • Examination of TRD (clinical severity) • Naturalistic (real-world treatment) 	<ul style="list-style-type: none"> • TRD definition is one of several in the literature (generalizability) 	Souery et al., 2007
ISPC	865	1.30	Asian, European	<ul style="list-style-type: none"> • Varied by site • In general, patients were: • Aged ≥18 years • MDD diagnosis (DSM-IV) • Met minimum HDRS score (8–18) 	citalopram, escitalopram, fluoxetine, fluvoxamine, paroxetine, sertraline, venlafaxine	3–8	HDRS	<ul style="list-style-type: none"> • ≥50% reduction in HDRS score after 4 weeks of treatment • n.r. 	48%	n.r.	<ul style="list-style-type: none"> • Mixed population can identify factors in both Asian and European populations 	<ul style="list-style-type: none"> • Mixed ancestry (population stratification) • Varied follow-up time across sites (limited analysis) 	Biernacka et al., 2015
MAKE BETTER	1,000	n.r.	Korean	<ul style="list-style-type: none"> • Aged >7 years • Diagnosis with MDD, dysthymic disorders, or depressive disorders not specified by the DSM-IV • HDRS score ≥14 	naturalistic bupropion, desvenlafaxine, duloxetine, escitalopram, fluoxetine, mirtazapine, paroxetine, sertraline, TCAs, venlafaxine, vortioxetine	104	HDRS, HADS	<ul style="list-style-type: none"> • ≥20% reduction in HDRS score after 2 weeks of treatment • HDRS score ≤ 7 after 12 weeks of treatment 	83%	43%	<ul style="list-style-type: none"> • Ethnically homogenous 	<ul style="list-style-type: none"> • Clinically heterogeneous 	Kang et al., 2020, 2018
MARS	842	1.32	European	<ul style="list-style-type: none"> • Aged 18–75 years • MDE, recurrent depression, or bipolar disorder (DSM-IV) 	naturalistic n.r., except in terms of ATD class: NaSSA, NRI, others, SSRI, SNRI, TCA	5	HDRS	<ul style="list-style-type: none"> • ≥50% reduction in HDRS after 5 weeks of treatment • HDRS score ≤7 	52%	28%	<ul style="list-style-type: none"> • Large cohort (real-world treatment) 	<ul style="list-style-type: none"> • Clinical stratification • Short follow-up 	Hennings et al., 2009

METADAP	624	2.20	Mixed (~90% European)	<ul style="list-style-type: none"> • Aged 18–65 years • Current MDE in MDD (DSM-IV) • HDRS score ≥ 18 	<p>naturalistic fluoxetine, paroxetine, sertraline, citalopram, escitalopram, fluvoxamine, venlafaxine, milnacipran, duloxetine, clomipramine, amitriptyline, imipramine, mianserin, mirtazapine, agomelatine, tianeptine, iproniazid, moclobemide, ECT, rTMS</p>	26	HDRS	<ul style="list-style-type: none"> • $\geq 50\%$ reduction in HDRS score • HDRS score ≤ 7 	53% ³	27% ³	Long-term follow-up	High dropout rate by last timepoint	Corruble et al., 2015
PGRN-AMPS	463	1.82	White	<ul style="list-style-type: none"> • Aged 18–84 years • MDE in MDD (DSM-IV) • HDRS score ≥ 14 	citalopram, escitalopram	8	HDRS, QIDS-C16	<ul style="list-style-type: none"> • $\geq 50\%$ reduction in QIDS-C16 score • QIDS-C16 score ≤ 5 	69%	50%	Findings generalizable to typical ATD treatments and STAR*D level 1	• Short follow-up time • Findings generalizable to SSRIs alone	Mrazek et al., 2014
PReDICT	344	1.32	Mixed (~48% White)	<ul style="list-style-type: none"> • Aged 18–65 years • MDE (DSM-IV) without prior treatment for MDD • HDRS score ≥ 18 at screening and ≥ 15 at baseline 	escitalopram, duloxetine	12	HDRS, QIDS-SR16, BDI	<ul style="list-style-type: none"> • $\geq 50\%$ reduction in HDRS score from baseline • HDRS score ≤ 7 at week 10 and week 12 • $\geq 50\%$ reduction in QIDS-SR16 at the last assessment • HDRS score ≤ 7 at exit 	64%	51%	Examination of first-time MDEs (clinical severity)	Small cohort, recruitment based on advertising and primary care referral (clinical severity)	Dunlop et al., 2017
STAR*D	2,876	1.76	Caucasian	<ul style="list-style-type: none"> • Aged 18–85 years • MDD (DSM-IV) • HDRS score ≥ 14 	(1) citalopram; (2) bupropion, sertraline, venlafaxine; (3) mirtazapine, nortriptyline; (4) tranylcypromine, venlafaxine+mirtazapine	14 ²	HDRS, QIDS-C16, QIDS-SR16	<ul style="list-style-type: none"> • $\geq 50\%$ reduction in QIDS-SR16 at the last assessment • HDRS score ≤ 7 at exit 	47%	28%	• Large cohort • Naturalistic treatment (real-world treatment strategies)	• Results of level 1 may not be generalizable to other treatments • High dropout rate by last timepoint	Rush et al., 2004; Trivedi et al., 2006

ATD: antidepressant drug; **BDI:** Beck Depression Inventory; **DSM:** Diagnostic and Statistical Manual of Mental Disorders; **ECT:** electroconvulsive therapy; **F:** female; **F/u:** follow-up; **HADS:** Hospital Anxiety and Depression Scale; **HDRS:** Hamilton Depression Rating Scale; **ICD:** International Classification of Diseases; **M:** male; **MADRS:** Montgomery and Asberg Depression Rating Scale; **MDD:** Major Depressive Disorder; **MDE:** major depressive episode; **N:** sample size; **NaSSA:** noradrenergic specific serotonergic antidepressant; **n.r.:** not reported; **NRI:** norepinephrine reuptake inhibitor; **QIDS-C16:** Quick Inventory of Depressive Symptomatology, clinician-rated; **QIDS-SR16:** Quick Inventory of Depressive Symptomatology, self-reported; **SSRI:** selective serotonin reuptake inhibitor; **SNRI:** serotonin-norepinephrine reuptake inhibitor; **TCA:** tricyclic antidepressant; **TRD:** treatment resistant depression

¹ ≥ 4 weeks in duration and dose \geq the lowest effective dose (product datasheet); ² in level 1 of the sequenced trials; ³ among the 326 measures available after 3 months of treatment

the synaptic concentrations of the monoamine neurotransmitters, serotonin [i.e., 5-hydroxytryptamine (5-HT)], dopamine, and noradrenaline (i.e., norepinephrine) (Malhi and Mann, 2018; Otte et al., 2016). Following the association of depleted monoamine neurotransmitter levels with depressive symptoms and the ability of early ATDs (i.e., TCAs and MAOIs) to enhance monoamine neurotransmission, these compounds were implicated in the pathophysiology of MDD and its treatment with ATDs (Otte et al., 2016).

Most ATDs influence monoamine neurotransmitter concentrations by directly blocking their reuptake into presynaptic neurons (e.g., SSRIs, SNRIs, and TCAs) or their enzymatic degradation (e.g., MAOIs) [see **Figure 1** (page 9) and **Table 2** (page 4)]. However, the true mechanistic action of ATDs remains largely unknown. Still, increased monoamine neurotransmitter levels are thought to (1) enhance monoamine neurotransmitter receptor signaling, (2) consequently stimulate second messenger pathways in postsynaptic neurons, and (3) cause downstream changes in gene expression, neurogenesis (i.e., the process of forming new neurons), and neuroplasticity (i.e., the ability of neurons to grow, adapt, and strengthen connections) (Malhi and Mann, 2018; Otte et al., 2016).

1.2. The search for biomarkers of antidepressant drug response

Although sociodemographic (e.g., education level) and clinical (e.g., baseline severity) associations are known (Jain et al., 2013; Rost et al., 2022), biological associations are also observed, including those with genomic (Singh et al., 2023), epigenomic (Engelmann et al., 2022; Webb et al., 2020; Zhou et al., 2021), transcriptomic (Belzeaux et al., 2018), proteomic (Gadad et al., 2018), and metabolomic factors (Duan and Xie, 2020).

These different “omics” approaches aim to capture variability at different biological levels with varied characteristics, ranging from fixed genetic variation at the level of DNA, the unchanging yet regulable genomic blueprint, to the concentrations of metabolites—constantly fluctuating materials closely associated with clinical phenotypes (Neavin et al., 2016). Pharmacogenetics (PGx) and pharmacometabolomics (PMx) seek to investigate how drug response is influenced by genetic variation and metabolite levels, respectively.

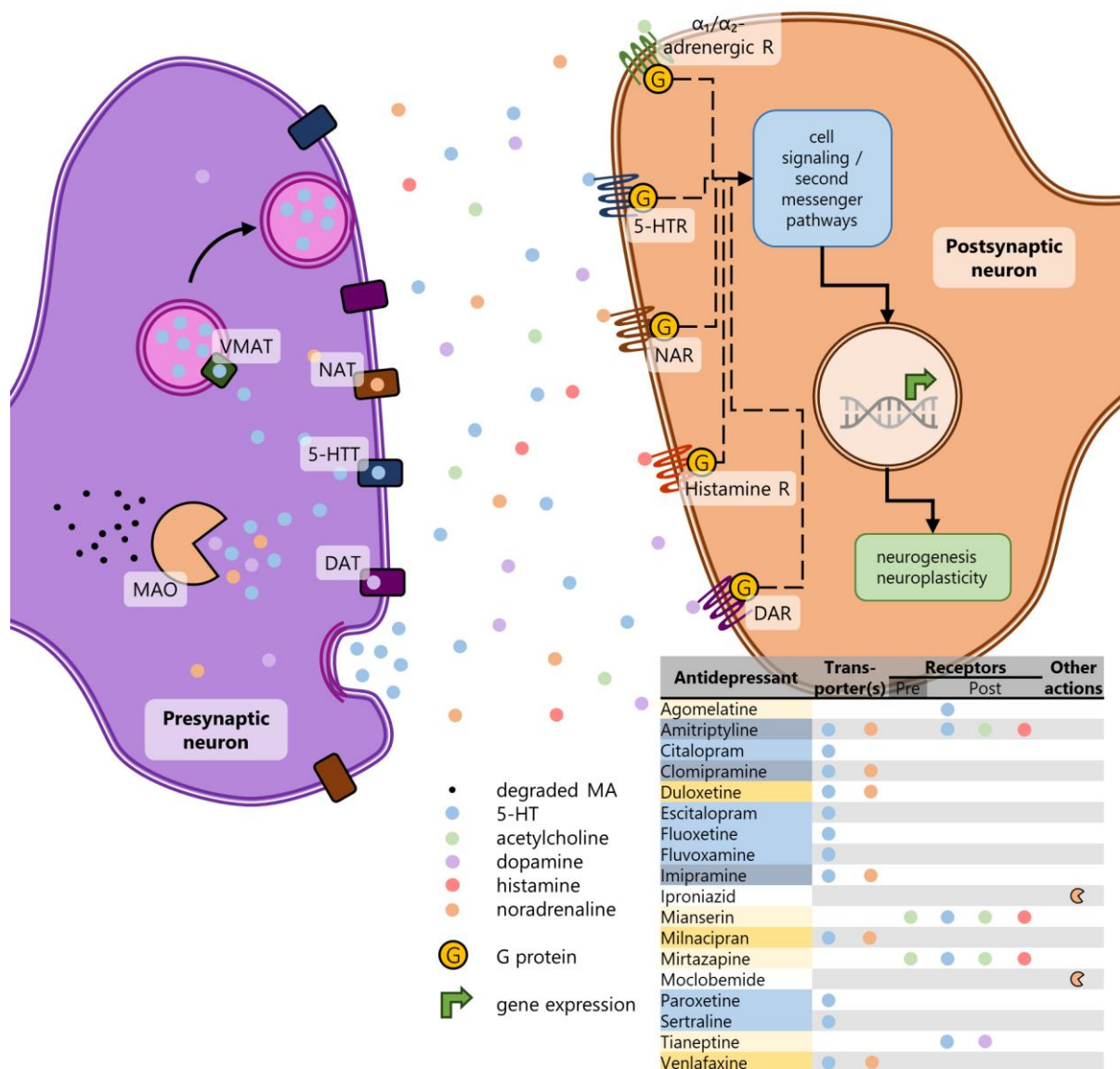


Figure 1: Antidepressant action at the synapse in the pharmacological treatment of depression

All available antidepressant drugs act on elements of monoamine neurotransmission on presynaptic (purple) and postsynaptic (orange) neurons (e.g., by altering neurotransmitter concentrations within the synapse). These changes lead to increased neurotransmission, including G protein-mediated signaling, consequent cell signaling and second messenger pathways, altered gene expression, and, ultimately, neural remodeling and neurogenesis. The table shows the specific receptor/transporter interactions of various antidepressant drugs, including SSRIs (light blue), SNRIs (yellow), TCAs (blue gray), other antidepressants (light yellow), and MAOIs (white). For example, agomelatine interacts with postsynaptic serotonergic receptors while duloxetine interacts with 5-HTT and NAT.

5-HT: serotonin; **5-HTR**: serotonin receptor; **5-HTT**: serotonin transporter; **DAR**: dopamine receptor; **DAT**: dopamine transporter; **NAR**: noradrenaline receptor; **NAT**: noradrenaline transporter; **R**: receptor; **MA**: monoamine; **MAO**: monoamine oxidase; **MAOI**: monoamine oxidase inhibitor; **TCAs**: tricyclic antidepressants; **SSRIs**: selective serotonin reuptake inhibitors; **SNRIs**: serotonin-noradrenaline reuptake inhibitors; **VMAT**: vesicular monoamine transporter

1.2.1. Pharmacometabolomics (PMx)

PMx is the study of metabolic profiles to better understand drug effects and their predictive potential for drug response. PMx can capture real-time treatment-related changes and provide insight into drug PK and PD, as well as interindividual and intergroup treatment response (Beger et al., 2020). Importantly, PMx may help to inform pharmacological interventions by, for example, classifying and stratifying responders and non-responders (Kaddurah-Daouk et al., 2014). Targeted and untargeted (i.e., global) approaches exist. Targeted approaches typically employ absolute quantification [i.e., metabolite concentrations (e.g., µg/mL)] while untargeted approaches are generally limited to relative quantification (i.e., peak intensities), which can differ between laboratories, batches, instrument parameterizations, and data processing methods (Castelli et al., 2022).

Several methods for biological metabolite measurement exist [see **Table 4** (page 11)] (Gamache et al., 2004; Lu et al., 2017). Comparatively, mass spectrometry (MS) benefits from improved accessibility and convenience thanks to commercially available kits. For example, the BIOCRATES AbsoluteIDQ® p180 kit is an MS-based targeted metabolomic assay for the identification and absolute quantification of 180 endogenous metabolites, including 5-HT (Laiakis et al., 2013). These kits have been used to discover metabolic signatures in disease, but also provide a means of standardization (Saigusa et al., 2021).

Many metabolic pathways are associated with the response to ATD treatment in depression, including lipid metabolism, as observed by our team through associations with changes in acetyl- and acyl-carnitines (Ait Tayeb et al., 2023, 2021) [see **Table 5** (page 13)]. Since monoamine neurotransmission is implicated in depression (Malhi and Mann, 2018; Otte et al., 2016), and since ATDs—which almost all target 5-HT levels—are relatively effective treatments (Jauhar et al., 2023), 5-HT remains the main metabolic target in the study of MDD and the response to its treatment with ATDs [see **Table 5** (page 13)].

Similar to other case-control studies of 5-HT levels (Holck et al., 2019; Sun et al., 2020), our team observed lower 5-HT levels in depressed patients compared to healthy controls (Colle et al., 2020). In depressed patients, higher levels of 5-HT at baseline and during ATD treatment have been associated with improved clinical outcomes (Bhattacharyya et al., 2019; Holck et al., 2019; Sun et al., 2020). Among 31 plasma metabolites, 5-HT levels at baseline and during treatment were also the most significantly associated with clinical response measures in the Pharmacogenomics Research Network-Antidepressant Medication Pharmacogenomics Study (PGRN-AMPS) cohort, with higher baseline levels and greater decreases during ATD treatment associating with improved outcomes (Gupta et al., 2016).

Table 4: Pharmacometabolomic approaches

Method	Abbreviation	Strengths	Limitations
Gas chromatography mass spectrometry	GC-MS	<ul style="list-style-type: none">• Can identify and quantify metabolites with highly variable concentrations in a sample• High-throughput (hundreds of samples per day)	<ul style="list-style-type: none">• Obtaining structural information is not straightforward or effective• Cannot be used for metabolites that cannot be volatilized (i.e., converted from liquid to gas)• Low mass accuracy (unless at the expense of cost or throughput)
Liquid chromatography electrochemical array	LCECA	<ul style="list-style-type: none">• Powerful tool for the analysis of redox metabolites (e.g., serotonin pathway metabolites)• High sensitivity (femtomolar range)• High precision• Low cost	<ul style="list-style-type: none">• Applicable only to electrochemically active metabolites• Low-throughput (~dozen samples per day)• Structural information is lacking
Liquid chromatography mass spectrometry	LC-MS	<ul style="list-style-type: none">• Can identify and quantify metabolites with highly variable concentrations in a sample• Highly flexible (applicable to most metabolites, adjustable accuracy and throughput)• High-throughput (hundreds of samples per day)• Exact metabolite composition possible	<ul style="list-style-type: none">• Obtaining structural information is not straightforward or effective• Variable precision
Nuclear magnetic resonance	NMR	<ul style="list-style-type: none">• Powerful tool for structural investigations• High-throughput (hundreds of samples per day)	<ul style="list-style-type: none">• Poor sensitivity compared to other methods• High cost

This latter PMx study also investigated how genetic variation may be associated with ATD response-associated plasma 5-HT levels (Gupta et al., 2016). In this PMx-informed genetic analysis, genetic variation within *TSPAN5* and *ERICH3* was associated with plasma 5-HT levels. Further functional analyses demonstrated that *TSPAN5* influenced the levels of serotonin pathway enzymes, while *ERICH3* more directly influenced 5-HT concentrations. One genetic variant of *ERICH3* in particular, rs11580409(A>C), was associated with (1) decreases in *ERICH3* levels, (2) variation in plasma 5-HT levels at baseline and during ATD treatment, and (3) clinical response in several depressed cohorts, including the STAR*D (Gupta et al., 2016; Liu et al., 2020).

These PMx-informed genetic analyses highlight the potential utility of combining different “omics” strategies to help elucidate or clarify the mechanisms underlying ATD treatment (Neavin et al., 2016). They equally underline the importance of the association of genetic variation with the response to ATD treatment.

1.2.2. Pharmacogenetics (PGx)

PGx is the study of how genetic variation influences drug response (Porcelli et al., 2011). Overall, PGx seeks to leverage genomic technology to identify genetic factors that can aid in drug development, selection, and optimization to maximize drug efficacy while minimizing toxicity (Pirmohamed, 2023).

Germline genetic variation is of particular importance to PGx (Ku et al., 2010) and includes both small- and large-scale variation. However, single nucleotide substitutions are the most frequently reported type of genetic variation with pharmacological consequences (Tafazoli et al., 2020). They are further classified by their population-level minor allele frequency (MAF), with single nucleotide variation (SNV) present in <1% and single nucleotide polymorphisms (SNP) present in $\geq 1\%$.

Genetic variation occurs between and within genes. Within-gene variation is further categorized into whether it occurs within protein-coding regions (i.e., exons), between them (i.e., introns), or in 3'- and 5'- untranslated regions (UTR). Depending on its effect on the encoded protein's amino acid structure, exonic single nucleotide substitutions can be synonymous (no alteration), missense (a substitution to a different amino acid), or nonsense (an alteration to an amino acid chain-terminating “stop” codon) [see **Figure 2** (page 15)].

Table 5: Pharmacometabolomic associations with clinical outcomes following antidepressant treatment

Reference	Sample source	Assay method	N	Treatment(s)	F/u (weeks)	Outcome(s)	Pathway(s)	Metabolite(s)	Association(s)
Ait Tayeb et al., 2021	Plasma	LC-MS (p180 Kit)	460	naturalistic fluoxetine, paroxetine, sertraline, citalopram, escitalopram, fluvoxamine, venlafaxine, milnacipran, duloxetine, clomipramine, amitriptyline, imipramine, mianserin, mirtazapine, agomelatine, tianeptine, iproniazid, moclobemide, ECT, rTMS	26	HDRS score change, response ¹ , remission ²	Lipid metabolism	acetyl-L-carnitines	↑ L-carnitine/acetyl-L-carnitine ratio in responders/remitters
Ait Tayeb et al., 2023	Plasma	LC-MS (p180 Kit)	460	citalopram, escitalopram	26	HDRS score change, response ¹ , remission ²	Lipid metabolism	acylcarnitines	↓ levels of medium- and long-chain acylcarnitines ∞ ↑ HDRS score ↘ levels ∞ greater score change greater ↘ in responders
Bhattacharya et al., 2019	Plasma	LCECA	290	citalopram, escitalopram	8	HDRS score change, response ¹	Tryptophan metabolism Serotonin pathway Norepinephrine metabolism	5-HT 5-HIAA 3-methoxy-4-hydroxyphenylglycol	↗ levels ∞ greater score change greater ↘ in responders
Caspani et al., 2021	Plasma	NMR	211	escitalopram	8	MADRS score change	Lipoprotein metabolism	apolipoprotein, HDL	↓ baseline levels in male responders
Czys et al., 2019	Plasma	LC-MS (p180 Kit)	159	escitalopram, bupropion + escitalopram, venlafaxine + mirtazapine	12	QIDS-SR16 score change HDRS score change, response ¹	Membrane structure Tryptophan metabolism	phosphatidylcholine diacyl C38:1	↑ baseline levels ∞ smaller score changes
Erabi et al., 2020	Plasma	LC-MS	62	escitalopram	6	% change QIDS-C score, response ³	Kynurenine pathway Tryptophan metabolism	kynurenic acid, kynurenine	↓ levels ∞ greater score changes ↓ baseline levels in responders
Gupta et al., 2016	Plasma	LCECA	306	citalopram, escitalopram	8	QIDS-C score, response ³	Serotonin pathway	5-HT	greater ↘ ∞ greater % change greater ↘ in responders/remitters

							remission ⁴			
Holck et al., 2019	Plasma	LC-MS	26	sertraline	8	response ¹	Serotonin pathway	5-HT	greater ∨ in responders	
Ji et al., 2011	Plasma	GC-MS	40	escitalopram	8	response ³	Nitrogen metabolism	glycine dihydroxyphenylacetic acid, 4-hydroxyphenyllactic acid, 5-HT, gamma tocopherol	↓ baseline levels in responders	
Kaddurah-Daouk et al., 2011	Serum	LCECA	43	sertraline	4	response ¹	Tryptophan metabolism Serotonin pathway	known chemicals with high VIP values to discriminate responders and non-responders		
Kaddurah-Daouk et al., 2013	Serum	GC-MS	43	sertraline	4	HDRS score change	Energy metabolism Signaling Protein synthesis	branched-chain amino acids	∨ levels of valine, leucine, and isoleucine ∞ greater score changes ↗ levels of C5-M-DC (acylcarnitine), histidine, proline, kynurenine, trans-5	
MahmoudianDehkordi et al., 2021	Plasma	LC-MS (p180 Kit)	136	citalopram, escitalopram	8	% change HDRS score	Lipid metabolism Tryptophan metabolism	acylcarnitines, amines, phosphatidylcholines	hydroxyproline (amines), and several phosphatidylcholines ∞ greater % change	
Sun et al., 2020	Plasma	LC-MS	161	desvenlafaxine, escitalopram	8	% change in HDRS score, response ¹ , remission ²	Tryptophan metabolism Serotonin pathway	5-HT	↑ baseline levels ∞ greater % change ↑ baseline levels in escitalopram responders	
						% change HDRS score, response ¹	Tryptophan metabolism Serotonin pathway	5-methoxytryptamine melatonin	↑ baseline levels ∞ greater score changes greater ∨ in responders greater ↗ in responders	
Zhu et al., 2013	Serum	LCECA	75	sertraline	4	HDRS score, response ¹	Methoxyindole pathway	5-HT	greater ∨ in responders	

5-HIAA: 5-hydroxyindoleacetic acid; **5-HT:** serotonin; **F/u:** follow-up; **GC:** gas chromatography; **HDRS:** Hamilton Depression Rating Scale; **LC:** liquid chromatography; **LCECA:** liquid chromatography electrochemical array; **MADRS:** Montgomery and Åsberg Depression Rating Scale; **MS:** mass spectrometry; **N:** sample size; **NMR:** nuclear magnetic resonance; **p180 Kit:** BIOCRATES AbsoluteIDQ® p180 kit; **QIDS-C16:** Quick Inventory of Depressive Symptomatology, clinician-rated; **QIDS-SR16:** Quick Inventory of Depressive Symptomatology, self-reported

¹ HDRS reduction of ≥50%; ² HDRS ≤7; ³ QIDS-C16 reduction of ≥50%; ⁴ QIDS-C16 ≤5; ↑ higher; ↓ lower; ∨ decrease(d); ↗ increase(d); ∞ associated with

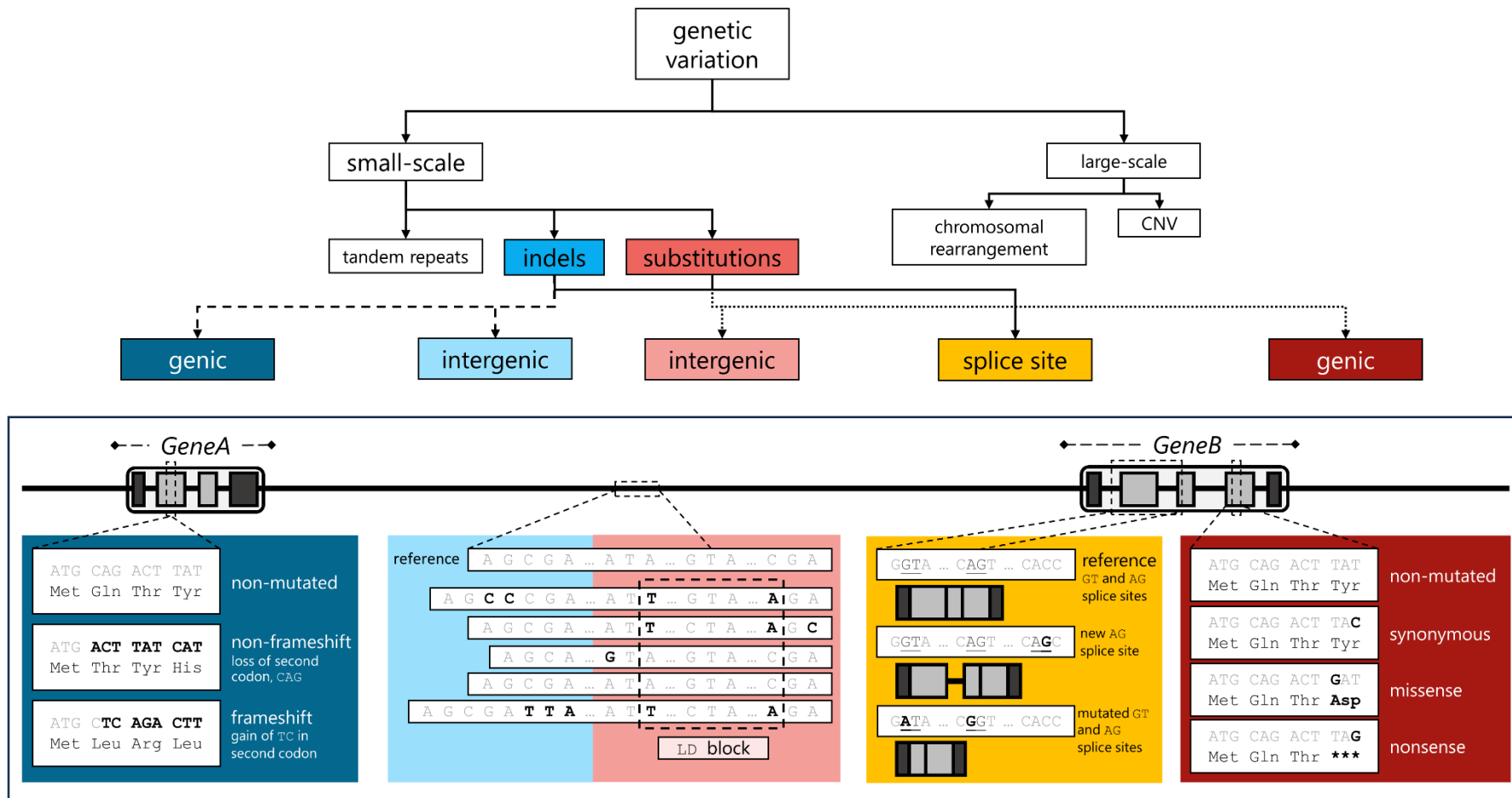


Figure 2: Genetic variation

Genetic variation can be both small- and large-scale. Single nucleotide substitutions and indels (black and bold **A, T, C, G**) can occur between genes, within them (gray boxes), or at splice sites. Intergenic and genic mutations resemble one another, but genic mutations within exons or UTRs (nested dark gray blocks) can have varied effects on the encoded amino acid. Splice site mutations can lead to exon exclusion or intron inclusion in the encoded amino acid.

CNV: copy number variation; **indel**: insertion/deletion; **LD**: linkage disequilibrium; **UTR**: untranslated region

Missense and nonsense mutations often have more straightforward effects on protein structure, protein levels, or downstream processes, and even synonymous variation is thought to disrupt splicing, transcription, protein folding, and mRNA stability (Zeng and Bromberg, 2019). Non-coding variation (i.e., intronic and intragenic)—which encompasses the vast majority of disease-associated genetic variation—has largely unknown consequences (Lin et al., 2019). This uncertainty is further confounded by (1) linkage disequilibrium (LD), the degree to which an allele of one genetic variant is inherited or correlated with an allele of another genetic variant (Myers et al., 2020) [see **Figure 2** (page 15)] and (2) specific disease-associated contexts (e.g., gene, cell type, and tissue) (Kundaje et al., 2015).

1.2.2.1. Pharmacogenetic approaches

Hypothesis-driven (i.e., leveraging prior knowledge to guide targeted genetic investigations) and hypothesis-generating (i.e., leveraging large datasets to find associations without any prior knowledge) approaches are used in PGx (Hulsen et al., 2019). Candidate gene (and pathway) studies and genome-wide association studies (GWAS) are commonly used hypothesis-driven and hypothesis-generating approaches, respectively; high-throughput sequencing (HTS) is applicable in each case. Each relies on different methodologies, and each has its strengths and limitations [see **Table 6** (page 17)].

Since the pathophysiology of MDD and the action of ATDs remain largely unknown, hypothesis-generating approaches offer a powerful means of genetic investigation. Indeed, hundreds of significantly associated genetic polymorphisms in hundreds of genes have been identified using GWAS (Singh et al., 2023). For example, a genome-wide significant polymorphism in *RNF219-AS1* was identified in a large population of self-reported 23andMe questionnaire responders [see **Table 7** (page 19)] (Li et al., 2020, 2016). Conversely, in the largest GWAS of clinical outcomes following ATD treatment in clinically diagnosed individuals to date, no genome-wide significant loci were identified (Pain et al., 2021). Although a powerful tool for discovery, GWAS is limited notably in the analysis of complex, heterogeneous diseases, like MDD.

The candidate approach, which leverages prior knowledge and often investigates PK- and PD-related genes (Alghamdi and Padmanabhan, 2014; Jorgensen et al., 2009), has also identified significant genetic associations with clinical outcomes following ATD treatment within several candidate genes (Kato and Serretti, 2010; Porcelli et al., 2011; Singh et al., 2023; Uher et al., 2009a). For example, associations have been identified with genetic

Table 6: Pharmacogenetic approaches

Approach	Abbrv	Methodology	Strengths	Limitations	References
Candidate gene (and pathway)	n.a.	Leverages prior knowledge to guide genetic investigation	Aims to target highly relevant genes and genetic variation, generally related to drug PK and PD	<ul style="list-style-type: none"> • More time-consuming in comparison to other approaches • Appropriate gene selection can be limited for complex, polygenic diseases that may remain poorly understood • Generates large volumes of data requiring dedicated workflows and staff 	Alghamdi and Padmanabhan, 2014; Jorgensen et al., 2009
Genome-wide association study	GWAS	Millions of preselected, common genetic markers across the genomes of many individuals are genotyped using microarray technology	<ul style="list-style-type: none"> • Allows for the simultaneous analysis of millions of genetic variants at once without any prior knowledge • Well-characterized methodology • Affordable • GWAS data applicable to other genetic analyses like PRS 	<ul style="list-style-type: none"> • Limited in the analysis of complex, polygenic diseases • Burdened by multiple testing correction and extreme statistical significance thresholds ($P < 0.00000005$) • Increased sample sizes to boost statistical power may introduce more heterogeneity and analytical confounding 	Moore, 2017; Tam et al., 2019; Uffelmann et al., 2021
High-throughput sequencing	HTS	Using the Illumina system, DNA is fragmented, amplified, and sequenced on flow cells (fluorescent-labeled nucleotides for each of the four nucleotides are added and recorded), then sequenced DNA is aligned to a reference genome and software is used to identify genetic variants	<ul style="list-style-type: none"> • Allows for the detection of <i>de novo</i> and rare genetic variation • Applicable to candidate, genome-wide, and exome-wide approaches • Continued improvements in accuracy, scalability, and flexibility • Prone to sequencing and analytical biases 	<ul style="list-style-type: none"> • Generates large volumes of data requiring dedicated workflows and staff • More expensive in comparison to other approaches • Less accurate in comparison to GWAS, especially in low-complexity and duplicated/deleted regions 	DePristo et al., 2011; Hong et al., 2013; Li et al., 2019; Slatko et al., 2018

n.a.: not applicable; **PD:** pharmacodynamic; **PK:** pharmacokinetic; **PRS:** polygenic risk score

variation in *MAOA* [encoding monoamine oxidase (MAO), which metabolizes monoamine neurotransmitters (Uzbekov, 2021)], *BDNF* [encoding brain derived neurotrophic factor (BDNF), which plays a role in regulating neurogenesis and neuroplasticity (Duman and Li, 2012; Holsboer, 2000)], and *ARRB1* [encoding β -arrestin 1, which has defined roles in receptor desensitization of many G protein-coupled receptor (GPCR) families, including monoamine neurotransmitter receptors (Kovacs et al., 2009; Lefkowitz and Shenoy, 2005)] [see **Table 7** (page 19)] .

Lastly, high-throughput sequencing (HTS) remains a particularly versatile approach to the analysis of genetic variation since it can examine genetic variation across the whole genome (or exome) or in target candidate regions. Indeed, genetic variants associated with the response to ATD treatment have been identified in both “ome”-wide and targeted HTS analyses [see **Table 7** (page 19)] (Levchenko et al., 2020; Park et al., 2021; Xu et al., 2020). HTS is also a powerful approach thanks to its ability to identify *de novo* and rare genetic variants. Importantly, although common genetic variation is estimated to account for 20%–42% of individual variation in ATD response (Pain et al., 2021; Tansey et al., 2013), rare genetic variation may account for some of the missing heritability (Pirmohamed, 2023). For example, up to 40% of functional variability in PK- and PD-related targets is suggested to be attributed to rare, non-synonymous genetic variation (Kozyra et al., 2017).

1.2.2.1. Novel approaches and in silico tools for pharmacogenetic analyses

1.2.2.1.1. New methods and analytical strategies

Various methods and analytical strategies for genetic analysis have evolved alongside approaches like GWAS and HTS. With the increased identification of rare genetic variation through HTS, these include methods for the generally underpowered analysis of rare genetic variation. These methods generally aggregate genetic variation into biological contexts (e.g., by gene) [see **Figure 3** (page 22)]. However, different methods make different assumptions, especially with respect to the presence, direction, and magnitude of rare genetic variation’s effect. For example, burden tests assume that all genetic variation (1) has an effect and (2) acts in the same direction (e.g., deleteriously). Conversely, the Sequence Kernel Association Test (SKAT) allows for mixing causal and non-causal variants, and variants with effects in opposing directions (Wu et al., 2011). However, these assumptions are not generally known, and so SKAT-O was developed to optimize test selection based on the power of each test (Lee et al., 2012b, 2012a). SKAT-O was also recently extended for use with repeated measures in longitudinal designs (Chen et al., 2019), making it particularly useful for the analysis of clinical outcomes following ATD treatment [see **Figure 3** (page 22)].

Table 7: Genetic associations with clinical outcomes following antidepressant treatment

Gene	Variant (RS)	Ref	Alt	Approach	Cohort(s)	N	Population	Treatment(s)	F/u (weeks)	Outcome	Risk group OR (NR)	[95%CI]	P value	Reference
pharmacokinetic factors														
<i>CYP2C8</i>	rs2071426	T	C	HTS (CAND)	-	530	Han Chinese	SSRI, SNRI, other	8	HDRS score	-	-	0.0000032	Xu et al., 2020
<i>CYP2D6</i>	rs1065852	C	T	CAND	-	94	Korean	escitalopram	12	response ¹	T	5.61 [2.32-13.59]	<0.001	Han et al., 2013
pharmacodynamic factors														
<i>monoamine metabolizing enzymes</i>														
<i>COMT</i>	rs6267	G	T	HTS (CAND)	MAKE BETTER	1,000	Korean males	naturalistic	12	early response/remission ⁸	G	2.83 [1.27-6.29]	0.015	Kang et al., 2020
<i>MAOA</i>	rs6323	G	T	CAND	-	116	European	mirtazapine	4	response ¹	G	-	0.02	Tadić et al., 2007a
<i>monoamine transporters</i>														
<i>SLC6A2</i>	rs2242446	C	T	CAND	-	96	Japanese	milnacipran	6	response ³	C	2.10 [1.08-4.11]	0.03	Yoshida et al., 2004
<i>SLC6A4</i>	5HTTLPR	Long	Short	CAND	-	940	Korean	fluoxetine, sertraline	6	response ¹	Long	3.90 [2.04-7.46]	0.000052	Myung et al., 2013
<i>monoamine receptors</i>														
<i>HTR2A</i>	rs6314	C	T	CAND	-	163	Caucasian	paroxetine, citalopram, imipramine, lofepramine, phenelzine	6	response ¹	GG	3.83 [1.25-11.76]	0.02	Wilkie et al., 2009
<i>HTR4</i>	rs1345697	T	G	CAND	META-DAP	492	Mixed	naturalistic	13	HDRS score	GG	-	0.0062	Poinsignon et al., 2022
<i>intracellular signaling</i>														
<i>ARRB1</i>	rs12274033	T	C	CAND	-	270	Korean	mirtazapine	4	remission ²	T	0.33 [0.12-0.88]	0.027	Chang et al., 2015
<i>ARRB2</i>	rs4790694	A	C	CAND	META-DAP	569	Mixed	naturalistic	26	response ¹	A	2.01 [1.15-3.51]	0.014	Petit et al., 2018
<i>GNAI5</i>	rs11671393	G	C	HTS (CAND)	-	530	Han Chinese	SSRI, SNRI, other	8	HDRS score	-	-	0.000000000005	Xu et al., 2020
<i>Other</i>														
<i>ACSS3</i>	rs10492002	C	T	GWAS	STAR*D, 1116, GENDEP	585	Mixed	citalopram, escitalopram, nortriptyline	12	sustained response vs. un-sustained response	T	0.61 [0.50-0.75]	0.0000045	Hunter et al., 2013
<i>AUTS2</i>	rs7785360	G	A	GWAS	-	711	Korean	escitalopram, sertraline, fluoxetine,	6	response ¹	A	0.31 [0.20-0.47]	0.000000016	Myung et al., 2015

paroxetine														
<i>BDNF</i>	rs6265	G	A	CAND	-	298	Han Chinese	paroxetine	6	response ¹	G	2.08 [1.43-3.01]	<0.0001	X.-C. Wang et al., 2014
<i>BMP7</i>	rs6127921	A	C	GWAS	STAR*D	1,491	Mixed	citalopram naturalistic,	14	response ⁴	C	0.57 [0.46-0.72]	0.00000345	Garriock et al., 2010
<i>CDH17</i>	rs6989467	A	G	GWAS	MARS	339	Caucasian	citalopram	5	response ⁶	-	-	0.00000076	Ising et al., 2009
<i>FKBP5</i>	rs1360780	T	C	CAND	STAR*D	1,809	White	citalopram	14	remission ⁷	CC	1.43 [1.07-1.91]	0.015	Lekman et al., 2008
<i>GPRIN3--[]--SNCA</i>	rs1908557	T	C	GWAS	23andMe	4,536	European	bupropion	-	response vs non-response ⁹	C	1.35 [1.21-1.50]	0.000000026	Li et al., 2016
<i>GRIA4</i>	rs11226856	G	A	CAND	STAR*D	1,541	Mixed	citalopram	2	response ⁵	A	1.55 [1.24-1.92]	0.000085	Fabbri et al., 2013
<i>GRIK4</i>	rs1954787	T	C	CAND	STAR*D	1,816	Mixed	citalopram	6	response ⁵	C	-	0.00023	Paddock et al., 2007
<i>IL11</i>	rs1126757	T	C	GWAS	GENDEP	706	Caucasian	escitalopram, nortriptyline	12	% change in MADRS	-	-	0.00049	Uher et al., 2010
<i>KRTAP1-1</i>	rs3213755	G	A	HTS (GWAS)	-	100	Korean	escitalopram	6	remission ²	G	1.86 [1.32-2.61]	0.00041	Park et al., 2021 Levchenko et al., 2020
<i>PIP4K2A</i>	rs61731109	C	T	HTS (CAND)	-	96	European	naturalistic SSRI, SNRI,	4	response ¹	C	-	0.000943	
<i>PPP2C8</i>	rs4733201	G	T	HTS (CAND)	-	530	Han Chinese	other	8	HDRS score	-	-	0.000000011	Xu et al., 2020
<i>PRNP</i>	rs1800014	G	A	HTS (CAND)	MAKE BETTER	1,000	Korean males	naturalistic	12	early response/ remission ⁸	G	3.78 [1.45-9.84]	0.0098	Kang et al., 2020
<i>RNF219-AS1</i>	rs4884091	G	A	GWAS	23andMe	12,537	European	SSRI	-	response vs non-response ⁹	-	1.21 1.52	0.000000024	Li et al., 2020
<i>RORA</i>	rs809736	A	G	GWAS	STAR*D	1,491	Mixed	citalopram paroxetine, fluvoxamine, nortriptyline,	14	response ⁴	-	1.52 [1.27-1.83]	0.00000819	Garriock et al., 2010
<i>SLIT3</i>	rs10516049	A	G	GWAS	-	96	Japanese	milnacipran	8	response ¹	G	10.3 [3.64-29.41]	0.00000043	Sasayama et al., 2013
<i>UST</i>	rs2500535	A	G	GWAS	GENDEP	706	Caucasian	escitalopram, nortriptyline	12	% change in MADRS	-	-	0.0012	Uher et al., 2010

Alt: alternative allele; **CAND:** candidate; **F/u:** follow-up; **GWAS:** genome-wide association study; **HDRS:** Hamilton Depression Rating Scale; **HTS:** high-throughput sequencing; **MADRS:** Montgomery and Asberg Depression Rating Scale; **N:** sample size; **NR:** non-response; **OR:** odds ratio; **QIDS-C16:** Quick Inventory of Depressive Symptomatology, clinician-rated; **QIDS-SR16:** Quick Inventory of Depressive Symptomatology, self-reported; **Ref:** reference allele; **RS:** reference SNP identifier; **SSRI:** selective serotonin reuptake inhibitor; **SNRI:** serotonin-noradrenaline reuptake inhibitor

¹ HDRS reduction of $\geq 50\%$; ² HDRS ≤ 7 ; ³ MADRS reduction $\geq 50\%$; ⁴ QIDS-SR16 reduction $\geq 50\%$; ⁵ QIDS-C16 reduction of $\geq 50\%$; ⁶ HDRS reduction $\geq 25\%$; ⁷ QIDS-C16 ≤ 5 ; ⁸ HDRS reduction $< 20\%$ at 2 weeks and remission² at 12 weeks; ⁹ 23andMe 'Antidepressant Efficacy and Side Effects' questionnaire

Methods aimed at reducing data dimensionality also exist for the analysis of common genetic variation, notably to help ease the statistical burden associated with GWAS. Genome-to-Phenome Sparse Regression (G2PSR), for example, aims to reduce data dimensionality by aggregating and jointly analyzing genetic variants according to defined biological contexts, such as genes [see **Figure 3** (page 22)] (Deprez et al., 2022). Other analyses, including polygenic risk scores (PRS), make use of data like GWAS summary statistics (e.g., effect sizes, standard errors, and *P* values). PRS are calculated as the sum of the number of risk alleles (i.e., 0, 1, or 2) for each selected genetic loci multiplied by their effect sizes (e.g., odds ratio or beta coefficients) (Lewis and Vassos, 2020). However, PRS have thus far demonstrated limited utility in association with the response to ATD treatment (García-González et al., 2017; Lewis and Vassos, 2020; Meerman et al., 2022; Ward et al., 2018). Still, higher PRS were generally associated with worse treatment response, suggesting an association between genomic burden and non-response to ATDs (Meerman et al., 2022).

Several candidate genes with associations to ATD response and/or MDD phenotypes, including *MAOA* (Tadić et al., 2007a) and *GRIA3* [encoding an α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) glutamate receptor subunit (de Sousa et al., 2017)], are located on the X chromosome. The X chromosome poses unique challenges for genetic analysis since (1) females possess two X chromosomes (and thus two gene copies and two alleles), while males possess only one, and (2) some genes may escape from X chromosome inactivation (XCI) (Berletch et al., 2011; Carrel and Willard, 2005), a biological mechanism that compensates for dosage differences in X-linked gene products between males and females by randomly inactivating one X chromosome in females (Johnston et al., 2008). Various strategies to account for X-linked factors exist (Chen et al., 2021; Clayton, 2008; Song et al., 2021; J. Wang et al., 2014), such as coding males like female heterozygotes (i.e., 1) or homozygotes (i.e., 2) to account for escape from XCI and random XCI, respectively (Clayton, 2009). XCI status is often unknown, however, and model misspecification can result in a loss of power. Recently, the inclusion of the sex \times genotype interaction was shown to eliminate model misspecification biases and preserve power under different XCI status assumptions (Chen et al., 2021; Song et al., 2021). Its inclusion in X-linked genetic analyses may thus help to elucidate dosage effects or sex-specific associations.

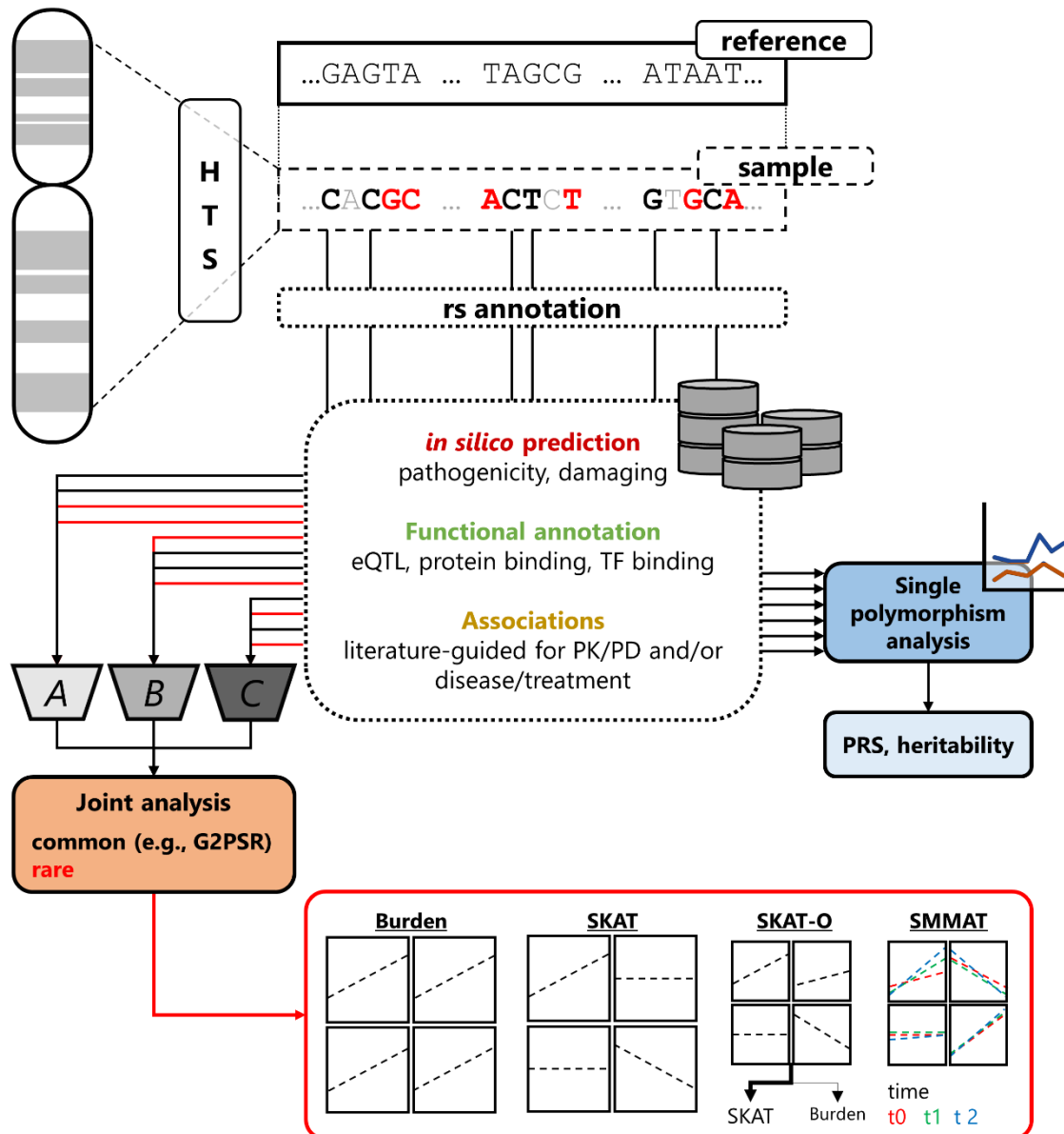


Figure 3: Strategies for the analysis of genetic variation

HTS can be used to identify common (**black**) and rare (**red**) genetic variation. Uniquely labeled genetic variation (e.g., by RS identifier) can be annotated with *in silico* predictions, functional annotations, and literature-based associations using various tools and databases (gray stacks). Genetic variation can be analyzed independently (**blue**), lending itself to other analyses (e.g., PRS), or jointly (**orange**) following aggregation into biological contexts (e.g., genes A, B, and C). Aggregation is also used to study rare genetic variation, though each method makes different assumptions on the presence, direction, and magnitude of the effect (dashed lines) of each rare genetic variant (individual boxes)

eQTL: expression quantitative trait loci; **HTS**: high-throughput sequencing; **G2PSR**: Genome-to-Phenome Sparse Regression; **PD**: pharmacodynamics; **PK**: pharmacokinetics; **PRS**: polygenic risk score; **rs**: reference SNP; **SKAT**: Sequence Kernel Association Test; **TF**: transcription factor

1.2.2.1.1. in silico tools for genetic variant prioritization

Although functional consequences for the majority of genetic variation remain to be elucidated, *in silico* predictive tools and functional databases can annotate genetic variants with informative data (Sefid Dashti and Gamielien, 2017). Many *in silico* tools exist to predict the impact of different types of genetic variation, including missense, synonymous, and intronic variants (Katsonis et al., 2022). By leveraging feature data (e.g., genomic, protein sequence, and evolutionary constraints) and training data (e.g., known polymorphism–Mendelian disease associations), these tools can calculate the probability that a genetic variant is damaging.

Often, a genetic variant identifier [e.g., Reference SNP (rs) identifier] is enough to access the data stored in functional databases, including *in silico* predictions, associations with gene expression, and annotations with transcription factor motif alteration or protein binding disruption [see **Figure 3** (page 22)]. Genetic variants for which different genotypes are associated with the expression of a target gene are known as expression quantitative trait loci (eQTL) (Ward and Kellis, 2016). The expression of both local (i.e., *cis*) and distant genes (i.e., *trans*) can be influenced by eQTL (Westra et al., 2013). Moreover, eQTL sometimes affect gene expression in a tissue-dependent manner (The GTEx Consortium, 2013), which can shift its relevance and interpretation depending on disease and/or treatment (Ramasamy et al., 2014). Importantly, since about 80% of the human genome, including non-coding variation, is suggested to fall within a functional region, functional annotations may help to hypothesize a genetic variant's impact (The ENCODE Project Consortium, 2012).

The exploration and analysis of eQTL have led to the development and curation of multiple eQTL databases, including those examining gene expression in blood (Westra et al., 2013), across tissues (The GTEx Consortium, 2013), and within various brain regions (Ramasamy et al., 2014). Other databases include these eQTL data and annotations of functional consequences [see **Table 8** (page 24)] (Rojano et al., 2019). RegulomeDB, for example, links gene expression and disrupted transcription factor motif and protein binding data to known genetic variants and ranks their likelihood to fall within a functional element (Boyle et al., 2012). Other databases, such as PharmGKB (Whirl-Carrillo et al., 2021) and PharmVar (Gaedigk et al., 2021), are specifically devoted to the curation of both PK- and PD-related genetic variation.

Table 8: Databases for the functional annotation of genetic variants

Database (version)	Annotation	Data sources	Scoring	Advantages	Reference
Annotation					
FORGEdb (v2)	Regulatory regions	ENCODE, Roadmap	2 points - DNase I hotspot (chromatin accessibility)	<ul style="list-style-type: none"> • Implements more and more updated data sources • Implements CADD data for protein-coding variation 	Breeze et al., 2022
	TF binding	Epigenomics, BLUEPRINT	2 points - Histone mark ChIP-seq broadPeak (regulatory states)		
	Target genes	CATO scores	1 point - TF motif		
HaploReg (v4.2)	Protein-level changes	ABC data, GTE _x , eQTLGen	1 point - CATO score (potential TF binding)	Provides variants in LD and detailed data source annotation information compared to RegulomeDB	Ward and Kellis, 2016
	Sequence conservation	CADD scores	2 points - Activity-by-contact (ABC) interaction (gene looping)		
	Regulatory regions	SiPhy, GERP	2 points - Expression quantitative trait locus (eQTL) (gene expression association)		
INFERNO	eQTL	ENCODE, Roadmap	n.a.	Provides context-specific annotation (e.g., gene, tissue, long non-coding RNA)	Amlie-Wolf et al., 2018
	LD	GTE _x , GEUVADIS	n.a.		
	Genomic position	1000 Genomes Project	n.a.		
RegulomeDB (v2.2)	Regulatory regions	UCSC knownGene	1 - Likely to affect binding and linked to expression of a gene target	Early functional annotation database integrating data from many sources and providing an annotation score for prioritization	Boyle et al., 2012
	TF binding	FANTOM5, Roadmap	2 - Likely to affect binding		
	eQTL	Epigenomics, HOMER, GENCODE	3 - Less likely to affect binding		
rVarBase (v2.0)	Pathway	GTE _x	4-6 - Minimal binding evidence	Provides variants in LD and detailed data source annotation information compared to RegulomeDB	Guo et al., 2016
	Risk polymorphisms	KEGG, Gene Ontology	n.a.		
	LD	PredictDB, MetaXcan	n.a.		

		GTEEx, Pan-canQTL 1000 Genomes Project NHGRI GWAS Catalog, GWASdb, GAD, GRASP, DisGeNet UCSC Browser		
	eQTL	ENCODE, FactorBook,		
	LD	HOCOMOCO, Homer		
	Risk	OncoBase		
	polymorphisms	PharmGKB		
	Sequence	Roadmap Epigenomics,	1 point - risk SNP	
	conservation	VISTA Enhancer	1 point - eQTL	
	Motif changes	Browser, Endb, HACER,	1 point - motif change	
	Somatic mutations	FANTOM5, GENCODE,	1 point - conservation	
	Drug-gene	TRInc, Remap, Cistrome,	1 point - enhancer/super enhancer	
	pairings	ChIP-Atlas, GTRD	1 point - promoter	
	Regulatory	KEGG, Reactome, Net-	1 point - TF binding	
	regions	Path, PANTHER, CTD,	1 point - ATAC accessible region	
VARAdb (v1.0)	Pathway	SMPDB	1 point - Hi-C	
Pharmacogenetics				
			1A - high level of evidence (variant-specific clinical guideline/FDA-approved annotation)	
			1B - high level of evidence (without clinical guidelines/FDA-approved annotation)	
			2A - moderate level of evidence (in Tier 1 "known" pharmacogenes)	
			2B - moderate level of evidence (not in Tier 1 pharmacogenes)	
			3 - low level of evidence	
			4 - unsupported	
PharmGKB	Clinical Drug label Variant	Published articles (e.g., pharmacogenomics journal)		Includes PK- and PD- associated genetic variation • Large community that leverages consortium members, expert panels, CPIC and PharmGKB data, and advisory boards to select robust pharmacogenes • Standardization of variant nomenclature
PharmVar (v6.0.2)	Variants Evidence level	Community and published data	0-100 points based on (a) gene curation level; (b) clinical annotation level; (c) pathogenicity of gene; (d) gene variation maintenance by other databases; (e) considerations such as part of pharmacogenetic testing or emergence of new data	Whirl-Carrillo et al., 2021 Gaedigk et al., 2021

Pharmacogenetics

			1A - high level of evidence (variant-specific clinical guideline/FDA-approved annotation)	
			1B - high level of evidence (without clinical guidelines/FDA-approved annotation)	
			2A - moderate level of evidence (in Tier 1 "known" pharmacogenes)	
			2B - moderate level of evidence (not in Tier 1 pharmacogenes)	
			3 - low level of evidence	
			4 - unsupported	
PharmGKB	Clinical Drug label Variant	Published articles (e.g., pharmacogenomics journal)		Includes PK- and PD- associated genetic variation • Large community that leverages consortium members, expert panels, CPIC and PharmGKB data, and advisory boards to select robust pharmacogenes • Standardization of variant nomenclature
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CPIC: Clinical Pharmacogenetics Implementation Consortium; **eQTL:** expression quantitative trait loci; **LD:** linkage disequilibrium; **n.a.:** not applicable; **PD:** pharmacodynamic; **PK:** pharmacokinetic; **SNP:** single nucleotide polymorphism; **TF:** transcription factor

1.2.2.1.1. New strategies for PGx analysis of ATD response

PGx has provided valuable insight into the variability of the response to ATD treatment in MDD. Indeed, early evidence suggests that PGx-guided treatment leveraging genetic variation in several genes (e.g., *CYP2D6*, *CYP2C19*, and *HTR2A*) may improve clinical outcomes (Bousman et al., 2023; Brown et al., 2022; Tafazoli et al., 2021).

Despite these promising findings, and increasingly evolving and accessible data of genetic variation, the clinical application of PGx to ATD treatment response remains elusive. Indeed, hundreds of associations have been observed in just the last couple decades, but robust and replicated associations are rare (Kendall et al., 2021; Pain et al., 2021; Singh et al., 2023). These findings are likely due in part to the fact that the genetic basis of MDD and the response to its treatment with ATDs is not yet fully understood (Corponi et al., 2019).

ATDs designed to target monoamine neurotransmission remain modestly effective despite decades of prescription and use (Otte et al., 2016; Trivedi et al., 2006). New avenues related to monoamine neurotransmission are still being discovered, like *ERICH3*—a previously uncharacterized protein that influences 5-HT levels (Gupta et al., 2016; Liu et al., 2020)—and “biased” pharmacological agents that preferentially activate G protein-dependent or -independent pathways (Bond et al., 2019). The monoamine synapse—including genes encoding proteins involved in its function—thus remains an interesting target for genetic analysis. As our understanding of it improves, precision medicine—including the use of biomarkers—to treat MDD with ATDs is sure to advance as well (Gadad et al., 2018; Hassan et al., 2022).

1.3. Objectives and hypotheses

The overall objective of this thesis project was to use novel strategies to analyze the association of genetic variation within candidate genes with plasma metabolite concentrations and/or clinical outcomes following ATD treatment in 6-month ATD-treated individuals suffering from a MDE in the context of MDD. More specifically, we sought to:

(1) replicate the association of the *ERICH3* rs11580409(A>C) genetic polymorphism, previously identified by the Mayo Clinic (Gupta et al., 2016), with clinical outcomes and plasma 5-HT levels;

(2) examine the association of genetic variation in the X chromosome-linked genes, *MAOA* and *MAOB* (encoding MAO), with clinical outcomes and plasma metabolite levels;

(3) investigate the association between clinical outcomes following ATD treatment and genetic variation within *ARRB1* (encoding β -arrestin 1), including both common genetic variation with potential functional consequences on gene expression and rare genetic variation.

These targets are shown in the context of ATD treatment in **Figure 4** (page 28).

We also sought to analyze HTS-identified genetic variation of a candidate gene panel using the polymorphism-gene constraint method, G2PSR, to identify which candidate genes were most associated with clinical outcomes following ATD treatment.

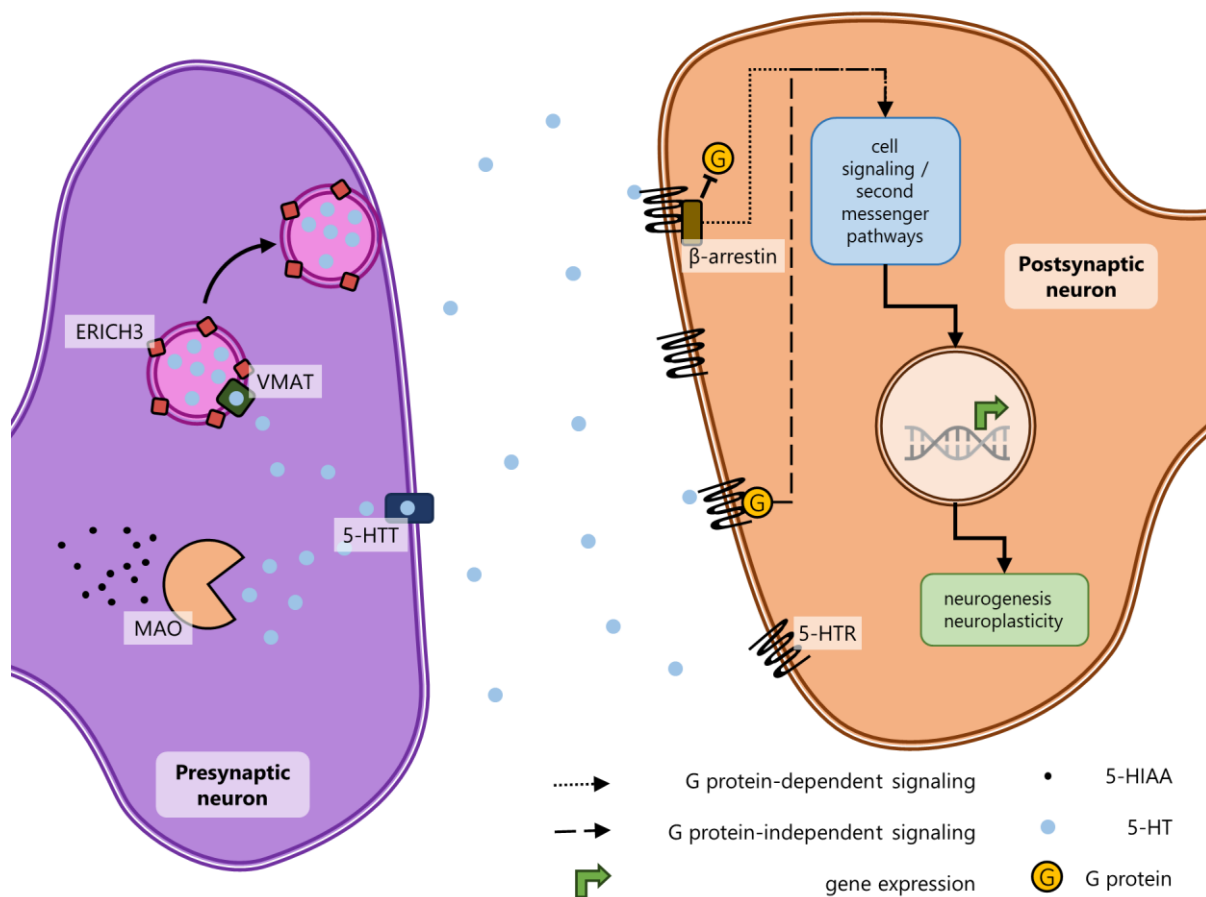


Figure 4: Research targets in the context of antidepressant drug treatment

The first three targets of this thesis project (i.e., ERICH3, MAO, and β -arrestin) are shown in their biological contexts with respect to antidepressant drug treatment for MDD. The levels of 5-HT (blue dot) in the synaptic cleft are influenced by factors in the presynaptic neuron (purple) including MAO [an enzyme that metabolizes 5-HT into 5-HIAA (black dot)] and ERICH3 (a protein hypothesized to be involved in 5-HT trafficking). On the postsynaptic neuron (orange), 5-HT binds to 5-HT receptors (5-HTR) and activates G protein-dependent signaling (dashed arrow), leading to cell signaling and second messenger pathways, downstream gene expression, and consequent neurogenesis and neuroplasticity. β -arrestin can bind to 5-HTRs and block G protein-mediated signaling and/or promote G protein-independent signaling (dotted arrow).

5-HIAA: 5-hydroxyindoleacetic acid; **5-HT:** serotonin; **5-HTT:** serotonin transporter; **MAO:** monoamine oxidase; **MDD:** Major Depressive Disorder; **VMAT:** vesicular monoamine transporter

II. Patients, Materials, and Methods

II.1. The METADAP cohort

II.1.1. Study design

The work presented in this manuscript was carried out in depressed patients of the METADAP cohort (Corruble et al., 2015). METADAP (Do Antidepressants Induce Metabolic Syndromes METADAP Study; ClinicalTrials.gov Identifier: NCT00526383) is a prospective, open-label, multicenter, naturalistic, 6-month follow-up cohort of patients with a current MDE in the context of MDD requiring the introduction of an ATD treatment.

The primary objective of METADAP was to evaluate the development of metabolic syndromes under ATD treatment. However, it was designed in such a way as to be able to pursue broader explorations. The principal investigator of METADAP is Pr. Emmanuelle Corruble. The pharmacology laboratory (under the direction of Pr. Laurent Becquemont and Pr. Céline Verstuyft) oversaw the METADAP biobank and PGx and PMx analyses. The endocrinologist, Pr. Bruno Fève, was the expert for the diagnosis of metabolic syndromes. The psychiatric department and these investigators were involved in the design and management of the study. Four phases of evaluation were carried out with patients over the course of the study: at inclusion and the introduction of the ATD treatment (M0) and after 1 month (M1), 3 months (M3), and 6 months (M6) of ATD treatment. Patients were included from November 2009 to March 2013. The study was registered by the French Agency for the Safety of Medicines and Health Products and the Commission Nationale de l'Informatique et des Libertés (CNIL). It was approved by the ethics committee (personal protection committee) of Paris-Boulogne and complied with international ethical standards. The Assistance Publique – Hôpitaux de Paris (AP-HP) promoted the study. METADAP is funded by two national Clinical Research Programs (PHRC) (AOM06022, AOR 10 071). The patients included were clinically evaluated using both clinician-rated scales and self-report questionnaires. A serum biobank was constructed from routine blood samples collected at each evaluation phase. Biological data were generated from METADAP biobank samples for genetic and metabolomic analyses.

II.1.2. Study population

II.1.2.1. Inclusion criteria

Patients were aged 18 to 65 years of age and were either hospitalized or consulted in the psychiatric departments of the following university hospitals: (1) University Hospital Center (CHU) of Bicêtre; (2) CHU Saint-Antoine; (3) Fernand Widai University Hospital; (4)

Grenoble University Hospital; (5) University Hospital of Lille; (6) University Hospital of Besançon.

Patients presented with a current MDE in the context of MDD according to DSM-IV-TR criteria, as assessed by the standardized Mini International Neuropsychiatric Interview (MINI) (Sheehan et al., 1998), and had a total score of ≥ 18 on the French translation of the 17-item Hamilton Depression Rating Scale (HDRS) (Hamilton, 1960). Patients required the introduction of an ATD treatment approved by the European authorities at a dose recommended by the Autorisation de Mise sur le Marché (AMM). Treatment resistance, a suicide attempt prior to inclusion, and the presence of suicidal ideation were not criteria for exclusion. Patients gave written informed consent for study participation and genetic analysis, including pharmacogenetic analyses related to the response to ATD treatment.

II.1.2.2. Exclusion criteria

Patients presenting with an MDE with psychotic symptoms (DSM-IV-TR), bipolar disorder (DSM-IV-TR), psychotic disorder (DSM-IV-TR), or alcohol or drug dependence (DSM-IV-TR)—except for tobacco—were excluded. Patients treated with a mood stabilizer (e.g., lithium, valproate, lamotrigine, or carbamazepine) or antipsychotics for ≥ 4 months during the year before inclusion were also excluded. Patients with psychiatric symptoms of organic origin, unstable medical pathologies, or who were currently pregnant were also excluded. These features were evaluated by the clinician. Patients involuntarily admitted to the hospital or who were under guardianship could not be included.

II.1.3. Treatments

II.1.3.1. Evaluated treatments

Evaluated treatments were prescribed naturalistically (i.e., in real-world prescription conditions) at the dose recommended by the Autorisation de Mise sur le Marché (AMM). These treatments included the following: (1) SSRIs [fluoxetine (20-60 mg/day), paroxetine (20-50 mg/day), sertraline (50-200 mg/day), citalopram (20-60 mg/day), escitalopram (10-20 mg/day), fluvoxamine (100-300 mg/day)]; (2) SNRIs [venlafaxine (75-375 mg/day), milnacipran (50-100mg/day), duloxetine (60-120mg/day)]; (3) TCAs [clomipramine (50-150mg/day), amitriptyline (50-150mg/day), imipramine (50-150 mg/day)]; (4) Other [mianserin (30-90 mg/day), mirtazapine (15-45 mg/day), agomelatine (25-50 mg/day), tianeptine (25-37.5 mg/day), iproniazid (50-100 mg/day), moclobemide (300-600 mg/day)]; electroconvulsive therapy (ECT); transcranial magnetic stimulation (rTMS). Pharmacological treatment levels were measured, but administration times were not precisely collected since

patients needed to fast before taking the treatment. Still, patients treated with citalopram and escitalopram showed a good level of compliance (unpublished data).

II.1.3.2. Tolerated psychotropic treatments

Benzodiazepines were tolerated and were prescribed at the minimum effective dose for the minimum duration. Cyamemazine and hydroxyzine were tolerated at minimal effective doses and for the shortest possible duration for anxiolytic/hypnotic purposes. Their prescription was not to exceed 14 days.

II.1.4. Clinical evaluation

Two clinician-rated depression rating scales were used to assess depressive symptoms in METADAP patients: the 17-item HDRS and the Clinical Global Impressions (CGI) – Severity (CGI-S) and Improvement (CGI-I) scales. Measures were taken at baseline (M0), M1, M3, and M6. The HDRS is the reference scale for assessing MDE severity and for evaluating the evolution of depressive symptoms and response/remission during and after ATD treatment (Carrozzino et al., 2020). Response and remission were defined according to the recommendations of the American College of Neuropsychopharmacology (ACNP) Task Force (Rush et al., 2006). Response was defined as a $\geq 50\%$ decrease in the HDRS score from baseline (M0). Remission was defined as an HDRS score ≤ 7 after ≥ 4 weeks of ATD treatment.

II.1.5. General description of the cohort

Six hundred twenty-four patients were included in METADAP. The mean age was 45.6 years (SD=13.2) and 433 patients (69.4%) were female. Among these 624 patients, 566 (90.7%) were Caucasian, 40 (6.4%) were African, 8 (1.3%) were Asian, 7 (1.1%) were of mixed ancestry, and 3 (0.5%) were of unknown ethnicity. Information on ethnicity was reported by the patient. The definitions used for ethnic groups are comparable to those of the United States Census Bureau. Caucasian ethnicity was defined as having Caucasian parents, African as having Sub-Saharan African and/or Afro-Caribbean parents, and Asian as having East Asian, Central Asian, and/or South Asian parents (Morales et al., 2018). All patients provided written informed consent for study participation (Corruble et al., 2015).

At baseline, the mean HDRS score was 24.7 (SD=5.0). Among the 624 patients, 243 (38.9%) received an SSRI, 240 (38.5%) an SNRI, 55 (8.8%) a TCA, and 86 (13.8%) another treatment, including MAOIs and ECT. Three hundred eighty-seven patients (62.0%) left the study before the end of follow-up. Among them, 214 (55.3%) were lost to follow-up, 135 (34.9%) stopped or changed treatment, 21 (5.4%) were removed due to the appearance of an exclusion criterion, 15 (3.9%) were removed due to the use of an unauthorized treatment, and

2 (0.5%) died. At M1, 182 patients (39.4%) were responders to treatment and 87 (18.8%) were in remission. At M3, 162 patients (49.6%) were responders and 85 (26.1%) were in remission. At M6, 159 patients (67.1%) were responders and 101 (42.6%) were in remission. No evidence of an effect of recruitment site on response and remission following treatment was observed.

II.2. Biological sample obtention and conservation

All biological data analyzed in this work is derived from blood samples, which allowed for the construction of a biobank of plasma, serum, and DNA samples. Fasting whole blood samples (1 mL) were collected between 8 a.m. and 10 a.m.—before taking any treatment—and stored in EDTA tubes for plasma analysis (Trabado et al. 2017). Lymphocytic DNA was extracted using the Puregene kit (Gentra Systems) and stored at -20°C at the Centre de Ressources Biologiques (CRB) Paris-Saclay for subsequent genetic analyses.

II.3. Plasma assay

All plasma metabolite assays presented within this work are derived from the AP-HP–Institut Servier (Plateforme Technologie Servier, Orléans, France) consortium validated by a convention signed by the Direction de la Recherche Clinique et de l’Innovation (DRCI) of the AP-HP, the study investigators, and Servier.

II.3.1. Serotonin pathway metabolite measurement

Fifty µL of plasma stored at -80°C was used to prepare metabolite extracts via protein precipitation with 250 µL of acetonitrile, evaporation under nitrogen, and reconstitution in 50 µL of water. Reference human plasma samples were prepared 3 times in all analytical batches as a system control within and between analyses. Ultrapformance liquid chromatography coupled with mass spectrometry (UPLC-MS) was used via a Waters Acquity UPLC system coupled to a ThermoScientific Q Exactive mass spectrometer. Values below the lower limit of quantification (LLOQ) were imputed as $\frac{LLOQ}{\sqrt{2}}$ for statistical analyses (Colle et al., 2020).

II.4. Genotyping

II.4.1. Genotyping of the ERICH3 rs11580409 polymorphism

Genotyping of the *ERICH3* rs11580409(A>C) polymorphism was performed by a technician under the supervision and validation of Pr. Céline Verstuyft at the pharmacology laboratory of the Université Paris-Saclay’s Faculté de Médecine. Genotyping was performed blind to clinical evaluations and was performed using TaqMan technology (Thermofisher®, Courtaboeuf, France) according to the manufacturer’s instructions. Allelic discrimination was

performed using the QuantStudio™ 7 Flex Real-Time PCR System (ThermoFisher®, Les Ulis, France). This technique allowed for the identification of the 3 possible genotypes: AA homozygotes, AC heterozygotes, and CC homozygotes. Genotypes were determined using the forward (5'-CTGTTTCCCCTTCTTCCGCT-3') and reverse (5'-GAGGGCAGCCCTGAGGA-3') primers. The A allele was detected using a VIC-fluorescent probe (5'-TCTCTTGCTAAATCTAATTC-3') while the C allele was detected using a FAM-fluorescent probe (5'-CTCTTGCTACATCTAATT-3').

II.4.2. High-throughput sequencing

Genotyping of genetic variants within *ARRB1*, *MAOA* and *MAOB*, and other genes involved in mood disorders and ATD metabolism of the targeted METADAP gene panel [see **Table 9** (page 34)] was performed under the supervision of Pr. Céline Verstuyft and in collaboration with Pr. Jérôme Bouligand using HTS on an Illumina MiSeq benchtop sequencer in the Génétique Moléculaire–Pharmacogénétique–Hormonologie service at Hôpital Bicêtre. Sequencing of exonic and flanking regions (± 50 base pairs) was performed for all genes of the targeted METADAP gene panel except for *ARRB1* and *ARRB2* which were whole-gene sequenced, including exons, introns, and 5'- and 3'-UTRs. Sequencing data were aligned to the hg19 human reference genome using BWA-MEM 0.7.10 (Li, 2013). Variant calling was performed using the GATK (v3.4-46). Variant calls were stored in Variant Call Format (VCF) files (Chappell et al., 2021).

II.4.2.1. Genetic variant selection

Variant Call Format data were loaded into R (v4.1.0) (R Core Team., 2020). Variant calls with a sequencing depth < 20 , a quality score < 275 (for SNPs) or < 770 (for indels), or an allele balance < 0.34 or > 0.79 (for heterozygous calls) or < 0.96 (for homozygous calls) were annotated as poor-quality variant calls. Genetic variants identified through HTS with a call rate $< 95\%$ were removed from analyses of *ARRB1* and *MAOA* and *MAOB*. Genetic variants were classified by their frequency. Frequent genetic variants were defined by a $MAF \geq 5\%$, while rare genetic variants were defined by a $MAF < 5\%$.

II.4.2.2. Linkage analysis

LD was assessed using HaploView (v4.2) (Barrett et al., 2005) or the *ld* function from the *snpStats* package in R (Clayton, 2023) in the subpopulation of Caucasian patients. Variants with a $r^2 \geq 0.8$ were considered to be in LD. The genetic variant with the highest MAF among those in LD was selected as a proxy for further analysis.

Table 9: METADAP gene panel for high-throughput sequencing

Gene symbol	Gene name (Entrez)	Chr	Size (bp)	Max CDS size (bp)	Strand	Coverage (bp)	Function (Entrez)
ionotropic glutamate receptor							
<i>GRIA1</i>	glutamate ionotropic receptor AMPA type subunit 1	5	323,200	2,751	+	5,639	
<i>GRIA2</i>	glutamate ionotropic receptor AMPA type subunit 2	4	145,956	2,652	+	5,509	
<i>GRIA3</i>	glutamate ionotropic receptor AMPA type subunit 3	X	306,636	2,685	+	5,983	
<i>GRIA4</i>	glutamate ionotropic receptor AMPA type subunit 4	11	372,018	2,709	+	5,563	
<i>GRIK1</i>	glutamate ionotropic receptor kainate type subunit 1	21	403,061	2,850	-	5,820	ionotropic glutamate receptor involved in excitatory neurotransmission
<i>GRIK2</i>	glutamate ionotropic receptor kainate type subunit 2	6	676,375	2,727	+	6,176	
<i>GRIK3</i>	glutamate ionotropic receptor kainate type subunit 3	1	238,989	2,760	-	4,920	
<i>GRIK4</i>	glutamate ionotropic receptor kainate type subunit 4	11	477,159	2,871	+	6,480	
<i>GRIK5</i>	glutamate ionotropic receptor kainate type subunit 5	19	71,883	2,946	-	5,872	
<i>GRIN2B</i>	glutamate ionotropic receptor NMDA type subunit 2B	12	444,266	4,455	-	5,880	
monoamine transmembrane transport							
<i>SLC22A1</i>	solute carrier family 22 member 1	6	36,904	1,665	+	3,180	transport of endogenous organic cations and drugs/toxins
<i>SLC22A2</i>	solute carrier family 22 member 2	6	42,067	1,668	-	3,180	
<i>SLC22A3</i>	solute carrier family 22 member 3	6	104,200	1,674	+	3,329	
<i>SLC29A4</i>	solute carrier family 29 member 4	7	23,970	1,593	+	2,880	transport of monoamines into presynaptic neurons
<i>SLC6A2</i>	solute carrier family 6 member 2	16	50,205	1,887	+	4,680	transports norepinephrine into presynaptic neurons
<i>SLC6A3</i>	solute carrier family 6 member 3	5	52,647	1,863	-	4,020	transports dopamine into presynaptic neurons
<i>SLC6A4</i>	solute carrier family 6 member 4	17	41,379	1,893	-	3,840	transports serotonin into presynaptic neurons
circadian rhythm							
<i>ARNTL/BMAL1</i>	bHLH ARNT like 1	11	110,615	1,881	+	6,787	bHLH protein that forms a heterodimer with CLOCK and activates transcription of circadian rhythm-related genes
<i>CLOCK</i>	clock circadian regulator	4	119,007	2,541	-	6,174	bHLH protein that forms a heterodimer with BMAL1 and activates transcription of circadian rhythm-related genes
<i>CRY1</i>	cryptochrome circadian regulator 1	12	102,186	1,761	-	3,969	component of the circadian core oscillator complex that regulates the

<i>CRY2</i>	cryptochrome circadian regulator 2	11	36,127	1,845	+	4,730	circadian clock
<i>NPAS2</i>	neuronal PAS domain protein 2	2	176,691	2,670	+	6,300	bHLH protein that may function as a part of a molecular clock
<i>PER2</i>	period circadian regulator 2	2	44,567	3,768	-	7,819	component of circadian rhythms for locomotor activity, metabolism, and behavior
cytokine activity							
<i>CXCL8</i>	C-X-C motif chemokine ligand 8	4	3,148	300	+	1,233	
<i>IL18</i>	interleukin 18	6	20,835	582	-	1,200	important mediator of the inflammatory response
<i>IL1B</i>	interleukin 1 beta	2	7,029	810	-	1,740	
<i>IL6</i>	interleukin 6	7	4,799	759	+	1,440	
<i>TNF</i>	tumor necrosis factor	6	2,772	702	+	1,854	proinflammatory cytokine involved in cell proliferation, differentiation, and apoptosis
regulation of metabolic processes							
<i>ADIPOR2</i>	adiponectin receptor 2	12	97,605	1,161	+	1,980	receptor for globular and full-length adiponectin
<i>AKT1</i>	AKT serine/threonine kinase 1	14	26,400	1,443	-	4,080	key component of many signaling pathways involving the binding of membrane-bound ligands including receptor tyrosine kinases and GPCRs
<i>INS</i>	insulin	11	1,431	333	-	1,320	peptide hormone that regulates carbohydrate and lipid metabolism
<i>LEP</i>	leptin	7	16,352	504	+	780	protein that plays role in the regulation of energy homeostasis
<i>LEPR</i>	leptin receptor	1	220,908	3,498	+	6,853	receptor for leptin involved in regulation of fat metabolism
synapse assembly regulation							
<i>BDNF</i>	brain derived neurotrophic factor	11	67,138	990	-	1,920	protein that promotes neuronal survival in the adult brain
<i>NGFR</i>	nerve growth factor receptor	17	19,716	1,284	+	2,040	receptor for factors including BDNF with roles in neuronal survival
<i>NLGN3</i>	neuroligin 3	X	26,361	2,547	+	3,709	may act as ligands for neuroligins and be involved in the formation and remodeling of central nervous system synapses

<i>NRXN1</i>	neurexin 1	2	1,113,630	4,644	-	11,205	cell-surface receptor that binds neuroligins to form synaptic complexes in the central nervous system that are required for efficient neurotransmission
<i>NTRK2</i>	neurotrophic receptor tyrosine kinase 2	9	358,533	2,517	+	5,468	receptor for neurotrophins including BDNF
neurotransmitter receptor activity							
<i>DRD2</i>	dopamine receptor D2	11	65,794	1,338	-	2,400	encodes the D2 subtype of the GPCR receptor for dopamine
<i>HTR1A</i>	5-hydroxytryptamine receptor 1A	5	4,572	1,269	-	1,380	
<i>HTR4</i>	5-hydroxytryptamine receptor 4	5	203,496	1,287	-	4,074	encodes a GPCR for serotonin
<i>HTR5A</i>	5-hydroxytryptamine receptor 5A	7	17,069	1,074	+	1,380	
organic anion transmembrane transport							
<i>SLC22A6</i>	solute carrier family 22 member 6	11	8,371	1,692	-	2,999	integral membrane protein involved in the transport and excretion of organic anions
<i>SLCO1A2</i>	solute carrier organic anion transporter family member 1A2	12	155,035	2,013	-	4,320	
<i>SLCO1B1</i>	solute carrier organic anion transporter family member 1B1	12	108,603	2,076	+	3,720	sodium-independent transporter that mediates cellular uptake of organic anions in the liver
<i>SLCO1B3</i>	solute carrier organic anion transporter family member 1B3	12	106,207	2,247	+	4,800	
xenobiotic transmembrane transport							
<i>ABCC1</i>	ATP binding cassette subfamily C member 1	16	193,438	4,626	+	8,820	
<i>ABCC2</i>	ATP binding cassette subfamily C member 2	10	69,955	4,368	+	8,997	transports various molecules across extra-and intra-cellular membranes including xenobiotics across the blood-brain barrier
<i>ABCG2</i>	ATP binding cassette subfamily G member 2	4	141,363	1,968	-	4,620	
arrestin family activity							
<i>ARRB1</i>	arrestin beta 1	11	91,540	1,257	-	81,947	participates in agonist-mediated desensitization of GPCRs
<i>ARRB2</i>	arrestin beta 2	17	10,866	1,293	+	10,226	
gap junction assembly							
<i>GJA1</i>	gap junction protein alpha 1	6	14,082	1,149	+	1,260	component of gap junctions that allow for the diffusion of material between

<i>GJB6</i>	gap junction protein beta 6	13	10,358	786	-	900	cells
kynurenine metabolism							
<i>IDO1</i>	indoleamine 2,3-dioxygenase 1	8	14,900	1,212	+	2,625	catalyzes the first and rate-limiting step in tryptophan catabolism to N-formyl-kynurenine
<i>IDO2</i>	indoleamine 2,3-dioxygenase 2	8	81,742	1,263	+	3,900	
neurotransmitter metabolism							
<i>MAOA</i>	monoamine oxidase A	X	91,811	1,584	+	3,840	catalyzes the oxidative deamination of amines such as dopamine, norepinephrine, and serotonin
<i>MAOB</i>	monoamine oxidase B	X	115,840	1,563	-	3,780	
xenobiotic metabolism							
<i>CYP2C19</i>	cytochrome P450 family 2 subfamily C member 19	10	92,867	1,473	+	2,700	catalyzes many reactions involved in drug metabolism
<i>CYP2D6</i>	cytochrome P450 family 2 subfamily D member 6	22	4,312	1,494	-	3,000	
arrestin family activity/stress response/synapse assembly regulation/Wnt signaling							
<i>CREB1</i>	cAMP responsive element binding protein 1	2	76,027	1,026	+	2,700	leucine zipper TF that activates gene transcription in response to hormonal stimulation of the cAMP pathway

bp: base pairs; **bHLH**: basic helix-loop-helix; **CDS**: coding sequence; **ER**: endoplasmic reticulum; **GPCR**: G protein-coupled receptor; **HPA**: hypothalamic-pituitary-adrenal; **TF**: transcription factor

II.1. Statistical analyses

The statistical analyses presented in this work were performed with R (v4.1.0) (R Core Team., 2020) using the integrated development environment, RStudio (v1.3.1093 or later) (RStudio Team, 2020). Data were managed using Excel (Microsoft Office 2019, Microsoft Corporation). Univariate analyses were performed for sociodemographic, clinical, and biological data and are presented as the mean (m) and standard deviation (SD) for quantitative variables and as the number of subjects (n) and percentage (%) for qualitative variables. Quantitative data were analyzed according to groups and normality, which was assessed visually and using the Shapiro-Wilk test. In the case of normally distributed data, the Student *t* test (for 2 groups) or the analysis of variance (ANOVA) test (for ≥ 3 groups) were performed. In the case of non-normally distributed data, the nonparametric Wilcoxon test (for 2 groups) or the Kruskal-Wallis test (for ≥ 3 groups) were performed. Qualitative data were analyzed using the chi-squared test or the Fisher Exact Test if the number of subjects in any group was fewer than 20. Multivariate analyses were based on linear regression models when the response (i.e., dependent) variable was quantitative and logistic regression models when the response variable was qualitative. Model covariates were chosen *a priori* according to the literature and/or hypotheses or *a posteriori* if a factor was observed to significantly (i.e., $P < 0.05$) differ between groups.

Mixed-effects models were created to analyze longitudinal (i.e., repeated measures) data. Linear mixed-effects models (for quantitative variables) and generalized logistic mixed-effects models (for qualitative variables) were constructed with the lme4 package (Bates et al., 2015). Mixed-effects models are also robust to missing data (e.g., due to loss to follow-up) and can estimate missing values from available data (Mallinckrodt et al., 2003). Significance of fixed effects was assessed with the Satterthwaite method in linear mixed-effects models and with the Wald chi-square test in generalized linear mixed-effects models (Luke, 2017). A significance threshold of $P < 0.05$ was considered significant by default and in the case of exploratory analyses. Otherwise, a Bonferroni correction for multiple comparisons was applied.

Rare genetic variation was analyzed through a variant set analysis using the SMMAT function of the GMMAT package (v1.3.2) in R, which accounts for repeated measures in longitudinal data and can analyze both continuous and binary variables (Chen et al., 2019). Null models were constructed to include both *a priori* and *a posteriori* covariables as discussed above. Gaussian distributions with an identity link function were used to model quantitative variables, while binomial distributions with a logit link function were used to

model qualitative variables. Genomic relationship matrices were constructed using the `snpgdsGRM` function of the `SNPRelate` package (v1.26.0) (Zheng et al., 2012). Default settings for `SMMAT` were used, except that the “`use.minor.allele`” argument was set to `True`—corresponding to the use of the minor allele as the coding allele rather than the alternative allele—and the “`MAF.range`” was set to 0.00-0.50. The SKAT-O test, corresponding to a linear combination of the burden and SKAT statistics, was used (Lee et al., 2012a). The significance level was adjusted using the Bonferroni correction as needed, according to the number of genes analyzed.

II.2. Whole-panel analysis using Genome-to-Phenome Sparse Regression

In collaboration with the Inria Epione team of Nice (Dr. Marie Deprez, Dr. Marco Lorenzi, and Dr. Susanne Thümmel), Genome-to-Phenome Sparse Regression (G2PSR) was used to analyze the association between all common genetic variation (i.e., $MAF \geq 5\%$) within genes of the targeted METADAP gene panel [see **Table 9** (page 34)] according to their gene context and several clinical variables during ATD treatment. Data at M1, M3, and M6 for (1) the HDRS total score, (2) the percent change in the HDRS total score relative to baseline (M0), (3) the CGI-S score, and (4) the CGI-I score were analyzed. The performance of G2PSR improves with the inclusion of redundant data like those analyzed (personal communication). Missing data were imputed as the median.

For different analyses with G2PSR, genetic variation within *ARRB2* and/or *ARRB1* was altered as follows: (1) genetic variation within *ARRB1* was split into eight groups of 8–9 genetic polymorphisms to better resemble the number of genetic polymorphisms within other panel genes (range: 1–13 polymorphisms); (2) genetic variation was limited to exonic and 5'- and 3'-UTR genetic polymorphisms to better resemble the sequenced regions of other panel genes (i.e., exonic regions) and to reduce the potential bias introduced by the greater number of genetic polymorphisms identified in these genes.

Call rates for each genetic variant were calculated as the number of poor-quality variant calls divided by the total number of variant calls; poor-quality variant calls were annotated by: a sequencing depth < 20 , SNPs with a quality score < 275 , indels with a quality score < 770 , heterozygous variant calls with an allele balance < 0.34 or > 0.79 , and homozygous variant calls with an allele balance < 0.96 . Genetic variants with a call rate $< 89.12\%$ (the lower quintile) were excluded. Genetic variation within *CYP2D6* was removed from analysis due to high sequence homology between *CYP2D6*, *CYP2D7*, and *CYP2D8*, consequent read alignment confounding across these three regions and, likely as a result, lower overall call quality in *CYP2D6* genetic variation (mostly with respect to allele balance).

LD between genetic variants was assessed using the *ld* function from the *snpStats* package in R. For those genetic variants in strong LD (i.e., $r^2 \geq 0.8$), those with the higher MAF were selected for further analysis as proxies.

G2PSR was run on Python 3 in a Jupyter environment (<https://jupyterhub.ijclab.in2p3.fr/>). Three matrices were generated to use G2PSR: (1) a sample \times phenotype matrix, containing data for each of the analyzed phenotypic variables; (2) a genetic variant \times associated gene matrix, containing either a 1 (if the genetic variant was associated with the gene) or a 0 (if the genetic variant was not associated with the gene); (3) a genetic variant \times sample matrix, containing the number of alternative alleles (0, 1, 2). G2PSR was parameterized to 50,000 epochs (i.e., iterations) at 1,000 steps and run 10 times to obtain means and SDs for the loss value, sigma, and α parameters. The early stopping strategy was applied to determine the optimal epoch to identify relevant genes (i.e., $\alpha < 0.05$) (Deprez et al., 2022).

III. Results

III.1. The ERICH3 rs11580409 genetic polymorphism is associated with clinical improvement following 6 months of antidepressant treatment in depressed patients

The *ERICH3* rs11580409(A>C) genetic polymorphism was recently identified in a GWAS of plasma 5-HT levels before and after citalopram treatment in the PGRN-AMPS cohort and shown to be associated with 5-HT levels and clinical response in several depressed cohorts, including the STAR*D. We hypothesized that the *ERICH3* rs11580409 genetic polymorphism would be associated with clinical outcomes and plasma 5-HT levels following ATD treatment in patients of the METADAP cohort.

III.1.1. Overview of patients, materials, and methods

A total of 377 Caucasian patients with ≥ 1 follow-up measure during the study were analyzed for HDRS score changes and response and remission rates after 1, 3, and 6 months of ATD treatment. Among them, 150 patients were analyzed for associations with plasma 5-HT levels at baseline and after 3 and 6 months of ATD treatment. The *ERICH3* rs11580409(A>C) genetic polymorphism was genotyped from leukocytic DNA using TaqMan technology. 5-HT levels were measured from fasting plasma samples using UPLC-MS and imputed where needed. Linear mixed-effects models were constructed in R and adjusted *a priori* for age, sex, and ATD class, as well as ATD-naïve status in the analysis of plasma 5-HT levels. Plasma 5-HT levels were log-transformed for analysis.

III.1.2. Principal results

Among the 377 patients analyzed for clinical associations, the frequency of the rs11580409 C allele was 36.7%. The time \times rs11580409 interaction was significantly associated with the HDRS score. After 6 months of ATD treatment, CC homozygotes had a significantly lower HDRS score compared to AC heterozygotes. Under a recessive genetic model (CC versus AA/AC), the time \times rs11580409 interaction was significantly associated with the HDRS score and response rates. After 6 months of ATD treatment, CC homozygotes had a significantly lower HDRS score and significantly higher response rate compared to A allele carriers.

Among the 150 patients analyzed for associations with plasma 5-HT levels, 21.33% of all plasma 5-HT levels were imputed. Baseline plasma 5-HT levels were significantly higher compared to levels after 3 and 6 months of ATD treatment. No association between the rs11580409 genetic polymorphism and plasma 5-HT levels was observed, even when imputed values were removed from the analysis. Plasma 5-HT levels were not associated with clinical outcomes.

III.1.3. Summary of the discussion

Our analysis is the first investigation of the *ERICH3* rs11580409 genetic polymorphism with clinical outcomes following ATD treatment in depressed patients independent of those led by the Mayo Clinic. Compared to other cohorts of depressed patients in which the rs11580409 polymorphism was analyzed, we observed significant differences in clinical outcomes between rs11580409 genotypes after 6 months of ATD treatment; treatment duration or depression severity may moderate the association of rs11580409 with clinical outcomes following ATD treatment in depression.

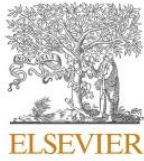
The *ERICH3* rs11580409(A>C) genetic polymorphism is not genome-wide significant, but functional analyses led by the Mayo Clinic have suggested it has a role in 5-HT trafficking and is intimately tied to 5-HT levels. It thus remains an intriguing target for continued analysis in the treatment of depression with ATDs. We did not replicate the association of rs11580409 with plasma 5-HT levels. However, the method used for plasma 5-HT quantification was less sensitive compared to previous studies, and imputed values were used. This study was limited by its high dropout rate, the use of imputed plasma 5-HT values, and its sample size, especially in the analysis of plasma 5-HT levels.

III.1.4. Conclusion

We observed an association of the *ERICH3* rs11580409 (A>C) genetic polymorphism with clinical improvement following 6 months of ATD treatment, which, along with its other associations in similar contexts, argues for its continued study.

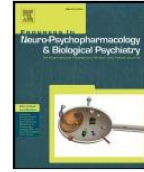
III.1.5. Publication

Our results were published as an original research article in *Progress in Neuro-Psychopharmacology & Biological Psychiatry* (2023 impact factor: 5.201) (Chappell et al., 2022b). Findings were presented as an oral communication at the Congrès de la Société Française de Pharmacologie et de Thérapeutique (SFPT) in Lille, France (June 14–16, 2022).



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The *ERICH3* rs11580409 polymorphism is associated with 6-month antidepressant response in depressed patients

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ABSTRACT

Introduction: Major Depressive Disorder (MDD) is the current leading cause of disability worldwide. The effect of its main treatment option, antidepressant drugs (AD), is influenced by genetic and metabolic factors. The *ERICH3* rs11580409(A > C) genetic polymorphism was identified as a factor influencing serotonin (5HT) levels in a pharmacometabolomics-informed genome-wide association study. It was also associated with response following AD treatment in several cohorts of depressed patients.

Objective: Our aim was to analyze the association of the *ERICH3* rs11580409(A > C) genetic polymorphism with response following AD treatment and plasma 5HT levels in METADAP, a cohort of 6-month AD-treated depressed patients.

Methods: Clinical ($n = 377$) and metabolic ($n = 150$) data were obtained at baseline and after 3 (M3) and 6 months (M6) of treatment. Linear mixed-effects models and generalized logistic mixed-effects models were used to assess the association of the rs11580409 polymorphism with the Hamilton Depression Rating Scale (HDRS) score, response and remission rates, and plasma 5HT levels.

Results: The interaction between the *ERICH3* rs11580409 polymorphism and time was an overall significant factor in mixed-effects models of the HDRS score ($F_{3,370} = 3.35$, $P = 0.019$). At M6, CC homozygotes had a significantly lower HDRS score compared to A allele carriers (coefficient = -3.50 , 95%CI $[-6.00, -0.99]$, $P = 0.019$). No association between rs11580409 and 5HT levels was observed.

Conclusion: Our results suggest an association of rs11580409 with response following long-term AD treatment. The rs11580409 genetic polymorphism may be a useful biomarker for treatment response in major depression.

1. Introduction

Major Depressive Disorder (MDD) is the current leading contributor

to disability worldwide (World Health Organization, 2017). It is associated with increased all-cause mortality and suicide (Chesney et al., 2014), as well as an overall increased risk to develop other health

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conditions (Penninx et al., 2013). The most common treatment option for MDD is antidepressant drugs (AD). However, only about one-third of AD-treated patients achieve remission (Trivedi et al., 2008). This is likely explained, at least in part, by the heterogeneity of depression, including environmental and biological contributions (Cai et al., 2020). The identification of subtypes and biomarkers to predict treatment response is thus an important clinical challenge.

Genetic polymorphisms have been associated with response following AD treatment in the context of MDD (Colle et al., 2015a; Laje et al., 2009; Noordam et al., 2016). However, despite research efforts including candidate gene and genome-wide association studies (GWAS), results have been limited in their utility due to the diversity of studied populations, variability of the methods used, and the complexity of MDD and its treatment (Lin and Lane, 2015; Srivastava et al., 2019).

In recent years, pharmacometabolomic studies have observed associations between various blood plasma and serum metabolites of the tryptophan metabolic pathways and MDD, MDD severity, and/or response to AD treatment (Colle et al., 2020; Liu et al., 2018; Sakurai et al., 2020). Differences in plasma serotonin (5HT) levels have been especially associated with response (Bhattacharyya et al., 2019; Gupta et al., 2016; Holck et al., 2019; Sun et al., 2020). 5HT is synthesized by the hydroxylation of tryptophan to 5-hydroxytryptophan, which is then decarboxylated to 5HT. 5HT is itself metabolized by monoamine oxidase to yield 5-hydroxyindoleacetic acid (Höglund et al., 2019). By combining both genetics and metabolomics, a GWAS of 5HT levels revealed significant associations with polymorphisms in the *ERICH3* gene (Gupta et al., 2016).

ERICH3 encodes for Glutamate-rich protein 3 (ERICH3), a previously uncharacterized protein. Recently, Liu et al. showed that ERICH3 is required for the maintenance of 5HT levels in neuronal cell lines, a process involving various proteins of clathrin-mediated vesicular trafficking (Kaksonen and Roux, 2018; McMahon and Boucrot, 2011), and is expressed predominantly in neurons compared to other cell types in the brain (Liu et al., 2020). Indeed, ERICH3 was found to interact with vesicle-related proteins and strongly colocalized with clathrin and 5HT in vitro. Furthermore, its knockdown led to signal loss of both ERICH3 and 5HT in immunofluorescence studies (Liu et al., 2020). These findings suggest that ERICH3 has a likely role in neurotransmitter vesicle formation and/or trafficking, notably in neuronal cells.

The *ERICH3* rs11580409(A > C) genetic polymorphism was associated with a ~ 80% decrease in ERICH3 concentration through proteasomal degradation in HEK-293 T/17 cells (Gupta et al., 2016). Furthermore, it was nominally associated (i.e., $P < 0.05$) with response after 6 weeks of AD treatment in the STAR*D cohort, 4 weeks of treatment in the ISPC cohort, and 12 weeks of treatment in the PRiEDICT cohort, but not after 8 weeks of treatment in the PGRN-AMPS cohort (Gupta et al., 2016; Liu et al., 2020). A meta-analysis of the association between rs11580409 and response following AD treatment including these 4 clinical cohorts revealed an overall significant association (odds ratio (OR) = 1.21, $P = 0.00074$). However, rs11580409 was not identified in a larger GWAS of the response to AD treatment that analyzed thousands of patients from several clinical cohorts that were treated up to 12 weeks by various ADs (Pain et al., 2021).

Given the genome-wide association of rs11580409 with 5HT levels in a pharmacometabolomics-informed GWAS (Gupta et al., 2016) and its association with response following various durations of AD treatment (Liu et al., 2020), our objective was to analyze the association of the *ERICH3* rs11580409(A > C) genetic polymorphism with response after AD treatment, as well as plasma 5HT levels, in a cohort of 6-month AD-treated individuals with MDD.

2. Materials and methods

2.1. Study design

Do Antidepressants Induce Metabolic Syndromes (METADAP) is a 6-

month prospective, naturalistic treatment, multicentric cohort study carried out in a psychiatric setting (Corruble et al., 2015). Patients with a current major depressive episode (MDE) were treated as usual and were assessed before and during AD treatment. This study was registered by the French National Agency for Medicine and Health Products Safety (ANSM) and the Commission Nationale de l'Informatique et des Libertés (CNIL). It was approved by the Ethics Committee of Paris-Boulogne (France) and conformed to international ethical standards (ClinicalTrials.gov identifier: NCT00526383).

2.2. Patients

Males and females without a serious medical or inflammatory condition, between the ages of 18 and 65, were recruited. Inclusion criteria were presentation with a MDE in the context of MDD (DSM-IV-TR), as assessed by the Mini International Neuropsychiatric Interview (MINI), need for consultation or hospitalization, a score ≥ 18 on the 17-item Hamilton Depression Rating Scale (HDRS) (Hamilton, 1960), and need for a new AD treatment. Individuals with other mental disorders—such as psychotic disorder, bipolar disorder, alcohol or drug dependence, or an eating disorder—were excluded. Measures and samples were obtained prior to beginning AD treatment (M0), and after 1 month (M1), 3 months (M3), and 6 months (M6) of treatment.

Of the 643 individuals included in METADAP, 19 had major protocol deviations and were excluded. Of the 624 available for analysis, 519 individuals provided samples for genetic studies. Given our objective to examine clinical response following AD treatment, 104 individuals without follow-up measures (i.e., at M1, M3, and M6) were removed. In total, 377 Caucasians were analyzed in this ancillary study (genetic cohort). Information about ancestry was self-reported. Caucasian ethnicity was defined as having parents of European descent, African as having parents of Sub-Saharan African and/or Afro-Caribbean descent, and Asian as having parents of East Asian, Central Asian, and/or South Asian descent (Morales et al., 2018). Dropouts occurred mainly because of AD changes, use of unauthorized drugs, or loss to follow-up. In the original metabolite analysis of METADAP (Colle et al., 2020), 173 individuals were analyzed because they were study completers. Among them, 150 were present within the genetic cohort and were analyzed (metabolic cohort). All patients provided written informed consent for study participation and for genetic analyses (Corruble et al., 2015).

2.3. Antidepressant treatment

AD monotherapies were prescribed by a psychiatrist in a “real world” psychiatric treatment setting as previously described (Corruble et al., 2015). ADs belonged to one of four classes: selective serotonin reuptake inhibitors (SSRI), serotonin norepinephrine reuptake inhibitors (SNRI), tricyclic antidepressants (TCA), or other AD treatments. In this ancillary study of 377 individuals, 141 (37%) were prescribed an SSRI, 159 (42%) an SNRI, 28 (8%) a TCA, 30 (8%) another AD treatment, and 19 (5%) received electroconvulsive therapy (ECT). If a change in AD treatment was required during follow-up, the subject was dropped from the study.

2.4. Assessment of response following antidepressant treatment

The HDRS was used to assess depression severity at baseline (M0) and response following AD treatment at M1, M3, and M6. Responders to AD treatment were classified by an improved HDRS score of $\geq 50\%$ relative to baseline. Remitters were defined by a HDRS score ≤ 7 after at least 4 weeks of treatment. Clinical assessments were performed blind to genotyping results. Each interview and diagnostic assignment was reviewed by a senior psychiatrist. For each subject, all visits were reviewed by the same psychiatrist.

2.5. Genotyping

5 mL of whole blood was collected at baseline. Leukocytic DNA was extracted from 1 mL of blood using a Puregene Kit (Gentra systems, Minneapolis, USA) and stored at -20°C . Genotyping was performed with a TaqMan SNP genotyping assay (ThermoFisher®, Courtaboeuf, France) according to the manufacturer's instructions. Allelic discrimination was performed with the QuantStudio 7 Flex Real-Time PCR System (ThermoFisher®, Les Ulis, France), as previously described (Colle et al., 2015b; Coulbault et al., 2006; Jousseveur et al., 2016). The *ERICH3* rs11580409(A > C) genotype was determined with the forward (5'-CTGTTTCCCTTCTTCCGCT-3') and reverse (5'-GAGGGCAGCCCT-GAGGA-3') primers. The A allele was detected using a VIC-fluorescent probe (5'-TCTCTTGCTAAATCTAATTC-3') while the C allele was detected using a FAM-fluorescent probe (5'-CTCTTGCTACATCTAATTC-3'). Genotyping was performed blind to clinical data. For each batch, samples were genotyped with 3 negative controls (sterile water) and 2 positive controls for each *ERICH3* rs11580409 genotype (i.e., AA, AC, and CC). Positive controls were previously sequenced. Average genotyping quality was 98.83 ± 0.18 . One sample was initially undetermined but successfully genotyped in a subsequent batch. See Supplementary Fig. S1 for an example of genotyping calls.

2.6. Metabolite measurement

Methods to determine metabolite levels were as previously described (Colle et al., 2020). Briefly, 50 μL of fasting plasma stored at -80°C was used to prepare metabolite extracts via protein precipitation with 250 μL of acetonitrile, evaporation under nitrogen, and reconstitution in 50 μL of water. Reference human plasma samples were prepared 3 times in all analytical batches as a system control within and between analyses. Ultra-performance liquid chromatography coupled with mass spectrometry (UPLC-MS) was used via a Waters Acquity UPLC system coupled to a Thermo Scientific Q Exactive mass spectrometer. 21.33% of all 5HT values (M0: 13.3%; M3: 28.7%; M6: 22%) were below the lower limit of quantification (LLOQ, 0.00113 μM) and were imputed as $\text{LLOQ}/\sqrt{2}$ (0.000799 μM) for statistical analyses, as previously described (Colle et al., 2020). Non-imputed 5HT levels ranged from 0.00114 to 0.123 μM .

2.7. Data analysis

All statistical analyses were performed using R (v4.1.0) (R Core Team., 2020). Hardy-Weinberg equilibrium was assessed using the chi-square test. For demographic data, the nonparametric Kruskal-Wallis test was used to compare quantitative variables, while the Fisher Exact test was used to compare qualitative variables. 5HT levels across time were analyzed with a nonparametric Friedman rank sum test to account for repeated measures within each individual. Nonparametric Wilcoxon signed-rank tests were used to compare 5HT levels between 2 time-points. Linear mixed-effects models and generalized logistic mixed-effects models were constructed with the lme4 package (Bates et al., 2015). The main variable to explain was the HDRS score. Other variables to explain were the response and remission rates and plasma 5HT levels. Plasma 5HT levels were \log_2 transformed due to non-normality (see Supplementary Fig. S2). The *ERICH3* rs11580409(A > C) polymorphism and its interaction with time were the main explanatory variables examined. Age, sex, and AD class were chosen a priori as fixed-effects covariables. Given the influence of ADs on 5HT levels, AD-naïve status was also included in models of 5HT levels. Variables that significantly differed (i.e., $P < 0.05$) between genotype groups were also included as fixed-effects covariables. Individual was included as the random-effect to account for repeated measures. A significance threshold of $P < 0.05$ was considered significant. Significance of fixed-effects was assessed with the Satterthwaite method in linear mixed-effects models and with the Wald chi-square test in generalized linear mixed-effects models. Post hoc comparisons were carried out with the emmeans package (Lenth,

2022).

Power analyses were carried out using the genpwr package assuming an additive genetic model (Moore et al., 2019). A minor allele frequency of 38% for rs11580409, as observed in Non-Finnish Europeans of gnomAD (Karczewski et al., 2020), an alpha level of 5%, and a power of 80% were used. In the genetic cohort, we had sufficient power to detect an OR of 1.88 among the 200 individuals at M6 (responder rate = 66.5%). In the metabolic cohort, we had sufficient power to detect an $R^2 = 5.1\%$ for baseline 5HT level ($\text{SD} = 1.97$) variation among the 150 individuals.

3. Results

3.1. Patient demographics

Sociodemographic characteristics of the whole genetic cohort and according to *ERICH3* rs11580409 genotypes are given in Table 1. The mean age was 46.2, 66% of subjects were females, 47% had a university-level education and 40% were current smokers at baseline. The missing-to-follow-up rate relative to baseline was 6% at M1, 32% at M3, and 47% at M6. The allele frequency of the rs11580409 variant C allele was 36.7%. It did not significantly deviate from Hardy-Weinberg equilibrium ($P = 0.09$). No significant differences in any of the sociodemographic factors were observed between *ERICH3* rs11580409 genotype groups in the genetic cohort (see Table 1).

3.2. Clinical response according to *ERICH3* rs11580409 genotypes

After controlling for age, sex, and AD class, the interaction between rs11580409 and time was a significant factor in the mixed-effects model of the HDRS score ($F_{6,063} = 2.69$, $P = 0.014$) (see Table 2), corresponding to a difference in the association between the HDRS score and the rs11580409 genotype over time. At M6, significantly lower HDRS scores in CC homozygotes (8.4 ± 1.22) compared to AC heterozygotes (12.5 ± 0.72) were observed (coefficient = -4.04 , 95%CI [-1.38 – 2.97], $P = 0.027$) (see Fig. 1A and Supplementary Table S1).

Given the significant difference in the HDRS score between CC homozygotes and AC heterozygotes, a recessive genetic model was examined. After controlling for the same variables, the interaction between rs11580409 and time was still an overall significant factor in the mixed-effects model of the HDRS score ($F_{3,870} = 3.35$, $P = 0.019$), and was additionally a significant factor in the model of response rates ($\chi^2 = 6.22$, $\text{df} = 2$, $P = 0.045$) (see Table 2). At M6, the HDRS scores in CC homozygotes (see above) compared to A allele carriers (11.93 ± 0.56) were significantly lower (coefficient = -3.50 , 95%CI [-6.00 – 0.99], $P = 0.019$) (see Fig. 1B and Supplementary Table S1). Additionally, a significantly higher response rate in CC homozygotes (89%, $n = 29$) compared to A allele carriers (59%, $n = 171$) was observed at M6 (OR = 5.81, 95%CI [1.45 – 23.36], $P = 0.039$) (see Fig. 2 and Supplementary Table S1).

3.3. 5HT levels according to *ERICH3* genotypes

We then investigated the association of rs11580409(A > C) with plasma 5HT levels (as quantified by UPLC-MS), as well as the association of plasma 5HT levels with clinical outcomes. Sociodemographic characteristics of the metabolic cohort according to *ERICH3* rs11580409 genotypes are given in Table 1. Whole sample 5HT levels significantly differed with time ($\chi^2(2) = 40.6$, $P \leq 0.001$). Baseline levels (mean = 0.015 μM , $\text{SD} = 0.024$) were significantly higher than levels after 3 (mean = 0.0070 μM , $\text{SD} = 0.017$; $z = 7702$, $P \leq 0.001$) and 6 (mean = 0.0076 μM , $\text{SD} = 0.017$; $z = 7320$, $P \leq 0.001$) months of AD treatment. 5HT levels at M3 and M6 did not significantly differ ($z = 4080$, $P = 0.26$). After controlling for age, sex, AD class, tobacco consumption (in pack years), and AD-naïve status, no significant association between 5HT levels (as quantified by UPLC-MS) and the rs11580409 polymorphism was observed in mixed-effects models of 5HT levels ($F_{2,55} =$

Table 1
Demographic characteristics according to *ERICH3* rs11580409 genotypes.

Genetic cohort	Patient total	<i>ERICH3</i> rs11580409				P
		AA	AC	CC	P	
	n = 377	n = 159	n = 159	n = 59		
Age (in years) (m ± sd)	46.2 ± 13.1	47 ± 14.2	45.5 ± 12.5	45.8 ± 11.7	0.52	
Female (n(%))	249(66)	111 (70)	100 (63)	38 (64)	0.40	
Education level (n(%))	Primary	31(8)	13(8)	13(8)	5(8)	0.99
	High school	168(45)	70 (44)	73 (46)	25 (42)	
	University	177(47)	76 (48)	72 (45)	29 (49)	
Current smoker (n(%))	151(40)	68 (43)	63 (40)	20 (34)	0.50	
Tobacco consumption (pack years) (m ± sd)	15.7 (14.6)	15.4 ± 12.6	15.7 ± 17.1	16.6 ± 12.3	0.49	
Recurrent MDE (n(%))	289(77)	126 (79)	118 (74)	45 (76)	0.58	
Onset age MDE (m ± sd)	35.3 ± 14.6	36.2 ± 15.3	34.5 ± 14.2	34.9 ± 13.9	0.65	
Baseline HDRS (m ± sd)	24.7 ± 4.9	24.1 ± 4.3	25.3 ± 5.4	24.7 ± 4.9	0.32	
Prescribed therapy (n(%))	SSRI	141(37)	(39)	(37)	20 (34)	0.12
	SNRI	159(42)	70 (44)	61 (38)	28 (47)	
	TCA	28(7)	13(8)	14(9)	1(2)	
	Other AD	30(8)	11(7)	12(8)	7(12)	
	ECT	19(5)	3(2)	13(8)	3(5)	
Missing to follow-up (n (%))	M1	24(6)	9(6)	10(6)	5(8)	0.75
	M3	119(32)	56 (35)	42 (26)	21 (36)	
	M6	177(47)	77 (48)	70 (44)	30 (51)	
Metabolic cohort	n = 150	n = 59	n = 68	n = 23		
Age (in years) (m ± sd)	46.7 ± 12	48.6 ± 11.3	46.1 ± 12.9	43.3 ± 10.2	0.15	
Female (n(%))	96(64)	36 (61)	46 (68)	14 (61)	0.70	
Current smoker (n(%))	54(36)	23 (39)	26 (38)	5(22)	0.32	
Tobacco consumption (pack years) (m ± sd)	14.5 (11)	19.5 ± 11.4	10.5 ± 9.4	14.2 ± 10.6	0.013*	
Recurrent MDE (n(%))	103(69)	45 (76)	45 (66)	13 (57)	0.19	
Onset age MDE (m ± sd)	36.3 ± 14.8	38.6 ± 14.1	34.8 ± 15.3	35 ± 14.8	0.30	
Baseline HDRS (m ± sd)	23.3 ± 4.1	23.5 ± 3.8	23.2 ± 4	23.4 ± 5.1	0.77	
AD-naïve (n(%))	54(36)	9(39)	22 (32)	23 (39)	0.83	
Prescribed therapy (n(%))	SSRI	56(37)	26 (44)	21 (31)	9(39)	0.45
	SNRI	65(43)	24 (41)	29 (43)	12 (52)	
	TCA	13(9)	5(8)	7(10)	1(4)	

Table 1 (continued)

Genetic cohort	Patient total	<i>ERICH3</i> rs11580409			
		AA	AC	CC	P
Other AD	11(7)	4(7)	6(9)	1(4)	
ECT	5(3)	0(0)	5(7)	0(0)	

Sociodemographic characteristics of Caucasian METADAP patients are shown for the whole sample and according to *ERICH3* rs11580409 genotypes for the cohorts that underwent genetic and metabolic analyses. Kruskal-Wallis tests were used to compare age, tobacco consumption, onset age of MDE, baseline HDRS, and baseline metabolite ratios, presented as mean ± standard deviation. Fisher Exact tests were used to compare sex, education level, smoking status at baseline, MDE recurrence, AD-naïve status, prescribed AD drug, and missing-to-followup rates over time, presented as the group number and percentage. **Bold text** and *: $P < 0.05$. AD: antidepressant drug; ECT: electroconvulsive therapy; HDRS: 17-item Hamilton Depression Rating Scale; m: mean; M1: after 1 month of treatment; M3: after 3 months of treatment; M6: after 6 months of treatment; MDE: major depressive episode; n: number of patients; P: P-value; sd: standard deviation; SNRI: serotonin norepinephrine reuptake inhibitor; SSRI: selective serotonin reuptake inhibitor; TCA: tricyclic antidepressant.

0.47, $P = 0.63$) (see Table 3). Removing imputed values did not affect this association ($F_{2,55} = 0.36$, $P = 0.70$). Examining baseline 5HT levels alone after adjusting for these same factors was also not significant ($F_{2,54} = 2.25$, $P = 0.12$), even without imputed values ($F_{2,51} = 0.67$, $P = 0.52$). Additionally, no significant associations between clinical outcomes and baseline 5HT levels or 5HT levels across time were observed, either with or without imputed values (see Supplementary Table S2).

4. Discussion

In the present ancillary study of METADAP, the *ERICH3* rs11580409 (A > C) genetic polymorphism was analyzed. The allele frequency of the variant C allele was comparable between METADAP (36.7%), PGRN-AMPS (35%) (Gupta et al., 2016), and Non-Finnish Europeans of gnomAD (38%) (Karczewski et al., 2020). We observed a significant association between rs11580409 and the HDRS score and response rates, with a significantly lower HDRS score and significantly higher response rate after 6 months of AD treatment in CC homozygotes compared to A allele carriers.

The association of rs11580409 with response following AD treatment varies between association studies in clinical cohorts (Gupta et al., 2016; Liu et al., 2020) and GWAS (Li et al., 2020; Pain et al., 2021). With respect to the former, rs11580409 was associated with response following 4 and 6 weeks of treatment in the ISPC (OR = 1.26, $P = 0.022$) and STAR*D (OR = 1.17, $P = 0.046$) cohorts, respectively (Gupta et al., 2016). However, as at least the *TSPAN5* rs11947402 polymorphism was also examined, these results may not have survived multiple testing correction. Although rs11580409 was identified in a GWAS of 5HT levels in the PGRN-AMPS cohort, it was not significantly associated with 8-week response in PGRN-AMPS (OR = 1.13, $P = 0.46$). A significant association in PREDICT following 12 weeks of treatment (OR = 2.12, $P = 0.029$) was also observed. In a meta-analysis of these 4 cohorts, rs11580409 was overall associated with response following these various treatment durations (OR = 1.21, $P = 0.00075$) (Liu et al., 2020).

Regarding results from large-scale GWAS, the *ERICH3* rs11580409 polymorphism did not reach genome-wide—nor nominal (i.e., $P < 0.05$)—significance in a large ($n = 5316$) GWAS of percentage improvement (OR = 0.99, $P = 0.63$) and remission (OR = 1.06, $P = 0.25$) combining 10 clinically-assessed European cohorts (Pain et al., 2021). Additionally, rs11580409 was not identified in a large ($n > 48,000$) GWAS of self-reported antidepressant efficacy using data obtained from 23andMe (Li et al., 2020). Despite the lack of replication in GWAS, we would like to argue that the *ERICH3* rs11580409 (A > C)

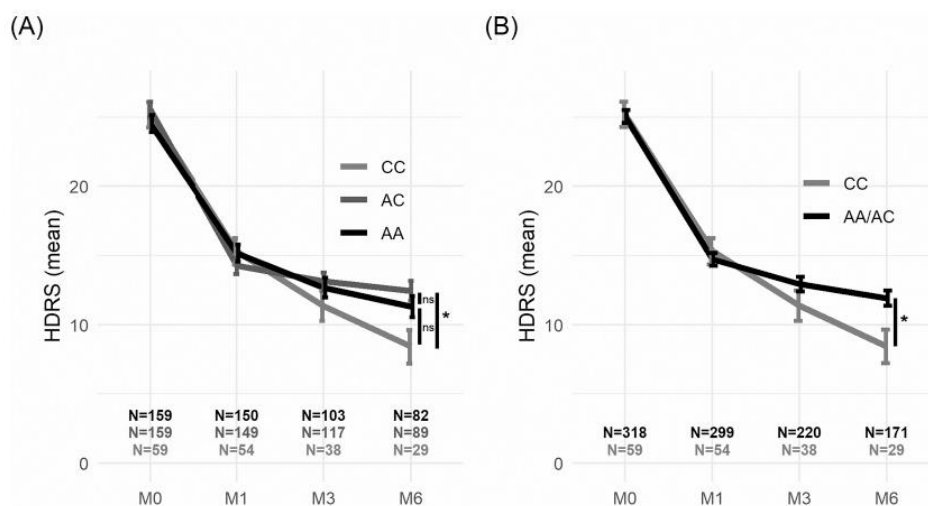


Fig. 1. HDRS scores according to *ERICH3* rs11580409 genotypes. The HDRS score (y-axis) at different study time points (x-axis) are shown for the different genotypes of the rs11580409(A > C) polymorphism (see legends) in both an additive genetic model (A) and a recessive genetic model (B). *: $P < 0.0056$ in (A) to account for the 9 Bonferroni-corrected comparisons and $P < 0.0167$ in (B) to account for the 3 Bonferroni-corrected comparisons during antidepressant treatment (M1-M3-M6).

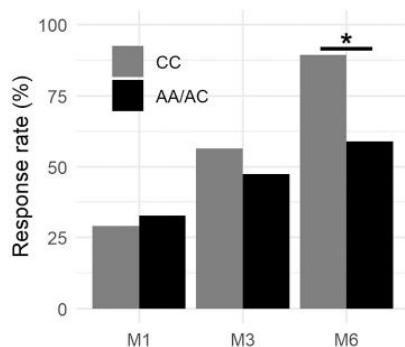


Fig. 2. Response rates of *ERICH3* rs11580409 C allele homozygotes and A allele carriers. The response rates (y-axis) at different study time points (x-axis) are shown for rs11580409 CC homozygotes (gray) and A allele carriers (black) *: $P < 0.0167$ to account for the 3 Bonferroni-corrected comparisons.

polymorphism remains an interesting target to consider in association with AD response.

First, well-characterized candidates are rarely—if ever—replicated in GWAS, though biologically functional roles may be observed and replicated, as with *FKBP5* (Moore, 2017). The rs11580409 polymorphism was originally identified from a pharmacometabolomics-informed GWAS of 5HT levels and later observed to be associated with drastic decreases (~80%) in *ERICH3* protein levels (Gupta et al., 2016). *ERICH3*, a previously uncharacterized protein, was proposed to have a role in 5HT trafficking through its interactions with vesicle proteins, including clathrin, and was linked to 5HT concentrations, as its knockout led to drastic decreases in 5HT levels (Liu et al., 2020). As 5HT is a common target of conventional AD therapies that modulate its synaptic levels in the brain, factors that intrinsically alter 5HT levels may thus be interesting candidates in the study of the response to AD treatments.

Second, there is often a tradeoff between statistical power and phenotypic homogeneity (Moore, 2017). Complex traits, such as depression, are heterogenous in their etiology and responses to

treatment (Fried and Nesse, 2015). Numerous factors—such as development, severity, measurement, symptomology, comorbidities, and gene-environment interactions—can lead to fundamentally different depressive subtypes (Cai et al., 2020). In their analysis of European populations, the second largest cohort examined by Pain et al., comprising roughly 20% of their sample, was the GDRS. This cohort had a high proportion of treatment-resistant depression patients (~40%) compared to the other cohorts (Pain et al., 2021). It remains possible that these treatment-related factors may have influenced the analysis. Stratification of these populations according to more-refined depressive subtypes may thus reveal novel associations.

The biggest difference between METADAP and the other cohorts investigating rs11580409 in the response to AD therapy is treatment duration. Indeed, treatment duration is at least double in METADAP (6 months) compared to the other cohorts (maximum 12 weeks) (Liu et al., 2020; Pain et al., 2021). Different treatment options were prescribed in the other cohorts, but the vast majority were prescribed SSRIs. In our analysis, the majority (42%) were prescribed an SNRI, while 37% were prescribed an SSRI. Each cohort, except the PGRN-AMPS cohort, which used the QIDS-C16, used the HDRS, though the minimum score for inclusion ranged from 10 to 18 (as in METADAP). Moreover, most individuals enrolled in METADAP were in-patients and patients treated by psychiatrists, while a mix of in- and out-patients were enrolled by the other cohorts, though PRoDICT recruited through advertising or physician referrals (Dunlop et al., 2017). Potential differences in terms of the diagnosis of depression and its severity may exist between cohorts that could help explain the different response times. Depression severity and treatment duration may be important factors influencing the effect of rs11580409 on response to AD treatments.

The levels of 5HT were also analyzed with respect to rs11580409, since rs11580409 was previously associated with lower 5HT levels (Gupta et al., 2016). From the 173 previously analyzed study completers (Colle et al., 2020), we examined 150 Caucasians genotyped for rs11580409. However, we did not observe an association between rs11580409 and 5HT levels. Several factors may have influenced this result. First, to assay plasma 5HT concentrations, our analysis employed UPLC-MS while Gupta et al. (2016) assayed the PGRN-AMPS cohort using high-performance liquid chromatography (HPLC) electrochemical (EC) coulometry. HPLC-EC detection is often more sensitive than MS

Table 2
Clinical associations with *ERICH3* rs11580409 genotypes.

	HDRS		df (num)	df (den)	F	P	Response			Remission		
	SS	MS					χ^2	df	P	χ^2	df	P
Additive genetic model												
(Intercept)							4.18	1	0.041	15.11	1	<0.001
Age	0.51	0.51	1	365.88	0.02	0.89	0.90	1	0.34	0.58	1	0.45
Sex	37.66	37.66	1	358.61	1.28	0.26	3.76	1	0.05	4.57	1	0.032
AD Class	178.42	44.60	4	371.51	1.52	0.20	7.52	4	0.11	7.55	4	0.11
rs11580409	79.21	39.60	2	385.37	1.35	0.26	2.17	2	0.34	1.90	2	0.39
Time	30,059.81	10,019.94	3	864.12	341.61	<0.001	17.21	2	<0.001	14.62	2	<0.001
rs11580409:Time	473.89	78.98	6	862.87	2.69	0.014*	8.96	4	0.06	4.86	4	0.30
Recessive genetic model												
(Intercept)							4.08	1	0.043	15.16	1	<0.001
Age	0.39	0.39	1	366.60	0.01	0.91	0.99	1	0.32	0.73	1	0.39
Sex	38.40	38.40	1	359.74	1.30	0.25	3.94	1	0.047	4.88	1	0.027
AD Class	195.65	48.91	4	372.21	1.66	0.16	7.43	4	0.11	7.33	4	0.12
rs11580409	64.85	64.85	1	393.50	2.20	0.14	0.20	1	0.65	0.31	1	0.58
Time	20,382.03	6794.01	3	869.90	230.50	<0.001	17.20	2	<0.001	14.63	2	<0.001
rs11580409:Time	296.04	98.68	3	870.27	3.35	0.019*	6.22	2	0.045*	3.53	2	0.17

ANOVA results for mixed-effects models of the HDRS score, response, and remission. HDRS was assessed using the Satterthwaite method while response and remission were assessed using the Wald chi-square method. The association of rs11580409 with clinical outcomes was assessed in both additive and recessive models. **Bold text** and *: $P < 0.05$. den: denominator; df: degrees of freedom; MS: mean squares; num: numerator; P: P-value; SS: sum of squares; χ^2 : chi-square.

Table 3
Mixed-effects model results of the association between rs11580409 and 5HT levels.

	SS	MS	df (num)	df (den)	F	P
Age	1.57	1.57	1	55.72	0.77	0.38
Sex	1.05	1.05	1	56.45	0.51	0.48
AD Class	72.19	18.05	4	56.77	8.82	<0.001
Tobacco consumption (pack years)	6.74	6.74	1	55.75	3.29	0.075
AD-naive status	11.55	11.55	1	177.42	5.64	0.019
Time	27.70	13.85	2	133.03	6.77	0.0016
rs11580409	1.92	0.96	2	55.54	0.47	0.63
rs11580409:Time	11.78	2.94	4	125.01	1.44	0.22

ANOVA results for mixed-effects models of 5HT levels, assessed using the Satterthwaite method. den: denominator; df: degrees of freedom; MS: mean squares; num: numerator; P: P-value; SS: sum of squares.

methods, especially for the detection of electroactive compounds including 5HT, and is capable of detection at nano-, pico-, and femtomolar concentrations (Kristal et al., 2007; Matson et al., 1984). Still, we did not observe an association between the rs11580409 polymorphism and 5HT levels—either across time or at baseline—even after removing imputed values from our analysis. Second, our sample ($n = 150$) was nearly half the size of the PGRN-AMPS cohort ($n = 289$) and was sufficiently powered to detect an $R^2 = 5.1\%$ to explain baseline 5HT level variation relative to rs11580409. Conversely, the PGRN-AMPS observed an $R^2 = 18.8\%$ between rs11580409 and baseline 5HT levels.

In addition to the differences in quantification between HPLC-EC and MS methods, sample collection and preparation can also influence 5HT measurement (Morgadinho et al., 2004), especially since the vast majority of peripheral 5HT is stored in platelets, while the rest is degraded in the intestine, liver, and lungs (Colle et al., 2020). These and other variables can thus affect 5HT quantification and make associations of 5HT levels with clinical outcomes more ambiguous. While different genotyping methods also exist, genotyping results (i.e., homozygous wild-type, heterozygous, or homozygous mutant) are often simpler and more easily comparable between methods relative to metabolite quantification. As such, and at least when considering current limitations, genotyping may have more utility in clinical settings compared to metabolite concentration measurement.

Most ADs, both in the brain and in the periphery, target monoamine

transporters and block 5HT reuptake (Artigas et al., 2002). The blocking action of ADs on platelet 5HT transporter (SLC6A4) likely contributes to the decreased peripheral plasma 5HT levels observed after beginning AD therapy. *ERICH3* is expressed notably in neurons, but is also expressed in platelets (Liu et al., 2020). Further research would help validate its utility as a peripheral biomarker for brain *ERICH3* activity. As rs11580409 has been associated with decreased *ERICH3* levels and decreased 5HT levels (Gupta et al., 2016), it may also be a useful biomarker for 5HT levels. Although its associations with reduced 5HT levels and improved response following AD treatment may appear counterintuitive, the precise mechanisms through which rs11580409 and *ERICH3* may affect 5HT levels and elicit an improved response to AD therapy remain to be elucidated.

There are several limitations to our study. First, the dropout rate was relatively high. However, at 6 months the dropout rate (47%) was comparable to that of STAR*D at 12 weeks (Muthén et al., 2011; Trivedi et al., 2006). Second, imputed data was used in the analysis of 5HT levels. However, as has been reported elsewhere, we also observed higher baseline 5HT levels followed by subsequent decreases after beginning AD treatment (Gupta et al., 2016; Holck et al., 2019; Sun et al., 2020). Third, our analysis of 5HT levels was underpowered to detect an effect as strong as previous reports (Gupta et al., 2016). Future studies would benefit from a larger sample with both genetic and metabolic data. This study benefits from its prospective and naturalistic design, as well as from its 6-month treatment duration, which allows for the analysis of treatment response across a longer period and better reflects “real-world” clinical practice.

5. Conclusion

In conclusion, we observed an association of the *ERICH3* rs11580409 (A > C) genetic polymorphism with 6-month depressive symptom improvement following AD treatment in a cohort of 6-month AD-treated Caucasians with depression. Given its previously nominal associations with response after different durations of AD treatment, continued study of rs11580409 in diverse cohorts of individuals with major depression may be warranted. Stratification by symptom severity and treatment duration may help to reveal further associations between rs11580409 and response, and perhaps point to mechanisms through which this polymorphism may act. Current knowledge of *ERICH3* suggest that it may be an interesting clinical target in the treatment of MDD.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.pnpbp.2022.110608>.

Human rights

This study was registered by the French National Agency for Medicine and Health Products Safety (ANSM) and the Commission Nationale de l'Informatique et des Libertés (CNIL). It was approved by the Ethics Committee of Paris-Boulogne (France) and conformed to international ethical standards ([ClinicalTrials.gov](https://www.clinicaltrials.gov) identifier: NCT00526383). All patients provided written informed consent for study participation and for genetic analyses.

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Ethical statement

I, Kenneth Chappell, consciously assure that the following is fulfilled for the manuscript entitled *The ERICH3 rs11580409 polymorphism is associated with 6-month antidepressant response in depressed patients*:

- 1) This material is the authors' own original work, which has not been previously published elsewhere.
- 2) The paper is not currently being considered for publication elsewhere.
- 3) The paper reflects the authors' own research and analysis in a truthful and complete manner.
- 4) The paper properly credits the meaningful contributions of co-authors and co-researchers.
- 5) The results are appropriately placed in the context of prior and existing research.
- 6) All sources used are properly disclosed (correct citation). Literally copying of text must be indicated as such by using quotation marks and giving proper reference.
- 7) All authors have been personally and actively involved in substantial work leading to the paper, and will take public responsibility for its content.

CRedit authorship contribution statement

Kenneth Chappell: Conceptualization, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Romain Colle**: Writing – review & editing. **Abd El Kader Ait Tayeb**: Formal analysis. **Jérôme Bouligand**: Resources, Writing – review & editing. **Khalil El-Asmar**: Formal analysis. **Eric Deflesselle**: Writing – review & editing. **Bruno Fève**: Funding acquisition, Writing – review & editing. **Laurent Becquemont**: Writing – review & editing. **Emmanuelle Corruble**: Funding acquisition, Writing – review & editing. **Céline Verstuyft**: Conceptualization, Resources, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

None.

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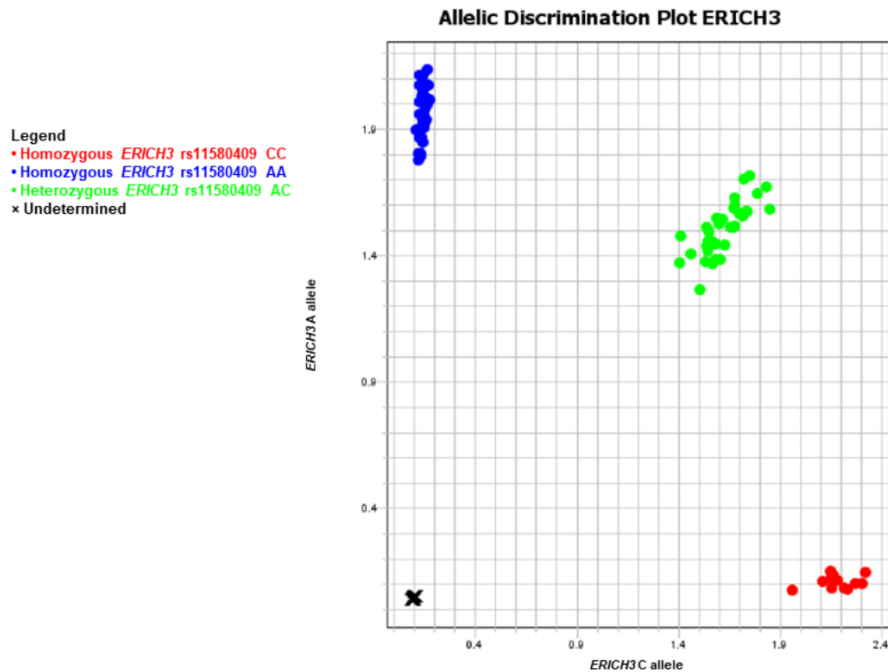
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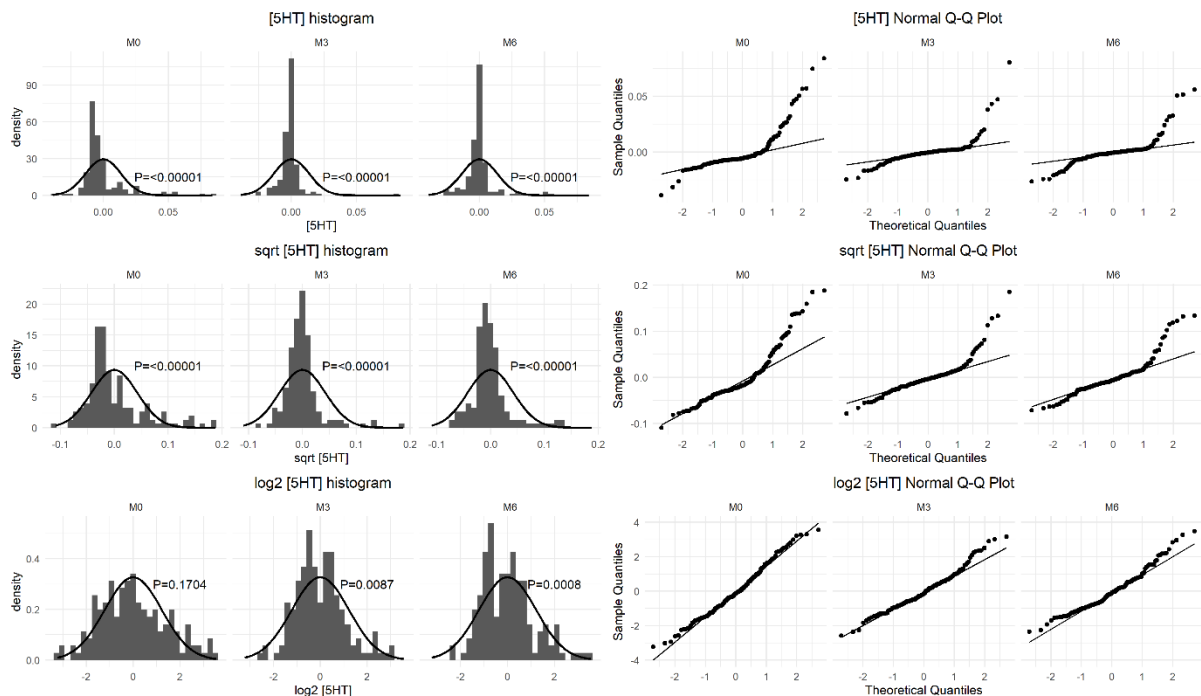
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III.1.6. Supplementary Materials



Supplementary Figure S1

An example of the genotype calling with the TaqMan SNP genotyping assay. Allelic discrimination of the ERICH3 rs11580409 A allele (y-axis) and the C allele (x-axis) allow for genotyping of each sample (red, blue, or green) relative to negative controls (black X's, see legend).



Supplementary Figure S2

Histograms [left; density (y-axis) and value (x-axis)] and normal QQ plots [right; sample quantile (y-axis) and theoretical quantile (x-axis)] are shown for serotonin (5HT) values at M0 (left), M3 (middle), and M6 (right) when untransformed (top), square root-transformed (middle), and log₂-transformed (bottom). *P* values from Shapiro-Wilk normality tests are shown with each corresponding histogram.

Supplementary Table 1: Post-hoc comparisons of significant mixed-effects models

Shown are the emmeans contrasts obtained from mixed-effects models. *: $P < 0.05$. P -values are adjusted by Bonferroni corrections (0.05/9 in the additive genetic model, 0.05/3 in the recessive genetic model). **95%CI**: 95% confidence interval; **AD**: antidepressant drug; **df**: degrees of freedom; **ECT**: electroconvulsive therapy; **M1**: after 1 month of AD treatment; **M3**: after 3 months of AD treatment; **M6**: after 6 months of AD treatment; **OR**: odds ratio; P : P -value; **Padj**: Bonferroni-adjusted P -value; **se**: standard error

Additive genetic model								
contrast	coefficient	se	df	95%CI	t	P	Padj	
HDRS								
M1								
CC - AC	1.05	1.05	956.58	-1.01 – 3.12	1.00	0.32	1	
CC - AA	0.12	1.05	955.14	-1.94 – 2.19	0.12	0.91	1	
AC - AA	-0.93	0.77	943.25	-2.44 – 0.59	-1.20	0.23	1	
M3								
CC - AC	-1.77	1.21	1084.89	-4.14 – 0.61	-1.46	0.15	1	
CC - AA	-1.33	1.23	1093.09	-3.74 – 1.08	-1.08	0.28	1	
AC - AA	0.44	0.88	1072.27	-1.29 – 2.16	0.50	0.62	1	
M6								
CC - AC	-4.04	1.36	1149.45	-6.71 – -1.38	-2.97	0.0030	0.027*	
CC - AA	-2.91	1.37	1151.37	-5.60 – -0.22	-2.12	0.034	0.31	
AC - AA	1.14	0.98	1140.15	-0.78 – 3.05	1.16	0.25	1	
Recessive genetic model								
contrast	coefficient	se	df	95%CI	t	P	Padj	
HDRS								
M1								
CC - AA/AC	0.59	0.98	964.43	-1.33 – 2.51	0.60	0.55	1	
M3								
CC - AA/AC	-1.55	1.14	1096.77	-3.79 – 0.68	-1.37	0.17	0.52	
M6								
CC - AA/AC	-3.50	1.28	1156.32	-6.0 – -0.99	-2.74	0.0062	0.019*	
Response								
contrast	OR	se	df	95%CI	z	P	Padj	
M1								
CC - AA/AC	0.84	0.32	Inf	0.40 – 1.78	-0.45	0.65	1	
M3								
CC - AA/AC	1.43	0.65	Inf	0.59 – 3.47	0.79	0.43	1	
M6								
CC - AA/AC	5.81	4.12	Inf	1.45 – 23.36	2.48	0.013	0.039*	

Supplementary Table 2: Mixed-effects model results of clinical outcomes with 5HT levels

ANOVA results for mixed-effects models of the HDRS score, response, and remission. HDRS was assessed using the Satterthwaite method while response and remission were assessed using the Wald chi-square method. The association of 5HT levels both across time and baseline 5HT levels alone with clinical outcomes was assessed. **den**: denominator; **df**: degrees of freedom; **MS**: mean squares; **num**: numerator; **P**: P-value; **SS**: sum of squares.

	HDRS						Response			Remission		
	SS	MS	df (num)	df (den)	F	P	Chisq	df	P	Chisq	df	P
All values												
5HT across time												
Age	1.760	1.760	1	144.107	0.075	0.784	0.766	1	0.382	0.180	1	0.671
Sex	21.178	21.178	1	143.454	0.904	0.343	1.928	1	0.165	1.966	1	0.161
AD Class	148.852	37.213	4	147.501	1.589	0.180	7.734	4	0.102	2.377	3	0.498
5HT	33.775	33.775	1	436.317	1.442	0.230	0.203	1	0.652	1.245	1	0.265
Time	14766.573	7383.286	2	308.304	315.221	<0.001	1.255	1	0.263	4.283	1	0.038
5HT:Time	10.053	5.027	2	348.246	0.215	0.807	0.000	1	0.995	0.752	1	0.386
Baseline 5HT												
Age	1.738	1.738	1	142.000	0.075	0.784	0.829	1	0.362	0.128	1	0.721
Sex	24.550	24.550	1	142.000	1.062	0.305	1.980	1	0.159	2.108	1	0.147
AD Class	164.709	41.177	4	142.000	1.781	0.136	7.612	4	0.107	2.129	3	0.546
Baseline 5HT	41.243	41.243	1	142.000	1.784	0.184	0.004	1	0.949	0.134	1	0.714
Time	17248.218	8624.109	2	296.000	373.016	<0.001	1.280	1	0.258	4.848	1	0.028
Baseline 5HT:Time	94.948	47.474	2	296.000	2.053	0.130	1.141	1	0.286	0.543	1	0.461
No imputed values												
5HT across time												
Age	0.622	0.622	1	141.445	0.024	0.878	1.053	1	0.305	0.355	1	0.551
Sex	25.605	25.605	1	138.350	0.972	0.326	1.374	1	0.241	2.671	1	0.102
AD Class	166.043	41.511	4	134.057	1.575	0.184	6.847	4	0.144	3.048	4	0.550
5HT	17.121	17.121	1	338.576	0.650	0.421	0.435	1	0.509	0.385	1	0.535
Time	12295.410	6147.705	2	249.811	233.295	0.000	1.406	1	0.236	3.771	1	0.052
5HT:Time	19.865	9.933	2	283.272	0.377	0.686	0.189	1	0.664	2.781	1	0.095
Baseline 5HT												
Age	0.518	0.518	1	136.707	0.020	0.888	1.294	1	0.255	0.314	1	0.575
Sex	34.931	34.931	1	136.439	1.338	0.249	1.559	1	0.212	2.617	1	0.106
AD Class	190.356	47.589	4	128.826	1.823	0.128	6.423	4	0.170	2.864	4	0.581
Baseline 5HT	63.660	63.660	1	141.331	2.438	0.121	0.139	1	0.709	0.249	1	0.618
Time	13982.669	6991.334	2	238.675	267.752	0.000	1.331	1	0.249	4.484	1	0.034
Baseline 5HT:Time	45.154	22.577	2	248.735	0.865	0.422	1.028	1	0.311	0.072	1	0.789

III.2. The MAOA rs979605 genetic polymorphism is differentially associated with clinical improvement following antidepressant treatment in depressed males and females

MAO metabolizes monoamine neurotransmitters [e.g., 5-HT to 5-hydroxyindoleacetic acid (5-HIAA)], whose concentrations are targeted by first-line ATDs. Compared to healthy individuals, MAO activity is increased in depressed patients. Genetic variation within *MAOA* and *MAOB* (encoding MAO) is associated with clinical outcomes following ATD treatment in depressed patients. These genes are located on the X chromosome, which poses unique statistical challenges for genetic analysis since (1) females possess twice the number of X chromosomes compared to males and (2) some X chromosomal regions escape from X chromosome inactivation, which corrects for dosage differences between the sexes. Strategies to control for X-linked factors have been developed. We hypothesized that *MAOA* and *MAOB* genetic variation would be associated with clinical outcomes and the plasma 5-HIAA/5-HT ratio (as an estimate of MAO activity) following ATD treatment in patients of the METADAP cohort.

III.2.1. Overview of patients, materials, and methods

A total of 378 patients with ≥ 1 follow-up measure during the study were analyzed for HDRS score changes and response and remission rates after 1, 3, and 6 months of ATD treatment. Among them, 148 patients were analyzed for associations with the plasma 5-HIAA/5-HT ratio. *MAOA* and *MAOB* genetic variation was genotyped from leukocytic DNA using HTS. Variant calls were subjected to quality control and LD analysis. Common genetic variation ($MAF \geq 5\%$) was retained for analysis. 5-HIAA and 5-HT levels were measured from fasting plasma samples using UPLC-MS and imputed where needed. Linear mixed-effects models were constructed in R and adjusted *a priori* for age, sex, ATD class, and smoking status, as well as ATD-naïve status in the analysis of the plasma 5-HIAA/5-HT ratio. The sex \times genotype interaction and a dominance term were included to control for X-linked factors. The plasma 5-HIAA/5-HT ratio was log-transformed for analysis.

III.2.2. Principal results

Among the 378 patients, 6 common ($MAF \geq 5\%$) genetic polymorphisms were identified: 5 in *MAOA* and 1 in *MAOB* [rs1799836(T>C)]. The 5 *MAOA* genetic polymorphisms were in strong LD, so the rs979605(A>G) genetic polymorphism was selected as a proxy. Thus, rs979605 and rs1799836 were analyzed. The sex \times rs979605 interaction was significantly associated with the HDRS score. Compared to G allele carriers, sex significantly associated with the HDRS score among A allele carriers, suggesting a

differential association of the rs979605 A allele with the HDRS score depending on sex. Female AA homozygotes had a significantly higher HDRS score compared to male A carriers after 6 months of ATD treatment. The rs1799836 genetic polymorphism was associated with the plasma 5-HIAA/5-HT ratio. Overall, CC/C females/males had a lower plasma 5-HIAA/5-HT ratio compared to TT/T females/males.

III.2.3. Summary of the discussion

To date, this is the largest analysis of *MAOA/MAOB* genetic variation with clinical outcomes following ATD treatment in depressed patients. Previous analyses of *MAOA/MAOB* genetic variation with clinical outcomes following ATD treatment in depressed patients were performed in males and females separately. Our combined analysis allowed for the identification of a sex \times genotype interaction and a potential sex-dependent association of the *MAOA* rs979605 genetic polymorphism.

MAO activity is influenced by many factors that we attempted to control for in our analysis of the association between the rs1799836 genetic polymorphism and the plasma 5-HIAA/5-HT ratio. This analysis was limited by its high dropout rate, its sample size, and the fact that the 5-HIAA/5-HT ratio is an estimate of MAO activity calculated from peripheral metabolite levels, which may differ from those of the brain.

III.2.4. Conclusion

Female homozygotes of the *MAOA* rs979605(A>G) A allele had significantly worse clinical improvement following 6 months of ATD treatment compared to male A carriers, a finding that suggests a potential moderating role of sex in this association that warrants further investigation.

III.2.5. Publication

Our results were published as an Article in the International Journal of Molecular Sciences (2023 impact factor: 6.208) (Chappell et al., 2023). Findings were presented as a poster at the Congrès de la Société Française de Pharmacologie et de Thérapeutique (SFPT) in Limoges, France (June 12–14, 2023) and at the 7th International Congress of the European Society for Pharmacogenomics and Personalised Therapy (ESPT) in Copenhagen, Denmark (October 25–28, 2023).

Article

The MAOA rs979605 Genetic Polymorphism Is Differentially Associated with Clinical Improvement Following Antidepressant Treatment between Male and Female Depressed Patients

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Abstract: Major depressive disorder (MDD) is the leading cause of disability worldwide. Treatment with antidepressant drugs (ATD), which target monoamine neurotransmitters including serotonin (5HT), are only modestly effective. Monoamine oxidase (MAO) metabolizes 5HT to 5-hydroxy indoleacetic acid (5HIAA). Genetic variants in the X-chromosome-linked MAO-encoding genes, *MAOA* and *MAOB*, have been associated with clinical improvement following ATD treatment in depressed patients. Our aim was to analyze the association of *MAOA* and *MAOB* genetic variants with (1) clinical improvement and (2) the plasma 5HIAA/5HT ratio in 6-month ATD-treated depressed individuals. Clinical ($n = 378$) and metabolite ($n = 148$) data were obtained at baseline and up to 6 months after beginning ATD treatment (M6) in patients of METADAP. Mixed-effects models were used to assess the association of variants with the Hamilton Depression Rating Scale (HDRS) score, response and remission rates, and the plasma 5HIAA/5HT ratio. Variant \times sex interactions and dominance terms were included to control for X-chromosome-linked factors. The *MAOA* rs979605 and *MAOB* rs1799836 polymorphisms were analyzed. The sex \times rs979605 interaction was significantly associated with the HDRS score ($p = 0.012$). At M6, A allele-carrying males had a lower HDRS score ($n = 24$, 10.9 ± 1.61) compared to AA homozygous females ($n = 14$, 18.1 ± 1.87 ; $p = 0.0067$). The rs1799836 polymorphism was significantly associated with the plasma 5HIAA/5HT ratio ($p = 0.018$). Overall, CC/C females/males had a lower ratio ($n = 44$, 2.18 ± 0.28) compared to TT/T females/males ($n = 60$, 2.79 ± 0.27 ; $p = 0.047$). The *MAOA* rs979605 polymorphism, associated with the HDRS score in a sex-dependent manner, could be a useful biomarker for the response to ATD treatment.

Keywords: pharmacogenetics; major depressive disorder; serotonin; MAO; antidepressant drugs

1. Introduction

Major depressive disorder (MDD) is the current leading cause of disability worldwide [1]. Differences between sexes exist, too, as adult females are twice as likely to develop MDD and have a greater risk of experiencing longer and more severe depressive episodes compared to men [2]. Antidepressant drugs (ATD), which modulate monoamine neurotransmitter levels, remain a common treatment option for MDD. However, only about one-third of ATD-treated individuals achieve remission [3]. Identifying biomarkers to predict treatment response is thus an important clinical challenge.

Monoamine oxidase (MAO) metabolizes monoamine neurotransmitters, including serotonin (5HT) [4]. Two MAO proteins have been characterized, namely MAOA and MAOB, encoded by the neighboring X chromosome (Xp11.23) genes, *MAOA* and *MAOB*, respectively [4]. MAOA preferentially metabolizes 5HT and noradrenaline, while MAOB preferentially metabolizes phenylethylene and benzylamine [4,5]. MAO expression changes with age [4] and differs between males and females [5]. Smoking also decreases MAO activity [5] although former smokers exhibit increased platelet MAO activity [6]. MAOB is more abundant in the brain, and *MAOB* expression occurs mainly in serotonergic and histaminergic neurons and astrocytes, while *MAOA* is expressed in dopaminergic and noradrenergic neurons [4,7]. In the periphery, *MAOA* is predominantly expressed in fibroblasts, while MAOB is the sole MAO in platelets [4].

MAOA and *MAOB* density in various brain regions was increased in the context of a medication-free major depressive episode (MDE) compared to healthy controls [8,9]. *MAOA* density remained elevated in MDD individuals following treatment with a selective serotonin reuptake inhibitor (SSRI), although it was lower in individuals achieving remission compared to recurrent MDE sufferers [10]. Increased levels of plasma 5-hydroxyindoleacetic acid (5HIAA)—the main metabolite of 5HT—were also observed in unmedicated depressed individuals compared to healthy controls, suggesting greater 5HT metabolism in the context of MDD [11]. Since direct evaluation of MAO activity in the human brain is difficult, peripheral 5HT and 5HIAA levels may serve as a viable proxy [12]. Given their role in monoamine metabolism, *MAOA* and *MAOB* are interesting targets in neurological disorders [4,13]. As such, factors influencing their expression or function are of similar interest.

Genetic variants within the MAO-encoding genes have been studied in various psychiatric contexts, including depression, bipolar disorder, and schizophrenia [4,14]. Studies examining ATD response in MDD have focused notably on the upstream variable number tandem repeat (uVNTR) within the *MAOA* promoter. While some report no association with response [15–18], others report that the short-form uVNTR is associated with improved clinical outcomes [19–21]. Regarding *MAOA* polymorphisms, the rs6323 (NM_000240.4:c.891G>T) synonymous polymorphism has been the most analyzed. In several studies, the rs6323(T) allele was associated with improved treatment response [16,22,23] although in Han Chinese females with depression, it was closely associated with poorer response to venlafaxine [24]. The *MAOA* rs979605 (NM_000240.4:c.1262+69A>T/G) intronic polymorphism and rs6323 were also associated with reduced MAO enzymatic activity [5,25]. Regarding *MAOB*, it was observed that female TT homozygotes for the rs1799836 (NM_000898.5:c.1348-36A>G) intronic polymorphism responded better to treatment [26]. Lower MAO activity was also observed in Swedish rs1799836(T) allele carriers [27].

Different factors complicate the analysis of X-chromosomal variants. For one, females carry two gene copies (and thus two alleles), while males carry one. However, X-chromosome inactivation (XCI) silences 1 of the gene copies in females. Still, factors such as XCI skewness (i.e., imbalanced allelic expression) and escape from XCI (i.e., expression from both silenced and active chromosomes) [28]—for which conflicting findings for *MAOA/MAOB* have been reported [29–31]—may influence *MAOA/MAOB* expression. Due to this complexity, X- and Y-linked variants are sometimes omitted from genome-wide association studies of ATD response [32,33]. Those that include them [34,35], however, may

not adjust for these factors. As such, associations between X-chromosomal variants and clinical improvement after ATD treatment would benefit from controlling for these factors.

The associations of *MAOA/MAOB* genetic variants with clinical improvement after ATD treatment and MAO activity, which modulates monoamine concentrations—common targets of ATD therapy—qualify them as potential biomarkers for clinical improvement following ATD treatment in MDD. Given their X-chromosomal location, improved analysis of these genetic factors may help to explain sex differences in the context of MDD and its treatment. We attempted such an analysis by examining the association of *MAOA/MAOB* genetic variants with (1) clinical improvement following ATD treatment and (2) the plasma 5HIAA/5HT ratio as an estimate of MAO activity in a cohort of 6-month ATD-treated individuals with a current MDE in the context of MDD.

2. Results

2.1. Patient Demographics

Sociodemographic characteristics for the whole clinical and metabolomic cohorts and with respect to sex are shown in Table 1. For males ($n = 119$) and females ($n = 259$) of the clinical cohort, respectively, the mean age was 45.9 and 45 years, and 96% and 88% of subjects were Caucasian. Missing at follow-up rates relative to baseline were 4% and 6% after 1 month of ATD treatment (M1), 28% and 33% after 3 months (M3), and 46% and 47% after 6 months (M6).

2.2. Genetic Variant Selection

A total of 29 *MAOA* (26 single-nucleotide polymorphisms (SNP) and 3 insertion/deletions) and 25 *MAOB* (25 SNPs) genetic variants were identified. Among these, five *MAOA* SNPs and one *MAOB* SNP had a call rate $\geq 95\%$ and a minor allele frequency (MAF) $\geq 5\%$ (see Table 2). None significantly deviated from Hardy–Weinberg equilibrium (HWE). The five *MAOA* SNPs were in linkage disequilibrium (LD) (see Figure S1). The *MAOA* rs979605(A > G) genetic polymorphism was selected as a *MAOA* haplotype proxy for further analysis. The rs979605(G) allele was correlated with the rs6323(T) allele. Allelic frequencies did not significantly differ between males and females.

Table 1. Sociodemographic characteristics according to males and females.

		Total	Male	Female
		Clinical cohort		
		$n = 378$	$n = 119$	$n = 259$
Age (in years) (m \pm SD)		45.3 \pm 13.3	45.9 \pm 12.8	45 \pm 13.6
Education (n(%))	Primary	35(9)	11(9)	24(9)
	High school	166(44)	45(38)	121(47)
	University	176(47)	62(52)	114(44)
Ethnicity (n(%))	Caucasian	343(91)	114(96)	229(88)
	African	24(6)	1(1)	23(9)
	Mixed	10(3)	4(3)	6(2)
Smoking status (n(%))	Current	144(38)	48(40)	96(37)
	Former	48(13)	22(18)	26(10)
	Non	186(49)	49(41)	137(53)
Pack years (m \pm SD)		14 \pm 13.5	14.4 \pm 12	13.8 \pm 14.3
Recurrent MDE (n(%))		278(74)	79(66)	199(77)
Onset age MDE (in years) (m \pm SD)		35.2 \pm 14.4	36.6 \pm 15.1	34.5 \pm 14
Baseline HDRS (m \pm SD)		24.7 \pm 4.9	24.8 \pm 5	24.7 \pm 4.8
Therapy (n(%))	SSRI	156(41)	49(41)	107(41)
	SNRI	152(40)	49(41)	103(40)
	TCA	24(7)	6(5)	18(7)
	Other	34(9)	9(8)	25(10)
	ECT	12(3)	6(5)	6(2)
Missing at follow-up (n(%))	M1	20(5)	5(4)	15(6)
	M3	119(31)	33(28)	86(33)
	M6	176(47)	55(46)	121(47)

Table 1. Cont.

		Total	Male	Female
		<i>n</i> = 148	<i>n</i> = 52	<i>n</i> = 96
Metabolomic cohort				
Age (in years) (m ± SD)		46.2 ± 12.2	45.6 ± 11.8	46.5 ± 12.4
Education (n(%))	Primary	15(10)	5(10)	10(10)
	High school	58(39)	15(29)	43(45)
	University	74(50)	31(60)	43(45)
Ethnicity (n(%))	Caucasian	139(94)	49(94)	90(94)
	African	7(5)	1(2)	6(6)
	Mixed	2(1)	2(4)	0(0)
Smoking status (n(%))	Current	53(36)	22(42)	31(32)
	Former	15(10)	9(17)	6(6)
	Non	80(54)	21(40)	59(61)
Pack years (m ± SD)		13.6(11.3)	14.6(11.9)	12.8(10.9)
Recurrent MDE (n(%))		100(68)	30(58)	70(73)
Onset age MDE (in years) (m ± SD)		36.7 ± 14.4	37.5 ± 15.7	36.2 ± 13.7
Baseline HDRS (m ± SD)		23.4 ± 4.1	23.6 ± 4.5	23.3 ± 3.9
ATD-naive (n(%))		59(40)	22(42)	37(39)
Therapy (n(%))	SSRI	61(41)	20(38)	41(43)
	SNRI	61(41)	25(48)	36(38)
	TCA	12(8)	3(6)	9(9)
	Other	12(8)	4(8)	8(8)
	ECT	2(1)	0(0)	2(2)

Sociodemographic characteristics of the whole sample and in males and females of the clinical and metabolomic cohorts. ATD, antidepressant drug; ECT, electroconvulsive therapy; HDRS, 17-item Hamilton Depression Rating Scale; m, mean; M1, after 1 month of treatment; M3, after 3 months of treatment; M6, after 6 months of treatment; MDE, major depressive episode; n, number of individuals; SD, standard deviation; SNRI, serotonin norepinephrine reuptake inhibitor; SSRI, selective serotonin reuptake inhibitor; TCA, tricyclic antidepressant.

Table 2. Genomic and haplotypic information of the 6 selected MAOA and MAOB variants.

Rs#	Region	Variant Effect	RefSeq	Major Allele	Minor Allele	HWE _F	MAF _{Total} (%)	MAF _F (%)	MAF _M (%)	<i>p</i>
<i>MAOA</i>										
rs6323	Exon 8	p.Arg297Arg	NM_000240	T	G	0.56	32.7	32.9	36.8	0.32
rs2235186	Intronic			G	A	0.56	32.9	32.9	38.2	0.32
rs979606	Intronic			T	C	0.51	32.8	32.8	37.8	0.32
rs979605	Intronic			G	A	0.78	34.8	33.6	38.2	0.62
rs1137070	Exon 14	p.Asp470Asp	NM_000240	C	T	0.63	34.0	33.8	36.8	0.59
				Haplotype frequency						
				65.6%	33.7%					
<i>MAOB</i>										
rs1799836	Intronic			T	C	0.99	45.1	45.8	38.7	0.08

For each variant, we show the rs identifier (rs#), its genomic region, its protein-level effect and the corresponding RefSeq accession, the major and minor alleles, HWE (in females), the total and female- and male-specific MAFs, and the *p*-value of the χ^2 test comparing male and female frequencies. For MAOA variants, we also show the frequency of the major and minor allele haplotypes. F, female; HWE, Hardy–Weinberg equilibrium; M, male; MAF, minor allele frequency; *p*, *p*-value.

Sociodemographic characteristics with respect to sex and MAOA rs979605 and MAOB rs1799836 genotypes are shown in Tables 3 and 4, respectively. Significant differences in the missing at follow-up rate (M1) and in age and the prescribed therapy were observed between female MAOA rs979605 genotypes of the clinical and metabolomic cohorts, respectively (see Table 3). Likewise, significant differences in ethnicity and in smoking status were observed between female MAOB rs1799836 genotypes of the clinical and metabolomic cohorts, respectively (see Table 4).

Table 3. Sociodemographic characteristics according to MAOA rs979605 genotypes in males and females.

		MAOA rs979605						
		Male			Female			
		G	A	<i>p</i>	GG	GA	AA	<i>p</i>
		Clinical Cohort						
		<i>n</i> = 76	<i>n</i> = 43		<i>n</i> = 119	<i>n</i> = 108	<i>n</i> = 32	
Age (in years) (m ± SD)		46.8 ± 12.8	44.4 ± 12.9	0.28	43.3 ± 14.1	45.6 ± 13.5	49.3 ± 11	0.055
Education (n(%))	Primary	7(9)	4(9)	0.90	10(8)	10(9)	4(12)	0.24
	High school	27(36)	18(42)		48(40)	58(54)	15(47)	
	University	41(54)	21(49)		61(51)	40(37)	13(41)	
Ethnicity (n(%))	Caucasian	72(95)	42(98)	1.00	103(87)	99(92)	27(84)	0.46
	African	1(1)	0(0)		12(10)	8(7)	3(9)	
	Mixed	3(4)	1(2)		3(3)	1(1)	2(6)	
Smoking status (n(%))	Current	29(38)	19(44)	0.77	48(40)	37(34)	11(34)	0.52
	Former	14(18)	8(19)		14(12)	8(7)	4(12)	
	Non	33(43)	16(37)		57(48)	63(58)	17(53)	
Pack years (m ± SD)		14.1 ± 12	14.9 ± 12.3	0.92	13.6 ± 16.9	13.3 ± 12	16.6 ± 9.7	0.29
Recurrent MDE (n(%))		54(71)	25(58)	0.16	88(74)	87(81)	24(75)	0.46
Onset age MDE (in years) (m ± SD)		36.9 ± 14.2	36.1 ± 16.9	0.81	34.4 ± 14.8	33.9 ± 13	36.4 ± 14.4	0.70
Baseline HDRS (m ± SD)		25.3 ± 5.1	23.9 ± 4.8	0.13	24.5 ± 4.8	24.9 ± 5.1	24.9 ± 4.3	0.77
Therapy (n(%))	SSRI	30(39)	19(44)	0.81	49(41)	47(44)	11(34)	0.87
	SNRI	30(39)	19(44)		46(39)	43(40)	14(44)	
	TCA	4(5)	2(5)		8(7)	8(7)	2(6)	
	Other	7(9)	2(5)		13(11)	7(6)	5(16)	
	ECT	5(7)	1(2)		3(3)	3(3)	0(0)	
Missing at follow-up (n(%))	M1	4(5)	1(2)	0.65	2(2)	10(9)	3(9)	0.017 *
	M3	23(30)	10(23)	0.52	43(36)	30(28)	13(41)	0.26
	M6	36(47)	19(44)	0.85	59(50)	44(41)	18(56)	0.21
		Metabolomic Cohort						
		<i>n</i> = 19	<i>n</i> = 33		<i>n</i> = 42	<i>n</i> = 42	<i>n</i> = 12	
Age (in years) (m ± SD)		45.3 ± 11	45.8 ± 12.5	0.91	44 ± 12.6	46.7 ± 12.4	54.2 ± 8.9	0.023 *
Education (n(%))	Primary	3(16)	2(6)	0.26	3(7)	6(14)	1(8)	0.69
	High school	3(16)	12(36)		17(40)	20(48)	6(50)	
	University	13(68)	18(55)		22(52)	16(38)	5(42)	
Ethnicity (n(%))	Caucasian	19(100)	30(91)	0.70	39(93)	39(93)	12(100)	1.00
	African	0(0)	1(3)		3(7)	3(7)	0(0)	
	Mixed	0(0)	2(6)		0(0)	0(0)	0(0)	
Smoking status (n(%))	Current	9(47)	13(39)	0.93	14(33)	13(31)	4(33)	0.97
	Former	3(16)	6(18)		3(7)	2(5)	1(8)	
	Non	7(37)	14(42)		25(60)	27(64)	7(58)	
Pack years (m ± SD)		15.8 ± 13.3	13.9 ± 11.4	0.70	7.5 ± 8.2	15.7 ± 11.5	19.8 ± 10.3	0.51
Recurrent MDE (n(%))		9(47)	21(64)	0.38	30(71)	31(74)	9(75)	1.00
Onset age MDE (in years) (m ± SD)		38.7 ± 16.8	36.8 ± 15.3	0.60	36.6 ± 14	35.1 ± 12.9	38.9 ± 16.2	0.80
Baseline HDRS (m ± SD)		22.5 ± 3.5	24.2 ± 4.9	0.30	23.6 ± 4.2	23.1 ± 3.8	23.4 ± 3.5	0.86
ATD-naïve (n(%))		6(32)	16(48)	0.46	14(33)	20(48)	3(25)	0.28
Therapy (n(%))	SSRI	6(32)	14(42)	0.79	14(33)	25(60)	2(17)	0.038 *
	SNRI	11(58)	14(42)		18(43)	13(31)	5(42)	
	TCA	1(5)	2(6)		5(12)	2(5)	2(17)	
	Other	1(5)	3(9)		3(7)	2(5)	3(25)	
	ECT	0(0)	0(0)		2(5)	0(0)	0(0)	

Sociodemographic characteristics of males and females of the clinical and metabolomic cohorts according to MAOA rs979605 genotypes. Males are hemizygous for either allele G or A. Females are either GG homozygotes, GA heterozygotes, or AA homozygotes. Wilcoxon rank-sum tests were used to compare age, pack years, onset age of MDE, and baseline HDRS (presented as mean ± standard deviation) between males, while Kruskal–Wallis tests were used to compare these variables between females. Fisher’s exact tests were used to compare education status, ethnicity, smoking status at baseline, MDE recurrence, prescribed therapy, missing at follow-up rates over time, and ATD-naïve status (presented as the group number and percentage). * *p* < 0.05. ATD, antidepressant drug; ECT, electroconvulsive therapy; HDRS, 17-item Hamilton Depression Rating Scale; m, mean; M1, after 1 month of treatment; M3, after 3 months of treatment; M6, after 6 months of treatment; MDE, major depressive episode; n, number of individuals; *p*, *p*-value; SD, standard deviation; SNRI, serotonin norepinephrine reuptake inhibitor; SSRI, selective serotonin reuptake inhibitor; TCA, tricyclic antidepressant.

Table 4. Sociodemographic characteristics according to *MAOB* rs1799836 genotypes in males and females.

		<i>MAOB</i> rs1799836						
		Male			Female			
		T	C	<i>p</i>	TT	TC	CC	<i>p</i>
		Clinical Cohort						
		<i>n</i> = 71	<i>n</i> = 45		<i>n</i> = 70	<i>n</i> = 126	<i>n</i> = 61	
Age (in years)	(m ± SD)	45.6 ± 12.7	46.8 ± 13.4	0.69	44.2 ± 13.7	45.4 ± 13.4	45.6 ± 14	0.85
Education (n(%))	Primary	7(10)	4(9)	1	5(7)	13(10)	6(10)	0.5
	High school	27(38)	17(38)		38(54)	52(41)	30(49)	
	University	36(51)	24(53)		27(39)	61(48)	25(41)	
Ethnicity (n(%))	Caucasian	68(96)	43(96)	0.57	66(94)	114(90)	47(77)	<0.001 *
	African	0(0)	1(2)		2(3)	7(6)	14(23)	
	Mixed	3(4)	1(2)		1(1)	5(4)	0(0)	
Smoking status (n(%))	Current	26(37)	21(47)	0.6	26(37)	43(34)	25(41)	0.19
	Former	14(20)	8(18)		10(14)	8(6)	8(13)	
	Non	31(44)	16(36)		34(49)	75(60)	28(46)	
Pack years (m ± SD)		16 ± 14.1	12.5 ± 9.3	0.73	12.5 ± 12.3	17 ± 17.3	10.2 ± 9.4	0.10
Recurrent MDE (n(%))		48(68)	30(67)	1	53(76)	94(75)	50(82)	0.54
Onset age MDE (in years) (m ± SD)		36.6 ± 15.1	36.4 ± 15.7	0.91	35.9 ± 13.3	34.2 ± 14.4	34 ± 14.1	0.60
Baseline HDRS (m ± SD)		24.2 ± 4.6	25.5 ± 5.6	0.23	24.3 ± 4.9	25.1 ± 4.7	24.4 ± 5.1	0.31
Therapy (n(%))	SSRI	31(44)	17(38)	0.24	27(39)	54(43)	25(41)	0.37
	SNRI	26(37)	22(49)		30(43)	52(41)	20(33)	
	TCA	6(8)	0(0)		5(7)	10(8)	3(5)	
	Other	5(7)	3(7)		7(10)	7(6)	11(18)	
	ECT	3(4)	3(7)		1(1)	3(2)	2(3)	
Missing at follow-up (n(%))	M1	2(3)	2(4)	0.64	3(4)	9(7)	3(5)	0.78
	M3	16(23)	16(36)	0.14	28(40)	36(29)	21(34)	0.25
	M6	23(51)	23(51)	0.44	35(50)	56(44)	29(48)	0.75
		Metabolomic Cohort						
		<i>n</i> = 33	<i>n</i> = 19		<i>n</i> = 27	<i>n</i> = 44	<i>n</i> = 24	
Age (in years)	(m ± SD)	43.5 ± 12	49.2 ± 11	0.91	44.3 ± 13	48.3 ± 12.6	46.5 ± 10.7	0.50
Education (n(%))	Primary	3(9)	2(11)	0.26	3(11)	5(11)	2(8)	0.050
	High school	10(30)	5(26)		11(41)	15(34)	17(71)	
	University	19(58)	12(63)		13(48)	24(55)	5(21)	
Ethnicity (n(%))	Caucasian	31(94)	18(95)	0.70	27(100)	41(93)	21(88)	0.16
	African	0(0)	1(5)		0(0)	3(7)	3(12)	
	Mixed	2(6)	0(0)		0(0)	0(0)	0(0)	
Smoking status (n(%))	Current	14(42)	8(42)	0.93	8(30)	11(25)	11(46)	0.018 *
	Former	5(15)	4(21)		3(11)	0(0)	3(12)	
	Non	14(42)	7(37)		16(59)	33(75)	10(42)	
Pack years (m ± SD)		14.5 ± 13.4	14.8 ± 10.1	0.70	10.5 ± 10.6	17.7 ± 12.2	11.8 ± 9.8	0.054
Recurrent MDE (n(%))		20(61)	10(53)	0.38	19(70)	30(68)	20(83)	0.39
Onset age MDE (in years) (m ± SD)		36 ± 15.7	40.2 ± 15.8	0.60	36.9 ± 12.7	37 ± 14.1	34.8 ± 14.5	0.77
Baseline HDRS (m ± SD)		23.5 ± 4.3	23.8 ± 4.9	0.30	22 ± 3.2	23.7 ± 3.8	23.9 ± 4.5	0.15
ATD-naïve (n(%))		12(36)	10(53)	0.46	11(41)	18(41)	8(33)	0.70
Therapy (n(%))	SSRI	13(39)	7(37)	0.79	9(33)	20(45)	12(50)	0.14
	SNRI	14(42)	11(58)		13(48)	15(34)	7(29)	
	TCA	3(9)	0(0)		2(7)	7(16)	0(0)	
	Other	3(9)	1(5)		2(7)	2(5)	4(17)	
	ECT	0(0)	0(0)		1(4)	0(0)	1(4)	

Sociodemographic characteristics of males and females of the clinical and metabolomic cohorts according to *MAOB* rs1799836 genotypes. Males are hemizygous for either allele T or C. Females are either TT homozygotes, TC heterozygotes, or CC homozygotes. Wilcoxon rank-sum tests were used to compare age, pack years, onset age of MDE, and baseline HDRS (presented as mean ± standard deviation) between males, while Kruskal–Wallis tests were used to compare these variables between females. Fisher’s exact tests were used to compare education status, ethnicity, smoking status at baseline, MDE recurrence, prescribed therapy, missing at follow-up rates over time, and ATD-naïve status (presented as the group number and percentage). * *p* < 0.05. ATD, antidepressant drug; ECT, electroconvulsive therapy; HDRS, 17-item Hamilton Depression Rating Scale; m, mean; M1, after 1 month of treatment; M3, after 3 months of treatment; M6, after 6 months of treatment; MDE, major depressive episode; n, number of individuals; *p*, *p*-value; SD, standard deviation; SNRI, serotonin norepinephrine reuptake inhibitor; SSRI, selective serotonin reuptake inhibitor; TCA, tricyclic antidepressant.

2.3. Clinical Improvement According to Variant Genotypes

We first examined associations of the MAOA rs979605 and MAOB rs1799836 genetic polymorphisms with clinical measures using the proposed full model for analyzing X-chromosomal variants, including the dominance term and polymorphism × sex interaction. In the model of the 17-item Hamilton Depression Rating Scale (HDRS) score, the MAOA rs979605 genetic polymorphism × sex interaction was significant after controlling for other factors ($F_{1,367} = 6.32, p = 0.012$), corresponding to a difference in the association of rs979605 with the HDRS score depending on sex (see Table 5). The MAOB rs1799836 genetic polymorphism was not a significant factor in models of clinical outcomes at a Bonferroni-corrected level of $p < 0.025$, and neither were its interactions with time or sex (see Table 5).

Table 5. Clinical associations with MAOA rs979605 and MAOB rs1799836 genotypes using the proposed full model for X-chromosomal variants.

	HDRS						Response			Remission		
	SS	MS	df (num)	df (den)	F	p	χ^2	df	p	χ^2	df	p
MAOA rs979605												
Age	0.44	0.44	1	369.04	0.015	0.90	2.68	1	0.10	0.78	1	0.38
Sex	149.07	149.07	1	385.70	4.97	0.026	1.44	1	0.23	2.84	1	0.092
ATD class	122.64	30.66	4	374.77	1.02	0.40	2.92	4	0.57	6.22	4	0.18
Loss to follow-up (M1)	86.28	86.28	1	442.40	2.88	0.091	1.56	1	0.21	0.32	1	0.57
Smoking status	19.39	9.70	2	369.28	0.32	0.72	3.70	2	0.16	0.40	2	0.82
Time rs979605 dominance term	28,305.80	9435.27	3	857.69	314.42	<0.001 *	35.66	2	<0.001 *	36.81	2	<0.001 *
rs979605	7.16	7.16	1	894.03	0.24	0.63	0.011	1	0.91	1.02	1	0.31
Time:rs979605	0.14	0.14	1	392.04	0.0048	0.94	0.12	1	0.73	0.025	1	0.88
Sex:rs979605	181.21	30.20	6	867.34	1.01	0.42	1.77	4	0.78	8.04	4	0.090
Sex:Time	189.65	189.65	1	367.50	6.32	0.012 *	2.81	1	0.094	3.27	1	0.071
Sex:Time	251.64	83.88	3	868.36	2.80	0.039	3.00	2	0.22	0.22	2	0.90
MAOB rs1799836												
Age	5.93	5.93	1	359.18	0.20	0.66	2.77	1	0.10	0.97	1	0.32
Sex	4.12	4.12	1	352.08	0.14	0.71	0.99	1	0.32	2.11	1	0.15
ATD class	156.33	39.08	4	368.79	1.29	0.27	3.69	4	0.45	6.46	4	0.17
Ethnicity	81.42	40.71	2	366.62	1.35	0.26	4.35	2	0.11	0.29	2	0.86
Smoking status	16.58	8.29	2	364.20	0.27	0.76	3.10	2	0.21	0.10	2	0.95
Time rs1799836 dominance term	35,802.44	11,934.15	3	853.23	395.00	<0.001 *	32.94	2	<0.001 *	39.08	2	<0.001 *
rs1799836	31.48	31.48	1	845.36	1.04	0.31	0.95	1	0.33	1.47	1	0.22
Time:rs1799836	119.36	119.36	1	356.46	3.95	0.048	1.52	1	0.22	2.17	1	0.14
Sex:rs1799836	121.70	20.28	6	856.73	0.67	0.67	4.45	4	0.35	1.85	4	0.76
Sex:Time	3.34	3.34	1	354.56	0.11	0.74	0.74	1	0.39	0.26	1	0.61
Sex:Time	138.63	46.21	3	855.42	1.53	0.21	4.23	2	0.12	1.11	2	0.57

Results of mixed-effects models of the HDRS score, response, and remission using the proposed full model. HDRS was assessed using the Satterthwaite method while response and remission were assessed using the Wald chi-square method. * $p < 0.025$. ATD, antidepressant drug; den, denominator; df, degrees of freedom; F, F statistic; HDRS, 17-item Hamilton Depression Rating Scale; MS, mean squares; num, numerator; p, p-value; SS, sum of squares; χ^2 , chi-square.

We further investigated the association between rs979605 and the HDRS score in A and G allele subgroups under different XCI status assumptions. When modeling the HDRS score in A allele carriers ($n = 183$) assuming random XCI (i.e., males coded the same as homozygous females), sex was a significant factor at a Bonferroni-corrected level of $p < 0.0125$ to account for the four models examined after controlling for other factors ($F_{1,187} = 7.66, p = 0.0062$), corresponding to a difference in the association of the MAOA rs979605 A allele with the HDRS score depending on sex (see Table S1). At M6, a significantly higher HDRS score was observed in female rs979605 AA homozygotes ($n = 14, 18.1 \pm 1.87$) compared to male A carriers ($n = 24, 10.9 \pm 1.61$) following Bonferroni correction (coef. = 7.21, 95%CI

[3.03–11.38], $p = 0.0067$) (see Figure 1). No other allele-specific associations with the HDRS score were observed.

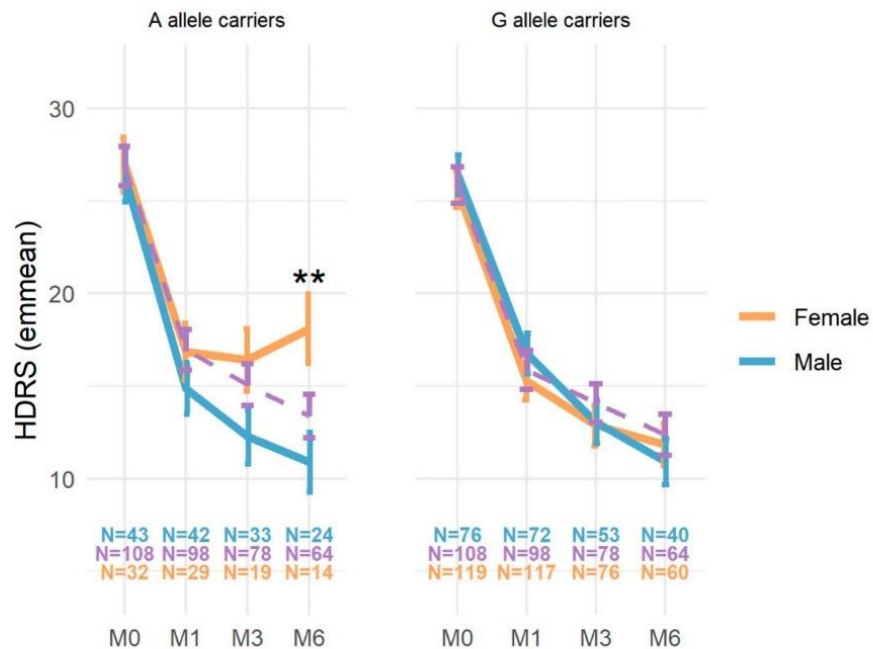


Figure 1. HDRS scores according to sex and *MAOA* rs979605 genotypes. Model estimates of the HDRS score (y-axis) at different study time points (x-axis) for *MAOA* rs979605 A allele female homozygotes (orange), female heterozygotes (purple dashed), and male carriers (blue). G allele carriers (right) are shown for comparison only. ** $p < 0.01$ following Bonferroni correction for the nine comparisons in A allele carriers. HDRS, 17-item Hamilton Depression Rating Scale.

We also examined the association of the *MAOA* rs979605 genetic polymorphism in male and female subgroups. However, neither it nor its interaction with time was a significant factor in either male or female populations after controlling for other factors.

2.4. MAO Activity Estimation According to Variant Genotypes

We also examined the associations of rs979605 and rs1799836 with the plasma 5HIAA/5HT ratio using the proposed full model. The plasma 5HIAA/5HT ratio was \log_2 transformed due to non-normality (see Figure S2). Baseline plasma 5HIAA/5HT ratios (mean = 8.84, SD = 11.15) were lower compared to ratios at M3 (mean = 15.02, SD = 12.75) and M6 (mean = 15.34, SD = 16.75). After controlling for other factors, including significant ones (i.e., age, ATD class, ATD-naïve status, and time), the plasma 5HIAA/5HT ratio was significantly associated with the *MAOB* rs1799836 genetic polymorphism ($F_{1,133} = 5.92$, $p = 0.016$) (see Table 6). In a model without the non-significant sex interactions, rs1799836 remained significant after controlling for the same factors ($F_{1,135} = 6.00$, $p = 0.016$) (see Table S2). Globally, TT/T females/males had a significantly higher 5HIAA/5HT ratio (2.79 ± 0.27) compared to CC/C females/males (2.18 ± 0.28) following Bonferroni correction for the three comparisons (coef. = 0.61, 95%CI [0.12–1.11], $p = 0.047$) (see Figure 2).

Table 6. Plasma 5HIAA/5HT ratio associations with MAOA rs979605 and MAOB rs1799836 genotypes.

	SS	MS	df (num)	df (den)	F	p
MAOA rs979605						
Age	11.61	11.61	1	135.08	5.99	0.016 *
Sex	0.33	0.33	1	136.39	0.17	0.68
ATD class	46.99	11.75	4	136.21	6.07	<0.001 *
Smoking status	2.66	1.33	2	136.27	0.69	0.50
ATD-naïve status	22.77	22.77	1	376.32	11.76	<0.001 *
Time	41.03	20.52	2	300.99	10.60	<0.001 *
rs979605 dominance term	0.02	0.02	1	330.21	0.0083	0.93
rs979605	2.55	2.55	1	135.88	1.32	0.25
Time:rs979605	0.26	0.07	4	285.41	0.034	1.00
Sex:rs979605	3.45	3.45	1	135.91	1.78	0.18
Sex:Time	3.81	1.91	2	285.33	0.98	0.38
MAOB rs1799836						
Age	15.53	15.53	1	133.66	8.02	<0.001 *
Sex	0.71	0.71	1	134.63	0.37	0.55
ATD class	42.30	10.58	4	134.71	5.47	<0.001 *
Smoking status	2.47	1.23	2	134.54	0.64	0.53
ATD-naïve status	23.74	23.74	1	372.91	12.27	<0.001 *
Time	40.46	20.23	2	298.70	10.45	<0.001 *
rs1799836 dominance term	0.00	0.00	1	313.20	0.0023	0.96
rs1799836	11.46	11.46	1	133.94	5.92	0.016 *
Time:rs1799836	2.91	0.73	4	282.18	0.38	0.83
Sex:rs1799836	0.25	0.25	1	134.42	0.13	0.72
Sex:Time	2.42	1.21	2	282.57	0.63	0.54

Results for mixed-effects models of the 5HIAA/5HT ratio assessed using the Satterthwaite method. * $p < 0.025$. ATD, antidepressant drug; den, denominator; df, degrees of freedom; F, F statistic; MS, mean squares; num, numerator; p, p-value; SS, sum of squares.

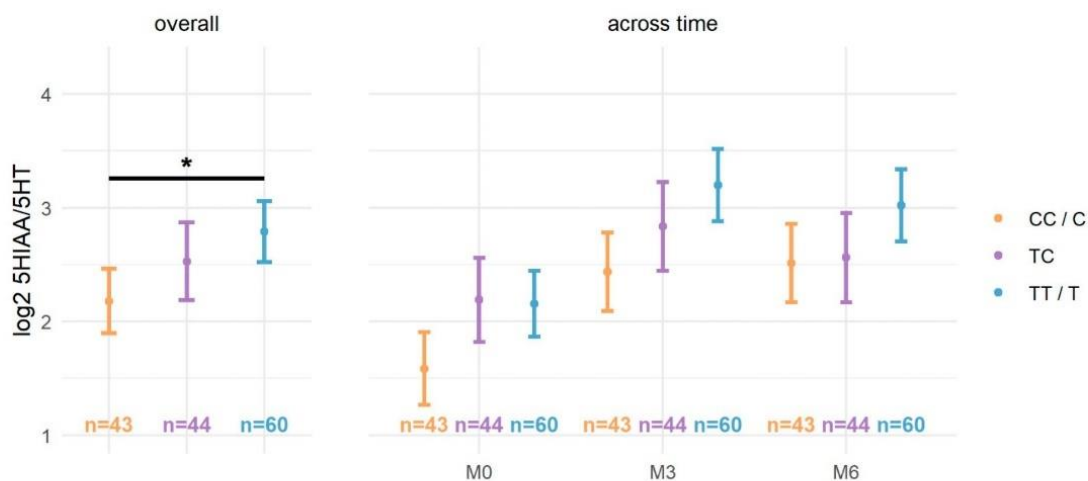


Figure 2. Log₂ 5HIAA/5HT ratios according to MAOB rs1799836 genotypes. The log₂ 5HIAA/5HT ratio (y-axis) according to rs1799836 genotypes (see legend) both overall (left) and across time (right). * $p < 0.05$ following Bonferroni correction for the three comparisons.

We also examined whether baseline or serial log₂-transformed plasma 5HIAA/5HT ratios were associated with clinical outcomes. However, neither 5HIAA/5HT ratios nor their interactions with rs979605 and rs1799836 genotypes or time were significant factors in models of clinical outcomes after controlling for other factors.

2.5. Power Analysis

We had sufficient power to detect an $R^2 = 3\%$ for the HDRS score ($SD = 7.48$) between genotypes for both rs979605 and rs1799836 among the 259 individuals at M3 and an $R^2 = 5.2\%$ for baseline plasma 5HIAA/5HT ratio ($SD = 1.97$) variation between genotypes for both polymorphisms among the 148 individuals of the metabolomic cohort.

3. Discussion

In this ancillary investigation of METADAP, we analyzed the association between the rs979605 and rs1799836 genetic polymorphisms of *MAOA* and *MAOB*—encoding for the monoamine oxidases—respectively, and clinical improvement following ATD treatment in depressed patients. The allele frequency of rs1799836(C) in METADAP (45.1%) was comparable to that of non-Finnish Europeans of gnomAD (45.6%) [36], but rs979605(A) was more frequent in METADAP (34.8%), notably in males (38.2%) compared to males of this same gnomAD population (29.7%). The *MAOB* rs1799836 genetic polymorphism was not significantly associated, and neither were its interactions with time or sex, with clinical outcomes. The rs979605 \times sex interaction was significantly associated with the HDRS score, and after 6 months of treatment, a significantly higher HDRS score was observed in female rs979605 AA homozygotes compared to male A carriers.

The *MAOA* rs6323 polymorphism—in LD with rs979605—was previously examined in association with response to ATD treatment in several contexts, including rapid versus delayed response and response in placebo- versus ATD-treated individuals [16,22]. Its analysis in two cohorts of depressed patients (baseline HDRS score ≥ 18) following several weeks of ATD treatment corresponds best with our own [23,24]. However, where Bi et al. observed that rs6323(T) was associated with poorer clinical outcomes in 6-week venlafaxine-treated Han Chinese females [24], Tadić et al. observed that, among females of their European population, TT homozygotes had significantly improved HDRS scores compared to G allele carriers after 4 weeks of mirtazapine treatment [23]. The former finding may be due to ethnic differences. Indeed, in our analysis of predominantly Caucasian (~90%) and 6-month SSRI- or SNRI-treated (81%) depressed patients (baseline HDRS score ≥ 18), we observed that females homozygous for rs979605(G)—in LD with rs6323(T)—had decreased HDRS scores compared to AA homozygotes although these differences were not significant.

Although we examined male and female populations separately like Tadić et al., associations with rs979605 only approached significance in these subpopulations. However, by analyzing males and females together, we observed a significant rs979605 \times sex interaction. This finding suggests that genetic variants of *MAOA* may have an influence on the response to ATD therapy in depressed patients in a sex-dependent manner. Importantly, this observation could extend to other X-chromosomal genetic polymorphisms involved in ATD pharmacodynamics.

We also examined the plasma 5HIAA/5HT ratio as an estimate of MAO activity. Decreased platelet MAO activity was previously reported in female *MAOA* rs979605(G) carriers [5] and male *MAOB* rs1799836(T) carriers [27]. We observed a significant association of the plasma 5HIAA/5HT ratio with rs1799836 but not rs979605. Overall, TT/T females/males had a significantly higher ratio compared to CC/C females/males, which could be indicative of increased MAO activity, a result that contradicts previous findings [27]. However, compared to this study, we analyzed a population of depressed individuals. Importantly, MAO activity was shown to differ in depressed individuals compared to healthy controls [8,9]. MAO expression and activity is influenced by demographic—including age, sex, and smoking [5]—and biological factors, including epigenetics [37], which, at least for *MAOB*, is influenced by smoking [6]. We were able to control for the former but not the latter (at least not directly and not completely). Additionally, we estimated MAO activity from peripheral plasma 5HT and 5HIAA levels measured using ultraperformance liquid chromatography coupled with mass spectrometry (UPLC-MS), while the previous studies measured MAO activity using radiometric assays. Of note, most peripheral 5HT is stored in platelets that only contain *MAOB*, which only weakly

metabolizes 5HT [7], while the rest is metabolized in peripheral organs [38]. Peripheral 5HT metabolism may differ from that of the brain. However, platelet MAO activity and MAOB density in the prefrontal cortex were both increased in depressed individuals [4,9], suggesting that MAO expression/activity in peripheral platelets may mirror that of the brain [4].

MAO expression is influenced by many factors. The sex hormones estrogen and progesterone can inhibit and promote 5HT degradation, respectively [39]. Glucocorticoids can also regulate brain MAOA activity [4,40]. *MAOA* and *MAOB* promoter regions contain binding elements for Sp1 [7], a transcription factor (TF) that regulates the expression of many genes. Altered Sp1 binding activity was observed in 3-week fluoxetine-treated rats [41], suggesting a role for Sp1 in the response to ATDs. At least for *MAOA*, members of the Sp family can bind to these Sp1-binding sites and either activate (Sp1, Sp4) or inhibit (Sp3) transcription [42]. Sp1 can also interact with transcription machinery and other TFs [42,43], including the glucocorticoid-regulated CDCA7L/R1 protein, and possibly the estrogen receptor [44]. The five *MAOA* genetic polymorphisms we identified were in LD. It is intriguing to speculate that some of these genetic polymorphisms are functional and may disrupt downstream processes linked to ATD therapy response, for example, through their interaction(s) with TFs. The potential and varied interactions between TFs and sex hormones in the context of MDD may contribute to the differential response to ATD treatment we observed between female rs979605(A) homozygotes and male A carriers.

Recent evidence suggests that pharmacogenomics-guided care improves treatment outcomes in depressed patients compared to treatment-as-usual, with 40% and 49% increases in response and remission, respectively [45]. It remains possible that an expanded genomic panel may further improve guided care. Polygenic risk scores also show promise as predictive tools although, to date, only nominal associations with antidepressant response in the context of MDD have been reported [46]. Given the differences between males and females in the context of depression [2], sex-specific factors may help improve the utility of each method. Although our results suggest a sex-dependent association of the *MAOA* rs979605 polymorphism with response following ATD treatment, this should be further replicated in an independent cohort of depressed individuals.

The present study has several limitations. First, the proportion of missing data was high. However, the proportion at M6 was comparable to that of the STAR*D cohort at 12 weeks [47]. Second, the genetic and metabolomic cohorts greatly differ in size. Studies with greater sample sizes and a balance of males and females would help corroborate our findings. Third, we only had access to peripheral metabolite levels, which may differ from those in the brain. This study benefits from its naturalistic and prospective design, which allows for the analysis of treatment response across a 6-month period in a “real-world” clinical setting. The modeling strategy by Chen et al. [48] also allowed us to more robustly analyze these X-chromosomal variants. To date, and to the best of our knowledge, this is the largest analysis of *MAOA/MAOB* variants in clinical response following ATD treatment in a predominantly Caucasian population of depressed patients.

4. Materials and Methods

4.1. Study Design

Do Antidepressants Induce Metabolic Syndromes (METADAP) is a 6-month prospective, multicentric, and observational cohort study carried out in a psychiatric setting [49]. Individuals with a current MDE were treated in naturalistic conditions and assessed before and during ATD treatment. This study was registered by the French National Agency for Medicine and Health Products Safety (ANSM) and the Commission Nationale de l'Informatique et des Libertés (CNIL). It was approved by the Ethics Committee of Paris-Boulogne (France) and conformed to international ethical standards (ClinicalTrials.gov identifier: NCT00526383).

4.2. Patient Population

Males and females 18–65 years of age without a serious medical or inflammatory condition were recruited. Inclusion criteria were presentation with a MDE in the context of MDD (DSM-IVTR)—assessed by the Mini International Neuropsychiatric Interview (MINI)—a score ≥ 18 on the HDRS [50], and need for a new ATD treatment. Individuals with psychotic disorder, bipolar disorder, alcohol or drug dependence, or an eating disorder were excluded, as previously described [49]. Measures and samples were obtained prior to beginning ATD treatment (M0) and at M1, M3, and M6.

Among the 643 individuals included in METADAP, 19 had major protocol deviations and were excluded. Of the 624 available for analysis, 519 individuals provided samples for genetic studies. From these, 400 underwent high-throughput sequencing. Nine samples were removed due to technical difficulties ($n = 2$), having a heterozygous call as a male ($n = 1$), or for having a missing genotype rate $> 50\%$ ($n = 6$). Ten samples without follow-up measures (i.e., M0 only) were removed given our objective to examine clinical response following ATD treatment. Three samples treated by MAO inhibitors were removed given our objective to examine the influence of *MAOA/MAOB* gene variants on clinical response, including their potential impact on MAO function. Thus, 378 individuals were analyzed (clinical cohort). Dropouts occurred mainly because of ATD changes, unauthorized drug use, or loss to follow-up. Ancestry was self-reported. Caucasian individuals were defined as having Caucasian parents; African individuals as having Sub-Saharan African and/or Afro-Caribbean parents; and Asian individuals as having East Asian, Central Asian, and/or South Asian parents [51]. In the original metabolite analysis of METADAP [38], 173 individuals were analyzed. Among them, 148 were present within the clinical cohort and analyzed (metabolomic cohort). All individuals provided written informed consent for study participation and genetic analyses [49].

4.3. Antidepressant Treatment

Monotherapies were prescribed by a psychiatrist in a “real world” psychiatric treatment setting as previously described [49] and belonged to one of five classes: SSRI; serotonin norepinephrine reuptake inhibitors (SNRI); tricyclic antidepressants (TCA); other ATDs; and electroconvulsive therapy (ECT). In this ancillary study of 378 individuals, 41% were prescribed an SSRI, 40% an SNRI, 7% a TCA, 9% another ATD treatment, and 3% received ECT. If a change in treatment was required during follow-up, the individual was dropped from the study.

4.4. Clinical Improvement after Antidepressant Treatment

The HDRS [50] was used to assess depression severity at M0 and clinical improvement after ATD treatment at M1, M3, and M6. Responders and remitters were defined by a decreased HDRS score $\geq 50\%$ relative to baseline and a HDRS score ≤ 7 after ≥ 4 weeks of treatment, respectively. Clinical assessments were performed blind to genotyping results. Each interview and diagnostic assignment was reviewed by a senior psychiatrist. For each individual, all visits were reviewed by the same psychiatrist.

4.5. High-Throughput Sequencing, Sequence Alignment, and Variant Calling

For sequencing, 5 mL of whole blood was collected at baseline. Leukocytic DNA was extracted from 1 mL of blood using a Puregene Kit (Gentra systems, Minneapolis, MN, USA) and stored at $-20\text{ }^{\circ}\text{C}$. DNA was sequenced using a targeted-exome panel of genes involved in mood disorders and ATD metabolism. The protocols for high-throughput sequencing [52] and information about the gene panel and variant calling [53] are described elsewhere.

4.6. Genetic Variant Selection

Variant call format data were loaded into R (v4.1.0) [54] using the vcfR package (v1.9.0) [55], as previously described [56]. Variant calls with a sequencing depth < 20 , SNPs

with a quality score (QUAL) < 275, insertion/deletions with a QUAL < 770, heterozygous calls with an allele balance (AB) < 0.34 or >0.79, and homozygous calls with an AB < 0.96 were annotated as poor-quality calls. Variants with a call rate (# poor-quality calls/# calls) < 95% were removed [57]. Variants with a MAF \geq 5% were selected for further analysis.

4.7. Haplotype Analysis, Genotype Imputation, and Functional Annotation

HWE and LD were assessed using Haploview (v4.2) [58]. Variants were considered to be in LD by a $r^2 \geq 0.8$, as assessed in the subgroup of Caucasians comprising 91% of the study population. The variant with the highest MAF was selected as a haplotype proxy for further analysis.

4.8. 5HT and 5HIAA Measurement

Methods for metabolite level determination were previously described [38]. Metabolite extracts were prepared from 50 μ L of fasting plasma stored at -80°C via protein precipitation with 250 μ L of acetonitrile, evaporation under nitrogen, and reconstitution in 50 μ L of water. Three reference human plasma samples were prepared in each analytical batch as a control within and between analyses. UPLC-MS was performed using a Waters Acquity UPLC system coupled to a Thermo Scientific Q Exactive mass spectrometer. A proportion of values were below the lower limits of quantification (LLOQ) for 5HT (21.4%; LLOQ: 0.00113 μM) and 5HIAA (3.6%; 0.0131 μM) and were imputed as LLOQ/ $\sqrt{2}$ (5HT:0.000799 μM ; 5HIAA:0.00926 μM), as previously described [38].

4.9. Data Analysis

Statistical analyses were performed using R (v4.1.0) [54]. Quantitative variables were analyzed using nonparametric Wilcoxon rank sum or Kruskal–Wallis tests. Qualitative variables were analyzed using Fisher’s exact tests. Linear mixed-effects models and generalized linear mixed-effects models were constructed using the lme4 package (v1.1-27.1) [59]. The main variable to be explained was the HDRS total score. Other variables to explain were the response and remission rates and the plasma 5HIAA/5HT ratio. Chen et al. recently proposed a method (referred to as the proposed full model) that is robust to XCI, XCI skewness, and escape from XCI [48]. Briefly, unknown XCI status and XCI skewness were accounted for by analytical equivalencies to the variant \times sex interaction and dominance term (i.e., female heterozygotes coded as 1 and all others as 0), respectively. Variant genotype and its interactions with time and sex were the main explanatory variables examined. Age, sex, the dominance term, time, and the sex \times time interaction were included a priori as fixed-effects covariables in all full models. ATD class was also included a priori in all models to control for potential differences in treatment efficacy (in clinical models) or the plasma 5HIAA/5HT ratio (in metabolomic models) between treatment classes (e.g., SSRIs and ECT). As smoking and smoking history impact MAO activity [5,6], smoking status (i.e., non-smoker, current smoker, or former smoker) was also included a priori in all models. Since ATDs alter 5HT levels, ATD-naïve status was also included as a fixed-effect covariable in models of the plasma 5HIAA/5HT ratio. Sociodemographic and baseline clinical variables that significantly differed (i.e., $p < 0.05$) between genotypes were also included as fixed-effects covariables. Individual was included as a random-effect to account for repeated measures. Significance of fixed effects was assessed using the Satterthwaite method in linear mixed-effects models and the Wald chi-square test in generalized linear mixed-effects models. As one MAOA SNP and one MAOB SNP were analyzed, a Bonferroni-corrected threshold of $p < 0.025$ ($0.05/2$) was considered significant. If variant \times sex interactions were significant in the full model, mixed-effects models were constructed in allelic subgroups under different XCI status assumptions (i.e., using different coding strategies). Post hoc comparisons were carried out using the emmeans package [60].

Power analyses were performed using the genpwr package assuming an additive genetic model [61]. MAFs of 29.8% (rs979605) and 45.6% (rs1799836) as observed in non-Finnish Europeans of gnomAD [36], an alpha level of 5%, and a power of 80% were used.

5. Conclusions

In conclusion, we observed that the *MAOA* rs979605(A>G) polymorphism was significantly associated with clinical improvement following ATD treatment in a sex-dependent manner. Female rs979605(A) homozygotes had higher HDRS scores compared to male A carriers after 6 months of treatment. Since rs979605 is linked to other *MAOA* genetic variants, this association may be due to the effect(s) of other linked variants. Given the role of *MAOA* in 5HT metabolism and previous associations of *MAOA* genetic variants with clinical improvement after ATD treatment, their potential as biomarkers for clinical improvement following ATD therapy should continue to be investigated.

Supplementary Materials: The supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/ijms24010497/s1>.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are under the protection of health data regulation set by the French National Commission on Informatics and Liberty (Commission Nationale de l'Informatique et des Libertés, CNIL) in line with European regulations, the Data Protection Act, and the Comité de protection des personnes (CPP, equivalent to the Research Ethics Committee). Data are not publicly available, as French law forbids free access to METADAP data. Data are available upon reasonable request to the principal investigator of the study (emmanuelle.corruble@aphp.fr).

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III.2.6. Supplementary Materials

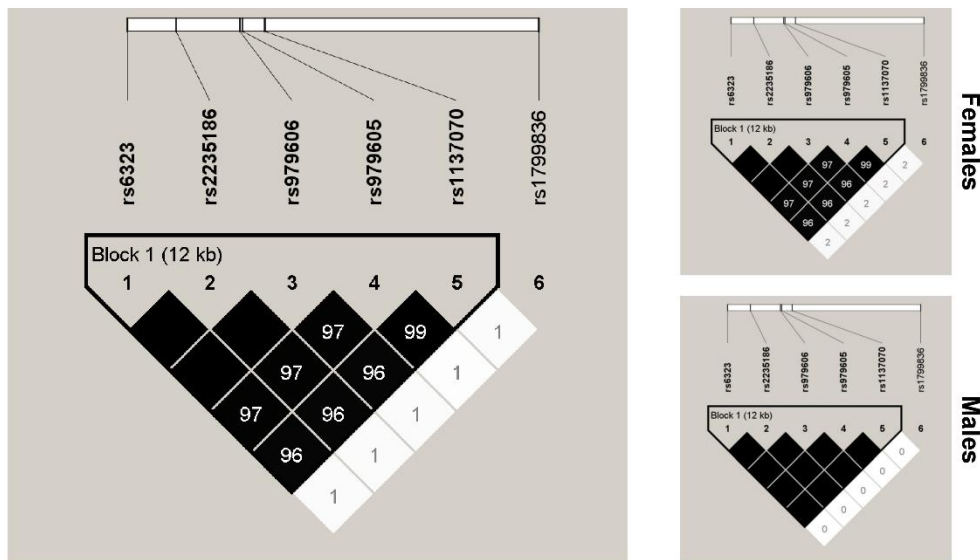


Figure S1: LD associations between MAOA and MAOB variants

For the 6 genetic variants of MAOA (rs6323, rs2235186, rs979606, rs979605, and rs1137070) and MAOB (rs1799836), we show the LD plots constructed using HaploView (v4.2) in the whole population as well as in males and females separately. Values within boxes correspond to r^2 values. Variants with an $r^2 > 80$ are considered in strong LD. Block 1 defines the haplotype block for variants observed to be in strong LD.

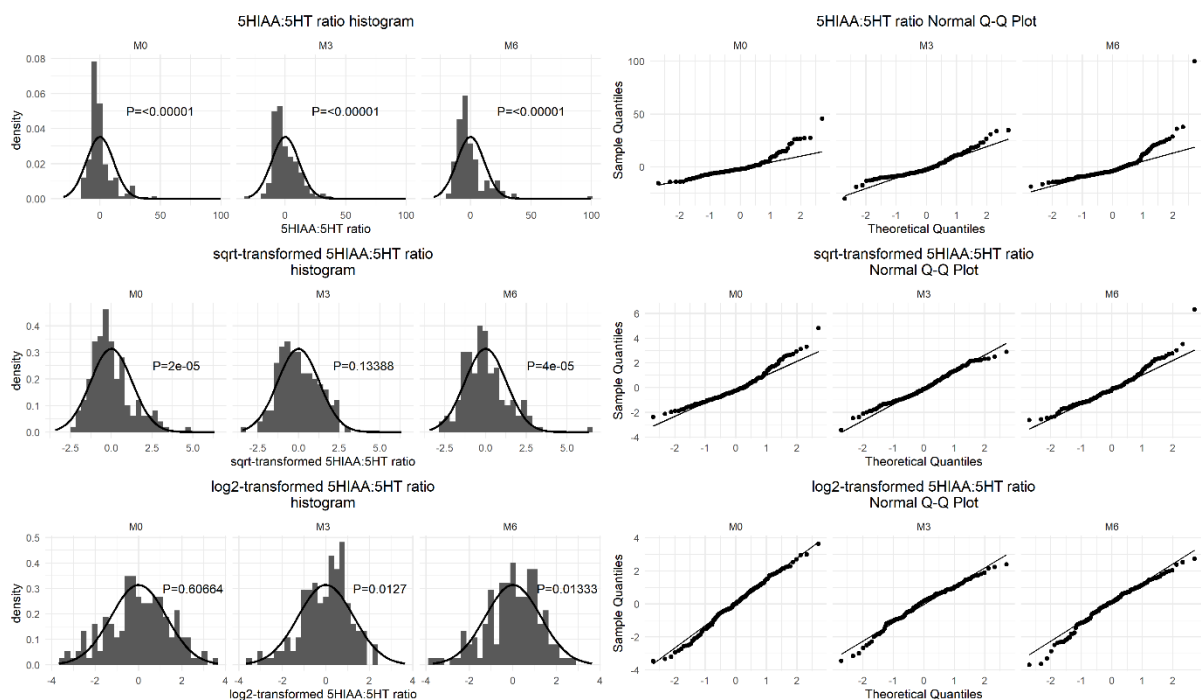


Figure S2: 5HIAA/5HT transformations

For each of the non-transformed (top), square-root-transformed (middle), and log₂-transformed (bottom) 5HIAA/5HT ratios at each time point (M0: left; M3: center; M6: right) are shown density histograms (left) and QQ plots (right). For histograms, 5HIAA/5HT ratios (x-axis) are plotted according to density (y-axis) in gray bars, while the normal distribution is shown as a black curve in histograms. *P*-values correspond to Shapiro-Wilk tests of normality.

Table S1: Associations of the HDRS score with MAOA rs979605 allelic subgroups

Results of mixed-effects models of the HDRS score in rs979605(A>G) allelic subgroups. Allelic subgroups were analyzed according to random XCI and escape from XCI status. HDRS was assessed using the Satterthwaite method. *: $P < 0.0125$ to account for the 4 analyses. **den**: denominator; **df**: degrees of freedom; **MS**: mean squares; **num**: numerator; **P**: P-value; **SS**: sum of squares; **XCI**: X-chromosome inactivation.

	Coding strategy	<i>A allele</i>						<i>G allele</i>					
		SS	MS	df (num)	df (den)	F	P	SS	MS	df (num)	df (den)	P	
		Age	17.26	17.26	1	175.27	0.64	0.43	4.56	4.56	1	295.99	0.14
Sex	208.15	208.15	1	187.82	7.66	0.0062*	20.52	20.52	1	289.49	0.65	0.42	
AD Class	124.54	31.14	4	181.84	1.15	0.34	123.44	30.86	4	303.02	0.98	0.42	
Loss to follow-up (M1)	Random XCI <i>Females (0, 1), Males (1)</i>	168.16	168.16	1	205.11	6.19	0.014	53.82	53.82	1	361.62	1.70	0.19
Smoking status		37.72	18.86	2	177.10	0.69	0.50	21.14	10.57	2	296.73	0.33	0.72
Time		13198.22	4399.41	3	410.00	161.92	<0.001*	29730.27	9910.09	3	694.95	313.17	<0.001*
rs979605		51.88	51.88	1	192.39	1.91	0.17	25.48	25.48	1	287.37	0.81	0.37
Sex:Time		258.50	86.17	3	418.07	3.17	0.024	87.66	29.22	3	699.71	0.92	0.43
Time:rs979605	183.20	61.07	3	421.42	2.25	0.082	21.93	7.31	3	697.86	0.23	0.87	
Age	17.26	17.26	1	175.27	0.64	0.43	4.56	4.56	1	295.99	0.14	0.70	
Sex	105.15	105.15	1	165.49	3.87	0.051	0.11	0.11	1	287.29	0.0035	0.95	
AD Class	124.54	31.14	4	181.84	1.15	0.34	123.44	30.86	4	303.02	0.98	0.42	
Loss to follow-up (M1)	Escape from XCI <i>Females (0, 1), Males (0)</i>	168.16	168.16	1	205.11	6.19	0.014	53.82	53.82	1	361.62	0.70	0.19
Smoking status		37.72	18.86	2	177.10	0.69	0.50	21.14	10.57	2	296.73	0.33	0.72
Time		16946.01	5648.67	3	411.24	207.90	<0.001*	29730.27	9910.09	3	694.95	313.17	<0.001*
rs979605		23.52	23.52	1	177.45	0.87	0.35	25.48	25.48	1	287.37	0.81	0.37
Sex:Time		79.88	26.63	3	411.19	0.98	0.40	120.40	40.13	3	698.27	1.27	0.28
Time:rs979605	183.20	61.07	3	421.42	2.25	0.082	21.93	7.31	3	697.86	0.23	0.87	

Table S2: Associations of the plasma 5HIAA/5HT ratio with MAOB rs1799836 without sex interactions

Results for mixed-effects models of the 5HIAA/5HT ratio without sex interactions included, assessed using the Satterthwaite method. *: $P < 0.05$. **den**: denominator; **df**: degrees of freedom; **MS**: mean squares; **num**: numerator; **P**: P -value; **SS**: sum of squares.

	SS	MS	df (num)	df (den)	F	P
Age	15.46	15.46	1	134.69	8.01	0.0054*
Sex	0.71	0.71	1	135.70	0.37	0.55
AD class	42.27	10.57	4	135.74	5.47	<0.001*
Smoking status	2.45	1.22	2	135.64	0.63	0.53
AD-naïve status	24.38	24.38	1	376.58	12.63	<0.001*
Time	40.30	20.15	2	301.02	10.44	<0.001*
rs1799836 dominance term	0.017	0.017	1	300.00	0.0086	0.93
rs1799836	11.59	11.59	1	135.00	6.00	0.016*
Time:rs17998361	5.03	1.26	4	284.31	0.65	0.63

III.3. The association of *ARRB1* genetic polymorphisms with response to antidepressant treatment in depressed patients

β -arrestin 1 is involved in receptor desensitization, protein scaffolding, and G protein-independent signaling. Compared to healthy individuals, β -arrestin 1 levels are lower in depressed patients but normalize after ATD treatment. Genetic variation within *ARRB1* (encoding β -arrestin 1) is associated with MDD and the response to ATD treatment in depressed patients. Advancements in genomic technology have allowed for functional annotation and rare variant detection. Functional annotation can help guide genetic analysis, while the analysis of rare genetic variation requires different analytical approaches. We hypothesized that common *ARRB1* genetic variation with potential functional consequences and the accumulation of rare *ARRB1* genetic variation would be associated with clinical outcomes following ATD treatment in patients of the METADAP cohort.

III.3.1. Overview of patients, materials, and methods

A total of 388 patients with ≥ 1 follow-up measure during the study were analyzed for HDRS score changes and response and remission rates after 1, 3, and 6 months of ATD treatment. *ARRB1* genetic variation was genotyped from leukocytic DNA using HTS. Variant calls were subjected to quality control and LD analysis and separated into rare ($MAF < 5\%$) and common ($MAF \geq 5\%$) genetic variation. Common genetic variation with a RegulomeDB score ≤ 2 was retained for analysis. Variant set analyses were used to examine the association of rare genetic variation with clinical measures and adjusted *a priori* for age, sex, and ATD class. Linear mixed-effects models were constructed in R and adjusted *a priori* for age, sex, and ATD class to analyze the association of the HDRS score and response and remission rates with common genetic variants and rare variant counts.

III.3.2. Principal results

Among the 388 patients analyzed, 953 *ARRB1* genetic variants were identified: 643 rare and 12 common with a RegulomeDB score ≤ 2 . Rare genetic variation was significantly associated with the HDRS score and remission rates in variant set analyses, while rare variant counts were significantly associated with the HDRS score and response and remission rates. Among the 12 common genetic variants, the time \times rs536852(A>G) and time \times rs553664(G>A) interactions were significantly associated with the HDRS score and remission. After 6 months of treatment, rs553664 AA homozygotes and rs536852 GG homozygotes had higher HDRS scores—and for rs536852, lower remission rates—compared to heterozygotes. rs536852 was not observed to be in LD with any genetic variants from data

of the 1000 Genomes Project. Several proteins, including FOS and JUN, were annotated as binding to regions containing rs536852 and rs553664.

III.3.3. Summary of the discussion

Our targeted analysis of *ARRB1* genetic variation with clinical outcomes following ATD treatment in depressed patients is the largest to date. The results suggest that the accumulation of rare *ARRB1* genetic variation and 2 common genetic variants of *ARRB1* are associated with clinical outcomes following ATD treatment in depressed patients. Which rare variants are responsible, and to what degree, remains to be elucidated.

The potential functional consequences of the significantly associated common genetic variation remains to be validated. However, their annotation with transcription factors such as FOS and JUN, which have been described in the context of ATD treatment for depression, offers a line of investigation. This analysis was limited by its dropout rate. The overlapping roles of β -arrestin 1 and 2 also confounds the interpretation of our results.

III.3.4. Conclusion

Both the accumulation of rare *ARRB1* genetic variation and common *ARRB1* genetic polymorphisms with potential functional consequences were associated with clinical improvement following ATD treatment, though these findings require replication and functional study.

III.3.5. Publication

Our results were published as an Original Research article in the journal *Frontiers in Pharmacology* (2023 impact factor: 5.988) (Chappell et al., 2022a). Early findings were presented as an electronic poster at the 11th Assises de Génétique Humaine et Médicale Couvent des Jacobins in Rennes, France (February 1–4, 2022).



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The association of *ARRB1* polymorphisms with response to antidepressant treatment in depressed patients

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Introduction: β -arrestin 1, a protein encoded by *ARRB1* involved in receptor signaling, is a potential biomarker for the response to antidepressant drug (ATD) treatment in depression. We examined *ARRB1* genetic variants for their association with response following ATD treatment in METADAP, a cohort of 6-month ATD-treated depressed patients.

Methods: Patients ($n = 388$) were assessed at baseline (M0) and after 1 (M1), 3 (M3), and 6 months (M6) of treatment for Hamilton Depression Rating Scale (HDRS) changes, response, and remission. Whole-gene *ARRB1* variants identified from high-throughput sequencing were separated by a minor allele frequency (MAF) $\geq 5\%$. Frequent variants (i.e., MAF $\geq 5\%$) annotated by RegulomeDB as likely affecting transcription factor binding were analyzed using mixed-effects models. Rare variants (i.e., MAF $< 5\%$) were analyzed using a variant set analysis.

Results: The variant set analysis of rare variants was significant in explaining HDRS score changes ($T = 878.9$; $p = 0.0033$) and remission ($T = -1974.1$; $p = 0.034$). Rare variant counts were significant in explaining response ($p = 0.016$), remission ($p = 0.022$), and HDRS scores at M1 ($p = 0.0021$) and M3 ($p < 0.001$). rs553664 and rs536852 were significantly associated with the HDRS score (rs553664: $p = 0.0055$ | rs536852: $p = 0.046$) and remission (rs553664: $p = 0.026$ | rs536852: $p = 0.012$) through their interactions with time. At M6,

significantly higher HDRS scores were observed in rs553664 AA homozygotes (13.98 ± 1.06) compared to AG heterozygotes (10.59 ± 0.86 ; $p = 0.014$) and in rs536852 GG homozygotes (14.88 ± 1.10) compared to AG heterozygotes (11.26 ± 0.95 ; $p = 0.0061$). Significantly lower remitter rates were observed in rs536852 GG homozygotes (8%, $n = 56$) compared to AG heterozygotes (42%, $n = 105$) at M6 ($p = 0.0018$).

Conclusion: Our results suggest *ARRB1* variants may influence the response to ATD treatment in depressed patients. Further analysis of functional *ARRB1* variants and rare variant burden in other populations would help corroborate our exploratory analysis. β -arrestin 1 and genetic variants of *ARRB1* may be useful clinical biomarkers for clinical improvement following ATD treatment in depressed individuals.

Clinical Trial Registration: clinicaltrials.gov; identifier NCT00526383

KEYWORDS

high-throughput sequencing, pharmacogenetics, major depressive disorder, β Arrestin, *ARRB1*

1 Introduction

Major Depressive Disorder (MDD) is the leading contributor to global disability (World Health Organization, 2017). Its main treatment option, antidepressant drugs (ATD), is only modestly effective, with more than 65% of ATD-treated patients failing to achieve remission (Trivedi et al., 2008). Given the rising global burden of MDD and the lack of effective treatments, it is necessary to identify robust biomarkers to predict treatment response.

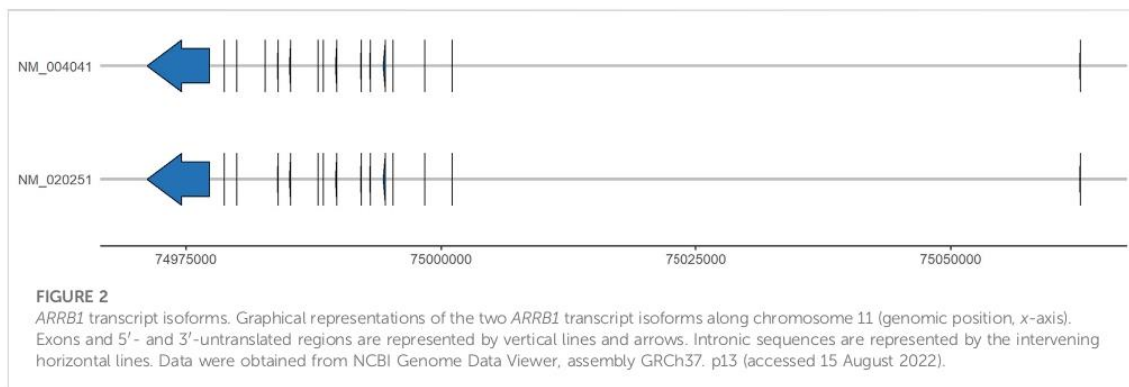
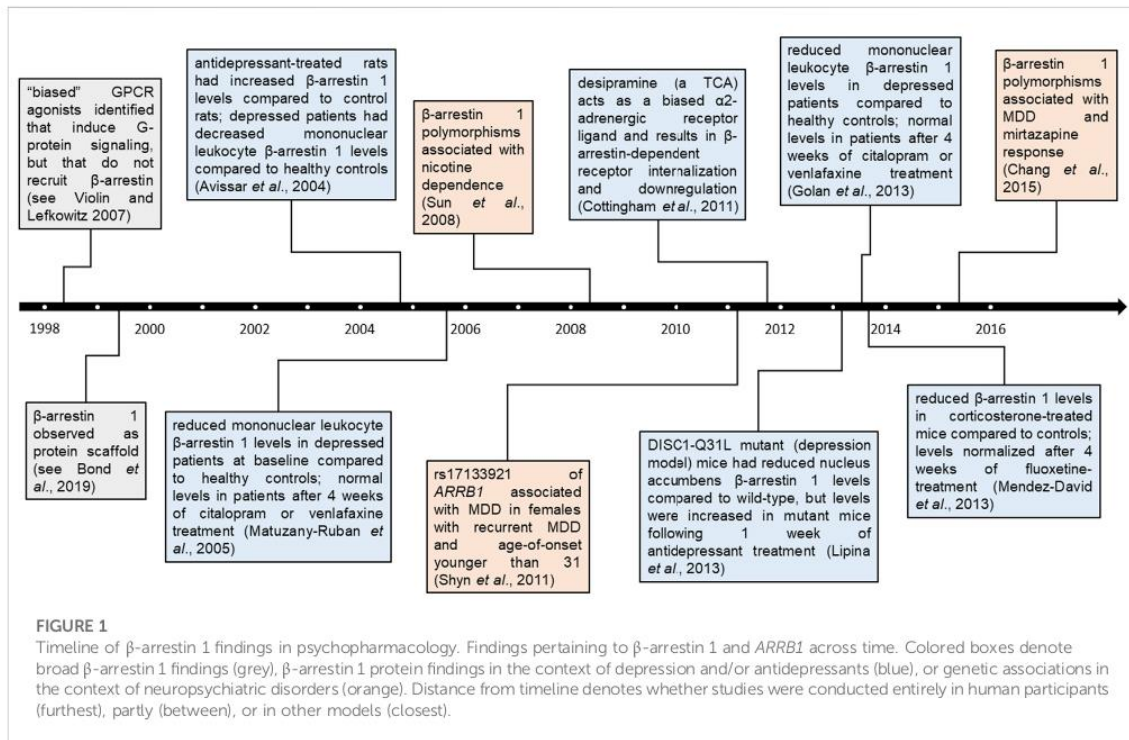
Two potential candidates, β -arrestin 1 and 2, are ubiquitously expressed proteins encoded by the *ARRB1* and *ARRB2* genes, respectively (Parruti et al., 1993; Lefkowitz and Shenoy, 2005). Both have functions in receptor desensitization, especially with many G protein-coupled receptor (GPCR) families, though roles in protein scaffolding, clathrin-mediated endocytosis, and G-protein-independent signaling are known (Shukla et al., 2011; Bond et al., 2019). Indeed, the β -arrestins serve as scaffolds for various proteins, including the phosphoinositide-3, extracellular signal-regulated 1 and 2 (ERK1/2), and several mitogen-activated protein (MAP) kinases, whose signaling pathways are implicated in the treatment of mood disorders (Golan et al., 2009; Shukla et al., 2011; Bond et al., 2019). Additionally, β -arrestin 1 has been observed to function as a cytoplasm-to-nucleus messenger and nuclear protein scaffold (Golan et al., 2009). Interactions with GPCRs include serotonin, dopamine, adrenergic, and melatonin receptors, which are associated with different brain pathways and implicated in the pathophysiology of MDD (Kovacs et al., 2009; Petit et al., 2018).

Furthermore, biased agonists can preferentially activate downstream pathways. With respect to GPCRs, this includes classical G-protein- and/or β -arrestin-mediated pathways. Importantly, biased signaling may lend itself to the develop of

novel therapeutic strategies in diverse pharmacological domains (Violin and Lefkowitz, 2007; Shukla et al., 2011; Bond et al., 2019). An example of biased agonism with respect to antidepressants was demonstrated with the tricyclic antidepressant, desipramine, a norepinephrine reuptake inhibitor and α 2-adrenergic receptor ligand that was observed to lead to β -arrestin-mediated α 2-adrenergic receptor internalization and downregulation in mouse embryonic fibroblasts (Cottingham et al., 2011).

Lower *ARRB1* mRNA and β -arrestin 1 protein levels have been observed in murine models of depression, as well as in human mononuclear leukocytes of depressed patients, compared to healthy controls (Avissar et al., 2004; Matuzany-Ruban et al., 2005; Golan et al., 2013; Lipina et al., 2013; Mendez-David et al., 2013). These levels returned to levels similar to those of healthy controls in both murine models and depressed patients following 4 weeks of antidepressant treatment, a change that preceded clinical improvement (Golan et al., 2013). A timeline of studies examining β -arrestin 1 and *ARRB1* in the context of depression and its treatment with ATDs is summarized in Figure 1. These associations suggest that β -arrestin 1 may be a promising candidate biomarker for ATD treatment response in MDD patients (Mendez-David et al., 2013). Factors that may alter its expression or function, such as genetic variants, are thus of interest.

ARRB1, located on chromosome 11q13 and comprising 16 exons, encodes for two splicing isoforms of β -arrestin 1 (Parruti et al., 1993; Calabrese et al., 1994; O'Leary et al., 2016) (see Figure 2). Single nucleotide polymorphisms (SNP) of *ARRB1* have been studied principally in the neuropsychiatric contexts of nicotine dependence and depression (Sun et al., 2008; Chang et al., 2015). In a European cohort, a haplotype constructed from 6 SNPs within *ARRB1* was significantly associated with nicotine dependence (Sun et al., 2008). In a meta-analysis of 3 cohorts of depressed individuals of European



ancestry, the intronic rs17133921 SNP was associated with MDD, specifically in the subgroup of females with recurrent MDD and an onset age of MDD prior to the age of 31 (Shyn et al., 2011). Furthermore, the rs12274033 SNP upstream of *ARRB1*, and a haplotype including it, were both associated with the response to mirtazapine treatment in a Korean population of MDD (Chang et al., 2015). These findings suggest that genetic polymorphisms of *ARRB1* may have an influence in different psychiatric contexts and could thus serve as potential candidate biomarkers for the response to ATD treatment in the context of MDD.

Advances in genetic analysis thanks to genome-wide association studies (GWAS) and high-throughput sequencing (HTS) technology have greatly increased the identification of genetic variants associated with disease. Furthermore, the Encyclopedia of DNA elements (ENCODE) project has mapped the vast majority of the human genome to functional elements involved in protein coding, transcription factor (TF) binding, and chromatin structure (The ENCODE Project Consortium, 2012). Importantly, these data can be used to annotate genetic

variants with regulatory information and offer insight into their potential functional consequences in various disease contexts. RegulomeDB is one such database that uses these and other predictive data to rank genetic variants on their likelihood to lie in a functional element and thus have a functional consequence (Boyle et al., 2012).

However, most variants detected by HTS have a minor allele frequency (MAF) less than the 5% threshold principally used to delineate common variants (Momozawa et al., 2016). The analysis of these rarer variants is thus generally more difficult and underpowered. One approach to this issue is grouping variants into sets—defined by genes or genomic regions—and analyzing the association between this cumulative variable and a trait of interest (Chen et al., 2019). Recently, the optimal unified sequence kernel association test (SKAT-O) variant set method was used to identify significant associations between rarer variants in *ADH1C* and *GSTO1* and mRNA expression from a set of 303 pharmacokinetic-related genes (Klein et al., 2019). Importantly, SKAT-O considers both burden and SKAT tests, which groups variants with respect to the mean or the variance, respectively. It thus optimizes test selection depending on whether most variants act in the same direction (burden) or in opposing directions, i.e., both protectively and deleteriously (SKAT) (Lee et al., 2012).

Given that both β -arrestin 1 levels and *ARRB1* genetic variants have been associated with MDD and ATD treatment response, the objectives of this study were to analyze the association of clinical response following ATD treatment in a cohort of 6-month ATD-treated MDD patients, with 1) the set of rare variants and 2) frequent variants with likely functional consequences, identified from exonic, intronic, and 5'- and 3'-untranslated regions of *ARRB1*.

2 Materials and methods

2.1 Study design

Do Antidepressants Induce Metabolic Syndromes (METADAP) is a 6-month prospective, multicentric, and observational cohort study carried out in a psychiatric setting (Corruble et al., 2015). Patients with a current major depressive episode (MDE) in the context of MDD were treated in naturalistic conditions and assessed before and after beginning a new ATD treatment. This study was registered by the French National Agency for Medicine and Health Products Safety (ANSM) and the Commission Nationale de l'Informatique et des Libertés (CNIL). It was approved by the Ethics Committee of Paris-Boulogne (France) and conformed to international ethical standards (ClinicalTrials.gov identifier: NCT00526383).

2.2 Patient population

Males and females 18–65 years of age without a serious medical condition were recruited. Inclusion criteria were presentation with a current MDE in the context of MDD (DSM-IVTR), as assessed by the Mini International Neuropsychiatric Interview (MINI) and a score ≥ 18 on the 17-item Hamilton Depression Rating Scale (HDRS) (Hamilton, 1960), as well as need for a new ATD treatment. Patients with psychotic symptoms or other mental disorders—such as psychotic disorder, bipolar disorder, alcohol or drug dependence, or an eating disorder—or those who were pregnant or who had organic brain syndromes or serious medical conditions, were excluded. Patients using antipsychotics or mood stabilizers before inclusion and/or for at least 4 months during the year before inclusion were also excluded. Concurrent use with antipsychotics, mood stabilizers, and/or stimulants was not allowed during the study. Benzodiazepines, at the minimum effective dose and for the minimum time period, and psychotherapies were allowed (Corruble et al., 2015). Measures and samples were obtained prior to beginning ATD treatment (M0), and after 1 month (M1), 3 months (M3), and 6 months (M6) of ATD treatment. Data were collected from November 2009 to March 2013 in 6 university hospital (CHU) psychiatry departments across France in Paris (CHU Fernand-Widal and CHU Saint-Antoine), Le Kremlin-Bicêtre (CHU Bicêtre), Grenoble (CHU Grenoble Alps), Lille (CHU Michel Fontan 1), and Besançon (CHU of Besançon) (Corruble et al., 2015).

Of the 643 patients included in METADAP, 19 had major protocol deviations and were excluded. Of the 624 available for analysis, 519 patients provided samples for genetic studies. From these, 400 have undergone HTS. Two samples were removed due to technical difficulties. As our objective was to examine clinical response following ATD treatment, 10 patients without follow-up measures (i.e., at M1, M3, and M6) were removed. Thus, 388 patients were analyzed. Genetic analyses may have included fewer than 388 patients if overall call quality was high, but individual calls were poor and removed (discussed further below). The reasons for dropout included loss to follow-up ($n = 87$, 48.6%), ATD changes ($n = 75$, 41.9%), use of unauthorized drugs, including antipsychotics, mood stabilizers, and stimulants ($n = 8$, 4.5%), the presence of an exclusion criterion (i.e., unstable medical condition, psychiatric disorder, substance abuse, or pregnancy) during follow-up ($n = 8$, 4.5%), or death ($n = 1$, 0.5%). Missing data at M1 ($n = 21$) included 8 dropouts (38.1%; 4 lost to follow-up and 4 due to ATD changes), while missing data at M3 ($n = 121$) included 110 dropouts (90.9%; 62 lost to follow-up, 36 due to ATD changes, 6 due to unauthorized drug use, 5 due to the presence of an exclusion

criterion, and 1 due to death. Information about ancestry was self-reported. Caucasian ethnicity was defined as having Caucasian parents, African as having Sub-Saharan African and/or Afro-Caribbean parents, and Asian as having East Asian, Central Asian, and/or South Asian parents (Morales et al., 2018). Three Asian patients were present within the analyzed population and were combined with the seven patients of mixed ethnicity. Education was classified into 3 levels: primary [e.g., elementary (≤ 5 years)]; secondary [e.g., middle school and high school (>5 and ≤ 12 years)]; tertiary [e.g., any college- or university-level education (>12 years)]. All patients provided written informed consent for study participation and for genetic analyses.

2.3 Antidepressant treatment

ATD monotherapies were prescribed by a psychiatrist in a “real world” psychiatric treatment setting as previously described (Corruble et al., 2015). ATDs belonged to 1 of 4 classes: selective serotonin reuptake inhibitors (SSRI), serotonin norepinephrine reuptake inhibitors (SNRI), tricyclic antidepressants (TCA), or other ATD treatments. In this ancillary study of 388 patients, 40% were prescribed an SSRI, 41% an SNRI, 6% a TCA, and 9% another ATD treatment; 4% received electroconvulsive therapy (ECT) instead of an ATD monotherapy. If a change in treatment was required during follow-up, the patient was dropped from the study.

2.4 Assessment of antidepressant treatment response

The HDRS was used to assess depression severity at M0 (i.e., baseline) and response to treatment at M1, M3, and M6. Responders to treatment were classified by an improved HDRS score of $\geq 50\%$ relative to baseline, remitters by a HDRS score ≤ 7 after at least 4 weeks of treatment, as recommended by the American College of Neuropsychopharmacology (ACNP) Task Force (Rush et al., 2006). Clinical assessments were performed blind to genotyping results. Each interview and diagnostic assignment was reviewed by a senior psychiatrist. For each patient, all visits were reviewed by the same psychiatrist.

2.5 High-throughput sequencing and sequence alignment

Patient DNA samples were sequenced using a targeted panel of genes involved in mood disorders and ATD metabolism. Sequencing of the whole *ARRB1* gene was performed, including exons, introns, and 5'- and 3'-untranslated regions. The HTS protocol and variant calling methods are as previously described (Bouali et al., 2017; Chappell et al., 2021).

2.6 Variant filtration and selection pipeline

Variant Call Format data were loaded into R (v4.1.0) (R Core Team, 2020) using the *vcfR* package (v1.9.0) (Knaus and Grünwald, 2017). Variant calls were annotated for call quality. Specifically, variant calls with a sequencing depth (DP) < 20 , SNPs with a quality score (QUAL) < 275 , insertions/deletions (indels) with a QUAL < 770 , heterozygous calls with an allele balance (AB) < 0.34 or > 0.79 , and homozygous calls with an AB < 0.96 , were annotated as poor-quality calls. Variants with a call rate (# poor-quality calls/# of calls) $< 95\%$ were removed (Anderson et al., 2010). Frequent and rare variants were defined by a MAF $\geq 5\%$ or $< 5\%$ (corresponding to both low-frequency and rare variants (Momozawa et al., 2016), but defined here as “rare” for simplicity), respectively. Frequent variants with a RegulomeDB category ranking of 1 or 2, corresponding to variants likely to directly affect TF binding—and in the case of rank 1, linked to expression of a gene target (Boyle et al., 2012)—were prioritized for individual analysis in association with clinical measures.

2.7 Variant set analysis

A variant set analysis was performed on all rare variants passing quality control (QC) using the *SMMAT* function from the *GMMAT* package (v1.3.2), which accounts for repeated measures in longitudinal data and can analyze both continuous and binary variables (Chen et al., 2019). The variables to be explained were the HDRS score, response rate, and remission rate. A null model was constructed including age, sex, ATD class, and visit (i.e., time) as covariables. Gaussian distributions with an identity link function were used to model the HDRS score, while binomial distributions with a logit link function were used to model response and remission rates. The genomic relationship matrix required by the *SMMAT* function was constructed using the *snpgdsGRM* function of the *SNPRelate* package (v1.26.0) (Zheng et al., 2012). The *SMMAT* function was run using default settings, except that the “use.minor.allele” argument was set to True—corresponding to the use of the minor allele as the coding allele rather than the alternative allele—and the “MAF.range” was set to 0.00-0.50. The SKAT-O test, corresponding to a linear combination of the burden and SKAT statistics, was used (Lee et al., 2012). As a single genomic region was analyzed, a threshold of $p < 0.05$ was considered significant.

2.8 Haplotype analysis and functional annotation of prioritized variants

Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) were assessed using Haploview (v4.2) in

the subgroup of Caucasian patients comprising 91% of the patient population (Barrett et al., 2005). Variants were considered to be in LD and assigned to a haplotype block as defined by an $r^2 \geq 0.8$. The genetic variant with the highest MAF from each haplotype block was selected as a proxy for further analysis. Genetic variants were analyzed according to an additive model. Data from RegulomeDB (v2.0.3) and HaploReg (v4.1), including LD information, chromatin state, bound proteins, and altered TF motifs obtained from sources including the 1000 Genomes Project, ENCODE, and JASPAR, were used to annotate variants (Boyle et al., 2012; Ward and Kellis, 2016). LD between analyzed variants and undetected variants or variants outside of our sequencing range were further assessed using LDLink (Machiela and Chanock, 2015).

2.9 Data analysis

Statistical analyses were performed in R (v4.1.0) (R Core Team, 2020). Quantitative variables were analyzed using nonparametric Kruskal–Wallis rank-sum tests, while qualitative variables were analyzed using Fisher Exact tests. Mixed-effects models were used to analyze these longitudinal data. Importantly, missing data (e.g., due to dropout) does not result in a complete loss of information as these models allow for an average estimate to be calculated from the non-missing data (Mallinckrodt et al., 2003). Linear mixed-effects models and generalized linear mixed-effects logistic models were constructed with the lme4 package (v1.1-27.1) (Bates et al., 2015). The main variable to explain was the HDRS score. Other variables to explain were response and remission rates. Age, sex, ATD class, and visit were included *a priori* as fixed-effect covariables in all models. Demographic variables that significantly differed between genotype groups (i.e., $p < 0.05$) were also added as fixed-effects covariables. Individual was added as the random effect to account for repeated measures. Rare variant counts, variant genotype, and its interaction with visit, were the main explanatory variables examined. Rare variant counts were calculated by summing the number of MAF<5% variants present (i.e., heterozygous or homozygous genotype) in each individual. Given the exploratory nature of our study, we considered $p < 0.05$ as significant (Bender and Lange, 2001). Significance of fixed effects was assessed with the Satterthwaite method in linear mixed-effects models and with the Wald chi-square test in generalized linear mixed-effects models. In the event of a significant main or interaction effect, *post hoc* comparisons were carried out by performing linear and logistic regressions at individual timepoints (i.e., M1, M3, and M6) or by using the emmeans package (Lenth, 2022). Briefly, models were input into the emmeans function (with the “type” argument set to response) to estimate emmeans (for the HDRS) and probabilities (for response and/or remission) according to variant genotypes

TABLE 1 Sociodemographic characteristics. Sociodemographic characteristics of METADAP patients are shown for the whole sample. Kruskal–Wallis tests were used to compare age, tobacco consumption, onset age of MDE, and baseline HDRS scores (presented as mean \pm standard deviation (m \pm sd)). Fisher Exact tests were used to compare sex, socio-education status, ethnicity, smoking status at baseline, MDE recurrence, prescribed ATD drug, and dropout rates across study time (presented as the number of patients and percentage). *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$. ATD, antidepressant drug; ECT, electroconvulsive therapy; HDRS, 17-item Hamilton Depression Rating Scale; m, mean; M1, after 1 month of treatment; M3, after 3 months of treatment; M6, after 6 months of treatment; MDE, major depressive episode; n, number of patients; p: p-value; SNRI, serotonin norepinephrine reuptake inhibitor; sd, standard deviation; SSRI, selective serotonin reuptake inhibitor; TCA, tricyclic antidepressant.

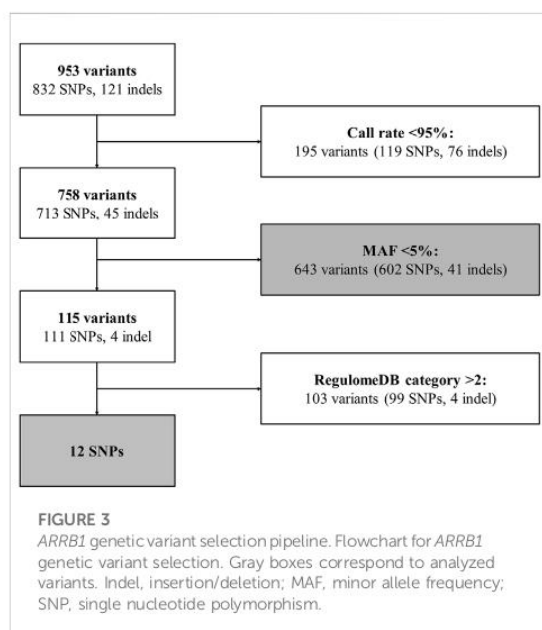
		Total sample $n = 388$
Age (in years) (m \pm sd)		45.4 \pm 13.3
Female [n (%)]		263 (68)
Education [n (%)]	Primary	36 (9)
	Secondary	168 (43)
	Tertiary	183 (47)
Ethnicity [n (%)]	Caucasian	353 (91)
	African	24 (6)
	Mixed	10 (3)
Current smoker [n (%)]		149 (38)
Pack years (m \pm sd)		15.2 \pm 14.9
Recurrent MDE [n (%)]		284 (73)
Onset age MDE (m \pm sd)		35.3 \pm 14.5
Baseline HDRS (m \pm sd)		24.8 \pm 4.9
Prescribed ATD [n (%)]	SSRI	157 (40)
	SNRI	158 (41)
	TCA	24 (6)
	Other	34 (9)
	ECT	15 (4)
Missing at follow-up [n (%)]	M1	21 (5)
	M3	121 (31)
	M6	179 (46)

after controlling for other covariables. Comparisons were performed with the *contrast* function (with the “method” argument set to pairwise and the “infer” argument set to True) using the *emmeans* output.

3 Results

3.1 Patient demographics

Sociodemographic characteristics for the whole cohort are shown in Table 1. The mean age was 45.4, 68% of subjects were women, and 91% of subjects were Caucasian. 38% were current smokers at baseline and 73% had previously experienced an MDE.



3.2 Genetic variant selection and linkage analysis

Genetic variant selection is summarized in Figure 3. A total of 953 unique genetic variants were identified from extracted Variant Call data, of which 832 were SNPs and 121 were indels (see Supplementary Table S1). 195 variants with call rates <95%

(119 SNPs and 76 indels) were removed. Thus, 758 variants were available for analysis. 643 variants were rare (i.e., $MAF < 5\%$) (602 SNPs and 41 indels), of which 610 had a $MAF < 1\%$ and 415 were identified in 1 individual (see Supplementary Table S1). Among the 115 frequent variants (i.e., $MAF \geq 5\%$) (111 SNPs and 4 indels), 12 SNPs had a RegulomeDB category rank of 1 or 2 [i.e., linked to expression of a gene target (rank 1) and likely to directly affect TF binding (ranks 1 and 2)] and were prioritized for individual analysis (see Figure 3). Information for each prioritized SNP is shown in Table 2. Prioritized SNPs did not significantly deviate from HWE. Ten of the twelve prioritized SNPs were intronic, eight of which were located in intron 1.

Among the 12 prioritized SNPs, 3 pairs were observed in LD. rs501372 and rs567807 comprised haplotype block 1 (ht1), rs553664 and rs506233 (ht2), and rs569796 and rs504683 (ht3) (see Table 2 and Supplementary Figure S1). rs501372, rs553664, and rs504683 were selected as proxy SNPs for further analysis from each haplotype block. Nine SNPs were thus analyzed.

3.3 Variant set and accumulation analyses of rare variants

SMMAT was used to assess the association of the 643 rare (i.e., $MAF < 5\%$) variants with clinical measures over time. The 643 rare variants were significant in explaining changes in the HDRS score over time in burden ($T = 878.9$; $\Phi = 89194.6$; $p = 0.0033$) and SKAT-O tests ($p = 0.0069$) and remission rates in the burden test ($T = -1974.1$; $\Phi = 869281.1$; $p = 0.034$), but not response rates, though a statistical trend was observed in the burden test ($T = -1773.9$; $\Phi = 937912.7$; $p = 0.07$) (see Table 3). To further

TABLE 2 Genomic and other information about the 12 prioritized frequent SNPs. For the prioritized SNPs (i.e., $MAF \geq 5\%$ and RegulomeDB category rank of 1 or 2), the rs#, genomic position relative to the human genome assembly, hg19, and genomic region are shown. RegulomeDB rankings correspond to likely to affect binding and linked to expression of a gene target (1b, 1f) and likely to affect binding (2b, 2c). The major and minor alleles and minor allele frequency (MAF (%)) with respect to the Caucasian subpopulation are also shown. Hardy-Weinberg equilibrium p -values (HWE), haplotype blocks (Haplotype block), and linkage disequilibrium r^2 values (LD (r^2)) relative to the Caucasian subpopulation are also given. HWE, Hardy-Weinberg equilibrium; MAF, minor allele frequency; LD, linkage disequilibrium.

rs #	Position	Region	RegulomeDB rank	Major	Minor	MAF (%)	HWE	Haplotype block	LD (r^2)
rs2279130	74977185	3'UTR	2b	C	T	7.2	0.5		
rs501372	74993593	intron 5	2b	C	A	38.2	0.24	ht1	0.96
rs877711	74994352	exon5	2b	G	A	11.8	0.18		
rs567807	75000189	intron 2	2b	A	G	37.2	0.13	ht1	
rs553664	75005209	intron 1	2b	G	A	44.4	0.58	ht2	0.98
rs506233	75012535	intron 1	1f	A	G	43.7	0.38	ht2	
rs536852	75017436	intron 1	2b	G	A	49.1	0.98		
rs1676887	75019296	intron 1	1f	G	A	10.4	0.38		
rs113636971	75033020	intron 1	2b	A	G	6.4	0.88		
rs569796	75050842	intron 1	1b	G	C	29.9	0.48	ht3	0.98
rs504683	75051233	intron 1	2c	A	G	30.2	0.39	ht3	
rs561923	75058004	intron 1	2b	C	A	25.4	0.8		

TABLE 3 SMMAT results. Data and results for variant set analyses of the association between rare (i.e., MAF<5%) variants passing quality control and HDRS scores, response rates, and remission rates. All models included age, sex, antidepressant class, and visit as covariables. Shown are the mean allele frequency, the burden test score statistic and variance, and the associated *p*-values of the burden, SKAT, and SKAT-O tests. bold text*: *p* < 0.05. AF, allele frequency; *p*: *p*-value.

Clinical measure	Minimum frequency	Mean frequency	Maximum frequency	Burden stat	Burden variance	Burden <i>p</i>	SKAT <i>p</i>	SKAT-O <i>p</i>
HDRS	0.00129	0.00442	0.5	878.9	89194.6	0.0033*	0.41	0.0069*
Response				-1773.9	937912.7	0.07	0.47	0.12
Remission				-1974.1	869281.1	0.034*	0.37	0.06

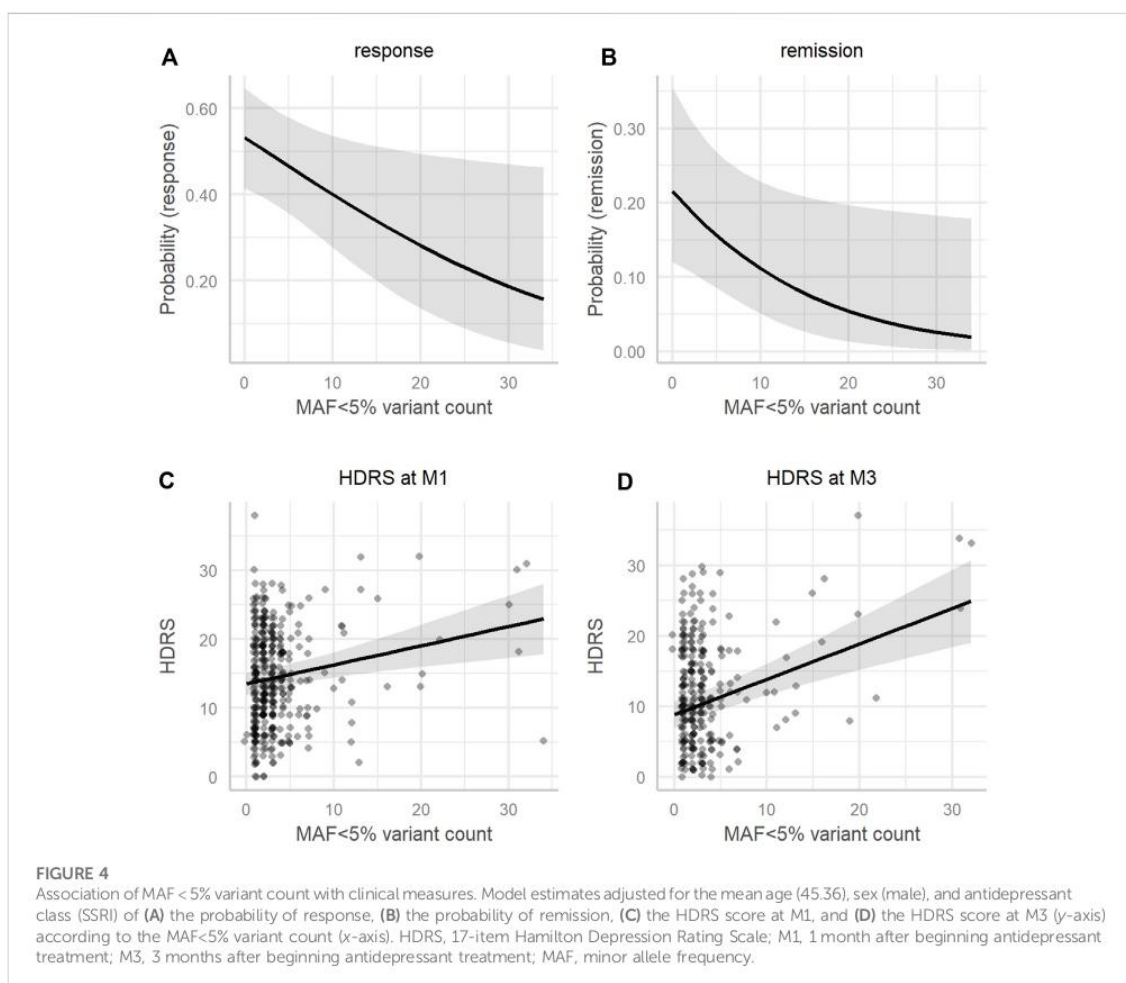
TABLE 4 Mixed-effects model results. Associations for each of the clinical measures (HDRS, response, and remission) with 1) the rare (i.e., MAF<5%) variant count and its interaction with visit (i.e., time) and 2) the 9 prioritized frequent (i.e., MAF≥5% and RegulomeDB rank of 1 or 2) SNPs analyzed and their interactions with visit. Significant (i.e., *p* < 0.05) associations are highlighted in dark gray and bolded. Significance of fixed effects was assessed with the Satterthwaite method in mixed-effects models of the HDRS score and with the Wald chi-square test in mixed-effects models of response and remission. All models were adjusted for age, sex, antidepressant class, and any sociodemographic characteristics that significantly (bold text and *: *p* < 0.05) differed between genotypes (see Supplementary Table S2).

	HDRS						Response			Remission		
	SS	MS	df (num)	df (den)	<i>F</i>	<i>p</i>	χ^2	df	<i>p</i>	χ^2	df	<i>p</i>
MAF<5% count												
MAF<5% count	804.264	804.264	1	438.056	26.87	<0.001*	5.81	1	0.016*	4.56	1	0.033
Visit:MAF<5% count	348.786	116.262	3	918.602	3.88	0.0090*	0.53	2	0.77	1.99	2	0.37
Prioritized variants (MAF≥5% and RegulomeDB rank ≤ 2)												
rs2279130	59.196	29.598	2	401.121	0.98	0.38	1.48	2	0.48	4.11	2	0.13
Visit:rs2279130	122.998	20.500	6	914.026	0.68	0.67	3.03	4	0.55	1.94	4	0.75
rs501372	5.277	2.638	2	371.393	0.09	0.92	1.16	2	0.56	1.50	2	0.47
Visit:rs501372	168.779	28.130	6	882.364	0.93	0.47	4.64	4	0.33	2.00	4	0.73
rs877711	29.797	14.898	2	411.229	0.50	0.61	1.17	2	0.56	1.10	2	0.58
Visit:rs877711	195.206	32.534	6	942.398	1.09	0.36	0.63	4	0.96	4.59	4	0.33
rs553664	147.286	73.643	2	399.038	2.51	0.08	0.49	2	0.78	3.76	2	0.15
Visit:rs553664	541.248	90.208	6	919.440	3.08	0.0055*	8.86	4	0.06	11.02	4	0.026*
rs536852	213.512	106.756	2	385.710	3.53	0.030*	0.85	2	0.66	3.85	2	0.15
Visit:rs536852	390.451	65.075	6	886.704	2.15	0.046*	7.11	4	0.13	12.84	4	0.012*
rs1676887	50.854	25.427	2	372.885	0.85	0.43	1.36	2	0.51	1.78	2	0.41
Visit:rs1676887	194.069	32.345	6	887.639	1.08	0.37	1.71	4	0.79	5.43	4	0.25
rs113636971	10.977	5.489	2	379.137	0.18	0.83	0.23	2	0.89	0.14	2	0.93
Visit:rs113636971	94.369	15.728	6	896.752	0.52	0.79	0.57	4	0.97	0.74	4	0.95
rs504683	64.300	32.150	2	373.296	1.05	0.35	2.47	2	0.29	2.47	2	0.29
Visit:rs504683	74.684	12.447	6	892.312	0.41	0.87	0.79	4	0.94	0.79	4	0.94
rs561923	17.422	8.711	2	366.270	0.28	0.75	1.03	2	0.60	0.31	2	0.86
Visit:rs561923	37.649	6.275	6	883.377	0.20	0.98	2.06	4	0.72	2.62	4	0.62

χ^2 , chi-square value; den, denominator; df, degrees of freedom; *F*, *F*-value; HDRS, 17-item Hamilton Rating Depression scale; MAF, minor allele frequency; MS, mean sum of squares; num, numerator; *p*, *p*-value; SS, sum of squares; ":" designates an interaction between model terms.

analyze the associations of rare variant accumulation with clinical measures, rare variant counts were calculated and analyzed. After controlling for age, sex, ATD class, and visit, a significant main effect of the rare variant count was observed in the mixed-effects models of response [χ^2 (df = 1, *n* = 388) = 5.81, *p* = 0.016] and remission [χ^2

(df = 1, *n* = 388) = 4.56, *p* = 0.033], as was a significant interaction with visit in the mixed-effects model of the HDRS score ($F_{3,918}$ = 3.88, *p* = 0.0090) (see Table 4). In simplified mixed-effects models (i.e., without interaction) controlling for the same factors, the rare variant count was indeed significant in models of response (odds

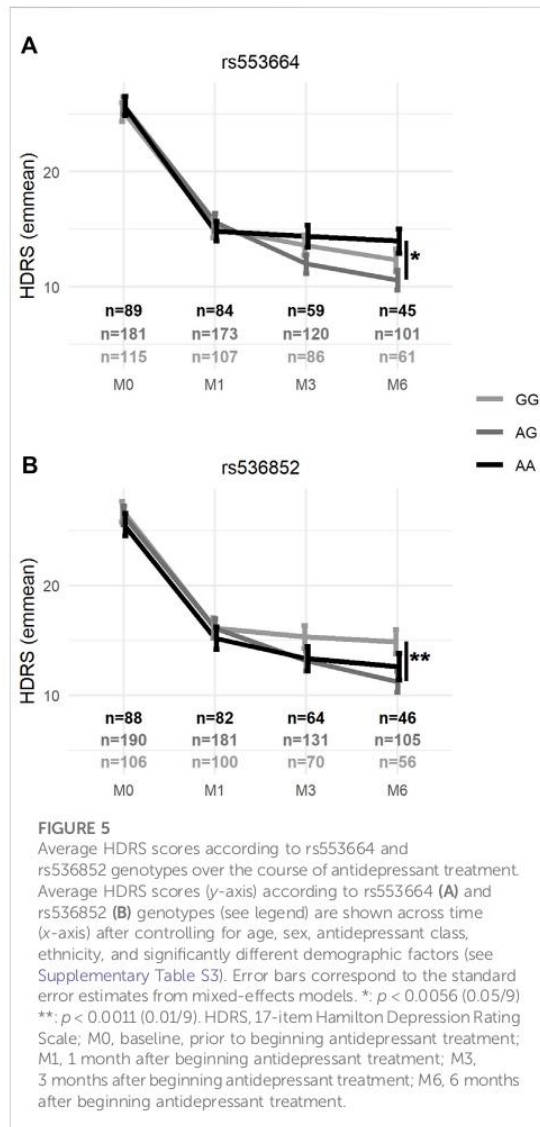


ratio (OR) = 0.95; 95% confidence interval (95%CI) [0.91–0.99]; $p = 0.016$) and remission (OR = 0.92; 95%CI [0.86–0.99]; $p = 0.022$) (see Figures 4A,B). Following Bonferroni correction, it was also a significant factor explaining the HDRS score in multiple linear regressions at M1 (coefficient = 0.26; 95%CI [0.11–0.41]; $p = 0.0021$) and M3 (coefficient = 0.46; 95%CI [0.28–0.64]; $p < 0.001$) (see Figures 4C,D), but not at M6 (coefficient = 0.25; 95%CI [–0.01–0.51]; $p = 0.19$).

3.4 Clinical response according to prioritized variants

Sociodemographic characteristics and significant differences between prioritized SNP genotypes are shown in Supplementary Table S2. Mixed-effects model associations of clinical measures with genotype and genotype \times visit interactions for the

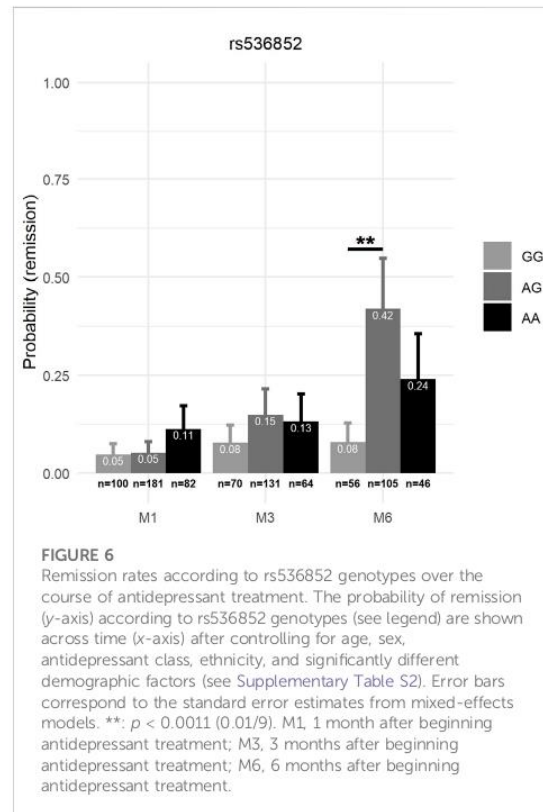
9 prioritized SNPs are summarized in Table 4. In mixed-effects models of the HDRS score controlling for age, sex, ATD class, ethnicity, and smoking status—and baseline HDRS scores for rs553664—the genotype \times visit interaction was a significant explanatory factor for rs553664 ($F_{6,919} = 3.08$, $p = 0.0055$) and rs536852 ($F_{6,886} = 2.15$, $p = 0.046$) (see Table 4). In mixed-effects models of remission controlling for these same variables, the genotype \times visit interaction was also significant for rs553664 [χ^2 (df = 4, $n = 388$) = 11.02, $p = 0.026$] and rs536852 [χ^2 (df = 4, $n = 388$) = 12.84, $p = 0.012$]. Following Bonferroni correction, a significantly higher HDRS score was observed in rs553664 AA homozygotes (13.98 ± 1.06) compared to AG heterozygotes (10.59 ± 0.86) at M6 (coefficient = 3.39, 95%CI [1.29–5.49], $p = 0.014$) (see Figure 5A) and in rs536852 GG homozygotes (14.88 ± 1.10) compared to AG heterozygotes (11.26 ± 0.95) at M6 (coefficient = 3.62, 95%CI [1.54–5.71], $p = 0.0061$) (see Figure 5B). A significantly lower remitter rate



was also observed in rs536852 GG homozygotes (8%, $n = 56$) compared to AG heterozygotes (42%, $n = 105$) at M6 (OR = 0.12, 95%CI [0.39–0.37], $p = 0.0018$) (see Figure 6). No significant differences in response rates, or in remitter rates between rs553664 genotypes, were observed.

3.5 Functional analysis of ARRB1 polymorphisms

Annotations from RegulomeDB (v2.0.3) and HaploReg (v4.1) for rs553664 and rs536852—associated with clinical



measures—and any variants in LD with them are shown in Table 5. rs553664 was observed to be in LD with rs506233 and an intronic deletion, rs35731045. rs536852 was not observed to be in LD with any variants within $\pm 500,000$ base pairs of its genomic position in non-Finnish European populations. It was most closely linked to rs553664 and rs506233, though these associations ($r^2 = 0.67$ and $r^2 = 0.68$, respectively) were weaker than in our sample (see Supplementary Figures S1, S2). rs553664, rs506233, and rs35731045 were annotated as binding FOS, NFIC, USF1, and IKZF1. rs536852 was annotated as binding POLR2A, FOS, and JUN. Altered TF binding motifs included the CCCTC-binding factor (CTCF). Each variant had at least one annotation with histone marks/chromatin state (see Table 5).

4 Discussion

The present study is an ancillary investigation of the METADAP cohort that aimed to analyze the association between genetic variants of *ARRB1* and response following ATD treatment. Overall, 758 genetic variants passed quality control measures and were further filtered based on allelic

TABLE 5 HaploReg and RegulomeDB annotations. For rs553664 and rs536852, the rs#'s in linkage disequilibrium (i.e., $r^2 > 0.8$) with the given SNP, the associated histone marks/chromatin state in brain tissues, the bound proteins, and known and discovered altered motifs, are given.

rs #	rs #'s in LD ($r^2 > 0.8$) ^a	Histone marks/ ^a chromatin state ^b (brain)	Bound proteins	Altered TF motifs
rs553664	rs506233, rs35731045	Strong transcription ^b	NFYA ^a , USF1 ^a , USF1 ^b , USF2 ^b , RAD51 ^b , NFYC ^b , FOS ^b , EMSY ^b , CBFA2T2 ^b , ZNF316 ^b	Brachyury_1 ^a , HP1-site-factor ^a , IRX3 ^b , TBXT ^b
rs506233	rs553664, rs35731045	Enhancer ^a , promoter ^a , enhancers ^b , strong transcription ^b	NFIC ^b	Myc_known ^a
rs35731045	rs553664, rs506233	Strong transcription ^b	IKZF1 ^b	ERalpha-a_disc1 ^a , ERalpha-a_disc2 ^a , ERalpha-a_known1 ^a , ERalpha-a_known4 ^a , Esr2, RAR ^a , RXRA_disc1 ^a , RXRA_known5 ^a , SF1 ^a , STAT_disc3 ^a
rs536852		Enhancer ^a , promoter ^a , enhancers ^b , strong transcription ^b	CFOS ^a , CJUN ^a , FOS ^b , JUN ^b , POLR2A ^b	CTCF_disc9 ^a , Irf_disc4 ^a , Pax-4_3 ^a , SP1_known2 ^a , TATA_disc4 ^a , TR4_disc3 ^a , PAX4 ^b

^aAnnotation from HaploReg v4.1.

^bAnnotation from RegulomeDB, v2.0.3. LD, linkage disequilibrium; TF, transcription factor.

frequency and their functional annotations as likely to affect TF binding. Analyses were carried out to assess the association of clinical response following ATD treatment with 1) the cumulative effect of rare (i.e., MAF<5%) variants and 2) frequent (i.e., MAF≥5%) variants with likely functional consequences. Twelve SNPs were prioritized, of which three pairs were observed to be in LD. Thus, 9 SNPs (3 proxies and 6 individual) were analyzed.

The variant set analysis using *SMMAT* (Chen et al., 2019) and leveraging the SKAT-O framework (Lee et al., 2012), suggests that the accumulation of rare *ARRB1* variants is associated with HDRS score changes and remission rates over the course of ATD treatment, as the burden test results were significant. As such, increased variant accumulation in *ARRB1* may have an impact on clinical response following ATD treatment. Furthermore, as reflected by the significant finding in the burden test, but not the SKAT test, a large proportion of these variants likely have causal effects on ATD response that, overall, act in the same direction (Lee et al., 2012). This was further highlighted by the significant association of greater rare variant counts with higher HDRS scores—notably at M1 and M3—and overall lower response and remission rates during treatment. Of note, certain rare variants in our cohort, including those in LD with the rs12274033 polymorphism that was previously associated with remission rates following mirtazapine treatment in a Korean population (Chang et al., 2015), may contribute to the observed associations with HDRS score changes and remission rates.

The 9 prioritized SNPs we analyzed have not been previously examined. They were prioritized for analysis from over 900 variants identified from HTS data spanning the *ARRB1* gene, including exons, introns, and 5'- and 3'-untranslated regions. Given that *ARRB1* mRNA and β -arrestin 1 protein levels have been previously associated with depression and

ATD treatment (Avisar et al., 2004; Matuzany-Ruban et al., 2005; Golan et al., 2013), variants annotated as likely to lie in a functional element—and thus be more likely to impact *ARRB1* expression or β -arrestin function—were prioritized using RegulomeDB (Boyle et al., 2012). RegulomeDB has previously been used to select and study genetic variants in various disease backgrounds (Lee et al., 2015; Hong et al., 2018; Huo et al., 2019). Specifically, variants with a category ranking of 1 or 2, corresponding to variants likely to directly affect TF binding, were prioritized for further analysis. To our knowledge, this is the first study to leverage functional annotations to prioritize variants for analysis in the context of MDD and its treatment with ATDs.

Protein binding annotations from HaploReg and RegulomeDB, utilizing ChIP-seq data from ENCODE, suggest that rs553664 (and its proxies, including rs506233) and rs536852, associated with the HDRS score and remission in METADAP, lie within functional elements that bind various proteins including USF1, NFIC, FOS, JUN, and POLR2A. Genetic risk factors that overlap with TF-binding sites for USF1, NFIC, and POLR2A have been identified in relation to schizophrenia and MDD (Huo et al., 2019; Li et al., 2020).

FOS and JUN can dimerize to form a member of the Activator Protein-1 (AP-1) TF family, a leucine-zipper TF shown to regulate proliferation, differentiation, and apoptosis (Garces de los Fayos Alonso et al., 2018). Both FOS and JUN are expressed in the brain where they have context-dependent influences on neurodegeneration and/or neuroprotection (Herdegen and Waetzig, 2001). Recently, AP-1 was observed to have a role in fluoxetine response in a murine model of heightened anxiety (Chottekalapanda et al., 2020), and was also found to be activated by other factors such as BDNF, which is itself a factor influencing the response to different ATD treatments (Colle et al., 2015). It is known that ATDs can

modify the activation of different genes through their modulation of serotonergic pathways (Lesch and Heils, 2000). Indeed, several TFs have been associated with ATD response or have been shown to be altered in depression (Pittenger and Duman, 2008; Sasayama et al., 2013). Moreover, the beta-arrestins act as scaffolds for kinases, including c-Jun N-terminal kinases of the JNK axis, which are implicated in neuronal plasticity, memory, and neuronal maturation (Herdegen and Waetzig, 2001; Shukla et al., 2011). As such, the potential impact rs553664 and/or rs536852 may have on AP-1 and/or the binding of other TFs could alter β -arrestin 1 expression and, consequently, any downstream functions of β -arrestin 1, including the transmission of receptor-mediated signals during ATD treatment or the scaffolding of proteins.

A putative binding motif for CTCF was also associated with rs536852. CTCF is a conserved zinc-finger TF with important roles in transcriptional regulation and 3D genome organization (Li et al., 2020). In a study of risk alleles for schizophrenia, SNPs lying in CTCF binding sites were enriched among those analyzed (Huo et al., 2019). Furthermore, in a study of MDD genetic risk factors, 11 of the 34 TF binding-disrupting SNPs analyzed were specific to CTCF binding sites, suggesting that disruption of CTCF binding may be a shared mechanism among genetic risk variants of MDD (Li et al., 2020). ATD-induced transcriptional alterations in the brain may also be influenced by CTCF (Piechota et al., 2015). Of course, whether CTCF binding is disrupted and, if so, the biological and clinical consequences of this, should be verified and further investigated.

Our findings demonstrate an association between genetic variants of *ARRB1* and clinical outcomes of response following ATD treatment. These findings suggest that these genetic factors might influence various mechanisms involved in the response to ATD therapy. Given that biased agonists can preferentially activate or bypass β -arrestin 1-mediated processes (Violin and Lefkowitz, 2007; Cottingham et al., 2011; Shukla et al., 2011; Bond et al., 2019), patients carrying either an excess of rare *ARRB1* variants or the rs553664 and rs536852 polymorphisms may benefit from the use of ATD treatments that 1) favor G-protein-mediated pathways and 2) circumvent β -arrestin-mediated pathways and the desensitization of GPCRs. Alternatively, these patients could also benefit from the addition of other therapeutical strategies, such as psychotherapies (i.e., talk therapies) or brain stimulation therapies, including ECT and repetitive transcranial magnetic stimulation.

Our study has several limitations. First, the proportion of missing data was relatively high, though the proportion at 6 months (46.1%) was comparable to the proportions in the STAR*D cohort at 9 (43%) and 12 weeks (61%) (Trivedi et al., 2006; Muthén et al., 2011). Additionally, mixed-effects models are a robust method to control for the bias imposed by these

missing data (Mallinckrodt et al., 2003). Second, hard filters were used to filter out poor-quality variants, which could have removed variants near the cutoff threshold. Importantly, these variants could have had significant associations with clinical measures. Third, it should be noted that the functions of β -arrestin 1 and 2 often overlap. Thus, any impact that variants of *ARRB1* may have on β -arrestin 1 expression or function may be compensated for by β -arrestin 2, at least within the cytoplasm (Shukla et al., 2011). The strengths of this study include its prospective and naturalistic design, which allows for the analysis of treatment response across a 6-month period and better reflects “real-world” clinical practice. Additionally, this study leveraged functional annotations to select and prioritize variants with likely functional consequences on *ARRB1* expression for analysis since reduced β -arrestin 1 levels have been previously associated with depression severity and the response to ATD treatment (Avisar et al., 2004; Matuzany-Ruban et al., 2005; Golan et al., 2013). Finally, to date, this is the largest cohort analysis of *ARRB1* variants in relation to response following ATD treatment in patients with MDD. The findings from this exploratory analysis should be replicated in independent cohorts of depressed individuals undergoing ATD treatment.

To conclude, *ARRB1* genetic variants were associated with clinical measures of response following ATD treatment in a cohort of ATD-treated depressed patients. Specifically, we observed associations between clinical measures and 1) rare variant accumulation and 2) two frequent variants with probable functional consequences, rs553664 and rs536852. These findings suggest that variants of *ARRB1*, and perhaps especially those within genomic regulatory regions, may contribute to clinical response following ATD treatment. Functional analyses would help confirm the potential effect(s) that these polymorphisms have on clinical improvement following antidepressant treatment. Further study in larger cohorts would also help corroborate the findings of our exploratory analysis.

Data availability statement

The data analyzed in this study is subject to the following licenses/restrictions: data are under the protection of health data regulation set by the French National Commission on Informatics and Liberty (Commission Nationale de l'Informatique et des Libertés, CNIL), in line with European regulations and the Data Protection Act, and the Comité de protection des personnes (CPP, equivalent to the Research Ethics Committee). The data can be available upon reasonable request to the principal investigator of the study (emmanuelle.corruble@aphp.fr). The French law forbids us to provide free access to METADAP data. Please feel free to contact us should you have any additional questions.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of Paris-Boulogne. The patients/participants provided their written informed consent to participate in this study.

Author contributions

KC, CV, and JB designed the study. EC, BF, LB, RC, DD, ST, and FG acquired the data. KC, AA, and KE-A analyzed the data. KC and CV wrote the article. All authors contributed to manuscript revision and read and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

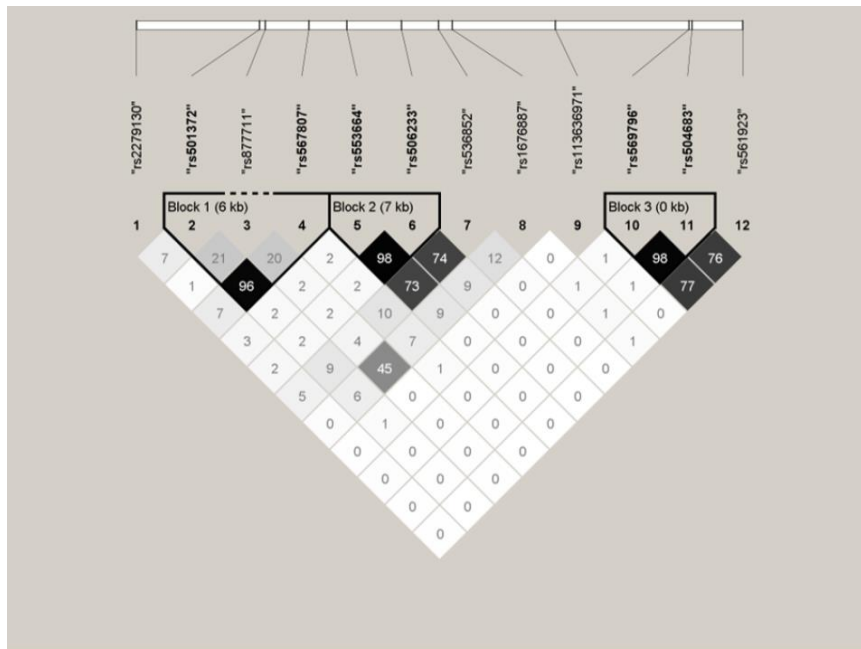
The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fphar.2022.974570/full#supplementary-material>

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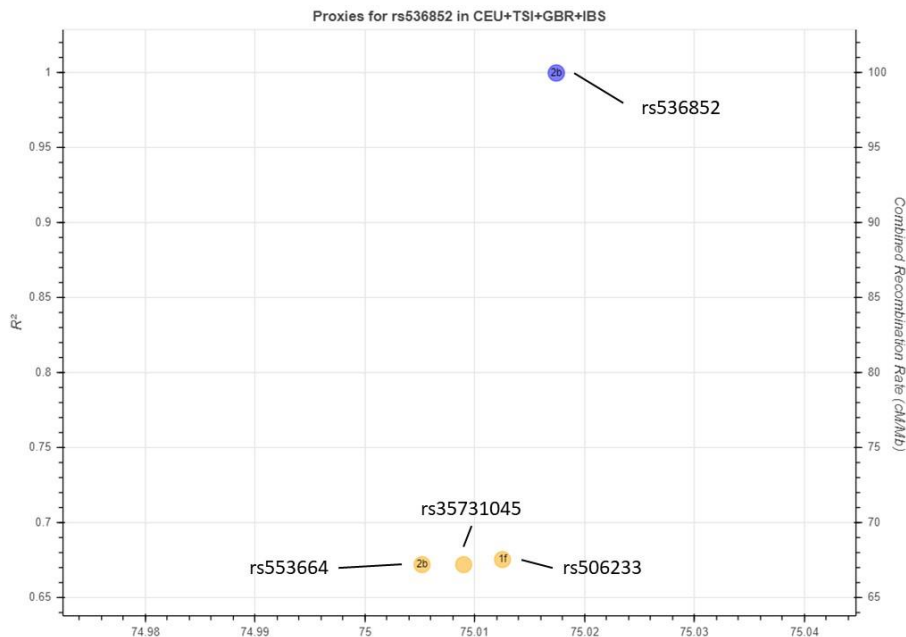
III.3.6. Supplementary Materials



Supplementary Figure 1: LD plot for the 12 prioritized frequent SNPs

Shown are the LD associations between each of the 12 prioritized SNPs. Haplotype blocks are defined by an $r^2 > 0.80$ (values shown in each square).

LD: linkage disequilibrium



Supplementary Figure 2: LD analysis of rs536852 using the 1000 Genomes Project

r^2 associations relative to rs536822 (y-axis) in non-Finnish European populations of the 1000 Genomes Project between rs536852, rs553664, and rs506233, and linked variants, plotted according to their hg19 genomic position (x-axis).

LD: linkage disequilibrium

	<i>African</i>	0(0)	12(7)	12(13)		11(10)	12(6)	0(0)		20(6)	4(6)	0(0)			
	<i>Mixed</i>	4(3)	2(1)	4(4)		3(3)	4(2)	3(3)		9(3)	1(1)	0(0)			
Current smoker (n(%))		55(48)	57(31)	35(39)	0.018*	40(38)	64(34)	44(50)		0.035*	113(36)	28(41)	5(83)	0.054	
Pack years (m±sd)		11.9±10.9	18.4±18.5	13.5±10.8		0.08 16.2±15.7	15.9±16.1	12.7±11.4		0.48 15.3±15.5	15.6±13.1	7.8±5.8		0.40	
Recurrent MDE (n(%))		84(73)	131(72)	67(75)		0.89 82(77)	136(72)	63(72)		0.54 224(72)	52(76)	5(83)		0.74	
Onset age MDE (m±sd))		34.1±13.8	36.1±14.6	35.1±15.2		0.52 35.1±14.8	36.7±14.7	32.3±13.3		0.06 35.3±14.7	35.7±13.5	33.5±17.7		0.87	
Baseline HDRS (m±sd))		23.6±4.4	25.2±4.7	25.2±5.4	0.025*	25.3±5.2	24.9±4.8	24±4.7		0.20 25.1±5	23.5±4.4	23.7±5		0.06	
Prescribed AD (n(%))	<i>SSRI</i>	46(40)	70(39)	41(46)		0.93 46(43)	72(38)	39(44)		0.66 124(40)	27(40)	5(83)		0.81	
	<i>SNRI</i>	48(42)	77(43)	30(34)		35(33)	84(44)	35(40)		127(41)	27(40)	1(17)			
	<i>TCA</i>	6(5)	11(6)	7(8)		9(8)	10(5)	5(6)		20(6)	4(6)	0(0)			
	<i>Other</i>	11(10)	16(9)	7(8)		10(9)	18(9)	6(7)		28(9)	6(9)	0(0)			
	<i>ECT</i>	4(3)	7(4)	4(4)		6(6)	6(3)	3(3)		11(4)	4(6)	0(0)			
Dropout (n(%))	<i>M1</i>	8(7)	8(4)	5(6)	0.62	6(6)	9(5)	6(7)		0.73 14(5)	6(9)	0(0)		0.44	
	<i>M3</i>	29(25)	61(34)	30(34)	0.26	36(34)	59(31)	24(27)		0.61 90(29)	27(40)	2(33)		0.22	
	<i>M6</i>	54(47)	80(44)	44(49)	0.71	50(47)	85(45)	42(48)		0.87 137(44)	39(57)	2(33)		0.11	
		rs113636971					rs504683					rs561923			
		AA	AG	GG	<i>P</i>	AA	AG	GG	<i>P</i>	CC	CA	AA	<i>P</i>		
		n=340	n=46	n=2		n=195	n=164	n=27		n=220	n=142	n=20			
Age (m±sd)		45.4±13.3	45.6±13.9	43±8.5		0.94 44.4±13.4	46.3±13	47.4±14.3		0.30 44.1±13.6	47±12.5	50.1±15.1		0.052	
Female (n(%))		233(69)	29(63)	1(50)		0.51 134(69)	108(66)	20(74)		0.68 150(68)	94(66)	14(70)		0.90	
Education (n(%))	<i>Primary</i>	32(9)	4(9)	0(0)		0.83 17(9)	16(10)	2(7)		0.95 20(9)	15(11)	1(5)		0.94	
	<i>High school</i>	147(43)	21(46)	0(0)		85(44)	73(45)	10(37)		91(41)	64(45)	9(45)			
	<i>University</i>	160(47)	21(46)	2(100)		92(47)	75(46)	15(56)		108(49)	63(44)	10(50)			
Ethnicity (n(%))	<i>Caucasian</i>	308(91)	43(93)	2(100)	0.92	167(86)	157(96)	27(100)		0.0044*	192(87)	136(96)	20(100)	0.032*	
	<i>African</i>	22(6)	2(4)	0(0)		19(10)	5(3)	0(0)		19(9)	4(3)	0(0)			
	<i>Mixed</i>	9(3)	1(2)	0(0)		9(5)	1(1)	0(0)		9(4)	1(1)	0(0)			
Current smoker (n(%))		126(37)	22(48)	1(50)	0.31	82(42)	59(36)	8(30)		0.32 90(41)	50(35)	6(30)		0.42	
Pack years (m±sd)		15.3±15.4	14±11.3	20±NA	0.72	14.4±14.5	16.7±15.1	13±17		0.26 14.3±14.3	17.6±16.2	11.1±12.5		0.24	
Recurrent MDE (n(%))		246(72)	36(78)	2(100)	0.67	138(71)	122(74)	23(85)		0.28 159(72)	105(74)	16(80)		0.80	
Onset age MDE (m±sd))		35.7±14.4	32.5±14.7	29±4.2	0.39	35±14	35.5±15.1	34.9±14.1		0.94 34.7±14	35.9±14.9	37±17.3		0.65	
Baseline HDRS (m±sd))		24.8±4.9	24.6±4.9	21.5±0.7	0.58	25.1±5.1	24.6±4.6	24.1±5.3		0.55 24.9±5.1	24.5±4.5	24.6±5.5		0.85	
Prescribed AD (n(%))	<i>SSRI</i>	139(41)	18(39)	0(0)	0.08	85(44)	64(39)	8(30)		0.41 90(41)	58(41)	7(35)		0.84	
	<i>SNRI</i>	139(41)	19(41)	0(0)		72(37)	72(44)	12(44)		86(39)	60(42)	9(45)			
	<i>TCA</i>	19(6)	5(11)	0(0)		12(6)	11(7)	1(4)		15(7)	8(6)	1(5)			
	<i>Other</i>	30(9)	2(4)	2(100)		20(10)	11(7)	3(11)		22(10)	10(7)	1(5)			
	<i>ECT</i>	13(4)	2(4)	0(0)		6(3)	6(4)	3(11)		7(3)	6(4)	2(10)			
Dropout (n(%))	<i>M1</i>	18(5)	3(7)	0(0)	0.76	9(5)	10(6)	2(7)		0.68 9(4)	10(7)	2(10)		0.21	
	<i>M3</i>	110(32)	10(22)	1(50)	0.23	63(32)	48(29)	10(37)		0.65 79(36)	34(24)	7(35)		0.046*	
	<i>M6</i>	158(46)	20(43)	1(50)	0.88	92(47)	74(45)	13(48)		0.91 108(49)	58(41)	10(50)		0.29	

III.4. Using Biological Constraint to Identify Relevant Genes in Association with Clinical Improvement Following Antidepressant Treatment in Depressed Patients

Numerous biological mechanisms are implicated in MDD and the response to its treatment with ATDs. As such, genes within these pathways remain candidate biomarkers. Genetic analysis for genome-wide approaches like GWAS and HTS is burdened by the number of statistical tests performed. Methods aimed at reducing data dimensionality can aid in reducing this statistical burden. The aggregation of genetic variation to their associated genes and their joint analysis is one example of this. We aimed to identify which genes from a targeted HTS gene panel were most relevant to clinical outcome measures following ATD treatment in patients of the METADAP cohort by using the biological constraint method, G2PSR.

III.4.1. Overview of patients, materials, and methods

A total of 394 patients with ≥ 1 follow-up measure during the study were analyzed. Genetic variation was genotyped from leukocytic DNA using HTS and a targeted gene panel of 72 candidate genes for MDD and ATD treatment outcomes. Variant calls were subjected to quality control. Common genetic variation ($MAF \geq 5\%$) was retained for analysis. Data were prepared in R. The HDRS score, percentage change, CGI-S, and CGI-I at M1, M3, and M6 were analyzed with G2PSR. G2PSR was run using Python 3 in a Jupyter environment. Linear mixed-effects models were constructed in R and adjusted *a priori* for age, sex, and ATD class to analyze the aforementioned clinical outcomes and response and remission rates.

III.4.2. Principal results

Among the 394 patients analyzed, 4,326 genetic variants were identified in 71 of 72 genes. Overall, 323 common genetic variants within 58 genes were analyzed. *SLC1A1* was the most relevant gene identified. Among the 7 *SLC1A1* genetic variants, the rs301435(T>C) genetic polymorphism was significantly associated with the HDRS and CGI-I scores, percentage change, and response and remission rates.

III.4.3. Summary of the discussion

Our whole METADAP gene panel analysis is the first application of G2PSR to targeted HTS data. The results suggest that genetic variation within *SLC1A1*—and especially the rs301435 genetic polymorphism—is associated with clinical outcomes following ATD treatment. This appears to be the first association of *SLC1A1* genetic variation with clinical outcomes following ATD treatment in depressed patients. However, *SLC1A1* has been previously associated with clinical outcomes following ATD treatment in patients with obsessive compulsive disorder (OCD). These and other data support the implication of the

glutamatergic system in the pathophysiology of MDD and its treatment with ATDs. The role of *SLC1A1* and the functional consequences of *SLC1A1* genetic variation in these contexts require further study. This analysis was limited by its dropout rate and various features of the analyzed data (i.e., relatively small volume, genetic variation distribution per gene, and enrichment of relevant genes).

III.4.4. Conclusion

G2PSR identified genetic variation within *SLC1A1* as associated with clinical outcomes following ATD treatment. Significant associations were observed with the rs301435(T>C) genetic polymorphism. This and previous findings between *SLC1A1* and pharmacological response in psychiatric disorders argue for continued study.

III.4.5. Publication

A formal manuscript of this work is in preparation. Herein, we present it in its current state.

Using Biological Constraint to Identify Relevant Genes in Association with Clinical Improvement Following Antidepressant Treatment in Depressed Patients

Abstract

Major Depressive Disorder (MDD) is the current leading cause of disability worldwide. Genetic variation can influence the effect of its main treatment option, antidepressant drugs (ATD). Genome-wide association studies aid genetic analysis but suffer notably from multiple testing; aggregating genetic variation to genes can reduce this burden. We aimed to analyze the association of gene-constrained genetic variation from a targeted panel with clinical outcomes in a cohort of 6-month ATD-treated individuals.

Clinical data were obtained at baseline and after 1 (M1), 3 (M3), and 6 (M6) months of ATD treatment in 394 patients of the METADAP cohort. Genetic data were obtained from high-throughput sequencing (HTS) using a targeted gene panel of 72 candidate genes. Genome-to-Phenome Sparse Regression (G2PSR) was used to analyze the association of gene-constrained genetic variation with the Hamilton Depression Rating Scale (HDRS) and Clinical Global Impressions (CGI) scores and the HDRS percentage change. Mixed-effects models were used to assess the association of genetic variation with these clinical measures and response and remission rates across time.

Among the 394 individuals, 323 genetic variants within 58 genes were analyzed. *SLC1A1* was identified in association with clinical outcomes. The *SLC1A1* rs301435(T>C) genetic polymorphism was significantly associated with the HDRS ($P=0.0077$) and CGI-Improvement scores ($P=0.014$), percentage change ($P=0.026$), and response ($P=0.038$) and remission rates ($P=0.016$). In each case, the C allele was associated with worse improvement. Our results demonstrate the utility of G2PSR in analyzing targeted HTS genetic data and an association of *SLC1A1* genetic variation with clinical improvement following ATD treatment in depressed patients. Further investigations of *SLC1A1* may demonstrate its utility as a biomarker in this context.

Keywords

Major Depressive Disorder, antidepressant, pharmacogenetics, biological constraint, *SLC1A1*, Excitatory Amino Acid Transporter 3

Introduction

Major Depressive Disorder (MDD) is the leading contributor to global disability ¹. Antidepressant drugs (ATD) remain the main treatment option for major depressive episodes (MDE), but only about half of ATD-treated patients respond to ATD therapy ². Clinical improvement is typically observed only after 4–6 weeks of treatment, prolonging the course of therapy and its cost ³. The complexity and heterogeneity of MDD likely contribute ^{4,5}.

Genetic variation, including single nucleotide polymorphisms (SNP), influences the response to ATD treatment ⁶. Hundreds of genetic associations have been identified ⁷, some of which are located in genes that belong to biological mechanisms implicated in the pathophysiology of MDD, including monoamine neurotransmission, glutamatergic signaling, hypothalamic–pituitary–adrenal (HPA) axis dysregulation, and inflammation ⁸.

Both candidate studies and large-scale genome-wide association studies (GWAS) have tried to identify common underlying genetic factors of the response to ATD treatment ⁷. In GWAS, independent tests for each genetic polymorphism (often millions) are performed for each phenotype of interest ⁹. Multiple testing correction helps limit false positives, but the high level of significance needed may also remove true positives ⁹. As a result, genome-wide significant associations are scarce among clinically diagnosed populations ^{7,10}.

Approaches like gene- or pathway-based association tests are proposed to help reduce the multiple testing burden. Often, these methods aggregate and jointly analyze genetic polymorphisms according to their associated genes or pathways ¹¹. For example, *ETV4*—a factor related to hippocampal dendrite development and plasticity—was identified in association with percentage improvement and remission in the largest clinical cohort analysis of ATD response in depression ¹⁰.

Like GWAS, high-throughput sequencing (HTS) can identify and analyze genome-wide genetic variation, though it can also be applied to the analysis of exomes (i.e., coding regions) and candidate regions ¹², often with improvements to cost and time ¹³. Gene panels for drug response generally target pharmacogenes that influence drug metabolism and/or efficacy ¹⁴. In the study of ATD outcomes in depression, genetic variation in several candidate genes is commonly explored, many of which relate to ATD metabolism, monoamine neurotransmission, glutamatergic signaling, the HPA axis, and inflammation ^{7,15}.

Given the diversity of genetic data—from genome-wide to targeted regions—and phenotypic features, gene-based analyses require varied flexibility, scalability, and performance. Genome-to-Phenome Sparse Regression (G2PSR) is one method that can analyze the association between large (i.e., ~100,000 SNPs in ~4,000 genes) and small (i.e., targeted regions) volumes of biologically-constrained genetic variation and several phenotypic features, alleviating the need for multiple testing ¹⁶. Importantly, its efficacy was demonstrated in the genetic analysis of clinical and imaging data in Alzheimer’s disease.

We sought to use G2PSR to explore which genes, if any, of a targeted panel of genes related to MDD and ATD treatment outcomes may be associated with clinical improvement outcomes following 6 months of ATD treatment in individuals with a current MDE in the context of MDD.

Patients, Materials, and Methods

Study design

The METADAP cohort is a 6-month prospective, naturalistic treatment, multicentric study carried out in a psychiatric setting ¹⁷. Patients with a current MDE were treated and assessed before and during ATD treatment. This study was registered by the French National

Agency for Medicine and Health Products Safety (ANSM) and the Commission Nationale de l'Informatique et des Libertés (CNIL). It was approved by the Ethics Committee of Paris-Boulogne (France) and conformed to international ethical standards ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00526383) identifier: [NCT00526383](https://clinicaltrials.gov/ct2/show/study/NCT00526383)).

Patients

Males and females 18–65 years of age without a serious medical or inflammatory condition were recruited. Inclusion criteria were presentation with a MDE in the context of MDD (DSM-IVTR), as assessed by the Mini International Neuropsychiatric Interview (MINI), a score ≥ 18 on the 17-item Hamilton Depression Rating Scale (HDRS)¹⁸, and need for a new ATD treatment. Patients with psychotic symptoms or other mental disorders—such as psychotic disorder, bipolar disorder, alcohol or drug dependence, or an eating disorder—or those who were pregnant or who had organic brain syndromes or serious medical conditions, were excluded. Measures and samples were obtained prior to beginning ATD treatment (M0), and after 1 (M1), 3 (M3), and 6 (M6) months of ATD treatment.

Of the 643 patients included in METADAP, 19 had major protocol deviations and were excluded. Of the 624 available for analysis, 519 patients provided samples for genetic studies. From these, 401 have undergone high-throughput sequencing. Two samples were removed due to technical difficulties. As the objective of this study was to examine clinical response following ATD treatment, 5 patients for whom no follow-up measures were available (i.e., at M1, M3, and M6) were removed. Thus, 394 patients were analyzed. Dropouts occurred mainly because of ATD changes, use of unauthorized drugs, or loss to follow-up. Information about ancestry was self-reported. Caucasian patients were defined as having Caucasian parents, African patients as having Sub-Saharan African and/or Afro-Caribbean parents, and Asian patients as having East Asian, Central Asian, and/or South Asian parents¹⁹. All individuals provided written informed consent for study participation and genetic analyses¹⁷.

Antidepressant treatment

ATD monotherapies were prescribed by a psychiatrist in a “real world” psychiatric treatment setting as previously described¹⁷. ATDs belonged to 1 of 4 classes: selective serotonin reuptake inhibitors (SSRI), serotonin norepinephrine reuptake inhibitors (SNRI), tricyclic antidepressants (TCA), or other ATD treatments. In this ancillary study of 394 patients, 40% (n=156) were prescribed an SSRI, 40% (n=158) an SNRI, 7% (n=26) a TCA, and 9% (n=34) another ATD treatment. If a change in treatment was required during follow-up, the patient was dropped from the study.

Assessment of antidepressant treatment response

The HDRS¹⁸ and Clinical Global Impressions (CGI)-Severity scale (CGI-S)²⁰ were used to assess depression severity at M0 (i.e., baseline). The HDRS and CGI-S, as well as the percentage change from the baseline HDRS score (hereafter referred to as percentage change) and CGI-Improvement scale (CGI-I)²⁰, were used to assess clinical improvement to ATD treatment at M1, M3, and M6. Responders were defined by an improved HDRS score $\geq 50\%$ relative to baseline, remitters by a HDRS score ≤ 7 after ≥ 4 weeks of ATD treatment, as recommended by the American College of Neuropsychopharmacology (ACNP) Task Force²¹. Clinical assessments were performed blind to genotyping results. Each interview and

diagnostic assignment was reviewed by a senior psychiatrist. For each patient, all visits were reviewed by the same psychiatrist.

High-throughput sequencing

Five mL of whole blood was collected at baseline. Leukocytic DNA was extracted from 1 mL of blood using a Puregene Kit (Gentra systems, Minneapolis, USA) and stored at -20°C. Patient DNA samples were sequenced using a targeted-exome panel of pharmacogenes involved in mood disorders and ATD metabolism. The protocols used for high-throughput sequencing and information about the gene panel and variant calling are described elsewhere²². Briefly, the METADAP gene panel targeted exons and intronic flanking regions (± 50 base pairs) of 72 candidate genes, though *ARRB1* and *ARRB2* were whole-gene sequenced.

Variant call data

Variant Call Format data were loaded into R (v4.1.0)²³. Individual variant calls were annotated for call quality as previously described²⁴. Briefly, variant calls with a sequencing depth <20 , SNPs with a quality score <275 , insertions/deletions with a quality score <770 , heterozygous variant calls with an allele balance <0.34 or >0.79 , and homozygous variant calls with an allele balance <0.96 were annotated as poor-quality calls. Call rates were calculated as the number of poor-quality calls divided by the total number of calls. Genetic variants with a call rate $<89.12\%$ (lower quintile) were excluded. Genetic variants within *CYP2D6* ($n=22$) were excluded due to (1) an overall low call rate (mean=66%), with 81.82% of variants below 89.12%, and (2) read alignment to regions of *CYP2D6*, *CYP2D7*, and *CYP2D8* due to their high sequence homology²⁵.

Linkage disequilibrium (LD) between genetic variants was assessed using the *ld* function from the *snpStats* package in R²⁶. For genetic variants in strong LD (i.e., $r^2 \geq 0.8$), those with the highest MAF were selected for analysis while the others were removed.

G2PSR data and parameterization

G2PSR was run using Python 3 in a Jupyter environment (<https://jupyterhub.ijclab.in2p3.fr/>). Three matrices were generated for use with G2PSR, as previously described¹⁶. The sample \times phenotype matrix contained data for each phenotypic variable (i.e., the HDRS score, percentage change, the CGI-S, and the CGI-I). The redundancy from including each improves accuracy and helps with the identification of relevant genes¹⁶. Data at M1, M3, and M6 were included. Missing clinical data were imputed as the median. The genetic polymorphism \times sample matrix contained the number of variant alleles carried (i.e., 0, 1, or 2) by each patient for each included genetic variant. The genetic polymorphism \times gene matrix contained polymorphism-gene constraints (i.e., 1 if polymorphism is associated with the gene, 0 otherwise).

Since *ARRB1* and *ARRB2* were whole-gene sequenced compared to the rest of the targeted gene panel, we created 3 datasets for use with G2PSR: (1) with all genetic variation constrained to their respective genes; (2) with genetic variation of *ARRB1* divided into 8 separate regions of 8–9 genetic variants to better match the number of variants of other genes; (3) with genetic variation of *ARRB1* and *ARRB2* limited to exonic and 5'- and 3'-UTR genetic variants only to better match the exonic sequencing of the other genes.

G2PSR was initialized to 50,000 epochs and 1,000 steps. G2PSR was run 10 times to obtain means and SDs for loss, sigma, and α parameters. Early stopping was used to determine the optimal epoch for the identification of relevant genes (i.e., $\alpha < 0.05$)¹⁶.

Data analysis

Statistical analyses were performed in R (v4.1.0)²³. Quantitative variables were non-normally distributed and analyzed using nonparametric Kruskal-Wallis (when comparing 3 groups) or Wilcoxon tests (when comparing 2 groups). Qualitative variables were analyzed using chi-square or Fisher Exact tests if >20% of expected cell counts were <5. Linear mixed-effects models and generalized linear mixed-effects regression models with the binomial link function were constructed using the lme4 package (v1.1-27.1)²⁷. The main variable to explain was the HDRS total score. Other variables to explain were the response and remission rates. The number of major alleles (i.e., 0, 1, or 2) was the main explanatory variable examined. Age, sex, and ATD class were included *a priori* as fixed-effects covariables. Sociodemographic and baseline clinical variables that significantly differed between genotypes (i.e., $P<0.05$) were also included as fixed-effects covariables. Individual was considered as a random intercept to account for the repeated measures. A significance threshold of $P<0.05$ was considered significant.

Results

Patient demographics

Sociodemographic characteristics for the whole cohort are shown in Table 1. The mean age was 45.4, 68% of subjects were female, and 91% of subjects were Caucasian. SSRIs or SNRIs were prescribed to 80% of subjects and 47% of subjects were missing at follow-up at M6.

Table 1: Sociodemographic characteristics

Sociodemographic characteristics for the whole sample. Age, onset age of MDE, and baseline HDRS are presented as mean±SD. Sex, education level, smoking status at baseline, MDE recurrence, prescribed ATD, and missing data during follow-up are presented as the group number and percentage.

ATD: antidepressant drug; **ECT:** electroconvulsive therapy; **HDRS:** 17-item Hamilton Depression Rating Scale; **M1:** 1 month after beginning antidepressant treatment; **M3:** 3 months after beginning antidepressant treatment; **M6:** 6 months after beginning antidepressant treatment; **MDE:** major depressive episode; **n:** number of patients; **SNRI:** serotonin norepinephrine reuptake inhibitor; **SSRI:** selective serotonin reuptake inhibitor; **TCA:** tricyclic antidepressant

	n=394
Age (in years) (m±sd)	45.4±13.3
Sex [n (%)]	268(68)
Education level [n (%)]	<i>Primary</i> 37(9)
	<i>Secondary</i> 171(43)
	<i>Tertiary</i> 185(47)
Ethnicity [n (%)]	<i>Caucasian</i> 358(91)
	<i>African</i> 25(6)
	<i>Mixed</i> 10(3)
Current smoker [n (%)]	149(38)
Recurrent MDE [n (%)]	289(73)
Onset age MDE (m±sd)	35.3±14.5
Baseline HDRS (m±sd)	24.8±5
Prescribed ATD [n (%)]	<i>SSRI</i> 158(40)
	<i>SNRI</i> 159(40)
	<i>TCA</i> 26(7)
	<i>Other</i> 34(9)
	<i>ECT</i> 17(4)
Missing at follow-up [n (%)]	<i>M1</i> 21(5)
	<i>M3</i> 124(31)
	<i>M6</i> 184(47)

Genetic variation selection

Among the 394 subjects, 4,326 genetic variants were identified in 71 of 72 gene panel genes, of which 600 (14%) were common ($MAF \geq 5\%$). Among these, 480 genetic variants (80%) were of good quality. Seventy-one genetic variants were in LD and removed. Thus, 323 genetic variants within 58 genes were analyzed. Among the 58 genes, almost 48% carried 3 or fewer genetic variants, while 1 (*ARRB1*) carried 67 (see Figure 1). When limiting *ARRB1* and *ARRB2* to exonic genetic variants only, 254 genetic variants within 58 genes were analyzed.

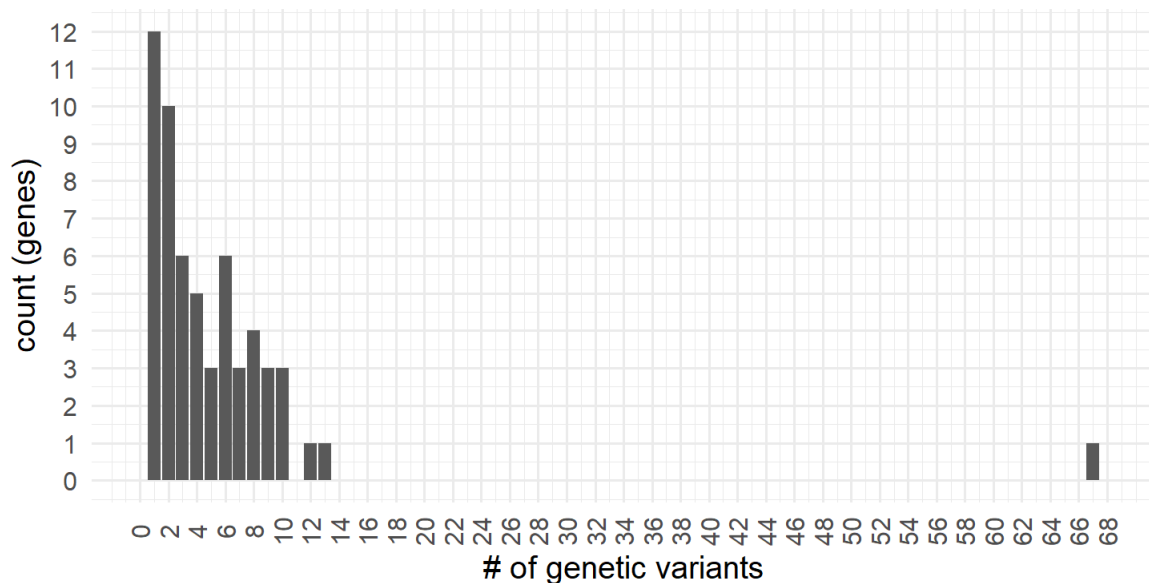


Figure 1: Gene counts according to number of genetic variants

Gene counts (y-axis) according to the number of genetic variants (x-axis) are shown.

G2PSR

The loss parameter began to normalize after ~20,000 epochs. Sigma parameters began to decrease around 10,000 epochs for CGI measures and 18,000 epochs for HDRS measures and normalize around 25,000 epochs (see Figure 2). We thus looked for relevant genes at 25,000 epochs. Here, *SLC1A1* ($\alpha=0.047$) and *ARRB1* ($\alpha=0.033$) were identified (see Figure 3A).

When genetic variation within *ARRB1* was divided into 8 separate regions, or when genetic variation within *ARRB1* and *ARRB2* were limited to exonic sequences only, loss and sigma parameters did not vary from the initial analysis (data not shown). We thus looked for relevant genes at 25,000 epochs in these analyses as well. In the divided *ARRB1* analysis, *NPAS2* ($\alpha=0.048$), *SLC6A3* ($\alpha=0.047$), and *SLC1A1* ($\alpha=0.042$) were identified (see Figure 3B). In the exonic *ARRB1* and *ARRB2* analysis, *SLC1A1* was the only gene identified ($\alpha=0.039$) (see Figure 3C).

Since *SLC1A1* was the only gene identified in each analysis, we selected it and its associated genetic variants for further analysis.

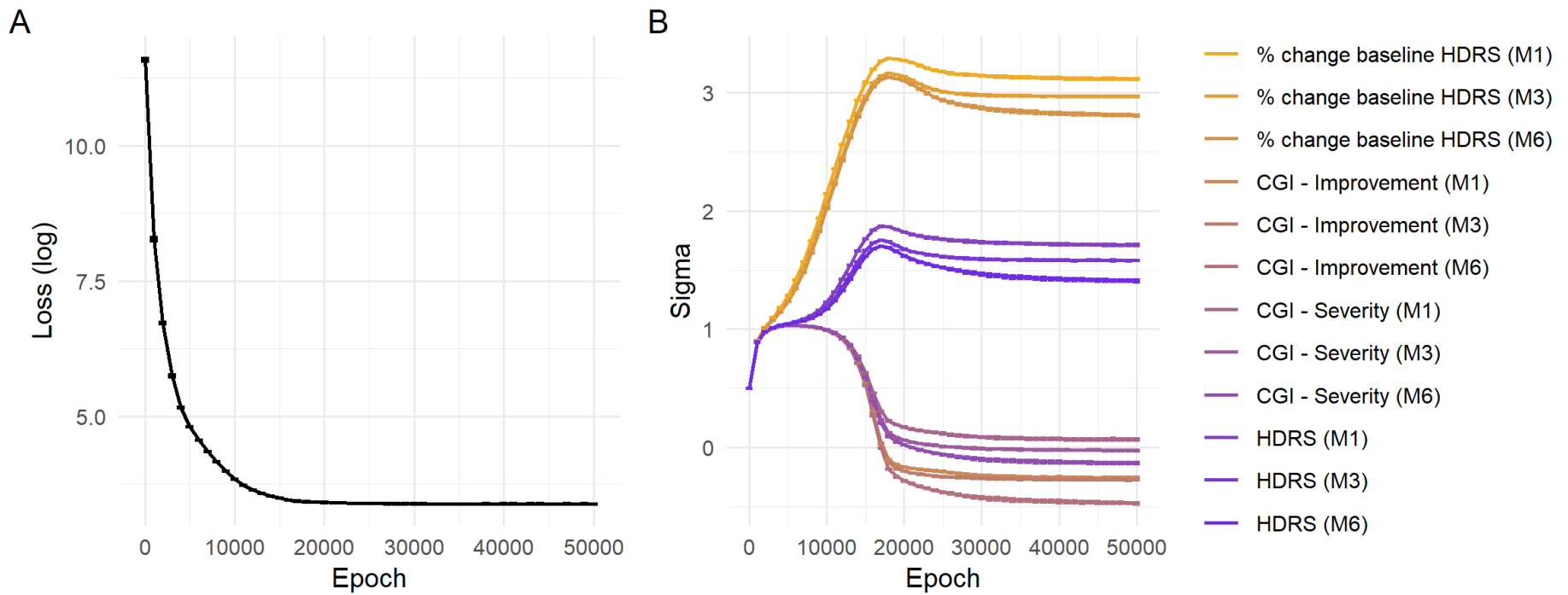


Figure 2: G2PSR loss and sigma parameters for optimization
 Log-transformed loss (A) and sigma (B) values (y-axes) are shown for each epoch/iteration (x-axes). Error-bars represent SD (10 replicate analyses).

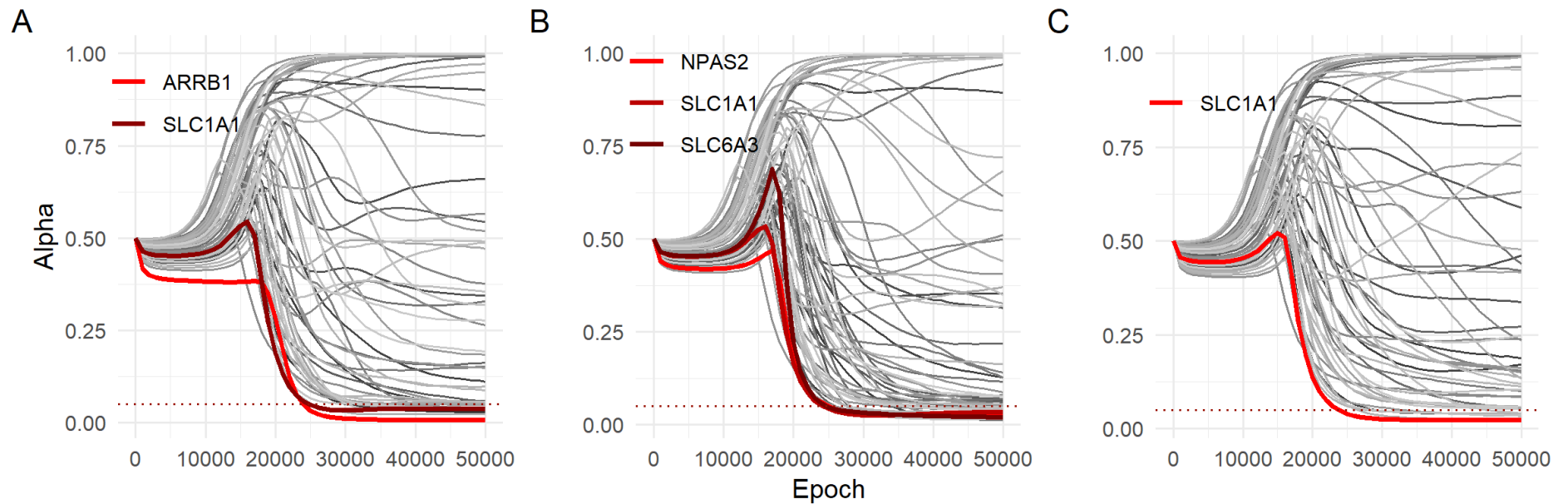


Figure 3: Relevant genes identified from G2PSR analyses

Relevant genes (red lines) identified in each of 3 G2PSR analyses: (A) all genetic variation constrained to their respective genes; (B) *ARRB1* genetic variation divided into subregions; (C) *ARRB1* and *ARRB2* genetic variation limited to exonic regions. The red, dashed horizontal line represents the significance threshold of $\alpha < 0.05$.

Association of genetic variation within G2PSR-identified genes with clinical outcomes

We next analyzed the association of the 7 *SLC1A1* genetic variants with the HDRS score using mixed-effects models. Among these 7 variants, and after controlling for age, sex, ATD class, time, and significant clinical and sociodemographic factors (see Supplementary Table 1), the rs301435(T>C) genetic polymorphism was significantly associated with the HDRS score, with the number of C alleles increasing with the HDRS score overall (coef=0.96, 95%CI [0.26–1.66], $P=0.0077$) (see Table 3). At M3, the number of C alleles was significantly associated with increased HDRS scores (coef=1.67, 95%CI [0.42–2.93], $P=0.0091$) (see Figure 4A).

The number of rs301435 C alleles was also associated with overall lower percentage change and overall higher CGI-I scores, but not with the CGI-S score (see Table 4 and Figure 2B-D). At M3, the number of C alleles was significantly associated with a lower percentage change (coef=-5.38, 95%CI [-10.3–-0.46], $P=0.032$) and higher CGI-I score (coef=0.26, 95%CI [0.068–0.45], $P=0.0081$) (see Figure 4B and 4D).

Lastly, we examined the association of rs301435 with response and remission rates. The number of rs301435 C alleles was associated with overall lower response and remission rates (see Table 4). The number of C alleles was significantly associated with decreased remission rates at M1 (OR=0.66, 95%CI [0.45–0.96], $P=0.032$) and with decreased response rates at M3 (OR=0.66, 95%CI [0.45–0.94], $P=0.023$) (see Figure 4E-F).

Table 3: Mixed-effects model results of the association of 7 *SLC1A1* genetic variants with the HDRS score For each *SLC1A1* genetic variant we show the results of the mixed-effects models of the HDRS total score. Models were adjusted for age, sex, ATD class, time, and significant clinical and sociodemographic factors (see Supplementary Table 1). **Bold text** and **: $P<0.01$.

95%CI: 95% confidence interval; **df:** degrees of freedom; **P:** P-value; **se:** standard error

term	estimate	se	stat	df	95%CI	P
rs45518336	-0.50	0.44	-1.12	375.05	-1.36–0.37	0.26
rs10974625	0.16	0.41	0.38	372.95	-0.64–0.95	0.70
rs2228622	-0.07	0.37	-0.20	381.27	-0.81–0.66	0.84
rs12682807	0.54	0.61	0.88	376.13	-0.67–1.74	0.38
rs301430	0.27	0.37	0.72	369.76	-0.46–0.99	0.47
rs1471786	-0.56	0.50	-1.10	377.06	-1.55–0.43	0.27
rs301435	0.96	0.36	2.68	373.80	0.26–1.66	0.0077**

Table 4: Mixed-effects model results of the association between the *SLC1A1* rs301435 genetic variant and clinical outcomes

Results for mixed-effects models examining the association of the *SLC1A1* rs301435 genetic polymorphism with the percentage change, CGI-S score, CGI-I score, and response and remission rates. Models were adjusted for age, sex, ATD class, and time. **Bold text** and (1) *: $P<0.05$; (2) **: $P<0.01$; (3) ***: $P<0.001$.

95%CI: 95% confidence interval; **CGI-I:** Clinical Global Impressions – Improvement scale; **CGI-S:** Clinical Global Impressions – Severity scale; **df:** degrees of freedom; **P:** P-value; **se:** standard error

clinical outcome	rs301435					
	estimate	se	stat	df	95%CI	P
Percentage change	-3.99	1.79	-2.23	352.00	-7.51–-0.47	0.026*
CGI-S	0.12	0.07	1.84	391.36	-0.01–0.25	0.066
CGI-I	0.16	0.06	2.47	358.10	0.03–0.28	0.014*
Response	0.75	0.10	-2.07	-	0.57–0.98	0.038*
Remission	0.61	0.12	-2.41	-	0.41–0.91	0.016*

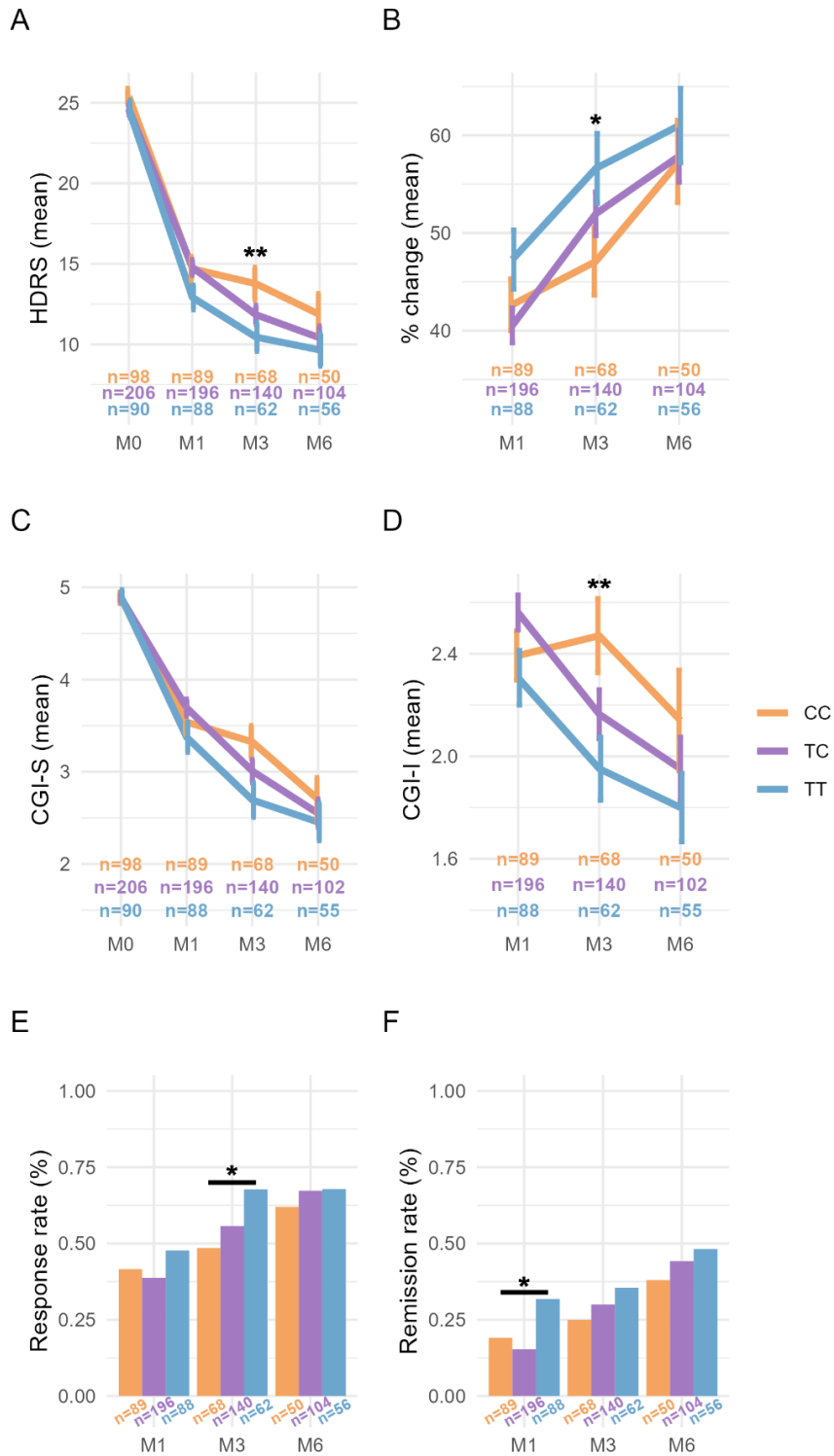


Figure 4: Clinical measures during 6 months of antidepressant treatment according to *SLC1A1* rs301435 genotypes

Average HDRS scores (A), percentage changes (B), CGI-S scores (C), CGI-I scores (D), response rates (E), and remission rates (F) (y-axes) across time (x-axes) between rs301435 genotypes (see legends). Error bars correspond to the standard error of the mean. *: $P < 0.05$; **: $P < 0.01$.

HDRS: 17-item Hamilton Depression Rating Scale; **M0:** baseline, prior to beginning antidepressant treatment; **M1:** 1 month after beginning antidepressant treatment; **M3:** 3 months after beginning antidepressant treatment; **M6:** 6 months after beginning antidepressant treatment; **n:** number of individuals

Discussion

In this ancillary investigation of the METADAP cohort, we analyzed genetic variation constrained to genes of a targeted HTS gene panel to identify genes associated with clinical outcomes following ATD treatment in individuals suffering from MDE in the context of MDD. To the best of our knowledge, this is the first application of G2PSR to the analysis of targeted HTS genetic data. Findings from G2PSR suggested that genetic variation within *SLC1A1* was strongly associated with clinical measures. We then observed significant associations between the rs301435(T>C) genetic polymorphism and the HDRS and CGI-I scores, percentage change, and response and remission rates. Overall, the rs301435 C allele was associated with worse improvement, especially after 3 months of ATD treatment.

G2PSR was previously used to explore genetic associations with Alzheimer's disease¹⁶. Among 104,854 SNPs across 3,953 genes, *APOE*—the principal genetic risk factor of Alzheimer's disease—and other genes associated with mechanisms of Alzheimer's disease onset or progression were identified in association with cognition scales and imaging data¹⁶. In comparison, our analysis of 323 genetic variants across 58 genes represents a 324- and 68-fold decrease in the number of genetic variants and genes, respectively. Despite the significantly smaller volume of data, a low significance threshold from performing tests for each of the 323 genetic variants would need to be applied (i.e., $P < 0.05/323 = 0.00015$), highlighting the interest of using G2PSR. In our analysis, G2PSR was able to identify *SLC1A1*, which follow-up analyses confirmed contained genetic variation associated with clinical outcomes following ATD treatment. Our findings suggest that G2PSR is a useful tool for genetic analysis when applied to targeted sequencing data.

Pharmacological investigations of *SLC1A1* genetic variation have been performed notably in the context of obsessive compulsive disorder (OCD)^{28–31}. In a population of 340 Han Chinese patients, the CC genotype of the rs301430(T>C) genetic polymorphism was associated with improved outcomes following 12 weeks of fluoxetine (an SSRI) treatment³⁰. Among 243 Iranian patients, rs2228622(G>A) and rs3780413(C>G) were associated with response following 12 weeks of fluvoxamine (an SSRI) treatment³¹. Lastly, in a study of 248 Spanish Caucasian patients, variant allele carriers of rs301434(C>T), rs301435(T>C), and rs3087879(G>C) were more resistant following more than 12 weeks of SSRI treatment²⁸.

In our analysis, no significant associations between the HDRS score and rs301430 or rs2228622 were observed. This may be due to ethnic differences. Indeed, Han Chinese and Iranian populations were previously examined^{30,31}, whereas our analysis was performed in a predominantly Caucasian population (~90%). The proportion of genotypes for each of these genetic polymorphisms also significantly differed with respect to ethnicity in our sample. The rs301435 genetic polymorphism was significantly associated with the HDRS and several other clinical outcomes. Here, the number of rs301435 C alleles was associated with worse improvement following ATD treatment, notably after 3 months of treatment. Our findings appear to agree with the study of Real *et al.*, wherein rs301435 variant allele carriers (i.e., the C allele) were more resistant to SSRI treatment in the context of OCD²⁸.

OCD and MDD share many characteristics and symptoms, and OCD is one of the most common comorbidities of MDD³². Alongside other psychiatric diseases, altered glutamatergic signaling is implicated and shared by OCD and MDD³³. *SLC1A1* encodes the neuronal glutamate transporter, Excitatory Amino Acid Transporter 3 (EAAT3), which aids in terminating glutamatergic excitatory signals through the cellular reuptake and synaptic removal of glutamate^{29,34}. The functional consequences of *SLC1A1* genetic variation remain, to the best of our knowledge, to be characterized and experimentally validated. However, our findings suggest that genetic variation within *SLC1A1*, which may alter glutamatergic processes, should continue to be investigated in association with ATD treatment outcomes.

Limitations

There are several limitations to our study. First, the proportion of missing data during follow-up is high. However, the use of mixed-effects models helps to control for the bias introduced by missing data³⁵. Second, the volume, distribution (i.e., 20% of genes with 1 polymorphism), and relevance (i.e., oversaturation of associated genes) of the genetic data used with G2PSR may represent several biases and limitations. Indeed, genes with more genetic variation may introduce selection bias, while the enrichment of relevant genes can negatively impact the performance of G2PSR¹⁶. As such, the number of relevant genes identified may have been limited, too. Third, despite the removal of non-relevant genes with G2PSR, the *SLC1A1* rs301435 genetic polymorphism does not survive multiple testing correction ($P < 0.05/7 = 0.0071$). We underline that this analysis remains exploratory in nature³⁶. Additionally, the association of the rs301435 genetic polymorphism with multiple clinical outcomes bolsters its potential as a genetic marker for the response to ATD treatment. This study benefits from its prospective and naturalistic design, which better reflects “real-world” clinical practice.

Conclusion

In conclusion, we identified genetic variation of *SLC1A1* as relevant in association with ATD treatment outcomes and, consequently, an association of the C allele of the rs301435(T>C) genetic polymorphism with worse clinical outcomes following ATD treatment. Previous associations of *SLC1A1* genetic variation with psychiatric disorders and with response to pharmacological treatment argue for its continued study in these contexts. Analysis in ethnically diverse and longitudinal studies would help confirm our findings.

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III.4.6. Supplementary Materials

Supplementary Table 1: Sociodemographic characteristics according to *SLC1A1* polymorphism genotypes

Sociodemographic characteristics of METADAP patients are shown according to genotype for each of the 7 *SLC1A1* polymorphisms. Kruskal-Wallis tests were used to compare age, onset age of MDE, and baseline HDRS scores (presented as mean±standard deviation). Chi square or Fisher Exact tests were used to compare sex, socio-education status, ethnicity, smoking status at baseline, MDE recurrence, prescribed ATD drug, and missing at follow-up rates (presented as the number of patients and percentage). *: $P<0.05$; ***: $P<0.001$. **ATD**: antidepressant drug; **ECT**: electroconvulsive therapy; **HDRS**: 17-item Hamilton Depression Rating Scale; **m**: mean; **M1**: after 1 month of treatment; **M3**: after 3 months of treatment; **M6**: after 6 months of treatment; **MDE**: major depressive episode; **n**: number of patients; **P**: *P*-value; **SNRI**: serotonin norepinephrine reuptake inhibitor; **sd**: standard deviation; **SSRI**: selective serotonin reuptake inhibitor; **TCA**: tricyclic antidepressant

	rs45518336				rs10974625				rs2228622				rs12682807				
	TT n=14	AT n=162	AA n=218	<i>P</i>	AA n=28	GA n=161	GG n=205	<i>P</i>	AA n=64	GA n=189	GG n=141	<i>P</i>	CC n=4	AC n=64	AA n=326	<i>P</i>	
Age (in years) (m±sd)	47.7±11.8	45±13.4	45.6±13.3	0.68	47.9±13.8	45.1±12.6	45.4±13.7	0.67	45.4±13.4	45.1±13.2	45.9±13.4	0.90	46.5±9.5	45.8±13.1	45.3±13.4	0.99	
Sex [n (%)]	9(64)	111(69)	148(68)	0.95	19(68)	110(68)	139(68)	0.99	41(64)	125(66)	102(72)	0.37	3(75)	43(67)	222(68)	0.95	
Education level [n (%)]																	
	<i>Primary</i>	2(14)	16(10)	19(9)	0.27	3(11)	12(7)	22(11)	0.69	6(9)	17(9)	14(10)	0.98	0(0)	4(6)	33(10)	0.68
	<i>Secondary</i>	8(57)	77(48)	86(39)		14(50)	74(46)	83(40)		29(45)	79(42)	63(45)		3(75)	31(48)	137(42)	
	<i>Tertiary</i>	4(29)	69(43)	112(51)		11(39)	75(47)	99(48)		29(45)	92(49)	64(45)		1(25)	29(45)	155(48)	
Ethnicity [n (%)]																	
	<i>Caucasian</i>	14(100)	149(92)	195(89)	0.72	24(86)	144(89)	190(93)	0.51	60(94)	182(96)	116(82)	<0.001***	3(75)	61(95)	294(90)	0.23
	<i>African</i>	0(0)	8(5)	17(8)		3(11)	12(7)	10(5)		1(2)	5(3)	19(13)		0(0)	2(3)	23(7)	
	<i>Mixed</i>	0(0)	4(2)	6(3)		1(4)	5(3)	4(2)		3(5)	1(1)	6(4)		1(25)	1(2)	8(2)	
Current smoker [n (%)]	9(64)	64(40)	76(35)	0.075	12(43)	61(38)	76(37)	0.84	26(41)	69(37)	54(38)	0.83	2(50)	29(45)	118(36)	0.34	
Recurrent MDE [n (%)]	11(79)	121(75)	157(72)	0.76	22(79)	122(76)	145(71)	0.45	48(75)	132(70)	109(77)	0.30	3(75)	51(80)	235(72)	0.45	
Onset age MDE (m±sd)	39.5±14.1	34.1±13.9	36±14.8	0.21	36.5±17.6	34.8±14.4	35.6±14.1	0.93	35.7±13.6	35.7±14.2	34.7±15.3	0.74	37.2±18.3	34.4±13.9	35.5±14.6	0.81	
Baseline HDRS (m±sd)	25.9±6	24.8±4.9	24.8±5	0.77	25.9±5.8	24.3±4.6	25.1±5.1	0.29	25.4±5.3	24.7±4.7	24.7±5.1	0.66	20.8±3	24.9±4.7	24.8±5	0.20	
Prescribed ATD [n (%)]																	
	<i>SSRI</i>	6(43)	66(41)	86(39)	0.51	14(50)	65(40)	79(39)	0.073	23(36)	69(37)	66(47)	0.14	1(25)	25(39)	132(40)	0.25
	<i>SNRI</i>	5(36)	66(41)	88(40)		4(14)	61(38)	94(46)		34(53)	77(41)	48(34)		1(25)	20(31)	138(42)	
	<i>TCA</i>	0(0)	15(9)	11(5)		4(14)	11(7)	11(5)		4(6)	14(7)	8(6)		1(25)	6(9)	19(6)	
	<i>Other</i>	0(0)	11(7)	23(11)		3(11)	16(10)	15(7)		2(3)	20(11)	12(9)		1(25)	6(9)	27(8)	
	<i>ECT</i>	3(21)	4(2)	10(5)		3(11)	8(5)	6(3)		1(2)	9(5)	7(5)		0(0)	7(11)	10(3)	
Missing at follow-up [n (%)]																	
	<i>M1</i>	0(0)	8(5)	13(6)	0.60	0(0)	11(7)	10(5)	0.30	5(8)	11(6)	5(4)	0.41	1(25)	2(3)	18(6)	0.16
	<i>M3</i>	4(29)	47(29)	73(33)	0.63	9(32)	47(29)	68(33)	0.72	23(36)	59(31)	42(30)	0.68	1(25)	20(31)	103(32)	1.00
	<i>M6</i>	6(43)	79(49)	99(45)	0.78	10(36)	69(43)	105(51)	0.14	33(52)	91(48)	60(43)	0.42	2(50)	27(42)	155(48)	0.78

	rs301430				rs1471786				rs301435				
	CC n=59	TC n=171	TT n=164	<i>P</i>	AA n=8	GA n=95	GG n=291	<i>P</i>	TT n=90	CT n=206	CC n=98	<i>P</i>	
Age (in years) (m±sd)	45.9±13.6	44.6±12.8	46.1±13.6	0.47	43.9±11.7	45.4±13.2	45.5±13.4	0.90	44.3±13.5	46.5±13.2	44.2±13	0.26	
Sex [n (%)]	42(71)	117(68)	109(66)	0.79	6(75)	66(69)	196(67)	0.85	64(71)	136(66)	68(69)	0.65	
Education level [n (%)]													
	<i>Primary</i>	11(19)	9(5)	17(10)	0.018*	1(12)	8(8)	28(10)	0.86	9(10)	19(9)	1.00	
	<i>Secondary</i>	28(47)	79(46)	64(39)		4(50)	45(47)	122(42)		40(44)	89(43)	42(43)	
	<i>Tertiary</i>	20(34)	83(49)	82(50)		3(38)	42(44)	140(48)		41(46)	97(47)	47(48)	
Ethnicity [n (%)]													
	<i>Caucasian</i>	43(73)	154(90)	161(98)	<0.001***	5(62)	85(89)	268(92)	0.082	86(96)	189(92)	83(85)	0.13
	<i>African</i>	13(22)	10(6)	2(1)		2(25)	7(7)	16(5)		3(3)	13(6)	9(9)	
	<i>Mixed</i>	3(5)	6(4)	1(1)		1(12)	3(3)	6(2)		1(1)	4(2)	5(5)	
Current smoker [n (%)]	20(34)	62(36)	67(41)	0.55	3(38)	37(39)	109(37)	0.95	36(40)	77(37)	36(37)	0.88	
Recurrent MDE [n (%)]	45(76)	126(74)	118(72)	0.81	5(62)	76(80)	208(71)	0.21	62(69)	156(76)	71(72)	0.46	
Onset age MDE (m±sd)	35.7±16.4	34.6±13.8	36±14.4	0.77	35.8±16.8	34.2±14.5	35.7±14.4	0.61	35.1±14.3	35.6±14.9	34.9±13.7	0.98	
Baseline HDRS (m±sd)	25.4±5.3	24.6±4.7	24.8±5.1	0.62	25.2±5.2	25.5±4.9	24.6±5	0.16	24.6±5.1	24.6±5	25.4±4.8	0.26	
Prescribed ATD [n (%)]													
	<i>SSRI</i>	29(49)	64(37)	65(40)	0.16	2(25)	40(42)	116(40)	0.084	36(40)	79(38)	43(44)	0.98
	<i>SNRI</i>	18(31)	65(38)	76(46)		2(25)	32(34)	125(43)		36(40)	86(42)	37(38)	
	<i>TCA</i>	5(8)	13(8)	8(5)		3(38)	5(5)	18(6)		6(7)	14(7)	6(6)	
	<i>Other</i>	4(7)	20(12)	10(6)		1(12)	10(11)	23(8)		8(9)	19(9)	7(7)	
	<i>ECT</i>	3(5)	9(5)	5(3)		0(0)	8(8)	9(3)		4(4)	8(4)	5(5)	
Missing at follow-up [n (%)]													
	<i>M1</i>	2(3)	8(5)	11(7)	0.55	2(25)	3(3)	16(5)	0.030*	2(2)	10(5)	9(9)	0.10
	<i>M3</i>	18(31)	48(28)	58(35)	0.35	1(12)	29(31)	94(32)	0.48	28(31)	66(32)	30(31)	0.97
	<i>M6</i>	24(41)	81(47)	79(48)	0.60	4(50)	42(44)	138(47)	0.86	34(38)	102(50)	48(49)	0.15

IV. Discussion

IV.1. Summary of findings

The main results in the context of ATD treatment are shown in **Figure 5**.

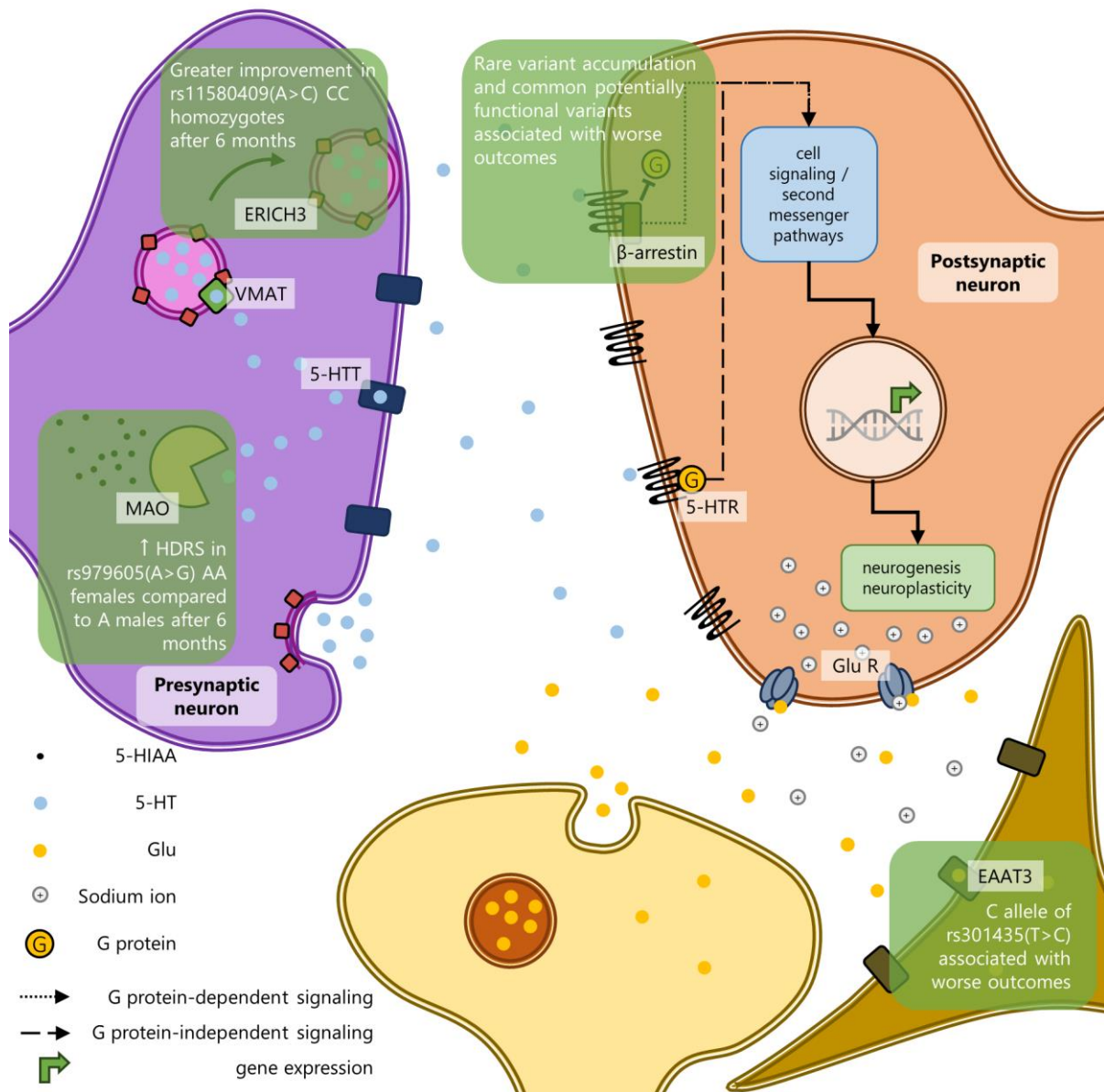


Figure 5: Research findings in the context of antidepressant drug treatment

The principal findings of the thesis project are shown in their biological contexts with respect to antidepressant drug treatment for MDD. The postsynaptic neuron (orange) receives input from presynaptic neurons, including serotonergic (purple) and glutamatergic (yellow) neurons. Glutamate (yellow dot) is removed from the synapse by EAAT3 located on glial cells (dark yellow). Significant associations (green zones) between clinical improvement following antidepressant treatment and genetic variation in four genes coding for ERICH3 (*ERICH3*), MAO (*MAOA*), β-arrestin 1 (*ARRB1*), and EAAT3 (*SLC1A1*) were identified.

↑: higher; **5-HIAA**: 5-hydroxyindoleacetic acid; **5-HT**: serotonin; **5-HTT**: serotonin transporter; **EAAT3**: Excitatory amino acid transporter 3; **Glu**: glutamate; **MAO**: monoamine oxidase; **MDD**: Major Depressive Disorder; **R**: receptor; **VMAT**: vesicular monoamine transporter

The overall objective of this thesis project was to use novel strategies to investigate the association between genetic variation within candidate genes and clinical outcomes, as well as metabolite levels, following ATD treatment in depressed individuals.

The first objective aimed to replicate the association of the *ERICH3* rs11580409(A>C) polymorphism with clinical outcomes and plasma 5-HT levels. CC homozygotes had a significantly improved HDRS score and response rate compared to A allele carriers after 6 months of ATD treatment. However, we did not observe an association between the rs11580409 polymorphism and plasma 5-HT levels.

The second objective was to investigate the association of genetic variation within the MAO-encoding genes, *MAOA* and *MAOB*, with clinical outcomes following ATD treatment and the plasma 5-HIAA/5-HT ratio as an estimate of peripheral MAO activity. We observed that the A allele of the *MAOA* rs979605(A>G) polymorphism was associated with greater HDRS score improvements in A allele-carrying males compared to AA homozygous females after 6 months of treatment. Although the 5-HIAA/5-HT ratio was not associated with clinical outcomes, CC/C females/males carrying the *MAOB* rs1799836(T>C) polymorphism had lower plasma 5-HIAA/5-HT ratios compared to TT/T females/males.

The third objective aimed to examine the association of genetic variation in *ARRB1* with clinical outcomes following ATD treatment. We used functional databases to prioritize frequent genetic variants with likely functional consequences for analysis using mixed-effects models, and gene burden methods to analyze the association of rare genetic variation. We observed that the *ARRB1* rs553664(G>A) and rs536852(A>G) genetic polymorphisms—with potential consequences on transcription factor binding—were significantly associated with HDRS score changes and remission rates, especially after 6 months of ATD treatment, while rare genetic variant accumulation was associated with poorer clinical outcomes.

Lastly, the final objective sought to analyze the entirety of the METADAP gene panel to find which genes were most strongly associated with clinical outcomes following ATD treatment. Using the biological constraint method, G2PSR, we observed that *SLC1A1* was most strongly associated with clinical measures including the HDRS. We then observed that the number of C alleles of the *SLC1A1* rs301435(T>C) genetic polymorphism was associated with poorer clinical improvement following ATD treatment.

IV.2. Is it still worth exploring factors of monoamine neurotransmission?

The association of depleted monoamine neurotransmitter levels with depressive symptoms, as well as the ability of early ATDs to enhance monoamine neurotransmission, pointed to a role for monoamines in the pathophysiology of depression (Malhi and Mann, 2018; Otte et al., 2016). Indeed, the efficacy of first-line ATD treatments such as SSRIs support the role for 5-HT in the pathophysiology of MDD (Jauhar et al., 2023). Still, response to ATD treatment remains modest. Although simplistic with regards to the adaptive effects of ATDs (Hyman and Nestler, 1996; Malhi and Mann, 2018; Otte et al., 2016), this variability in response suggests other factors may influence the effect of ATDs. Those with potential modulatory effects on monoamine neurotransmission thus remain interesting therapeutic targets in the treatment of MDD with ATDs.

IV.2.1. Monoamine neurotransmitter transport—a role for ERICH3?

The function of ERICH3 was unknown at the time of its identification in a PMx-informed PGx study of plasma 5-HT levels. Fortunately, the association of *ERICH3* genetic variation with decreased serotonin levels, especially the rs11580409(A>C) polymorphism, offered some insight (Gupta et al., 2016). Indeed, functional analyses revealed several interesting associations.

First, ERICH3 depletion in neuroblastoma cells via knockdown or knockout was associated with decreased 5-HT levels without altering the expression of serotonin pathway enzymes. Second, ERICH3 interacts with vesicle proteins, as observed in samples obtained from neuroblastoma cells, and may participate in the transport of monoamine neurotransmitters. Third, the rs11580409(A>C) polymorphism, which causes a leucine-to-valine substitution at position 1056 of ERICH3, was associated with proteasome-mediated decreases in ERICH3 levels in HEK-293T/17 cells. Fourth, rs11580409 was also associated with decreased plasma 5-HT levels, as observed in plasma samples of depressed patients. Interestingly, however, rs11580409 was also associated with improved ATD treatment response (Gupta et al., 2016; Liu et al., 2020) [see **Figure 6** (page 116)].

The novel characterization of ERICH3 as a potential factor in monoamine neurotransmission, as well as the intriguing association of rs11580409 with ATD treatment response, highlights the still incomplete picture of (1) monoamine neurotransmission and, by extension, (2) the mechanism of action of ATDs and how they relieve depressive symptoms. Since ATDs remain only modestly effective, it also argues for continued exploration to complete this picture.

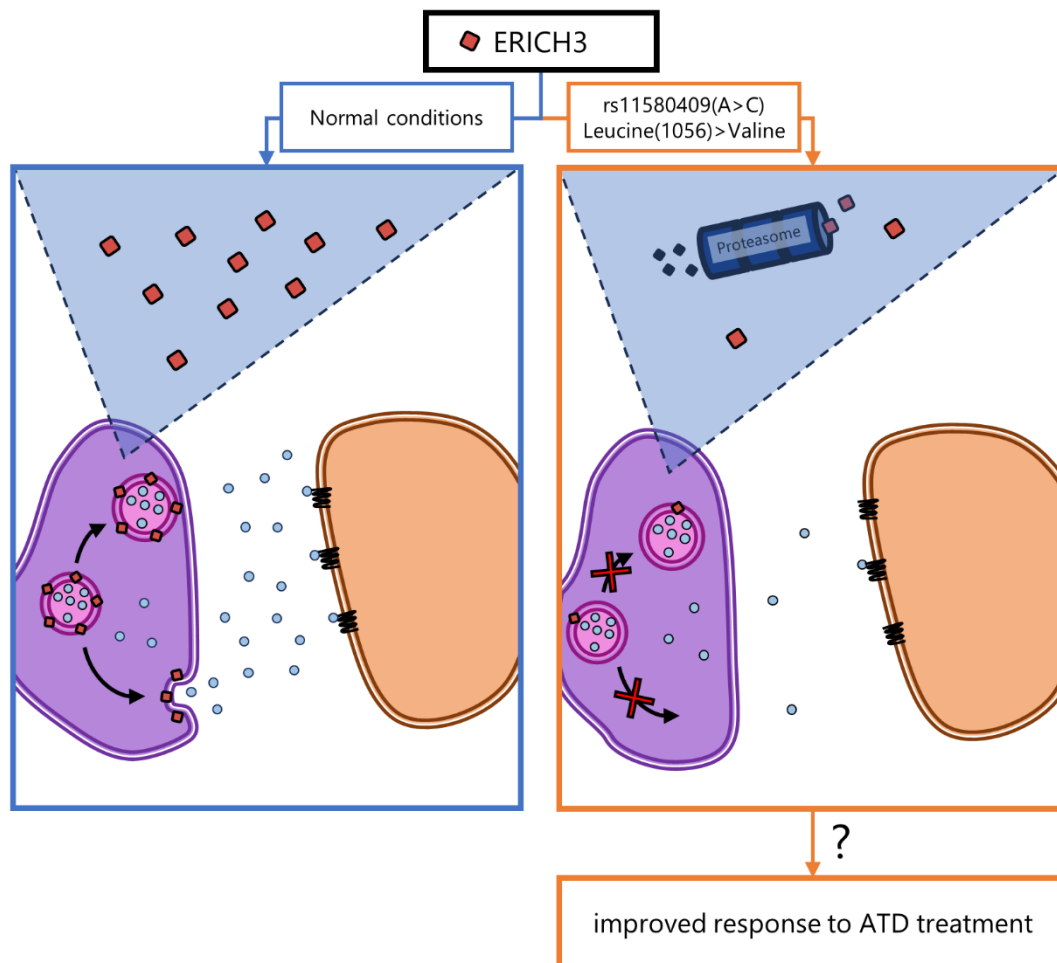


Figure 6: Proposed role of ERICH3 and consequence of the rs11580409 genetic polymorphism

Under normal conditions (blue path) ERICH3 is proposed to function in the trafficking of monoamine neurotransmitters by interacting with monoamine-transporting vesicles, allowing for the release of 5-HT (blue dots) into the synapse. The rs11580409(A>C) genetic polymorphism is associated with decreased (1) ERICH3 and (2) plasma 5-HT levels, while decreased ERICH3 levels are associated with decreased 5-HT levels. At neuronal synapses, the rs11580409 genetic polymorphism may cause decreases in presynaptic ERICH3 levels, decreased ERICH3-mediated 5-HT trafficking and, consequently, decreased synaptic 5-HT levels (orange path). However, rs11580409 is also associated with improved response to ATD treatment in depressed patients. Its synaptic consequences and how it may promote improved response to ATD treatment remain to be elucidated.

5-HT: serotonin; **ATD:** antidepressant drug

In our analysis of the *ERICH3* rs11580409 polymorphism, we observed that CC homozygotes had significantly better clinical outcomes compared to A allele carriers following 6 months of ATD treatment. The inclusion of the time \times rs11580409 interaction helped to identify the specificity of this association. Compared to other cohorts that examined rs11580409 in a similar context, this observation followed a much longer duration of ATD treatment. Continued studies may help explain this discrepancy, as well as why the

rs11580409 polymorphism was not significantly associated with response to ATD treatment in the PGRN-AMPS cohort in which it was originally identified (Gupta et al., 2016).

One of the biggest criticisms of significant findings in candidate studies is their lack of replication in larger and more highly powered GWAS, which suggests to some that these findings are false positives (Border and Keller, 2017). Indeed, robust efforts exploring the association between several depressed phenotypes and genetic variation in historical candidate genes—as well as interactions with various environmental factors—failed to identify any significant associations (Border et al., 2019). A similar exploration remains to be performed for clinical outcomes following ATD treatment in depressed individuals. Still, replication remains an important goal in genetic association studies (Kraft et al., 2009), and functionally relevant findings seem less likely to be due to chance alone. For example, the association between genetic variation within *FKBP5* and MDD has been independently replicated, linked to FKBP5 protein expression, and associated with environmental factors influencing its epigenetic regulation (Moore, 2017; Zannas et al., 2016). Similarly, *ERICH3* and the rs11580409 polymorphism are suggested to be functionally relevant to the action of ATDs. Even if the association of rs11580409 with ATD response never reaches genome-wide significance (Gupta et al., 2016; Liu et al., 2020), the fact that this association (1) has been replicated and (2) proceeds in the same direction argues that rs11580409 may be relevant to clinical improvement following ATD treatment in depressed individuals. Overall, genome-wide and candidate approaches are both likely to remain valuable for discovery and replication in genetic association studies analyzing the response to ATD treatment in MDD.

IV.2.2. Monoamine neurotransmitter metabolism and MAO

With respect to the modulation of presynaptic monoamine neurotransmitter levels and the response to ATD treatment, MAO is an interesting target for several reasons. First, MAO—especially and canonically MAOA—metabolizes the monoamine neurotransmitters (Uzbekov, 2021). Second, MAO expression and activity vary widely across the body, with brain *MAOA* and *MAOB* expression occurring primarily in catecholaminergic (e.g., dopaminergic and noradrenergic) neurons and histaminergic and serotonergic neurons, respectively—the latter being where 5-HT is produced and stored (Jones and Raghanti, 2021; Shoji et al., 2023). Beyond the gut, most peripheral 5-HT is stored in platelets (Colle et al., 2020), where only MAOB is expressed (Jansson et al., 2005). Since blood is a commonly analyzed biological material (Malsagova et al., 2020), measures of peripheral MAO activity may thus be representative of MAOB activity alone. The use of fibroblasts, which express *MAOA* (Hotamisligil and Breakefield, 1991; Pintar and Breakefield, 1982), may be useful to

examine peripheral MAOA activity in depression (Mesdom et al., 2020). Third, high levels of estrogen and progesterone, important hormones in the sexual and reproductive development of females, are also associated with lower and higher MAOA activity, respectively (Klaiber et al., 1979; Yonkers et al., 1992). Estrogen may influence MAOA expression through its interaction with Sp1, a transcription factor with known binding sites in both MAOA and MAOB promoters (Jones and Raghanti, 2021; Tipton et al., 2004). SRY, another transcription factor whose encoding gene is located on the Y chromosome, can also bind to the MAOA promoter and, through its interaction with Sp1, activate MAOA transcription (Wu et al., 2009). Male- and female-specific factors that influence MAOA activity may thus indirectly influence monoamine neurotransmission.

Finally, the location of MAOA and MAOB on the X chromosome poses unique analytical challenges since its XCI status is debated (Carrel and Willard, 2005; Stabellini et al., 2009; Tukiainen et al., 2017). Previous analyses of the association between MAOA genetic variation and the response following ATD treatment either did not adjust for X-linked biological factors (Müller et al., 2002; Peters et al., 2004; Tzeng et al., 2009; Yoshida et al., 2002) or were performed in male and female populations separately (Bi et al., 2021; Cusin et al., 2002; Domschke et al., 2008; Tadić et al., 2007a; Yu et al., 2005), likely resulting in a loss of power (Clayton, 2009). Only one study analyzing the MAOA rs6323(G>T) polymorphism included the sex \times genotype interaction (Leuchter et al., 2009). However, this interaction was not significant, though the main effect of rs6323 was. Still, this study analyzed the association of rs6323 with placebo response, whose mechanism of action likely differs from that of ATD treatment (Leuchter et al., 2002).

The significant association of the sex \times MAOA rs979605 genotype interaction with the HDRS score we observed may indicate either (1) a difference in the effect of the rs979605 polymorphism according to sex or (2) that the rs979605 polymorphism is in a region of escape from XCI (Song et al., 2021). Although we did not observe a significant association of the rs979605 polymorphism with the HDRS score in male and female subpopulations separately—likely due to a loss in power—the model coefficient was negative in males and positive in females, supporting the former hypothesis. This relationship needs to be further investigated. Indeed, our association study may support a sex-dependent association, but a dosage effect in females cannot be ruled out by our findings.

Sex-specific factors in depression and/or the response to its treatment with ATDs are plausible following several observations, including: (1) the higher prevalence of MDD in females compared to males (World Health Organization, 2017); (2) biological differences

between depressed males and females (e.g., estrogen levels, as discussed above) and at the genomic (Silveira et al., 2023) and transcriptomic levels (Labonté et al., 2017; Maitra et al., 2023); (3) the disproportionate prevalence of other neurological disorders between males and females, including anxiety, attention-deficit hyperactivity disorder, substance abuse disorders and autism spectrum disorder (Hübel et al., 2019; Jones and Raghanti, 2021). With respect to these latter two, sex-specific associations with *MAOA* genetic variation have also been observed (Fite et al., 2020; Verma et al., 2014). First, the rs6323 T allele (correlated with the rs979605 G allele) was associated with a higher risk of autism spectrum disorder in males, but not females; *in silico* analyses also suggested that the rs1137070 C allele (correlated with the rs979605 G allele) may delete a binding site for GATA-2, a transcription factor that interacts with SRY (Verma et al., 2014). Second, a sex-specific gene \times environment interaction was observed in association with polysubstance use—often intimately linked with MDD (Quello et al., 2005). Among university students with a history of childhood abuse, males carrying the low-activity allele of the *MAOA* upstream variable number tandem repeat (located within the *MAOA* promotor) and females homozygous for the high-activity allele reported the greatest amount of substances consumed (Fite et al., 2020).

Along with the possible influence of sex-specific factors (e.g., hormones, XCI, Y-linked SRY), these observations argue for likely sex differences in psychiatric disease—including MDD—and the consideration of more nuanced analyses of X-linked genetic variation. The inclusion of the sex \times genotype interaction, for example, may yield more meaningful results and help point towards potential dosage effects, sex-specific associations, or main effects (Chen et al., 2021; Song et al., 2021). For example, in addition to the association between the HDRS score and the sex \times rs979605 genotype interaction, we observed a nominally significant (i.e., $P < 0.05$) main effect for the *MAOB* rs1799836 polymorphism after controlling for X-linked factors. This nominal association between rs1799836 and HDRS score changes, its significant association with the plasma 5-HIAA/5-HT ratio in our sample, and its previous association with response following ATD treatment (Tadić et al., 2007b), suggest that it may also warrant further investigation. Importantly, these findings point to the utility of this method in identifying both sex-specific associations and main effects in the analysis of X-linked genetic variation. With respect to genetic variation in other X-linked genes of the METADAP gene panel, including *GRIA3*—for which genetic variation is associated with depressive phenotypes and treatment outcomes (de Sousa et al., 2017)—and *NLGN3*, the inclusion of the sex \times genotype interaction may help reveal similar associations.

IV.2.3. Is mediating the response to ATD treatment one of the many roles of β -arrestin 1?

Compared to the majority of the METADAP panel, the genetic data for *ARRB1* was expansive owing to its whole-gene sequencing. By using the functional database, RegulomeDB (Boyle et al., 2012), we limited the number of analytical targets by prioritizing genetic variants with likely functional consequences, a procedure that has been previously used with success (Hong et al., 2018; Huo et al., 2019; Lee et al., 2015). We identified two significant genetic associations but were unable to validate whether these genetic variants affect *ARRB1* expression since *ARRB1* RNA and β -arrestin 1 protein concentrations were not available. In addition to the functional validation of these genetic variants, our results require independent replication. Importantly, the interaction between β -arrestin 1 and monoamine neurotransmitter receptors and the potential of biased signaling should be explored in these contexts as well (Bond et al., 2019).

Although RegulomeDB aided our analysis, its use over other databases, as well as our selection criteria (i.e., RegulomeDB rank of 1 or 2), may not always be appropriate. Indeed, the effect of genetic variation may vary in different contexts, including disease and treatment, which may not be captured—at least presently—by these databases. Additionally, some genetic variants associated with ATD treatment response would not have been prioritized using our strategy, including genetic variants with known effects on encoded protein levels such as rs1360780 of *FKBP5* ([RegulomeDB: rs1360780](#)) and rs11580409 of *ERICH3* ([RegulomeDB: rs11580409](#)). As such, it remains possible that we filtered out *ARRB1* genetic variation potentially associated with clinical outcomes following ATD treatment. Importantly, the association of *ARRB1* with clinical outcomes in our G2PSR analysis may point to this possibility, as it remains unlikely that rs553664 and rs536852 alone, among a total of 67 analyzed genetic variants, account for this association.

Our findings also suggest that the accumulation of rare *ARRB1* genetic variation is associated with worse response to ATD treatment. Although rare genetic variation has been examined in association with MDD risk (Cheng et al., 2022), its association with the response to ATD treatment in the context of MDD remains largely unexplored. Still, associations at both gene and pathway levels have been observed (Fabbri et al., 2018). However, the effect of rare *OR4K2* genetic variation was in opposite directions in the two analyzed cohorts (i.e., the GENDEP and STAR*D cohorts), while the observed enrichment of rare missense genetic variation in the GO:0005694 chromosome pathway remains broad. Nevertheless, genes related to neurogenesis within this pathway and their association with ATD treatment were

discussed, but require validation (Fabbri et al., 2018). Whether genetic variation is rare or common also frequently depends on ethnicity. Indeed, this is the case for functional genetic variation of PK-related genes like *CYP2D6*, which, when rare in European populations, is often common in Asian populations (van Westrhenen et al., 2020).

For rare genetic variation, causality, direction, and magnitude are important factors to consider. Our analysis of rare *ARRB1* genetic variation suggests that the effect of its accumulation acts overall towards worse clinical improvement, however the question of causality and magnitude remain unanswered. Indeed, we did not investigate which rare genetic variants—whether only a few or many, and to what degree—were influencing this association with clinical outcomes to ATD treatment. Functional analyses may help respond to these questions, but, given the volume of rare genetic variation analyzed, would likely be too burdensome and costly. Our findings should be replicated before considering such investigations, but do support the continued study of rare genetic variation and its association with clinical improvement following ATD treatment.

IV.3. Glutamatergic signaling and ATD response—should we be excited?

Our findings, and many others, suggest that the monoaminergic system remains a suitable target for the continued analysis of treatment outcomes following ATD treatment in MDD. We also suggest that various factors may moderate these associations, including time and sex. Still, our analysis of the whole METADAP gene panel suggested a strong association between clinical outcomes following ATD treatment and genetic variation within *SLC1A1*, encoding the neuronal glutamate transporter, Excitatory Amino Acid Transporter 3 (EAAT3), which plays a role in glutamatergic signaling (Brandl et al., 2012; Lai, 2013). This finding thus joins other evidence implicating the glutamatergic system in MDD and the response to its treatment with ATDs (Sanacora et al., 2012; Sarawagi et al., 2021), including genetic associations with polymorphisms located within other genes of the glutamatergic system such as N-methyl-D-aspartate (NMDA), AMPA, and kainate receptor subunits (de Sousa et al., 2017).

However, genes encoding other glutamatergic system-related proteins were not strongly associated with clinical measures of ATD response alongside *SLC1A1*. This result may be due to the small size of our dataset compared to the previous application of G2PSR to the analysis of Alzheimer's disease (Deprez et al., 2022). Additionally, the analyzed genes were (1) heavily skewed towards containing fewer genetic variants and (2) candidate genes of MDD and ATD treatment outcomes, which may have enriched the number of relevant genes and thus negatively impacted performance (Deprez et al., 2022). Moreover, G2PSR is best

for the analysis of additive genetic effects, which may influence its ability to capture associations better characterized by a dominant or recessive genetic model, as well as the influence of any moderating factors, as we observed between *MAOA* and sex and *ARRB1* and *ERICH3* and time. As such, genes encoding components of the glutamatergic system—and beyond—may benefit from more nuanced analyses that consider different genetic models and interactions.

IV.4. Strengths

The analyses described in this thesis project benefit from several strengths. First, although smaller compared to other cohorts of depressed patients receiving ATD treatment, notably the STAR*D, the size of our sample remains large. Indeed, statistical power was high enough to identify significant genetic associations with reasonable confidence and to draw general conclusions from these findings. Second, the multicentric design of METADAP allows for a small degree of generalizability across depressed populations, though recruitment was greater at some sites compared to others. Third, the naturalistic design of METADAP resembles clinical practice more than controlled trials, allowing for a greater degree of generalizability. Although it also introduces potential confounding factors with respect to the different mechanisms of action of different ATDs, we attempted to adjust for this in our analyses. Fourth, and despite the likely highly variable symptomology between patients, METADAP represents a diagnostically homogeneous sample of individuals suffering from a severe depressive episode in the context of MDD. Lastly, compared to most other cohorts of depressed individuals receiving ATD treatment, the 6-month treatment duration in METADAP allows for analysis following a much longer treatment period, which can provide more insight into observed associations.

IV.5. Limitations

The analyses described in this thesis project are subject to several limitations. First, they are only association studies and thus do not offer any degree of causality when considering their biological contexts. Second, the rate of missing data due to dropouts, medication changes, unauthorized drug use, the presence of exclusion criteria, or death, increased with follow-up, nearly reaching 50% by the final timepoint. While we attempted to control for this in our analyses, the robustness of our results can still be challenged by the presence of these missing data. Third, metabolite concentrations were assayed from peripheral blood samples, which may not necessarily reflect concentrations in the central nervous system. This may especially be the case for 5-HT, which does not cross the blood–brain barrier (El-Merahbi et al., 2015). Fourth, METADAP comprises a mostly Caucasian

(>90%) sample of depressed individuals, which limits the generalizability of findings. Indeed, allele frequencies—and thus genetic associations—can vary greatly between ethnicities. Whether similar associations are observable in other ethnicities requires further examination. Fifth, treatment compliance was not considered in our analyses, which may have influenced our observations. Lastly, it remains plausible that the observed improvements in depressive symptoms may be confounded by one or several other factors, including the placebo effect, the effect of concurrent psychotherapy, the effect of any number of environmental factors, or the effect of other factors.

IV.6. Perspectives

IV.6.1. Does context matter?

Various context-specific associations between genetic variation and the response to ATD treatment have been described, including those that are ATD class-dependent (Colle et al., 2015), sex-dependent—both at a genome-wide level (Silveira et al., 2023) and in our analysis of the *MAOA* rs979605 polymorphism—and time-dependent, as suggested by our analyses of the *ERICH3* rs11580409 genetic polymorphism and genetic variation of *ARRB1*. Other context-dependent associations may exist and benefit from more nuanced analyses. As such, moderators of genetic associations should continue to be explored.

IV.6.2. Full METADAP gene panel analysis of other variables

We demonstrated the utility of G2PSR in the analysis of clinical measures following ATD treatment. G2PSR can be applied to analyze genetic associations with any quantitative data. In METADAP, this includes the body mass index, which relates back to the principal objective of the METADAP study: to examine the impact of ATD treatment on the development of metabolic syndromes (Corruble et al., 2015).

Although limited in scope compared to the PMx-informed PGx analyses performed by the Mayo Clinic (Gupta et al., 2016; Ji et al., 2011; Liu et al., 2018), it might be interesting to see if genetic variation within genes of the METADAP gene panel might be associated with plasma concentrations of the metabolites assayed by the BIOCRATES AbsoluteIDQ® p180 kit in METADAP. For example, recent analyses in METADAP suggest that acetyl-L-carnitine and acylcarnitine levels are associated with clinical outcomes following ATD treatment (Ait Tayeb et al., 2023, 2021).

IV.6.3. Is MDD precise enough for precision medicine?

The use of precision medicine—finding the “right” treatment for a patient—remains largely unsuccessful in the treatment of psychiatric disease (Sullivan and Geschwind, 2019). Despite hundreds of biological associations, few biomarkers—if any—are robust enough to

predict the response to ATD treatment. This is likely partly explained by the heterogeneity of MDD, including its many different clinical subtypes (Goldberg, 2011) with potentially little overlap between their underlying biological mechanisms (Cai et al., 2020). Several observations suggest the genetic analysis of MDD and its treatment with ATDs may benefit from more specificity.

First, biological associations vary between depressive subtypes like atypical and melancholic depression, notably with measures of HPA-axis, metabolic, and inflammatory dysregulation (Penninx et al., 2013). Second, stronger genetic associations have been identified in more homogenous populations (Cai et al., 2015), which argues for the application of similar strategies to other depressed populations. Third, there is extreme variability in the response to ATD treatment when analyzing total depression scale scores—the most common means to examine clinical improvement of depressive symptoms (Maslej et al., 2021)—that may be reduced by more symptomatic (e.g., clinical subtypes) and biological (e.g., biomarkers) specificity (Eiko I Fried and Nesse, 2015; Eiko I. Fried and Nesse, 2015).

Although a greater demand with respect to time, staff, and resources would be required to successfully recruit, diagnose, treat, follow, and analyze such a population, decades of conflicting results and research suggest that it may be worth the effort. However, the generalizability of findings may challenge the likely cost required. It seems unlikely that such an analysis would reveal any significant associations in METADAP given its already smaller sample size compared to cohorts like the STAR*D. Still, METADAP could perhaps help contribute to a broader yet more precise analysis as part of a consortium.

V. Conclusion

This thesis project aimed to leverage different and novel strategies in the exploration of the association between biological data—notably genetic variation in several candidate genes—and the response following ATD treatment in individuals suffering from a depressive episode in the context of MDD.

In our analyses of clinical outcomes following ATD treatment in depressed patients, we replicated the association of the *ERICH3* rs11580409 genetic polymorphism, suggested that the association of the *MAOA* rs979605 genetic polymorphism with clinical improvement may differ between males and females, observed associations with rare genetic variant accumulation and potentially functional genetic variants of *ARRB1*, and identified *SLC1A1* as a potential candidate gene for continued study.

These results require independent replication and functional validation in the case of genetic variation within *ARRB1*, *MAOA*, and *SLC1A1*. Still, these findings suggest that genetic variation in genes encoding biological factors linked to classical paradigms of monoamine neurotransmission and glutamatergic signaling may influence the response to ATD treatment in the context of MDD. Furthermore, these genetic variants may prove useful in the development of novel pharmacological treatments or in guiding therapeutic strategies for the treatment of MDD.

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