

# Understanding treatment-resistant depression using “omics” techniques: A systematic review

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## ARTICLE INFO

### Keywords:

Genome-wide

Genomics

Major depressive disorder (MDD) treatment

High-throughput “omics” techniques

Transcriptomics

Treatment-resistant depression (TRD)

## ABSTRACT

**Background:** Treatment-resistant depression (TRD) results in huge healthcare costs and poor patient clinical outcomes. Most studies have adopted a “candidate mechanism” approach to investigate TRD pathogenesis, however this is made more challenging due to the complex and heterogeneous nature of this condition. High-throughput “omics” technologies can provide a more holistic view and further insight into the underlying mechanisms involved in TRD development, expanding knowledge beyond already-identified mechanisms. This systematic review assessed the information from studies that examined TRD using hypothesis-free omics techniques.

**Methods:** PubMed, MEDLINE, Embase, APA PsycInfo, Scopus and Web of Science databases were searched on July 2022. 37 human studies met the eligibility criteria, totalling 17,518 TRD patients, 571,402 healthy controls and 62,279 non-TRD depressed patients (including antidepressant responders and untreated MDD patients).

**Results:** Significant findings were reported that implicate the role in TRD of various molecules, including polymorphisms, genes, mRNAs and microRNAs. The pathways most commonly reported by the identified studies were involved in immune system and inflammation, neuroplasticity, calcium signalling and neurotransmitters.

**Limitations:** Small sample sizes, variability in defining TRD, and heterogeneity in study design and methodology.

**Conclusions:** These findings provide insight into TRD pathophysiology, proposing future research directions for novel drug targets and potential biomarkers for clinical staging and response to antidepressants (citalopram/escitalopram in particular) and electroconvulsive therapy (ECT). Further validation is warranted in large prospective studies using standardised TRD criteria. A multi-omics and systems biology strategy with a collaborative effort will likely deliver robust findings for translation into the clinic.

## 1. Introduction

Despite antidepressant medications, such as selective serotonin reuptake inhibitors (SSRIs), being effective mainstay treatments for major depressive disorder (MDD) (Cipriani et al., 2018), approximately a third or more of people with MDD do not achieve an adequate clinical response, even after multiple treatments, and so are deemed as having treatment-resistant depression (TRD) (Fava and Davidson, 1996; Fekadu et al., 2009a). This is usually defined as failing to demonstrate significant clinical improvement after at least two adequate antidepressant trials of different classes, although its characterisation in the literature is conflicting (Berlim and Turecki, 2007; Sforzini et al., 2021). This lack of

a consensus definition for TRD poses a major challenge to advancing research in this area, leading to possible patient misclassification and highly varied samples that may weaken the replicability and comparability of results (Sforzini, 2022; Sforzini et al., 2021; Trevino et al., 2014).

TRD can lead to substantial financial burden and human suffering, including considerable medical care costs, elevated healthcare resource utilisation, lower quality of life, increased likelihood of relapse and worse mortality (Fekadu et al., 2009a; Jaffe et al., 2019; Johnston et al., 2019). Understandably, one of the worst TRD-related complications is the elevated risk of suicide (Bergfeld et al., 2018). Different factors besides an MDD/TRD diagnosis should be considered in evaluating this

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<https://doi.org/10.1016/j.jad.2022.09.011>

Received 28 April 2022; Received in revised form 26 August 2022; Accepted 7 September 2022

Available online 11 September 2022

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risk. As an example, lifetime suicide attempts have been reported to be independently and more strongly predicted by affective temperaments rather than a diagnosis of major affective disorder (Baldessarini et al., 2017). In addition, subjects at risk for suicide are likely to search online for information and news regarding self-harm and suicidal behaviors. Thus, a careful monitoring of online searches may represent an important preventive strategy by developing a supportive environment for suicidal individuals and everyone who is seeking help (Solano et al., 2016). Although various strategies exist for TRD management, including neurostimulation techniques such as electroconvulsive therapy (ECT) and emerging pharmacological interventions like ketamine and esketamine, a significant proportion of non-responders still remain (Al-Harbi, 2012; Dunner et al., 2006) and TRD-specific guidelines are limited (Sforzini et al., 2021; Voineskos et al., 2020; Wang et al., 2020). Therefore, there is an urgent need to elucidate the biological underpinnings that differentiate remitters/responders from resistant/non-responder individuals and develop new interventions for these individuals.

While there is evidence of biological heterogeneity between TRD and healthy controls or treatment-responsive MDD individuals (Cattaneo et al., 2013, 2016, 2020; Murphy et al., 2017), existing definitions and instruments for assessing TRD are mainly based on clinical observations and do not encompass the underlying neurobiology, despite suggestions being raised for incorporating information regarding pathogenetic mechanisms in future definitions, for more precise characterisation of this condition (Akil et al., 2018; Berlim and Turecki, 2007; Fabbri et al., 2019a; Sforzini et al., 2021). However, the multifactorial and highly polygenic nature of this disorder, with the contribution of multiple genetic, environmental and clinical factors to its aetiology (Fabbri et al., 2019a; Fabbri et al., 2021a; Murphy et al., 2017) complicates this endeavour (Pigoni et al., 2019; Sforzini, 2021).

Most biological studies have adopted a “candidate mechanism” approach in order to examine the molecular or genetic underpinnings of TRD, by investigating a restricted number of biomarkers, candidate mRNAs or genetic polymorphisms, selected based on prior knowledge. Yet considering the complexity of this disorder such studies do not capture the intricate contribution of multiple biological factors. Furthermore, the hypothesis-driven approach means potentially missing out novel molecules and mechanisms whose putative function or link to TRD or antidepressant action is not yet fully elucidated (Fabbri et al., 2019a).

Technological advances of the last two decades have led to a rise in the use of non-targeted high-throughput “omics” techniques, which have emerged as powerful tools to analyse large datasets generated from the wide-scale assessment of biological molecules to yield cost-efficient and more robust evidence during the study of complex diseases, including MDD (Gadad et al., 2018; Hasin et al., 2017; Manzoni et al., 2018). This global approach can provide a comprehensive view into the cellular pathophysiology of TRD in a multi-layer manner, allowing for integrated information ranging from genetic changes to products of metabolic functions; complemented by enrichment/pathway analysis for inferring associated biological functions of identified molecules (Manzoni et al., 2018). Furthermore, the untargeted nature of omics techniques, compared to candidate approaches, means that they could impart a more in-depth understanding of the biological basis of TRD through revealing potentially novel molecules and networks that may be dysregulated in this condition. Thus helping to generate hypotheses or inform the direction of future confirmatory candidate mechanistic studies regarding biomarker and drug target identification.

Current systematic reviews have looked at a specific omics technique in relation to MDD (Bharti et al., 2021; Knudsen et al., 2021; MacDonald et al., 2019; Sanada et al., 2020; Wittenberg et al., 2020) or antidepressant response prediction (Alladi et al., 2018; Webb et al., 2020). However, no review has yet appraised the results from omics studies focusing specifically on TRD. Therefore, to our knowledge, this is first systematic review that aims to search the scientific literature in order to

identify, evaluate and summarise the findings from relevant papers that utilised any hypothesis-free omics technique to investigate TRD in human subjects. While our review is more scoping in nature, we hypothesised differences in the omics profile of TRD patients from healthy and non-TRD individuals, implicating primarily the inflammatory system and hypothalamic-pituitary-axis (HPA) axis disturbance.

## 2. Methods

### 2.1. Search strategy

The systematic searches were conducted using the following electronic databases: PubMed (1975–July 2022), MEDLINE ([R] and Epub Ahead of Print, In-Process, In-Data-Review & Other Non-Indexed Citations and Daily; 1946 to July 20, 2022), Embase (1974 to 2022 July 20), APA PsycInfo (1806 to July Week 2 2022), Scopus and Web of Science Core Collection (1900–2022). The search string used was: (“treatment resistant depression” OR “antidepressant resistant” OR “pharmacotherapy resistant” OR “difficult-to-treat” OR “medication resistant” OR depress\* OR “major depressive disorder” OR MDD) AND (“transcriptome profile\*” OR transcriptom\* OR “bioinformatic analys\*” OR “whole genome\*” OR “whole exome\*” OR genome-wide OR RNAseq OR RNA-seq OR microarray OR proteomic\* OR genomic\* OR epigenomic\* OR metabolomic\* OR omic\*). An additional search was performed using the following string (“electroconvulsive therapy” OR ECT) AND (depress\* OR “major depressive disorder” OR MDD) AND (“transcriptome profile\*” OR transcriptom\* OR “bioinformatic analys\*” OR “whole genome\*” OR “whole exome\*” OR genome-wide OR RNA-seq OR microarray OR proteomic\* OR genomic\* OR epigenomic\* OR metabolomic\* OR omic\*) to identify studies on ECT-treated major depression, as the use of this treatment suggests a non-response. The final search was performed on July 21st, 2022. No filters or limits were applied.

### 2.2. Study selection

Rayyan Systems Inc. (Ouzzani et al., 2016) was used to manage the records obtained as well as to screen and select the relevant papers.

Eligible studies were required to be peer-reviewed data papers or publications (i.e., inclusion of a methods and results section) that investigated the use of any hypothesis-free “omics” technique, to study human patients with treatment-resistant or treatment-refractory depression that must include an MDD or unipolar depression sample. We included only TRD/refractory-related studies, comprising a section on ECT-treated MDD. All tissue and study types were acceptable, but only papers in the English language were considered. Those studies that solely used a hypothesis-driven or targeted approach or did not clearly focus on non-targeted “omics” techniques were not eligible for this review. Moreover, studies that didn't focus on TRD, including only MDD individuals or different/comorbid disorders (psychiatric or other) were excluded. Non-data papers, like conference abstracts and studies that investigated animal models, were not included.

### 2.3. Study characteristics

The systematic search obtained 52,889 records that eventually resulted in 37 studies being included in the review after the study selection process, that is visually represented in Fig. 1.

Details regarding the main study characteristics are displayed in Table 1. The papers identified were conducted or published between 2013 and 2022 and mainly in Europe or the US. 32 studies recruited participants with MDD only, whereas 5 studies reportedly also included bipolar patients (Bekhat et al., 2021; Clements et al., 2021; Foo et al., 2019; Guo et al., 2018; Pisanu et al., 2021). A total of 20 studies focused solely on TRD (Barakat et al., 2020; Cole et al., 2021; Fabbri et al., 2018, 2020; Fabbri et al., 2021c; Fabbri et al., 2019b, 2021b; Fabbri et al.,

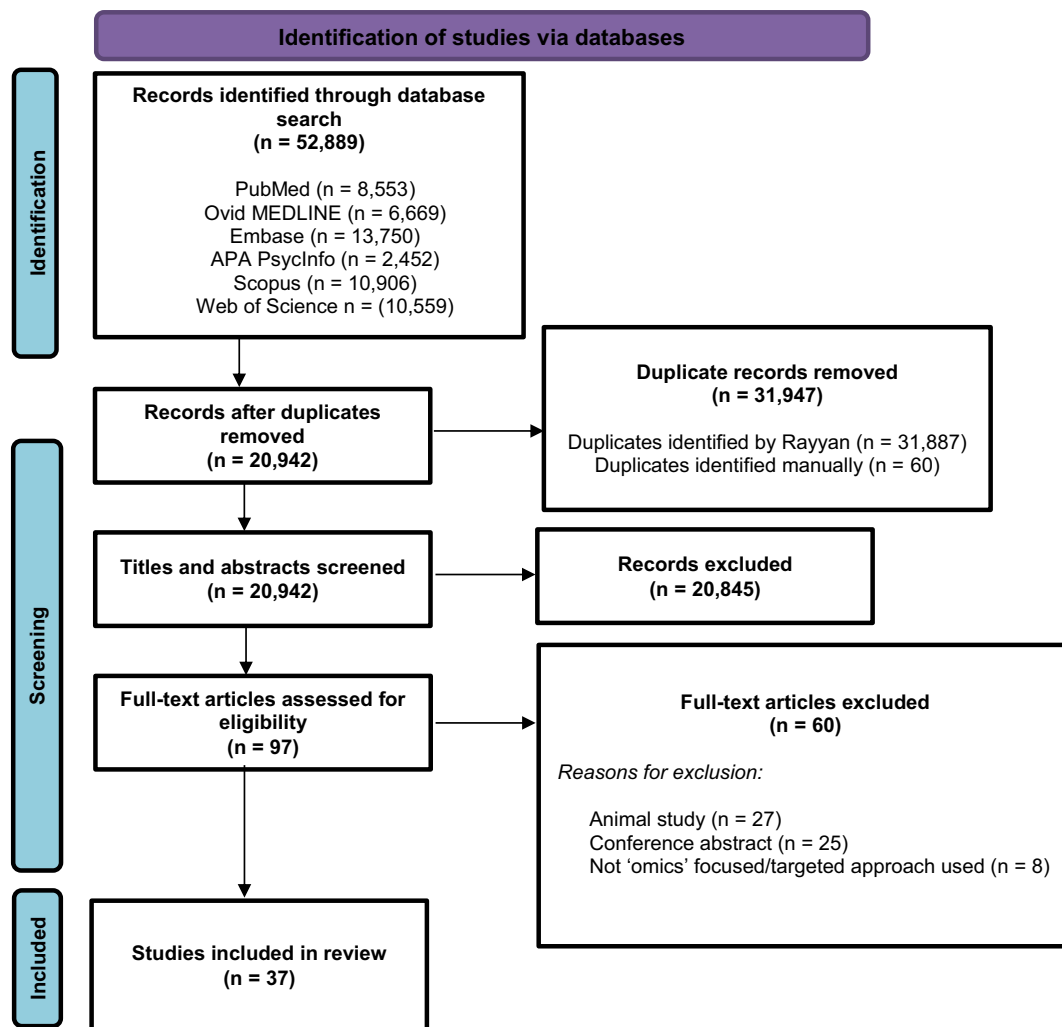


Fig. 1. Flow Diagram of study selection – adapted from the flow diagram template provided by PRISMA 2020 (Page et al., 2021).

2021a; Fanelli et al., 2021; Li et al., 2021; Li et al., 2016; Li et al., 2020a; McClain et al., 2020a; McClain et al., 2020b; O'Dushlaine et al., 2014; Pettai et al., 2016; Ruland et al., 2016; Vadodaria et al., 2019a; Vadodaria et al., 2019b; Wigmore et al., 2020), with two papers further differentiating between TRD and non-responders (Fabbri et al., 2020; Fabbri et al., 2019b). The remaining 17 studies examined treatment response in TRD (Bekhbat et al., 2021; Cathomas et al., 2022; Chen et al., 2021; Clements et al., 2021; Foo et al., 2019; Guo et al., 2018; Gururajan et al., 2016; Israel-Elgali et al., 2021; Li et al., 2020b; Maffioletti et al., 2020; Moschny et al., 2020; Pisanu et al., 2021; Rotroff et al., 2016; Ryan et al., 2017; Singh et al., 2022; Souza-Silva et al., 2020; Stelzhammer et al., 2013). Among these, 7 studies solely administered ECT (Clements et al., 2021; Foo et al., 2019; Maffioletti et al., 2020; Moschny et al., 2020; Pisanu et al., 2021; Ryan et al., 2017; Stelzhammer et al., 2013), with one incorporating antidepressants at a later time point; in 4 studies, patients received either ketamine or a different treatment that included esketamine, scopolamine, ECT or a placebo (Chen et al., 2021; Guo et al., 2018; Gururajan et al., 2016; Rotroff et al., 2016), while in two studies all TRD patients received ketamine (Cathomas et al., 2022; Singh et al., 2022); one study administered a different brain stimulation technique – repetitive transcranial magnetic stimulation (rTMS) (Souza-Silva et al., 2020); 2 studies combined antidepressants with esketamine or ketamine/ECT (Israel-Elgali et al., 2021; Li et al., 2020b); in one study patients were treated with infliximab, an anti-inflammatory monoclonal antibody directed against tumor necrosis factor (TNF) (Bekhbat et al., 2021). Articles mainly used a genome-wide association study (GWAS)

approach or data (n = 16) (Chen et al., 2021; Clements et al., 2021; Fabbri et al., 2018; Fabbri et al., 2021a; Fabbri et al., 2019b; Fanelli et al., 2021; Foo et al., 2019; Guo et al., 2018; Li et al., 2016; Li et al., 2020a; Li et al., 2020b; Maffioletti et al., 2020; O'Dushlaine et al., 2014; Pisanu et al., 2021; Souza-Silva et al., 2020; Wigmore et al., 2020), while 2 studies employed whole-exome sequencing (Fabbri et al., 2020; McClain et al., 2020b), one utilised comparative genomic hybridisation for analysing copy number variations (CNVs) (McClain et al., 2020a) and another was a pharmacogenomic study (Fabbri et al., 2021b) that utilised data from the study by Fabbri et al. (2020). Eleven studies employed transcriptomic techniques (Barakat et al., 2020; Bekhbat et al., 2021; Cathomas et al., 2022; Cole et al., 2021; Fabbri et al., 2021c; Gururajan et al., 2016; Israel-Elgali et al., 2021; Li et al., 2021; Pettai et al., 2016; Vadodaria et al., 2019a; Vadodaria et al., 2019b), which included microarray and sequencing technologies, with 3 studies using these methods to measure microRNAs (miRNAs) which can have an epigenetic effect on gene expression (Gururajan et al., 2016; Israel-Elgali et al., 2021; Li et al., 2021) and one study combined genomic and transcriptomic data for a transcriptome-wide association study (TWAS) approach (Fabbri et al., 2021c). Fewer articles focused on proteomics (n = 3) (Ruland et al., 2016; Ryan et al., 2017; Stelzhammer et al., 2013), epigenomics (n = 1) (Moschny et al., 2020) and metabolomics (n = 2) (Rotroff et al., 2016; Singh et al., 2022). A variety of samples were collected from participants. Most studies (n = 23) analysed blood (Barakat et al., 2020; Bekhbat et al., 2021; Cathomas et al., 2022; Clements et al., 2021; Cole et al., 2021; Fabbri et al., 2020; Foo et al.,

**Table 1**  
Study characteristics and results.

Study	Sample	Tissue type	Omics technique	Definition of treatment resistance	Main reported findings	Limitations
Li et al. (2016) USA	23andMe participants who completed the AESES – GWAS on 4 groups of phenotypes including NTRD (n = 7795) vs TRD (n = 1311), and compared with healthy controls (n = 192,178)  Subjects self-reported to take antidepressants for depression indication and were of European ancestry	Saliva	GWAS – platforms used for SNP genotyping were either variants of the Illumina HumanHap550+ BeadChip or based on the Illumina OmniExpress+ BeadChip or a fully custom array  Pathway/gene-set enrichment analysis – INRICH	Self-reported efficacy score of $\leq 1$ (i.e. a little/ to not at all) to at least 2 antidepressants and didn't report an efficacy score of $\geq 3$ (i.e. a fair amount/ a great deal) to any antidepressant	<ul style="list-style-type: none"> <li>No SNP reached genome-wide significance threshold* for TRD phenotype</li> <li><b>Strongest associations NTRD vs TRD</b></li> <li>rs1375194 (<i>FAM98A-MYADML</i>), rs190662943 (<i>DPP10</i>), rs75507262 (<i>RHOU-RAB4A</i>), rs10847303, rs73086581 (<i>RNF24</i>) <i>TRD vs healthy controls</i></li> <li>rs190544851 (<i>KCNJ15-ERG</i>), rs57043326 (<i>C6orf99</i>), rs12068879 (<i>KAZN</i>), rs1322281 (<i>PTPRD</i>), rs13418410 (<i>RASGRP3-FAM98A</i>)</li> <li><b>Enriched gene sets (corrected p &lt; 0.1) NTRD vs TRD</b></li> <li>Fatty acid metabolism, endonuclease activity, regulation of MAPKKK cascade <i>TRD vs healthy controls</i></li> <li>Alanine, aspartate and glutamate metabolism, activation of RAC, signalling by ROBO receptor</li> </ul>	<ul style="list-style-type: none"> <li>Phenotype data ascertained from self-report questionnaires – potential for recall bias</li> <li>Qualitative nature of outcome assessment (as opposed to clinical assessment) means that there may be a lack of diagnostic certainty</li> <li>Heterogeneity within self-reported samples due to potential inclusion of both MDD and minor depressive disorder individuals</li> <li>Uncertainty about whether patients were optimally dosed and compliant for minimal dose exposure</li> </ul>
Li et al. (2020a) USA	23andMe participants who completed the AES – GWAS on 4 groups of phenotypes including NTRD (n = 17,214) vs TRD (n = 3168)  23andMe participants who completed the AESES – GWAS on 4 groups of phenotypes including NTRD (n = 7795) vs TRD (n = 1311)  Healthy controls (n = 354,820) for comparison  Subjects self-reported to take antidepressants for depression indication  Meta-analysis for TRD phenotype (31,068 NTRD vs 5714 TRD)	Saliva	GWAS and meta-analysis – platforms used for SNP genotyping were either variants of the Illumina HumanHap550+ BeadChip or based on the Illumina OmniExpress+ BeadChip or a fully custom array  Gene-based and gene-set enrichment analysis – MAGMA	AES cohort – minimum of 2 antidepressants taken for $\geq 5$ –6 weeks and found overall treatment effect to be not “helpful or very helpful” or the medication did not help even if overall treatment effect was “helpful or very helpful”  AES cohort – self-reported efficacy score of $\leq 1$ (i.e. a little/ to not at all) to at least 2 antidepressants, but didn't report an efficacy score of $\geq 3$ (i.e. a fair amount/ a great deal) to any antidepressant	<ul style="list-style-type: none"> <li>No SNP reached genome-wide significance threshold* for NTRD vs TRD phenotype in AES or AESES cohorts (suggestive association signals for AESES shown above)</li> <li>Meta-analysis (NTRD vs TRD) → genomic region passed genome-wide significance threshold* - lead SNP rs150245813 (<i>ZNF37A-LINC00999</i>) in 10p11.1 (p = <math>8.07 \times 10^{-9}</math>) correlates with variation in expression of <i>ZNF48</i> gene as well as RP11-672F9.1 and RP11-258F22.1 lncRNAs</li> <li>For gene-based analysis, only <i>NCR3</i>, <i>LST1</i> and <i>LTB</i> genes in the AES cohort reached genome-wide significance (p = <math>2.64 \times 10^{-6}</math>)</li> <li>Gene-set enrichment analysis yielded non-statistically significant findings</li> </ul>	<ul style="list-style-type: none"> <li>Small sample size</li> <li>Use of self-reported questionnaire for TRD phenotype ascertainment – potential recall bias</li> <li>Participants may not be representative of all depression patients since they volunteered to provide biological samples</li> <li>Sample heterogeneity since participants were not necessarily diagnosed with MDD</li> </ul>
Fabbri et al. (2019b) UK	GWAS in GSRD MDD sample, and meta-analysis of GSRD, STAR*D and GENDEP MDD samples	N/A	GWAS and meta-analysis  • GSRD – Illumina Infinium PsychArray 24 BeadChip	Lack of response to $\geq 2$ antidepressant treatments	<ul style="list-style-type: none"> <li>No SNP/gene/loci reached genome-wide significance threshold* for association with the phenotypes of interest</li> </ul>	<ul style="list-style-type: none"> <li>Low power and limited sample sizes to detect single SNP/variant associations with TRD</li> <li>Heterogeneity in patients recruitment for 3 samples and clinical-</li> </ul>

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Table 1 (continued)

Study	Sample	Tissue type	Omics technique	Definition of treatment resistance	Main reported findings	Limitations
	<p>TRD (<i>n</i> = 1378)                      Responders (<i>n</i> = 934)                      Non-responders (<i>n</i> = 914)</p> <p>Phenotypes - TRD vs others (responders and non-responders to first antidepressant treatment) phenotype (<i>n</i> = 3225)                      TRD vs response (to first antidepressant treatment), as well as % symptom improvement in TRD and responders (to first antidepressant treatment) phenotypes (<i>n</i> = 2214)</p>		<ul style="list-style-type: none"> <li>STAR<sup>®</sup>D – Affymetrix Human mapping 500 K Array Set or Affymetrix Genome-Wide Human SNP Array 5.0</li> <li>GENDEP – Illumina Human610-quad bead chip</li> </ul> <p>Gene-level and gene-set (pathway) enrichment analysis – MAGMA</p>		<p><b>Single-variant analysis</b>                      GSRD</p> <ul style="list-style-type: none"> <li>rs7665833 intergenic SNP was closest to significance threshold (<math>p = 1.05 \times 10^{-7}</math>) for association with TRD vs others phenotype</li> <li>Genes in regions containing SNPs with suggestive <i>p</i>-values (<math>p &lt; 5 \times 10^{-6}</math>) were enriched in pathways related to intermediate filament cytoskeleton regulation</li> </ul> <p><b>Meta-analysis</b></p> <ul style="list-style-type: none"> <li>rs12160925 and rs12160621 intergenic SNPs (located ~30 kb upstream of <i>SEZ6L</i>) in complete LD were close to reaching a significant association with symptom improvement (<math>p = 9.14 \times 10^{-8}</math>)</li> <li>Genes in regions containing SNPs with suggestive <i>p</i>-values were enriched in pathways involved in transcription regulation, apoptosis, calcium signalling, synaptic transmission, second messenger cascades, secretion and response to hormones e.g. steroids</li> </ul> <p><b>Gene-level and gene-set analysis</b>                      GSRD</p> <ul style="list-style-type: none"> <li>GO:0043949 gene set (regulation of cAMP mediated signal) was found to be associated with TRD vs response phenotype (comparative corrected <math>p = 0.030</math>)</li> <li>Most significant genes in this set → <i>CRTC3</i> and <i>PDE10A</i></li> </ul> <p><b>Meta-analysis</b></p> <ul style="list-style-type: none"> <li>GO:0000183 gene set (chromatin silencing) was found to be associated with TRD vs others phenotype (comparative corrected <math>p = 0.027</math>)</li> <li>Most significant genes in this set → <i>HIST1H4E</i>, <i>BEND3</i> and <i>SIRT2</i></li> <li>No SNP reached genome-wide significance* for any of the phenotypes in the GWAS or meta-analysis</li> <li>No gene or gene-set was significantly associated with any of the phenotypes or passed multiple testing correction</li> </ul> <p><b>GS:SFHS – stages of resistance</b></p>	<p>demographic characteristics e.g. treatment</p> <ul style="list-style-type: none"> <li>Slight variations in the way phenotypes (including TRD) were defined across samples</li> </ul>
<p>Wigmore et al. (2020)                      UK</p>	<p>GWAS for stages of resistance (i.e. individuals on antidepressants) and antidepressant treatment resistance in population based cohort GS:SFHS (<i>n</i> = 3452, 250 TRD, 3202 NTRD)</p> <p>GWAS for</p>	Blood	<p><b>GWAS and meta-analysis</b></p> <ul style="list-style-type: none"> <li>GS:SFHS – Applied Biosystems OpenArray genotyping system</li> <li>GENDEP – Illumina Human610quad bead chip</li> </ul> <p>Gene and gene-set enrichment analysis –</p>	<p>GS:SFHS (using prescribing data) –</p> <ul style="list-style-type: none"> <li>Treatment resistance – individuals prescribed &gt;2 antidepressants at adequate dose and duration</li> <li>Stages of resistance – number of different antidepressants prescribed at adequate</li> </ul>	<ul style="list-style-type: none"> <li>No SNP reached genome-wide significance* for any of the phenotypes in the GWAS or meta-analysis</li> <li>No gene or gene-set was significantly associated with any of the phenotypes or passed multiple testing correction</li> </ul> <p><b>GS:SFHS – stages of resistance</b></p>	<ul style="list-style-type: none"> <li>Small sample size – underpowered to detect significant associations</li> <li>GS:SFHS participants may have been prescribed antidepressants for a condition other than MDD and may have had a misdiagnosis, but sample was considered</li> </ul>

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Table 1 (continued)

Study	Sample	Tissue type	Omics technique	Definition of treatment resistance	Main reported findings	Limitations
	antidepressant treatment resistance in GENDEP sample (n = 109 TRD, 668 NTRD)  Meta-analysis for antidepressant treatment resistance of GD:SFHS and GENDEP (n = 4213)		MAGMA  PRS analysis – to examine whether TRD indicated a higher genetic liability to other psychiatric disorders	dose and duration (i.e. 4 encodes individuals who were prescribed >4 different antidepressants). It was assumed that antidepressant switching meant lack of clinical response.  GENDEP – non-response to >2 antidepressant treatments	<ul style="list-style-type: none"> <li>• Most significant SNP → rs116902282 intergenic variant (<i>CI0orf35-COL13A1</i>) (<math>p = 1.5 \times 10^{-7}</math>)</li> <li>• Other SNPs with suggestive <i>p</i>-values → rs188352979 (<i>ACADSB-HMX3</i>), rs182041872 (<i>ACADSB-HMX3</i>) and rs4400118 (<i>DNAH5-TRIO</i>)</li> </ul> <p><b>Meta-analysis – treatment resistance</b></p> <ul style="list-style-type: none"> <li>• Most significant SNP → rs188352979 intergenic variant (<i>ACADSB-HMX3</i>) (<math>p = 3.25 \times 10^{-7}</math>)</li> <li>• Other SNPs with suggestive <i>p</i>-values → rs145842949 (<i>MON1B</i>), rs111968111 (<i>SRPK1-SLC26A8</i>) and rs138583130 (<i>CNTN1</i>)</li> </ul> <p><b>Most significant genes and gene-sets – treatment resistance</b></p> <ul style="list-style-type: none"> <li>• <i>AASDHPPT, PLOD2, NEUROG3</i></li> <li>• Sarcoplasmic reticulum calcium ion transport, regulation of protein oligomerisation and regulation of protein homo-oligomerisation</li> </ul> <p><b>Most significant genes and gene-sets – stages of resistance</b></p> <ul style="list-style-type: none"> <li>• <i>ZFP28, ZNF600, ZFR2</i></li> <li>• Innate immune response in mucosa, necroptotic process, and organ or tissue specific immune response</li> </ul> <p><b>PRS analysis</b></p> <ul style="list-style-type: none"> <li>• No result survived FDR correction</li> <li>• Antidepressant treatment resistance and stages of antidepressant resistance were positively and nominally associated with MDD-PRS.</li> <li>• Only antidepressant treatment resistance was positively and nominally associated with schizophrenia-PRS.</li> </ul>	<ul style="list-style-type: none"> <li>• genetically representative of MDD</li> <li>• Potential confounding due to not accounting for combination therapies e.g. antidepressants plus ECT or psychotherapy</li> <li>• Associations may not be specific to certain antidepressants due to integration of prescribing data regarding different antidepressant drugs and classes</li> <li>• Antidepressant switching as measure for staging resistance is not as comprehensive as other existing measures and is not in line with models used in other studies</li> </ul>
Guo et al. (2018) USA	326 subjects with TRD and a current MDE who were diagnosed with MDD or BD and who received single IV 0.5 mg/kg ketamine or 4 µg/kg scopolamine infusion	N/A	GWAS – 900,000 SNPs genotyped on Illumina Infinium OmniExpress and Illumina Infinium OmniExpressExome chips for ketamine-treated cohort  PRS analysis – to predict response to scopolamine	Ketamine-treated sample – current or past history of lack of response to at least 2 adequate antidepressant trials  Scopolamine-treated sample – failed at least 2 adequate treatment trials for depression	<p><b>Ketamine GWAS</b></p> <ul style="list-style-type: none"> <li>• No SNP reached genome-wide significance threshold*</li> </ul> <p><i>Antidepressant response to ketamine</i></p> <ul style="list-style-type: none"> <li>• 31 SNPs and 8 LD-independent loci with <i>p</i>-values <math>&lt;1 \times 10^{-5}</math> were found</li> </ul>	<ul style="list-style-type: none"> <li>• Heterogeneity of sample due to the inclusion of BD subjects – results not specific to MDD and may result in confounding and spurious associations</li> <li>• Small sample size – could increase the risk of type II error and reduce power for detecting true associations</li> </ul>

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Table 1 (continued)

Study	Sample	Tissue type	Omics technique	Definition of treatment resistance	Main reported findings	Limitations
Li et al. (2020b) UK	527 TRD European patients who had received intranasal esketamine combined with a newly initiated oral antidepressant treatment (SSRI or SNRI) in an open-labelled (SUSTAIN-2) or randomised (TRANSFORM-3) manner	Blood	GWAS – Illumina PsychArray genotyping data  Pathway enrichment analysis – MAGMA  PRS analysis – test for associations between esketamine treatment response outcome and genetic loading for psychiatric conditions/symptoms profiles	Non-response to ≥2 oral antidepressants (and ≤ 5 for TRANSFORM-3 cohort) in the current episode of depression that was assessed using the MGH-ATRQ (Fava, 2003)	<ul style="list-style-type: none"> <li>• Top-ranked SNP was rs55945116 (<math>p = 5.93 \times 10^{-7}</math>), which is found within <i>SEC11A</i> gene</li> <li>• Next highest ranked SNP was rs112647602 (<math>p = 4.82 \times 10^{-6}</math>), which is found close to <i>KRASPI</i> and <i>FAM83B</i> genes</li> </ul> <p><i>Dissociative side effects</i></p> <ul style="list-style-type: none"> <li>• 52 SNPs and 12 LD-independent loci with <math>p</math>-values <math>&lt; 1 \times 10^{-5}</math> were found</li> <li>• Top-ranked SNP was rs17211233 (<math>p = 1.9 \times 10^{-7}</math>), which is found within <i>RASGRF2</i> gene</li> </ul> <p><b>PRS analysis</b></p> <ul style="list-style-type: none"> <li>• 97,129 SNPs with <math>p &lt; 0.5</math> from ketamine GWA study explained 6 % of the variance in scopolamine response (PRS <math>p</math>-value = 0.19), but this was not statistically significant</li> </ul> <p><b>Genome-wide association analysis</b></p> <ul style="list-style-type: none"> <li>• Genome-wide significant association between exonic SNP rs11465988 (<i>IRAK3</i>) (<math>p = 3.57 \times 10^{-8}</math>) and percentage change in MADRS score (continuous variable)</li> <li>• No SNP reached genome-wide significance threshold* for responder and remission status (categorical variables)</li> </ul> <p><b>Gene-level association analysis</b></p> <ul style="list-style-type: none"> <li>• Significant association between <i>NME7</i> gene (<math>p = 1.73 \times 10^{-6}</math>) and percentage change in MADRS score</li> <li>• No association between percentage change in MADRS score and <i>BDNF</i> Val66Met/rs6265 polymorphism</li> </ul> <p><b>Pathway enrichment analysis – all nominal significance</b></p> <ul style="list-style-type: none"> <li>• Percentage change in MADRS score → enrichment of genes involved in negative regulation of glucocorticoid metabolic process and neuronal action potential</li> <li>• Responder status → enrichment of genes</li> </ul>	<ul style="list-style-type: none"> <li>• Small/modest sample size</li> </ul>

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Table 1 (continued)

Study	Sample	Tissue type	Omics technique	Definition of treatment resistance	Main reported findings	Limitations
Maffioletti et al. (2020) Italy	Genome-wide data for 71 MDD TRD patients treated with ECT	Whole (peripheral venous) blood	GWAS – Illumina Infinium Multi-Ethnic Genotyping Array (MEGA)  VEGF levels and SNPs were also measured	Stage III of Thase and Rush Staging Method (Thase and Rush, 1997) – non-response to an adequate TCA trial as well as to $\geq 2$ adequate trials of $\geq 2$ different antidepressant classes	involved in synaptic vesicle clustering, negative regulation of glucocorticoid metabolic process, regulation of synaptic vesicle clustering, anterior posterior axon guidance, and netrin mediated repulsion signals <ul style="list-style-type: none"> <li>Remission status → enrichment of genes involved in negative regulation of extrinsic apoptotic signalling pathway, NF-<math>\kappa</math>B canonical pathway, stress pathway, and TNFR1 induced proapoptotic signalling</li> </ul> <b>PRS analysis</b>  <ul style="list-style-type: none"> <li>No genome-wide significant associations.</li> <li>A suggestive negative correlation between depressive symptom-PRS and percentage change in MADRS score.</li> <li>A suggestive positive correlation between depressive symptom-PRS and esketamine responder status.</li> <li>Depressive symptoms-PRS and insomnia-PRS displayed suggestive positive correlations with esketamine remission status.</li> <li>Significant association between rs78355601 A allele (linked to lower VEGF levels) and ECT non-response (<math>p = 0.01</math>), particularly patients homozygous for risk allele (AA) vs patients carrying the protective allele (AG + GG) (<math>p = 0.026</math>), which was a significant finding</li> </ul>	<ul style="list-style-type: none"> <li>Small sample size</li> </ul>
Fabbri et al. (2020) UK	1209 MDD subjects from GSRD sample  <ul style="list-style-type: none"> <li>Training sample (<math>n = 847</math>) – 353 TRD, 203 responders, 291 non-responders</li> <li>Testing sample (<math>n = 362</math>) – 151 TRD, 86 responders, 125 non-responders</li> </ul>	Whole blood	<b>Whole exome sequencing</b> – Illumina HiSeq platform <b>Genome-wide genotyping</b> – Illumina Infinium PsychArray 24 BeadChip  Differential distribution of damaging variants between TRD vs responders vs non-responders was tested  Gene-based (including rare and common variants) and pathway-based scores (rare variants only or rare and common variants) were used to create models for predicting TRD, with the addition of clinical predictors  Replication of models	Lack of response to $\geq 2$ adequate antidepressant treatments during current MDE	<ul style="list-style-type: none"> <li>No difference between TRD patients, non-responders and responders in the distribution of damaging variants in individual genes or the whole exome</li> <li>Gene-based and pathway-based scores were not associated with TRD – no association survived Bonferroni correction</li> <li>Top genes for TRD vs non-response vs response in the whole sample were <i>NBN</i> and <i>ZNF418</i></li> </ul> <b>Predictive modelling</b>  <ul style="list-style-type: none"> <li>Only models using pathway-based scores (including only rare variants) and models using gene-based scores were able to significantly predict TRD vs response in the whole testing sample as</li> </ul>	<ul style="list-style-type: none"> <li>GSRD sample may have contained complex cases of MDD (i.e. highly impacted by clinical risk factors), thus potentially explaining why models did not perform better than those with only clinical predictors</li> <li>Differences in terms of the genetic data and clinical profile of patients and definition of TRD phenotype was ascertained slightly differently between GSRD, STAR*D and GENDEP samples</li> </ul>

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Table 1 (continued)

Study	Sample	Tissue type	Omics technique	Definition of treatment resistance	Main reported findings	Limitations
			was tested in the GENDEP and STAR*D samples		well as in patients treated with serotonergic antidepressants <ul style="list-style-type: none"> <li>• Adding clinical predictors improved the predictive performance of the models, but they did not perform better than clinical predictors alone</li> </ul> <b>Replication results</b> <ul style="list-style-type: none"> <li>• Only models including clinical predictors as well as pathway-based scores (including only rare genetic variants) or gene-based scores were able to significantly predict TRD vs response</li> <li>• Only exception was model that used pathway-based scores (including only rare variants) without clinical predictors that significantly predicted TRD in patients treated with serotonergic antidepressants in STAR*D</li> </ul>	
Fabbri et al. (2021b) UK	Utilised data from whole exome sequence study (Fabbri et al., 2020) – 504 TRD and 416 treatment-responsive MDD	N/A	<b>Pharmacogenomic study</b> – gene-sets previously demonstrated to predict TRD risk were compared with those coding for drug targets from Drug repurposing Hub, Drug-Gene Interaction database and DrugBank database	Lack of response to ≥2 adequate antidepressant treatments during current MDE	<ul style="list-style-type: none"> <li>• 542 compounds were identified to be enriched for genes in TRD-associated pathways (FDR &lt; 0.05).</li> <li>• Drugs with known therapeutic effect in TRD → lithium (FDR <math>p = 2.72 \times 10^{-4}</math>), tricyclic antidepressants (FDR <math>p = 9.83 \times 10^{-3}</math>) and ketamine (FDR <math>p = 1.84 \times 10^{-2}</math>).</li> <li>• Most common mechanisms of action of the 542 compounds were associated with → modulation of cell proliferation/survival (32 %), monoamine neurotransmission (14 %) and modulation of inflammation/immune response (16 %).</li> <li>• Other mechanisms of action → glycogen synthase kinase 3 beta (GSK3), vasopressin, angiotensin and/or oxytocin modulation, cholinergic neurotransmission, GABAergic/glutamatergic neurotransmission, calcium metabolism/signalling, angiogenesis modulation, sex hormone signalling.</li> </ul>	<ul style="list-style-type: none"> <li>• This approach does not distinguish the direction of the effect (therapeutic or noxious) of the compounds.</li> <li>• Heterogeneity in the study and characterisation of drug targets based on their duration in the market or availability in different countries may have influenced the results.</li> <li>• This approach may have led to over-representation of drugs targeting better studied genes that are in known pathways and genes that are not individually associated with TRD</li> <li>• Relatively limited sample size</li> </ul>
McClain et al. (2020b) USA	124 treatment-refractory MDD patients who belonged to BH4, CFD or AAP metabolic phenotypes/subgroups  6238 controls	Blood	<b>Whole exome sequencing and EWAS</b> – Illumina HiSeq 2500 system  Pathway analysis – filtration of exonic variants to retain damaging variants (i.e. SNPs with likely	Non-response to 3 medications at maximum dosage taken for ≥6 weeks each, which was ascertained by completing ATRQ (Fava, 2003)	<b>Exome-wide association study</b>  <ul style="list-style-type: none"> <li>• 3 SNPs/exonic variants were suggestive of association but did not reach genome-wide significance* → rs11913417 LARGE (chr2),</li> </ul>	<ul style="list-style-type: none"> <li>• Small sample size, thus do not have enough power to detect meaningful associations with SNPs or variants with very small effect sizes.</li> </ul>

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Table 1 (continued)

Study	Sample	Tissue type	Omics technique	Definition of treatment resistance	Main reported findings	Limitations
			deleterious outcomes) and test their association with biological pathways using MEGA-V		rs138627563 <i>KMT2C</i> (chr7) and <i>SNP ID not available ROBO2</i> (chr3) <b>Pathway analysis</b>	
			Rare variant analyses – identify rare variants in metabolic subgroups of these patients and those expressed in CNS		<ul style="list-style-type: none"> <li>• Examples of significant pathways enriched with rare damaging variants in metabolic subgroups and whole treatment-refractory MDD cohort → GABA receptor activation, potassium channels, transmission across chemical synapses, neuronal system; neurotransmitter receptor binding and downstream transmission in the post-synaptic cell, structural constituent of cytoskeleton</li> </ul>	
					<b>Rare variant analyses</b>	
					<ul style="list-style-type: none"> <li>• In each metabolic subgroup, several rare damaging variants were identified, a proportion of which were expressed in the CNS and many of the variants mapped to gene loci found to be associated with psychiatric disorders like MDD</li> </ul>	
McClain et al. (2020a) USA	125 treatment-refractory MDD patients who belonged to one or more of the following metabolomic phenotypes: 5-HIAA, HVA, 5-MTHF and/or BH4  26 healthy controls – only used to confirm absence of the 15q13.3 duplication CNV	Whole (peripheral) blood	<b>Genome-wide copy number analysis</b> – 180 K oligonucleotide-based comparative genomic hybridisation microarray	Non-response to ≥3 maximum-dose medication trials lasting ≥6 weeks each	<ul style="list-style-type: none"> <li>• Significant enrichment for 15q13.3 duplications in 5 patients (out of 125)</li> <li>• Most patients had a 5-HIAA or HVA deficiency</li> <li>• 3 patients had a 445 or 472 kb size duplication and this region contains the <i>CHRNA7</i> gene</li> <li>• 2 patients had a 796.6 kb size duplication and this region contains <i>CHRNA7</i> as well as <i>GOLGA8K</i>, <i>ULK4P3</i>, <i>ULK4P1</i>, <i>ULK4P2</i>, <i>WHAMMP1</i> and <i>LOC10996255</i> among others</li> <li>• Clinical characteristics shared among these patients → young age at MDD onset, presence of co-morbidities, past family history of MDD and other psychiatric disorder</li> </ul>	<ul style="list-style-type: none"> <li>• Small sample size</li> </ul>
O’Dushlaine et al. (2014) USA	778 European subjects in i2b2 sample (300 TRD, 478 SSRI-responsive individuals)  485 European subjects in STAR*D sample (152 TRD, 333 SSRI-responsive individuals)	i2b2 sample – blood  STAR*D sample – N/A	Analysis of <b>GWAS</b> data to identify <b>CNVs</b>  <ul style="list-style-type: none"> <li>• i2b2 sample – Illumina Omni MM or Omni Express array</li> <li>• STAR*D sample – Affymetrix Human mapping 500 K Array Set or Affymetrix Genome-Wide Human SNP Array 5.0</li> </ul>	i2b2 sample – received ≥2 antidepressants during MDE or received ECT after ≥1 documented antidepressant trial  STAR*D sample – score of ≥10 in the QIDS-SR after 2 antidepressant trials	<b>CNV analysis</b>  <ul style="list-style-type: none"> <li>• For both samples, there was nominally significant enrichment of 100–200 kb duplications intersecting genes in TRD vs SSRI-responsive individuals (permuted <math>p = 0.04</math>)</li> <li>• Nominally significant association between TRD and deletions spanning <i>PABPC4L</i> gene (4q28.3) (empirical <math>p = 0.02</math>) as well as in the 9p23 region (empirical <math>p = 0.03</math>) that</li> </ul>	<ul style="list-style-type: none"> <li>• Phenotypic heterogeneity between the 2 samples i.e. STAR*D used standard rating scales while i2b2 sample used data from electronic health records, which may have prevented the identification of strong and consistent associations</li> </ul>

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Table 1 (continued)

Study	Sample	Tissue type	Omics technique	Definition of treatment resistance	Main reported findings	Limitations
Chen et al. (2021) Taiwan	65 Taiwanese TRD patients who received 40 min intravenous infusion of 0.5 or 0.2 mg/kg of ketamine or saline placebo	N/A	Gene-based GWAS – all participants were genotyped for 684,616 SNPs using Illumina Human Omni Express Exome Bead Chips. Then 12 genes were selected for gene-based GWAS  Gene-based association and gene-set enrichment analyses	N/A	<p>doesn't have annotated genes</p> <p><b>Pathway analysis</b></p> <ul style="list-style-type: none"> <li>Nominally significant enrichment of CNVs (mainly duplications) intersecting 8 of 191 genes (including <i>ITGA8</i>, <i>ITGA10</i>, <i>RAK3</i>, <i>CYFIP1</i> and <i>CRKL</i>) related to regulation of actin cytoskeleton pathway in TRD cases from i2b2 sample, which was not replicated/found in the STAR*D sample</li> </ul> <p><b>SNP-based association analysis – ketamine response at different follow-up time points</b></p> <ul style="list-style-type: none"> <li>6 SNPs reached at least suggestive association threshold (<math>p &lt; 1.0 \times 10^{-2}</math>) for both HAMD and MADRS scoring systems → rs10217777 (<i>NTRK2</i>), rs16966731 and rs34413887 (<i>GRIN2A</i>) with antidepressant effect of ketamine at 40 min post-infusion, rs10868590 (<i>NTRK2</i>) with 240 min post-infusion, and rs79965951 (<i>GRIN3A</i>) and rs11055643 (<i>GRIN2B</i>) with day 14 post-infusion</li> </ul> <p><b>Gene-based association analysis – ketamine response at different follow-up time points</b></p> <ul style="list-style-type: none"> <li>Genes that reached at least suggestive association threshold (<math>p &lt; 5.0 \times 10^{-2}</math>) for both scoring systems → <i>GRIN2C</i> at day 2 post-infusion and <i>GRIN3A</i> at day 14 post-infusion (that are involved in the GABA and glutamate nerve terminal pathway that is related to antidepressant effect) and <i>BDNF</i> (involved in postsynaptic dendritic spine pathway that is related to antidepressant effect) at day 3, 6 and 7 post-infusion as well as <math>\geq 2</math> responses during day 2–5 post-infusion</li> <li><i>GRIN2C</i> reached significant association threshold (<math>p &lt; 1.0 \times 10^{-2}</math>) for both scoring systems at day 3 and 4 post-infusion</li> </ul> <p><b>Gene-set enrichment analysis – ketamine response</b></p>	<ul style="list-style-type: none"> <li>Associations may not be specific to ketamine, since patients were allowed to use their regular medication and ketamine as an add-on treatment</li> <li>Small sample size</li> <li>Sample consisted of only Taiwanese TRD patients, thus the generalisability of findings to other ethnic groups is uncertain</li> <li>SNP/gene associations with ketamine response were slightly inconsistent between the 2 different depressive symptom scoring systems used</li> <li>Did not provide a definition for treatment resistance</li> </ul>

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Table 1 (continued)

Study	Sample	Tissue type	Omics technique	Definition of treatment resistance	Main reported findings	Limitations
Fabbri et al. (2018) USA	STAR*D genome-wide data was used to replicate results from candidate-gene approach for TRD (i.e. response and remission in Level 2, n = 620) phenotype	N/A	<p><b>Genome-wide approach</b> – Affymetrix Human Mapping 500 K Array Set or Affymetrix Genome-Wide Human SNP Array 5.0</p> <p>Pathway enrichment analysis – MAGMA</p> <p>Predictive performance of SNPs from the top pathway was investigated using machine learning models – R cran Caret package</p>	STAR*D level 2 – patients that did not respond to citalopram (level 1), who then progressed to level 2 and were treated with either: a) citalopram combined with bupropion-SR, buspirone or CBT, or b) switching to sertraline, bupropion-SR, venlafaxine-XR or CBT.	<p>• Top significant pathways (<math>p &lt; 5 \times 10^{-2}</math>) include → CREB phosphorylation through the activation of CaMKII, Ras activation upon Ca<sup>2+</sup> influx through NMDA receptor, NOS1 pathway, CREB phosphorylation through the activation of Ras, post NMDA receptor activation events, activation of NMDA receptor upon glutamate binding and postsynaptic events, long-term potentiation, neurotransmitter receptor binding and downstream transmission in the postsynaptic cell, transmission across chemical synapses, ionotropic glutamate receptor activity, glutamate signalling pathway, glutamate receptor activity and calcium signalling pathway</p> <p><b>Replication results in STAR*D</b></p> <p>• Interesting finding → rs11062157 (<i>CACNA1C</i>) was in high LD with rs10848635 and was nominally associated with response in level 2 (i.e. TRD) (<math>p = 0.044</math>)</p> <p>• Nominal associations with response in level 2 (i.e. TRD) → rs10994180 (<i>ANK3</i>) in high LD with rs10740006, rs7912753 (<i>CACNB2</i>) in high LD with rs983048, and rs10221362 (<i>TCF4</i>) in LD with rs1261085</p> <p>• Nominal associations with remission in level 2 (i.e. TRD) → rs1222814, rs11062296 and rs11608296 (<i>CACNA1C</i>), rs7912753 (<i>CACNB2</i>) in high LD with rs983048, and rs1261085 and rs1787792 (<i>TCF4</i>)</p> <p>• No pathway survived multiple-testing correction, and the top pathway was regulation of striated muscle contraction for association with response in level 2 (i.e. TRD)</p> <p>• Genes included in this pathway and their role → <i>CACNA1C</i>, genes encoding ion channels, genes involved in neurogenesis, neural plasticity, synaptic transmission, long-term potentiation, and genes associated with MDD and antidepressant efficacy</p>	<ul style="list-style-type: none"> <li>• Only Caucasian participants were selected in STAR*D sample, thus limiting the generalisability of results to non-Caucasian samples</li> <li>• Models used for TRD prediction showed good sensitivity but the specificity was not very high, and replication was not performed</li> <li>• Limited number of clinical than genetic predictors included in models for TRD prediction</li> </ul>

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Table 1 (continued)

Study	Sample	Tissue type	Omics technique	Definition of treatment resistance	Main reported findings	Limitations
					<b>TRD prediction</b>	
					<ul style="list-style-type: none"> <li>• SNPs in abovementioned pathway predicted TRD with mean accuracy of 0.73, sensitivity of 0.83 and specificity of 0.56</li> </ul>	
<b>Clements et al. (2021)</b> Sweden	Severely ill cases from PREFECT cohort  o 2725 cases with a broad case definition who received ECT for a MDE in the context of MDD as well as other mood disorders o 1796 cases with a narrow case definition are a subset of the broad group who received ECT for a MDE in the context of MDD  3290 controls  964 mild-moderately ill MDD cases from iCBT cohort who were treated for MDD with internet-based CBT	Whole (peripheral) blood	<b>GWAS</b> – Illumina GSA-MD SNP arrays  GWAS was conducted with cases from PREFECT cohort compared to controls  Genetic risk score analysis – to compare severely ill PREFECT cases with mild-moderately ill cases receiving iCBT	ECT-treated MDD is considered TRD in real clinical practice	<p><b>GWAS</b></p> <ul style="list-style-type: none"> <li>• 3 SNPs reached genome-wide significance (<math>p &lt; 5 \times 10^{-8}</math>) for the broad case definition</li> <li>o rs114583506 (<math>p = 3.56 \times 10^{-8}</math>) – located in intron of <i>HLA-B</i> in the major histocompatibility region on chr 6</li> <li>o rs144631932 (<math>p = 1.51 \times 10^{-8}</math>) located in <i>ENPP2</i> gene on chr 8</li> <li>o rs142610580 (<math>p = 4.02 \times 10^{-10}</math>) on chr 7</li> <li>• rs142610580 (<math>p = 5.70 \times 10^{-10}</math>) reached genome-wide significance for the narrow case definition</li> </ul> <p><b>Genetic risk score comparison to mild and moderate MDD sample</b></p> <ul style="list-style-type: none"> <li>• Severely ill PREFECT cases (narrow case group) had slightly higher mean genetic risk score for MDD (<math>p = 0.02</math>) than the mild-moderately ill iCBT cases.</li> <li>• For both narrow and broad case groups, PREFECT cases carried a significantly higher genetic risk score burden for bipolar disorder and lower genetic risk scores for educational attainment and IQ compared to iCBT cases (<math>p &lt; 1 \times 10^{-4}</math>)</li> </ul>	<ul style="list-style-type: none"> <li>• Small sample size</li> <li>• Cases and controls were not well-matched on sex and age</li> <li>• Diagnoses were ascertained in a real-world clinical setting which may differ from diagnoses ascertained with structured research interviews</li> </ul>
<b>Foo et al. (2019)</b> Germany	51 patients with a MDE who were assigned to right unilateral brief pulse ECT treatment  Population-based sample – 3547 healthy controls and 426 individuals who self-reported depression  Genome-wide association data from Psychiatric Genomics Consortium MDD-working group were used to calculate PRS	Whole (venous) blood	<b>Genome-wide genotyping</b> – Illumina Global Screening Array  PRS analysis – test whether MDD-PRS is associated with MDD ECT case-control status	ECT-treated MDD is considered TRD in real clinical practice  A proportion of patients were assigned to ECT due to having TRD, defined as failing to respond to 2 antidepressant medications or psychotherapy of adequate dose and duration from different classes in the current MDE	<ul style="list-style-type: none"> <li>• Statistically significantly higher PRS found in ECT patients than controls (<math>p = 0.022</math>)</li> <li>• No statistically significant difference in PRS between ECT patients and self-reported depression individuals (<math>p = 0.237</math>), or self-reported depression individuals and controls (<math>p = 0.150</math>).</li> <li>• Trend of intermediate PRS in self-reported depression compared to ECT patients and controls</li> <li>• No statistically significant difference in PRS between responders and non-responders to ECT treatment, but trend towards non-responders to have higher PRS for MDD than responders</li> </ul>	<ul style="list-style-type: none"> <li>• Limited sample size</li> <li>• Heterogeneity in self-reported depression sample</li> <li>• ECT sample included both MDD and BD patients, thus may have introduced heterogeneity into the sample and so the results are not specific to MDD</li> </ul>

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Table 1 (continued)

Study	Sample	Tissue type	Omics technique	Definition of treatment resistance	Main reported findings	Limitations
Fanelli et al. (2021) UK	1148 MDD patients from the GSRD sample (479 TRD)  PRSs were calculated using data from genome-wide analyses and meta-analyses	N/A	<b>Genome-wide genotyping</b> – Illumina Infinium PsychArray 24 BeadChip  PRS analysis – to investigate whether PRSs for BD, MDD, neuroticism and schizophrenia are associated with resistance to antidepressants in MDD patients	Non-response to $\geq 2$ antidepressants of adequate dose and duration (i.e. 4 weeks)	<ul style="list-style-type: none"> <li>Statistically significant correlation between MDD-PRS and (clinical variable) alcohol dependence/abuse in ECT patient sample</li> <li>PRSs for BD, MDD, neuroticism and schizophrenia were not associated with resistance to antidepressants after correction for multiple testing.</li> <li>A weak nominal association between PRS for neuroticism and TRD (<math>p = 0.049</math>).</li> </ul>	<ul style="list-style-type: none"> <li>Small sample size</li> <li>PRSs only capture the effect of common genetic variants but not rare variants or interactive/epistatic effect between common variants in predicting shared genetic risk</li> </ul>
Fabbri et al. (2021a) UK	2430 TRD from UK Biobank cohort 15,951 non-TRD (MDD cases) from UK Biobank cohort	N/A	<b>Genome-wide genotyping</b> - Applied Biosystems UK BiLEVE Axiom Array and Applied Biosystems UK Biobank Axiom Array  PRS analysis – to test the association of PRSs for psychiatric disorders with TRD	Defined using primary care records in UK Biobank as MDD patients who had $\geq 2$ switches between different antidepressant drugs (independent from class) that were each prescribed for $\geq 6$ consecutive weeks and the time interval between prescription of 2 consecutive drugs was $\leq 14$ weeks	<ul style="list-style-type: none"> <li>No SNP reached genome-wide significance threshold for association with TRD vs non-TRD.</li> <li>ADHD-PRS was significantly associated with TRD vs non-TRD (<math>p = 4.38 \times 10^{-4}</math>), but other PRSs did not show an effect after Bonferroni correction.</li> </ul>	<ul style="list-style-type: none"> <li>Primary care records do not reflect complete information regarding antidepressant prescription</li> <li>Potential inclusion of cases with depressive disorders other than MDD due to lack of standardised diagnostic assessment</li> <li>Potential higher number of TRD patients than is the case, because of possible inclusion of patients receiving subtherapeutic doses due to unavailability of prescribed daily medication dose and thus excluded from TRD definition</li> <li>Inadequate power to detect variants associated with TRD vs non-TRD at genome-wide level</li> </ul>
Souza-Silva et al. (2020) Australia	99 TRD patients who received at least 18 sessions of 10 Hz at left dorsolateral prefrontal cortex repetitive TMS	N/A	<b>Genome-wide genotyping</b> – Illumina Infinium PsychArray-24 BeadChip  Functional enrichment analysis – STRING and Cytoscape	N/A	<ul style="list-style-type: none"> <li>53 significant SNP associations <math>\rightarrow</math> 11 SNPs were associated with treatment response and 42 SNPs were associated with non-responsiveness</li> <li>Enrichment analysis <math>\rightarrow</math> identified a synaptic plasticity regulation pathway containing the genes <i>SPPL2A</i> (associated with treatment response), <i>APP</i>, <i>EXOSC7</i>, <i>GRID2</i>, <i>ADGRB3</i>, <i>COL9A3</i>, <i>LY9</i> and <i>FOXN3</i> (associated with non-response)</li> </ul>	<ul style="list-style-type: none"> <li>Did not provide a definition for treatment resistance</li> </ul>
Pisanu et al. (2021) Italy	Genome-wide genotyping data was available for 107 TRD patients with MDD or BD who were treated with ECT  12 treatment-resistant MDD	N/A  PBMCs	Analysis of GWAS data – Illumina Infinium Multi-Ethnic Genotyping Array and Illumina Infinium PsychArray 24 BeadChip  Gene-based level analysis - MAGMA  <b>Global DNA methylation</b> – Illumina	Stage III of Thase and Rush Staging Method (Thase and Rush, 1997) – non-response to an adequate TCA trial as well as to $\geq 2$ adequate trials of $\geq 2$ different antidepressant classes  N/A	<ul style="list-style-type: none"> <li>No SNP or gene reached genome-wide significance threshold for association with response to ECT or leukocyte telomere length</li> <li>No significant overlap between SNPs or genes nominally associated with ECT response and leukocyte telomere length</li> <li>No significant difference in global DNA methylation</li> </ul>	<ul style="list-style-type: none"> <li>Small sample size</li> <li>Inclusion of BD subjects may have introduced heterogeneity into the sample and so the results are not specific to MDD</li> <li>Small sample size – not feasible to investigate</li> </ul>

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Table 1 (continued)

Study	Sample	Tissue type	Omics technique	Definition of treatment resistance	Main reported findings	Limitations
Moschny et al. (2020) Germany	patients who received ECT for 4 weeks		TruSeq Methyl Capture EPIC Library Kit and Illumina NextSeq 550 Sequencer		<ul style="list-style-type: none"> <li>between 4 measured time points (before and after first and last ECT) as well as between ECT responders and non-responders</li> <li>Methylation differences at 11 significant CpG probes/sites were found (when analysing each probe) between ECT responders and non-responders                             <ul style="list-style-type: none"> <li>5 novel protein-coding genes → 4 CpG sites in <i>RNF213</i> (chr17) and 1 CpG site in <i>RNF175</i> (chr4), <i>TBC1D14</i> (chr4), <i>TMC5</i> (chr16) and <i>WSCD1</i> (chr17)</li> <li>3 genes encoding lncRNAs → 1 CpG site in <i>AC018685.2</i> (chr2), <i>AC098617.1</i> (chr2) and <i>CLCN3P1</i> (chr9)</li> </ul> </li> <li>DNA methylation at 2 CpG sites located in <i>AQP10</i> and <i>TRERF1</i> genes changed significantly during the treatment period but did not affect clinical outcome</li> </ul>	<ul style="list-style-type: none"> <li>influence of clinical characteristics on DNA methylation</li> <li>The rarity of use of the specific platform makes comparisons with other DNA methylation studies investigating MDD subject unfeasible because the same CpG sites are not covered</li> <li>Did not provide a definition for treatment resistance</li> </ul>
Gururajan et al. (2016) Ireland	40 treatment-resistant MDD patients who received twice weekly brief-pulse bitemporal ECT ( <i>n</i> = 24) or 0.5 mg/kg <sup>-1</sup> intravenous ketamine infusion once a week for 3 sessions ( <i>n</i> = 16)  20 healthy controls	Blood	<p><b>miRNA microarray analysis</b> – Exiqon services</p> <p>Validation using qPCR</p> <p>Bioinformatic analysis of gene targets and pathways – MicroT-CDS and DIANA miRPath server</p>	Failure of ≥2 adequate antidepressant trials	<p><b>Microarray analysis</b></p> <ul style="list-style-type: none"> <li>Significant decrease in let-7b and let-7c expression after ECT treatment in TRD patients compared with healthy controls (i.e. in both ECT responders and non-responders), but this was not detected in qPCR analysis</li> <li>Did not identify any miRNA at baseline that predicted response to ECT or ketamine treatment</li> </ul> <p><b>Bioinformatic analysis</b></p> <ul style="list-style-type: none"> <li>Significant enrichment of 27 genes (targeted by let-7b and let-7c) that are involved in the intracellular PI3K-Akt signalling pathway, and 12 of which are involved in receptor activity and protein binding</li> </ul>	<ul style="list-style-type: none"> <li>Possible confounding of results – due to patients taking medication during the study, sample heterogeneity i.e. inclusion of melancholic and non-melancholic patients and predominantly older patients in ECT group who likely receive multiple medications for multiple conditions, as well as time differences in the collection of blood samples between ECT and ketamine treatment groups</li> <li>Fidelity issues between microarray and qPCR may explain inconsistent results</li> <li>Uncertainty as to whether changes in microRNA expression in peripheral blood are an accurate reflection of changes in brain tissue</li> </ul>
Li et al. (2021) China	4 TRD patients  4 healthy controls	Plasma	<p><b>Exosomal miRNA next-generation sequencing</b> – Qiagen exoRNeasy Midi Kit and Illumina HiSeq high-throughput sequencing</p> <p>GO functional enrichment analysis of miRNA target genes</p> <p>KEGG pathway enrichment analysis</p>	Recurrent depressive episodes in last 3 years after receiving ≥2 antidepressants	<ul style="list-style-type: none"> <li>Has-miR-335-5p was significantly upregulated (adjusted <i>p</i>-value = 0.0315) and has-miR-1292-3p was significantly downregulated (adjusted <i>p</i>-value = 0.0006) in TRD patients compared to controls</li> <li>GO analysis → enrichment of differentially expressed miRNA target genes in TRD that are involved in axonogenesis, regulation of postsynaptic density and</li> </ul>	<ul style="list-style-type: none"> <li>Small sample size</li> </ul>

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Table 1 (continued)

Study	Sample	Tissue type	Omics technique	Definition of treatment resistance	Main reported findings	Limitations
Israel-Elgali et al. (2021) Israel	47 TRD patients who received antidepressant drug treatment alone (i.e. TAU) (n = 16) or in combination with either unilateral ECT (n = 17) or ketamine (intravenous 0.2 mg/kg or intranasal 50 mg) (n = 14) twice weekly for 3 weeks  23 healthy controls	PBMCs	<b>mRNA and RNA sequencing as well as miRNA profiling</b> – MacroGen Inc. poly-A mRNA sequencing and Illumina True-Seq platform (n = 35) as well as multiplexed NanoString nCounter miRNA expression assay (n = 21)  Validation using qPCR	N/A	neuron differentiation, and post-transcriptional change  <ul style="list-style-type: none"> <li>KEGG analysis → enrichment of differentially expressed miRNA target genes in TRD that are involved in PI3K-Akt, Ras, MAPK, calcium and chemokine signalling pathways, complement and coagulation cascades and cytokine-cytokine receptor interaction</li> <li>Identified 14 putative target genes of differentially expressed miRNAs → including <i>P2RX7</i>, <i>CREB1</i> and <i>HTR1A</i> that are involved in MDD, synaptic synthesis and transport of neurotransmitters, and binding process with corresponding receptors</li> </ul> <b>TRD vs controls</b>  <ul style="list-style-type: none"> <li>Top 13 genes with adjusted p-value &lt;0.005 that showed differential expression between TRD patients and controls → <i>MGAM</i>, <i>SRSF5</i>, <i>PTGS1</i>, <i>ANKRD9</i>, <i>PCSK6</i>, <i>PEAR1</i>, <i>B2M</i>, <i>ARHGAP10</i>, <i>EGFL8</i>, <i>FKBP5</i>, <i>ITGA2B</i>, <i>SNN</i>, <i>NDST2</i></li> <li><i>B2M</i>, <i>FKBP5</i> and <i>ITGA2B</i> were selected for qPCR validation → finding of significant upregulation of <i>B2M</i> was not replicated, but significantly elevated expression of <i>FKBP5</i> and <i>ITGA2B</i> in TRD patients vs controls was replicated</li> </ul> <b>TRD treatment groups vs controls</b>  <ul style="list-style-type: none"> <li>Significantly higher <i>FKBP5</i> expression levels in ECT and ketamine groups compared to controls</li> <li>Significant correlation between <i>FKBP5</i> expression and serum cortisol for post-treatment samples of ECT responders and pre-treatment samples of TAU responders</li> </ul> <b>miRNA profiling</b>  <ul style="list-style-type: none"> <li>No statistically significant changes in top 10 miRNA expression levels (with pre-adjusted p &lt; 0.01) between pre- and post-ECT samples after adjustment of p-values</li> <li>miR-24-3p levels were not significantly changed between pre- and post-</li> </ul>	<ul style="list-style-type: none"> <li>Small sample size</li> <li>Did not provide a definition for treatment resistance</li> </ul>

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Table 1 (continued)

Study	Sample	Tissue type	Omics technique	Definition of treatment resistance	Main reported findings	Limitations
Cole et al. (2021) UK	94 MDD treatment-resistant, 47 MDD treatment-responsive and 46 MDD untreated patients  44 healthy controls	PBMCs	<b>RNA sequencing</b> – Illumina TruSeq stranded mRNA-seq library preparation kit and Illumina HiSeq 4000	Total HAMD score > 13 and had received a therapeutic dose of a monoaminergic drug for ≥6 weeks	treatment samples in responders and non-responders, but miR-24-3p was upregulated in ECT responders and down-regulated in ECT non-responders post-treatment <ul style="list-style-type: none"> <li>miR-24-3p regulates <i>ITGA2B</i> expression levels</li> <li>No significant differential gene expression signature between healthy controls and MDD treatment-resistant patients or other subgroups (significance threshold was adjusted <math>p &lt; 0.01</math>)</li> </ul>	<ul style="list-style-type: none"> <li>Heterogeneity typically seen in MDD</li> <li>Some heterogeneity due to retrospective self-reporting of prior medication use and potential recall bias</li> <li>Inclusion of patients with a lack of medical comorbidities could have reduced the representativeness of the MDD sample</li> <li>Small sample size</li> <li>Did not include a healthy control group receiving ketamine and so potential physiological fluctuations in transcriptional profiles over time were not controlled for</li> </ul>
Cathomas et al. (2022) USA	26 TRD patients who received a single 0.5 mg/kg ketamine infusion over 40 min  21 healthy controls	Blood	<b>Whole blood transcriptional signatures (RNA-seq)</b> – Illumina TruSeq Stranded mRNA library preparation kit and Illumina HiSeq machine  WGCNA - to identify clusters or 'modules' of intercorrelated genes associated with clinical features and biological pathways  IPA & GO analysis – David Bioinformatics Resource 6.8	Lifetime history of non-response to ≥2 antidepressant trials according to ATHF (Sackeim, 2001) and on average did not respond to 4.9 adequate trials	<b>Differential gene expression TRD vs healthy controls at baseline (pre-treatment)</b>  <ul style="list-style-type: none"> <li>560 differently expressed genes → 262 up- and 298 downregulated genes</li> <li>Biological pathway most significantly enriched in differentially expressed genes between TRD vs controls → type I interferon signalling &amp; was activated in TRD vs controls according to IPA</li> <li>WGCNA → one module significantly correlated with disease recurrence (as a clinical descriptor of illness course) and genes of the module were enriched for the interferon signalling pathway</li> </ul> <b>Ketamine responders vs non-responders at baseline</b>  <ul style="list-style-type: none"> <li>331 differentially expressed genes → 166 up- and 165 downregulated genes in responders compared to non-responders</li> <li>Pathways activated and significantly enriched in differentially expressed genes between responders vs non-responders → cAMP-mediate signalling and neuropathic pain signalling in dorsal horn neurons</li> <li><i>GRM2</i> and <i>GRIN2D</i> genes of abovementioned pathways (involved in glutamate signalling) were enriched in responders vs non-responders</li> </ul> <b>Transcriptional signatures associated with clinical improvement (24 h post-</b>	<ul style="list-style-type: none"> <li>Some changes in responders groups may be due to placebo effect</li> <li>No cell type-specific information on transcriptional changes, i.e. regarding cell types involved in mentioned biological processes and smaller biologically relevant changes in immune cells</li> <li>Sample was not very ethnically diverse which limits generalisability and representativeness</li> </ul>

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Table 1 (continued)

Study	Sample	Tissue type	Omics technique	Definition of treatment resistance	Main reported findings	Limitations
					<b>infusion)</b>	
					<ul style="list-style-type: none"> <li>• 464 significant differentially expressed genes → 233 genes showed downregulation of gene expression with clinical improvements and 231 genes showed upregulation of gene expression with clinical improvement</li> <li>• IPA → cAMP signalling and osteoarthritis pathways were associated with treatment response</li> <li>• No association between treatment response and anti-inflammatory gene expression signature or an effect on interferon pathway</li> </ul>	
Bekhbat et al. (2021) USA	Microarray results were confirmed and extended in 57 TRD patients (diagnosed with MDD or BD-current episode depressed) before and after anti-inflammatory challenge with infliximab (5 mg/kg) vs placebo infusions at baseline, week 2 and week 6, who had high or low baseline inflammation (i.e. plasma CRP > 5 vs ≤ 5 mg/L)	Whole (peripheral) blood	<p><b>Transcriptional signatures (microarray)</b> – Illumina Human HT-12 Expression BeadChips and Illumina HiScan</p> <p>Functional pathways examined using WikiPathways and KEGG databases</p> <p>WGCNA – to identify clusters or ‘modules’ of intercorrelated genes associated with psychomotor slowing in patients</p>	Non-response to antidepressant treatment by scoring ≥2 on the MGH-S method (Fava, 2003) in the current episode	<ul style="list-style-type: none"> <li>• Confirmatory analysis in TRD showed that genes associated with psychomotor slowing were nominally enriched for pathways related to inflammation and glucose metabolism (all <math>p &lt; 0.05</math>)</li> <li>• TRD patients with high inflammation → infliximab treatment correlated with reduced expression of a gene module that is significantly enriched for pathways related to oxidative stress and mitochondrial degradation (<math>p &lt; 0.05</math> and <math>q &lt; 0.1</math>) and nominally enriched for pathways related to immune function and cancer metabolism (<math>p &lt; 0.05</math>) at Week 2, and this was associated with faster psychomotor reaction times at Week 8. While, infliximab treatment correlated with increased expression of another gene module that is nominally enriched for immune-related pathways (<math>p &lt; 0.05</math>) at Week 2 and this was associated with improvements in psychomotor speed at Week 8</li> <li>• TRD patients with low inflammation → infliximab treatment correlated with increased expression of a gene module that is significantly enriched for pathways related to immune and mitochondrial function (<math>p &lt; 0.05</math> and <math>q &lt; 0.1</math>) at Week 2, and this was associated with smaller improvement in psychomotor speed at Week 8</li> </ul>	<ul style="list-style-type: none"> <li>• Heterogeneity of sample due to the inclusion of BD subjects – results not specific to MDD, and may result in confounding and spurious associations.</li> </ul>
Vadodaria et al.	MDD patients who received 20 mg	Skin biopsies to generate	<b>Whole transcriptome analysis (RNA-seq)</b> –	Extreme SSRI-non-remitter and SSRI-	<ul style="list-style-type: none"> <li>• Protocadherin <i>PCDHA6</i> and <i>PCDHA8</i> were the 2</li> </ul>	<ul style="list-style-type: none"> <li>• Small sample size due to use of the in vitro</li> </ul>

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Table 1 (continued)

Study	Sample	Tissue type	Omics technique	Definition of treatment resistance	Main reported findings	Limitations
(2019a) USA	citalopram or 10 mg escitalopram for 8 weeks  Skin biopsies were performed on extreme SSRI-remitters ( $n = 3$ ) and SSRI-non-remitters/SSRI-resistant subjects ( $n = 3$ ) that were selected from the whole cohort, as well as on neurotypical healthy controls ( $n = 3$ )	iPSCs and serotonergic neurons	Illumina TruSeq Stranded mRNA Sample Prep Kit and Illumina HiSeq 2500 platform	resistant terms were used interchangeably  Non-remitters were defined as demonstrating non-significant improvement in QIDS and HAMD scores after 8 weeks of SSRI treatment	most differentially expressed genes out of 5 significant differentially regulated genes that overlapped between the control vs non-remitter/resistant (25 genes) and non-remitter/resistant vs remitter (7 genes) groups <ul style="list-style-type: none"> <li>• <i>PCDHA6/A8</i> was significantly lower in non-remitters/resistant subjects compared to remitters and controls</li> <li>• Longer neurite length observed in serotonergic neurons derived from non-remitters/resistant subjects compared to remitters and controls</li> <li>• Regulation of neurite length of serotonergic neurons <i>PCDHA6/A8</i> was confirmed in <i>PCDHA6/A8</i> knockdown experiments</li> </ul>	iPSC low-throughout technology <ul style="list-style-type: none"> <li>• Uncertain whether in vitro observations will be reflected in patients in vivo</li> <li>• Definition for treatment resistance was not clearly stated</li> </ul>
Vadodaria et al. (2019b) USA	MDD patients who received 20 mg citalopram or 10 mg escitalopram for 8 weeks  Skin biopsies were performed on extreme SSRI-remitters ( $n = 3$ ) and SSRI-non-remitters/SSRI-resistant subjects ( $n = 3$ ) that were selected from the whole cohort, as well as on neurotypical healthy controls ( $n = 3$ )	Skin biopsies to generate iPSCs and forebrain neurons	<b>Whole transcriptome analysis (RNA-seq)</b> – Illumina TruSeq Stranded mRNA Sample Prep Kit and Illumina HiSeq 2500 platform	Extreme SSRI-non-remitter and SSRI-resistant terms were used interchangeably  Non-remitters were defined as demonstrating non-significant improvement in QIDS and HAMD scores after 8 weeks of SSRI treatment	<ul style="list-style-type: none"> <li>• 163 genes were found to be significantly differentially regulated between non-remitter/resistant and remitter groups → those with most significant adjusted <math>p</math>-values were <i>FAM19A4</i> (<math>3.5 \times 10^{11}</math>), <i>RSPO2</i> (<math>4.78 \times 10^{-11}</math>), <i>SLC7A8</i> (<math>1.14 \times 10^{-10}</math>)</li> <li>• Non-remitter/resistant forebrain neurons displayed significantly higher (neuronal) activity compared to remitter and control groups following exogenous 5-HT (serotonin) treatment, but no significant differences were observed between remitter and control groups.</li> <li>• <i>HTR7</i> expression significantly differed between non-remitter/resistant and remitter groups (adjusted <math>p = 0.021</math>)</li> <li>• 5-HT7 receptor protein was significantly higher in non-remitter/resistant forebrain neurons than remitter and control groups.</li> <li>• Pre-treatment with FDA-approved 5-HT7 receptor antagonist, Lurasidone, significantly reduced 5-HT-induced hyperactivity in non-remitter/resistant neurons. This was also observed for SB-269970, another 5-HT7 receptor antagonist.</li> </ul>	<ul style="list-style-type: none"> <li>• Small sample size due to use of the in vitro iPSC low-throughout technology</li> <li>• Uncertain whether in vitro observations will be reflected in patients in vivo</li> <li>• Definition for treatment resistance was not clearly stated</li> </ul>
Pettai et al. (2016) Estonia	MDD patients treated with 10–20 mg/day of escitalopram for 12 weeks  10 mg for first 4 weeks, then dosage	Whole blood	<b>Whole genome expression profiling (microarray)</b> – Illumina Human-6 v2 and HumanHT-12 v3 BeadChips ( $n = 87$ )	20 mg non-responders were referred to as being resistant to escitalopram  These non-responders were defined as patients who didn't show $\geq 50\%$	<ul style="list-style-type: none"> <li>• No significant difference in gene expression at baseline and week 12 when comparing 20 mg responders and 20 mg non-responders</li> </ul>	<ul style="list-style-type: none"> <li>• Relatively small sample size</li> <li>• Did not conduct validation e.g. using PCR</li> </ul>

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Table 1 (continued)

Study	Sample	Tissue type	Omics technique	Definition of treatment resistance	Main reported findings	Limitations
	increase to 20 mg for patients showing <50 % reduction in MADRS total score at week 4 or worsening of depressive symptoms, resulting in 10 mg responder ( <i>n</i> = 28), 20 mg responder ( <i>n</i> = 23) and 20 mg non-responder/resistant ( <i>n</i> = 36) groups		GO, KEGG, BIOCARTA pathway analysis – DAVID	reduction in both MADRS and HAMD total scores and didn't score ≤ 2 on the CGI improvement scale	<p><b>End of treatment – week 12</b></p> <ul style="list-style-type: none"> <li>Differential expression of 3 genes between all responders (10 and 20 mg) and 20 mg non-responders → decreased <i>FKBP1A</i> expression (increased in responders) as well as increased <i>NR2C2</i> and <i>ZNF641</i> expression in non-responders</li> <li>Differentially expressed genes between 10 mg responders and 20 mg non-responders are involved in cellular component organisation or biogenesis, regulation of metabolic processes, cell motility, chromosome/chromatin organisation</li> </ul> <p><b>Predictive gene expression profile – baseline</b></p> <ul style="list-style-type: none"> <li>7 differentially expressed genes between all responders and 20 mg non-responders – no enriched clusters observed</li> <li>40 differentially expressed genes between 10 mg responders and 20 mg non-responders → most interesting being <i>NLGN2</i> that had higher expression in 10 mg responders compared to 20 mg non-responders, but no enriched clusters observed</li> </ul> <p><b>Predictive gene expression profile – week 4</b></p> <ul style="list-style-type: none"> <li>74 differentially expressed genes between 20 mg responders and 20 mg non-responders are involved in cell motility, immune response, signal transduction, nervous system and neurotrophin pathway (<i>PLCG1</i>, <i>CDC42</i>, <i>MAPK14</i>)</li> <li>186 differentially expressed genes between 10 mg responders and 20 mg non-responders are involved in nucleotide binding, phosphatase activity, ribosomal biogenesis and protein phosphorylation</li> </ul>	<ul style="list-style-type: none"> <li>Definition for treatment resistance was not clearly stated</li> </ul>
Barakat et al. (2020) Germany	Identification of candidate genes using whole transcriptome analysis in SSRI-treated patients from MARS exploratory sample ( <i>n</i> = 17). Validation of these genes in TRD patients ( <i>n</i> = 20) and first-level responders ( <i>n</i> = 24) from the STAR*D sample	Patient-derived LCLs (that were incubated with citalopram for 24 and 48 h)	<p><b>Whole transcriptome/genome-wide expression analysis –</b> Agilent Single Color platform of 8 × 60 K microarrays (in MARS sample)</p> <p>Validation – qPCR (in STAR*D sample)</p>	STAR*D level-4-non-responders – lack of satisfactory therapeutic response to psychotropic drug or psychotherapy switching or augmentation strategies of previous levels and level 4 (Rush et al., 2004)	<ul style="list-style-type: none"> <li>8 identified candidate genes from the whole-transcriptome analysis in the MARS exploratory sample were selected for qPCR validation → <i>GAD1</i>, <i>FYB</i>, <i>RAMP1</i>, <i>TBC1D9</i>, <i>PITX1</i>, <i>NFIB</i>, <i>GRIN2A</i>, and <i>AADAT</i></li> <li>Multivariate analysis revealed marginal association between treatment resistance and <i>NFIB</i> (<i>p</i> = 0.068)</li> </ul>	<ul style="list-style-type: none"> <li>Uncertain whether in vitro observations will be reflected in patients in vivo</li> <li>Small sample size of TRD cohort</li> <li>Heterogeneity between MARS and STAR*D samples</li> </ul>

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Table 1 (continued)

Study	Sample	Tissue type	Omics technique	Definition of treatment resistance	Main reported findings	Limitations
Fabrizi et al. (2021c) UK	TRD (n = 2165) vs healthy controls (n = 11,188)	N/A	TWAS – imputed gene expression profiles by integrating genome-wide genotyping data and gene expression data to identify associations between imputed gene expression changes and TRD using FUSION software and compare imputed TRD-associated gene expression profiles with drug-induced gene expression profiles to identify compounds with opposite transcriptomic changes during drug repurposing analysis using the Connectivity Map (Cmap) Query tool	Defined using primary care records in UK Biobank as MDD patients who had ≥2 switches between different antidepressant drugs (independent from class) that were each prescribed for ≥6 consecutive weeks and the time interval between prescription of 2 consecutive drugs was ≤14 weeks	<ul style="list-style-type: none"> <li>No association between treatment resistance and <i>GAD1</i> (<math>p = 0.27</math>) and <i>TBC1D9</i> (<math>p = 0.23</math>) expression (which were significantly associated with response status, remission status and improvement in depression scale in MARS sample)</li> <li>No transcriptome-wide significant (<math>p = 1.37 \times 10^{-6}</math>) signals were identified for TRD</li> <li>Drug repurposing analysis in TRD identified 76 compounds whose mechanism of action mainly involved modulation of cell survival-proliferation-differentiation (13 %) and monoaminergic neurotransmission (7 %)</li> <li>The compounds with a significant permuted <math>p</math>-value <math>&lt; 0.05</math> were zamifenacin, a muscarinic M3 and M5 receptor antagonist, and two molecules with unknown activity. Dantrolene, a calcium channel blocker, was closest to the significance threshold</li> </ul>	<ul style="list-style-type: none"> <li>Relatively small sample size</li> <li>Drug-induced gene expression profiles determined in vitro and under heterogeneous experimental conditions thus uncertain whether this reflects in vivo gene expression changes</li> </ul>
Ruland et al. (2016) Germany	65 MDD patients who were staged for treatment resistance	Serum	Protein profiling – non-hypothesis-driven label-free LC-MS approach in addition to targeted approaches  GO term analysis	TRM (Thase and Rush, 1997) <ul style="list-style-type: none"> <li>Stage I – failing ≥1 adequate trial of ≥4 weeks at moderate dose of a major antidepressant class</li> <li>Stage II – failing ≥2 adequate trials of ≥2 different antidepressant classes</li> <li>Stage III – stage II and failing an adequate TCA trial</li> <li>Stage IV – stage III and failing an adequate MAOI trial</li> <li>Stage V – Stage IV and a bilateral ECT course</li> </ul> MSM (Fekadu et al., 2009b) <ul style="list-style-type: none"> <li>Staging was based on scores corresponding to mild (3–6), moderate (7–10) and severe (11–15) treatment resistance</li> <li>The scores were determined based on information about duration, symptom severity and use of antidepressants, augmentation and ECT</li> </ul>	<ul style="list-style-type: none"> <li>8 proteins that were significantly (<math>p &lt; 0.05</math>) different between TRM stage I and stage II groups → serum amyloid P-component, ficolin-3, C4b-binding protein beta and alpha chain, complement C1q subcomponent subunit C, histidine-rich glycoprotein, nuclear factor of activated T-cells and beta-ala-his dipeptidase</li> <li>10 proteins that were significantly (<math>p &lt; 0.05</math>) changed between MSM stage I and stage II groups → heparin cofactor 2, plasma serine protease inhibitor, anti-thrombin-III, interleukin-1 receptor accessory protein, complement factor D, haemoglobin subunit alpha and beta, putative post-meiotic segregation increased 2-like protein 11, calcium-binding protein 5 and cytosolic beta-glucosidase</li> <li>Common biological processes that the identified proteins are involved in → blood coagulation, complement activation and immune response.</li> </ul>	<ul style="list-style-type: none"> <li>Small sample size</li> <li>Sample not representative of all TRD stages (i.e. higher stages like failure of ≥5 trials) since participants were not specifically recruited to assess all stages</li> <li>Did not include a responder control group</li> <li>Only investigated 2 staging models</li> </ul>
	12 MDD patients resistant to	Serum		N/A	<ul style="list-style-type: none"> <li>Significantly (<math>p \leq 0.05</math>) changed levels of 12</li> </ul>	<ul style="list-style-type: none"> <li>Small sample size</li> </ul>

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Table 1 (continued)

Study	Sample	Tissue type	Omics technique	Definition of treatment resistance	Main reported findings	Limitations
Stelzhammer et al. (2013) Netherlands	antidepressants who received ECT for 4 weeks and at weeks 3 and 4 patients received a combination of ECT and antidepressants  Acute (6 h) and chronic (6 treatments/2 weeks) ECT ± antidepressant treatment		<b>Molecular profiling</b> – LC-MS (n = 10) and a targeted approach		<p>proteins after 1st acute treatment with ECT</p> <ul style="list-style-type: none"> <li>o Increased levels → neuromedin-U receptor 1, anti-thrombin-III, WD repeat-containing protein 17, apolipoprotein C2, UBP-7, ZSWM-4, extracellular matrix protein 1, apolipoprotein C3, apolipoprotein E, myosin-11 and histidine-rich glycoprotein</li> <li>o Decreased levels → platelet factor 4</li> </ul> <ul style="list-style-type: none"> <li>• Significant relationship between acute changes in platelet factor 4 and symptom improvement (p = 0.0241)</li> <li>• Significantly altered levels of 4 proteins after chronic ECT → increased levels of AACT, and decreased levels of apolipoprotein A2, serotransferrin and clusterin</li> <li>• Significantly altered levels of 6 proteins after chronic ECT and antidepressant combination treatment → increased levels of BIG-1 and CO7, and decreased levels of RhoG, C1QA, C1QC and RGAG1</li> <li>• LC-MS findings for apolipoproteins C2, A2 and E were validated</li> </ul>	<ul style="list-style-type: none"> <li>• Did not provide a definition for treatment resistance</li> </ul>
Ryan et al. (2017) Ireland	30 patients with an MDE who received ECT twice weekly with hand-held electrodes and were classified as remitters (i.e. 60 % decrease in baseline HAMD score and an end-of-treatment HAMD score ≤ 10 for two consecutive weeks)  Pre- and post-ECT samples were compared to identify proteome changes	Plasma	<b>Discovery-phase proteomics</b> – 2D-DIGE and mass spectrometry  GO analysis - DAVID	ECT-treated MDD is considered TRD in real clinical practice	<ul style="list-style-type: none"> <li>• 4 proteins identified in high-abundance gel spots and 32 proteins in low abundance gel spots that were significantly altered following ECT (p ≤ 0.001)</li> <li>• High abundance proteins → serotransferrin, serum albumin, Ig mu chain C region, Apolipoprotein A-I</li> <li>• Low abundance proteins → Complement C1r subcomponent, myosin light chain 3, tropomyosin alpha-1 chain, PEDF, complement factor I, serum amyloid P-component etc.</li> <li>• Common GO terms for low-abundance proteins → extracellular region/space, contractile fiber, actin cytoskeleton, actin binding, cytoskeletal protein binding, acute inflammatory response, complement activation, activation of immune response, complement and coagulation cascades, muscle tissue morphogenesis (p ≤ 0.001)</li> <li>• Common GO terms for high-abundance proteins → extracellular region/space, antigen binding and immune response (p ≤ 0.001)</li> </ul>	<ul style="list-style-type: none"> <li>• Findings may not be specific to ECT, since patients were also receiving pharmacological TAU during ECT.</li> <li>• Time point of plasma sample collection post-ECT ranged from 1 to 3 days which may have impacted the results</li> </ul>

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Table 1 (continued)

Study	Sample	Tissue type	Omics technique	Definition of treatment resistance	Main reported findings	Limitations
Singh et al. (2022) USA	9 TRD patients who received a single 40 min intravenous infusion of 0.5 mg/kg of ketamine	Plasma	<b>Metabolomic profiling</b> – LC-MS/MS (comprehensive non-targeted metabolomics platform) and a targeted approach	Failure of ≥2 previous antidepressant treatments within the current depressive episode  Failed antidepressant treatments can include: <ul style="list-style-type: none"> <li>o Antidepressant pharmacotherapy at adequate dose and ≥ 8 weeks</li> <li>o Acute series of ≥6 administrations of ECT</li> <li>o Acute series of TMS</li> </ul>	<ul style="list-style-type: none"> <li>• Validation → PEDF concentrations increased following ECT (using immunoassay; <math>p = 0.003</math>), PEDF levels were significantly higher in MDD patients compared with healthy controls (<math>p &lt; 0.05</math>), PEDF mRNA levels significantly increased in the hippocampus (<math>p = 0.02</math>) and dentate gyrus (<math>p = 0.03</math>) of rats treated with chronic ECS</li> </ul> <p><b>Early changes (baseline to end of infusion/40 min) in peripheral metabolites</b></p> <ul style="list-style-type: none"> <li>• Significant increase in 20 metabolites within 40 min → including 10 ceramides, AABA, putrescine, fatty acid, cortisol, 2 long chain acylcarnitines, 2 glycosylceramides, cholesterol ester, glycerophospholipid</li> <li>• Significant decrease in → acylcarnitines, 93 triacylglycerols, trigonelline, 5 amino acids or related, Ind-SO<sub>4</sub>, Choline, 2 cholesterol esters, glycerophospholipids</li> </ul> <p><b>Late changes (100 min to 24 h post-infusion) in peripheral metabolites</b></p> <ul style="list-style-type: none"> <li>• Significant increase in → indole-3-acetate, indole-3-propionate, GABA, kynurenine, 9 acylcarnitines, 15 amino acids (including glutamine), 2 biogenic amines, 1 bile acid 27 sphingolipids/glycerophospholipids, 77 triacylglycerols, 25 ceramides/glycosylceramides and 12 cholesterol esters</li> <li>• Kynurenine, choline, acylcarnitines and ceramides significantly correlated with percentage change in MADRS following ketamine infusion</li> <li>• No significant association between baseline metabolite level and ketamine and esketamine response</li> <li>• 52 metabolites were significantly altered after ketamine treatment</li> <li>o 31 known metabolites → including indole-3-acetate (decreased), 3-hydroxybutyric acid, arachidonic</li> </ul>	<ul style="list-style-type: none"> <li>• Small sample size</li> <li>• Lack of control group</li> <li>• Potential confounding – all the analyses were non-adjusted for covariates</li> <li>• Findings may not be specific to ketamine, since patients received prescribed medications</li> </ul>
Rotroff et al. (2016) USA	Treatment-refractory MDD patients who received intravenous 0.20 or 0.40 mg/kg <sup>-1</sup> esketamine ( $n = 20$ ) or 0.5 mg/kg <sup>-1</sup> ketamine ( $n = 33$ ) or saline placebo ( $n = 22$ )	Plasma	<b>Metabolite profiling</b> – GC-TOF (untargeted approach) and a targeted approach	MGH-ATRQ (Fava, 2003) was used to confirm that patients had an inadequate response to ≥1 antidepressant in their current MDE and to ≥1 other antidepressant in their current or previous MDE	<ul style="list-style-type: none"> <li>• Kynurenine, choline, acylcarnitines and ceramides significantly correlated with percentage change in MADRS following ketamine infusion</li> <li>• No significant association between baseline metabolite level and ketamine and esketamine response</li> <li>• 52 metabolites were significantly altered after ketamine treatment</li> <li>o 31 known metabolites → including indole-3-acetate (decreased), 3-hydroxybutyric acid, arachidonic</li> </ul>	<ul style="list-style-type: none"> <li>• Small sample size – low power and high risk of false negatives</li> </ul>

(continued on next page)

Table 1 (continued)

Study	Sample	Tissue type	Omics technique	Definition of treatment resistance	Main reported findings	Limitations
					acid, lactic acid, methionine, mannose, fructose, gluconic acid, glyceric acid, isothreonic acid and glutamic acid (increased) <ul style="list-style-type: none"> <li>o 21 unknown metabolites → including unknown metabolite-18,225</li> <li>• Significant reduction of unknown metabolite-18,225 with esketamine treatment, but was significantly increased following ketamine exposure</li> <li>• Indole-3-lactate and indole-3-acetate (tryptophan metabolites) were also significantly reduced with esketamine treatment</li> </ul>	

**Abbreviations:** N/A, not available or not applicable; PBMCs, peripheral blood mononuclear cells; SSRIs, selective serotonin reuptake inhibitors; iPSCs, induced pluripotent stem cells; LCLs, lymphoblastoid cell lines; GWAS, genome-wide association study; SNP, single nucleotide polymorphism; TRD, treatment-resistant depression; NTRD, non-treatment-resistant depression; AESES, antidepressant efficacy and side effects survey; AES, antidepressant efficacy survey; GSRD, European Group for the Study of Resistant Depression; STAR\*D, sequenced treatment alternatives to relieve depression; GENDEP, genome-based therapeutic drugs for depression; LD, linkage-disequilibrium; GS:SFHS, Generation Scotland: Scottish Family Health Study; MDE, major depressive episode; MDD, major depressive disorder; BD, bipolar disorder; PRS, polygenic risk score; SNRI, serotonin-norepinephrine reuptake inhibitor; ECT, electroconvulsive therapy; EWAS, exome-wide association study; CNV, copy number variants; CSF, cerebrospinal fluid; BH4, clinically low CSF tetrahydrobiopterin; CFD, clinically low CSF 5-methyltetrahydrofolate; AAP, abnormal acylcarnitine profile in peripheral blood; 5-HIAA, deficient CSF levels of 5-hydroxyindoleacetic acid; HVA, deficient CSF levels of homovanillic acid; 5-MTHF, deficient CSF levels of 5-methyltetrahydrofolate; CBT, cognitive behavioural therapy; MADRS, Montgomery-Asberg Depression Rating Scale; MARS, Munich Anti-depressant Response Signatures; LC-MS, liquid chromatography mass spectrometry; LC-MS/MS, liquid chromatography with tandem mass spectrometry; GC-TOF, gas chromatography-time-of-flight mass spectrometry; MGH-ATRQ, Massachusetts General Hospital-Antidepressant Treatment Response Questionnaire; TRM, Thase and Rush staging model; MSM, Maudsley Staging Model; TCA, tricyclic antidepressants; MAOI, monoamine oxidase inhibitors; QIDS-SR, Quick Inventory of Depressive Symptomatology-Self-Report; QIDS, Quick Inventory of Depressive Symptomatology; HAMD, Hamilton Depression Rating Scale; CGI, Clinical Global Impression scale; GO, gene ontology; KEGG, Kyoto encyclopedia of genes and genomes; TWAS, transcriptome-wide association study; qPCR, real time quantitative polymerase chain reaction; CRP, C-reactive protein; WGCNA, weighted gene co-expression network analysis; MGH-S, Massachusetts General Hospital Staging; GABA, gamma-aminobutyric acid; lncRNA, long non-coding RNAs; TAU, treatment as usual; 2D-DIGE, Two-Dimensional Difference Gel Electrophoresis; PEDF, pigment epithelium-derived factor; ECS, electroconvulsive stimulation; TMS, transcranial magnetic stimulation; EXCEED, Extended Cohort for E-health, Environment and DNA; PREFECT, Predictors For ECT; FDR, false discovery rate; ATHF, Antidepressant Treatment History Form; IPA, Ingenuity Pathway Analysis.

\*Genome-wide significance threshold for SNPs →  $p < 5 \times 10^{-8}$ .

2019; Gururajan et al., 2016; Israel-Elgali et al., 2021; Li et al., 2021; Li et al., 2020b; Maffioletti et al., 2020; McClain et al., 2020a; McClain et al., 2020b; Moschny et al., 2020; O'Dushlaine et al., 2014; Pettai et al., 2016; Rotroff et al., 2016; Ruland et al., 2016; Ryan et al., 2017; Singh et al., 2022; Stelzhammer et al., 2013; Wigmore et al., 2020), which included whole blood, peripheral blood mononuclear cells (PBMCs) ( $n = 3$ ), serum ( $n = 2$ ) and plasma ( $n = 4$ ) samples, in addition to one study obtaining patient-derived lymphoblastoid cell lines (LCLs) (Barakat et al., 2020). Two studies obtained skin biopsies to generate induced pluripotent stem cells (iPSCs) (Vadodaria et al., 2019a; Vadodaria et al., 2019b); 2 studies collected saliva samples (Li et al., 2016; Li et al., 2020a); and 10 did not specify the tissue type (Chen et al., 2021; Fabbri et al., 2018; Fabbri et al., 2021a; Fabbri et al., 2019b, 2021b; Fabbri et al., 2021c; Fanelli et al., 2021; Guo et al., 2018; Pisanu et al., 2021; Souza-Silva et al., 2020).

### 3. Results

Findings are discussed below, divided in sections according to the identified pathways and the techniques used. All studies are described in Table 1.

#### 3.1. Immune system, inflammation and HPA axis

Across all studies, the most replicated findings were linked with the immune system, inflammation and the HPA axis (see also Table 1).

##### 3.1.1. GWAS

The *LTB* gene, which induces the inflammatory response system, passed genome-wide significance during gene-based analysis (Li et al., 2020a) and the innate immune response pathway was closest to the significance threshold for association with stages of resistance (Wigmore et al., 2020). In patients treated with esketamine augmentation, there was a genome-wide significant association between percentage change in depression, severity score and exonic SNP rs11465988 located in the *IRAK3* gene, that is involved in Toll-like receptor signalling in the innate immune system, with genes enriched in immune signalling being nominally associated with esketamine remission status (Li et al., 2020b).

Studies also implicated the stress response system/HPA axis. For example, genes enriched for pathways related to glucocorticoid receptor/stress response have been reported to be nominally associated with percentage change in depression severity score as well as remission and responder status in TRD patients who received esketamine augmentation (Li et al., 2020b). Furthermore, the SNP rs188352979, that spans *ACADSB-HMX3*, was close to the significance threshold in a meta-analysis for the association with treatment resistance (Wigmore et al., 2020); *HMX3* is a transcription factor that is implicated in the HPA axis (Wang et al., 2004), while *ACADSB* is involved in fatty acid metabolism (Rozen et al., 1994). The fatty acid metabolism pathway, which has been linked to inflammation (Borsini et al., 2021), was reported to be an enriched gene-set for the non-TRD vs TRD phenotype (Li et al., 2016).

##### 3.1.2. Microarray/sequencing

Whole genome mRNA expression profiling and functional annotation

clustering revealed 74 differentially expressed genes in clusters that included pathways involved in immune response when comparing responders and resistant individuals to 20 mg/day escitalopram after 4 weeks of treatment, while at week 12 only the expression of *NR2C2*, *ZNF641* and *FKBP1A* was altered between resistant patients and responders (to both 10 mg and 20 mg/day) (Pettai et al., 2016); *FKBP1A* is relevant to immunoregulation (Kang et al., 2008). Similarly, 163 genes were found to be significantly differentially regulated between resistant patients compared to citalopram/escitalopram remitters, with *FAM19A4*, which is involved in macrophage chemotaxis and phagocytosis (Wang et al., 2015), having the most significant adjusted *p*-value (Vadodaria et al., 2019b). RNA sequencing and bioinformatic analysis revealed the type I interferon signalling pathway to be the most significantly enriched in differentially expressed genes between TRD patients vs healthy controls at baseline (Cathomas et al., 2022). Furthermore, genes targeted by miRNAs that were significantly differentially expressed between TRD and controls, including has-miR-335 and has-miR-1292, were enriched for pathways relevant to complement cascade and immune function (Li et al., 2021); miRNAs are small non-coding RNAs that regulate the expression of the genes they target.

Interestingly, Bekkhat et al. (2021) found that immune and metabolic pathways were implicated in psychomotor slowing in TRD patients. In particular, they observed that treatment with the anti-inflammatory medication infliximab was linked to changes in the expression of genes enriched for pathways including immune function, and that in turn these changes were associated with psychomotor speed improvements in patients with high inflammation (but smaller improvements in patients with low inflammation). However, Cole et al. (2021) did not find any differences in gene expression in PBMCs of TRD patients compared with healthy controls, and they postulate that the immunobiological markers in their sample of TRD patients do not derive from PBMCs.

Barakat et al. (2020) found *NFIB* to be the only gene that approached the significance threshold for association with treatment resistance after multivariate analysis during quantitative polymerase chain reaction (qPCR) of TRD patient-derived LCLs out of 8 candidate genes identified through whole transcriptome microarray analysis. This gene is implicated in HPA axis function, and its expression in rats is reversed by antidepressant treatment after being altered by chronic mild stress (Orsetti et al., 2009). Israel-Elgali et al. (2021) reported *FKBP5*, which is involved in immune and HPA axis regulation, to be one of the top 13 genes identified as showing significantly elevated expression in TRD individuals compared to controls; and more specifically in TRD patients that received combined antidepressant therapy with unilateral ECT or intravenous/intranasal ketamine. Moreover, the authors found that *FKBP5* expression significantly correlated with serum cortisol levels for TRD patients that responded to ECT and antidepressant treatment alone.

### 3.1.3. Other omics techniques

Cortisol was observed to be significantly increased within 40 min of ketamine treatment in TRD patients through metabolomic profiling (Singh et al., 2022). Moschny et al. (2020) reported differential methylation between ECT responders and non-responders at significant CpG sites located in protein-coding genes like *RNF213*, which is implicated in the immune system. A proteomics study identified several proteins involved in complement activation and immune response that were significantly different between TRD staging groups I and II using the Maudsley Staging Method and the Thase and Rush staging model, which correspond to different degrees of treatment resistance based on the number of failed antidepressant trials and classes or the total score obtained after evaluating various TRD-related factors (Ruland et al., 2016). Fabbri et al. (2021b) conducted a pharmacogenomic study by comparing genes in pathways found to predict TRD risk in a whole exome sequence study (Fabbri et al., 2020) with known drug targets from Drug repurposing Hub, Drug-Gene Interaction database and DrugBank database. The authors identified 542 compounds enriched for

genes in TRD-associated pathways, with a common mechanism of action being inflammation/immune response modulation.

## 3.2. Neuroplasticity

There is evidence supporting the involvement of molecules related to various neuronal and synaptic functions. Several studies implicated components of the TrkB signalling pathway, involved in different neuronal activities including hippocampal long-term potentiation and synaptic plasticity (Minichiello, 2009) (Table 1).

### 3.2.1. GWAS

Enrichment analysis identified the regulation of MAPKKK cascade gene-set as relevant to TRD (Li et al., 2016) and long-term potentiation, neurotransmitter receptor binding and synaptic transmission were found to be some of the top significant pathways associated with ketamine response in a recent GWAS (Chen et al., 2021). Additionally, suggestive associations were found between ketamine response and SNPs/genes involved in BDNF-TrkB signalling like *NTRK2* and *BDNF* (Chen et al., 2021) as well as rs112647602 located close to *FAM38D*, a gene that is involved in PI3k-Akt and MAPK signalling (Guo et al., 2018).

Genes enriched in the neuronal action potential, synaptic vesicle clustering, and axon guidance pathways, were also associated with esketamine response phenotypes with nominal significance (Li et al., 2020b). In another study, the pathway closest to being associated with TRD after multiple-testing correction was enriched with genes related to neurogenesis, neuroplasticity, long-term potentiation and synaptic transmission (Fabbri et al., 2018), with the last found to be a gene-set that was enriched with variants with suggestive *p*-values in a separate study (Fabbri et al., 2019b). More recently, Souza-Silva et al. (2020) reported that enrichment analysis implicated a synaptic plasticity regulation pathway, which included the genes *APP*, *EXOSC7*, *GRID2*, *ADGRB3*, *COL9A3*, *LY9* and *FOXN3* that were found to be significantly associated with non-response to rTMS, and *SPPL2A* that was significantly associated with rTMS response in TRD patients.

### 3.2.2. Microarray/sequencing

Gururajan et al. (2016) analysed the expression of miRNAs, and reported a significant decrease in let-7c and let-7b miRNA expression following twice weekly brief-pulse bitemporal ECT in TRD patients compared with healthy controls, while at baseline no miRNA was predictive of response to ECT or intravenous ketamine. However, pathway analysis did uncover that genes targeted by these miRNAs, in addition to those found to be differentially expressed by Li et al. (2021), were enriched in the Ras, MAPK and/or PI3k-Akt signalling pathway, whose downstream target, mTOR signalling pathway, has shown to be dysregulated in MDD (Jernigan et al., 2011) and is related to ketamine's antidepressant effects (Li et al., 2010). Moreover, let-7c targets the abovementioned *RNF213* gene (Zhao et al., 2015). Pettai et al. (2016) carried out microarray analysis and found that genes involved in the neurotrophin pathway, such as *PLCG1*, *CDC42* and *MAPLK14*, were differentially expressed between responders and resistant patients to 20 mg escitalopram at week 4.

McClain et al. (2020b) performed whole exome sequencing and compared exonic variant allele frequencies between treatment-refractory MDD patients and healthy controls. Exonic SNPs with potential deleterious effects on the resulting protein, like loss of a termination codon, were described as rare “damaging” variants and were tested for association with biological pathways. This pathway analysis uncovered significant gene-sets including synaptic transmission, neuronal system and neurotransmitter receptor binding that were enriched with these rare “damaging alleles”. Similarly, Li et al. (2021) found that differentially expressed miRNA target genes were enriched in pathways related to regulation of postsynaptic density and axonogenesis. Additionally, they separately identified putative miRNA target genes that were involved in synaptic synthesis, neurotransmitter



transport and receptor binding. Israel-Elgali et al. (2021) observed that one of the top 13 genes showing expression changes in TRD compared with controls was *ITGA2B*, which is involved in maintaining synapses and synaptic plasticity, and whose expression was significantly elevated. The authors also demonstrated that *ITGA2B* is a direct target of miR-24-3p, which was upregulated in TRD patients who responded to ECT and downregulated in those who did not respond to ECT.

### 3.2.3. Other omics techniques

Despite no transcriptome-wide significant results in comparing TRD patients with healthy controls, Fabbri et al. (2021c) conducted drug repurposing analysis by screening compounds from the Connectivity Map database showing an opposite gene expression profile to their top TWAS results for the purpose of identifying molecules that could reverse the pathogenetic changes in TRD. The authors found 76 compounds with mechanisms of action mostly involving modulation of cell survival-proliferation-differentiation, which is the main function of the aforementioned signalling pathways, and monoaminergic neurotransmission, that may potentially restore the expression of genes that are dysregulated in TRD. A comparable study also reported these two mechanisms of action to be the most common for compounds enriched for genes in TRD-associated pathways (Fabbri et al., 2021b).

## 3.3. Calcium signalling

Studies also discovered the potential role of second messenger cascades in TRD, including calcium signalling (Table 1).

### 3.3.1. GWAS

Fabbri et al. (2019b) reported the enrichment of regions comprising suggestive variants in calcium signalling in a meta-analysis of TRD, while gene-level and pathway analysis revealed the regulation of cAMP signalling to be associated with TRD vs response in a separate GWAS. Calcium signalling was also found to be one of the significant pathways associated with ketamine response in TRD patients after gene-set enrichment analysis (Chen et al., 2021). Furthermore, the sarcoplasmic reticulum calcium ion transport pathway was found to be the closest to significance threshold for association with treatment resistance (Wigmore et al., 2020).

### 3.3.2. Microarray/sequencing

Functional enrichment analysis of the genes targeted by differentially expressed exosomal miRNAs between TRD patients and health controls, identified through next-generation sequencing, revealed their involvement in the calcium signalling pathway (Li et al., 2021). McClain et al. (2020a) also reported the presence of the heterozygous 15q13.3 duplication in TRD patients, which encompasses the *CHRNA7* gene and can lead to the downregulation of calcium signalling cascades modulated by  $\alpha 7$ -nAChR (encoded by *CHRNA7*) (Gillentine et al., 2017). The aforementioned *FKBP1A* gene, whose expression was decreased in resistant individuals compared to all escitalopram responders at week 12 (Pettai et al., 2016), also functions in intracellular  $Ca^{2+}$  release (Zalk et al., 2007). The same authors further identified differentially expressed genes involved in signal transduction between resistant patients and 20 mg responders at week 4. Cathomas et al. (2022) reported neuropathic pain signalling and cAMP-mediated signalling as the biological pathways that were activated and significantly enriched in differentially expressed genes between ketamine responders vs non-responders prior to ketamine administration. The latter process along with the osteoarthritis pathway were also associated with clinical improvements 24 h post ketamine infusion.

## 3.4. Neurotransmitters

GABA/glutamate signalling has also been implicated in TRD as well as treatment response (Table 1).

### 3.4.1. GWAS

Li et al. (2016) reported the enrichment of suggestive variants in glutamate metabolism for the TRD vs controls phenotype. In a recent GWAS, the authors compared TRD patients who were administered either ketamine or placebo (Chen et al., 2021). In the association with ketamine response, pathways related to NMDA receptor function and GABA and glutamate nerve terminals survived Bonferroni correction, and the *GRIN2C* gene reached significance threshold at day 3 and 4 post-ketamine infusion (Chen et al., 2021). In addition, the authors found suggestive non-significant associations between ketamine response and SNPs/genes involved in glutamatergic and GABAergic systems (*GRIN2A*, *GRIN3A*, *GRIN2B*, *GRIN2C*) (Chen et al., 2021).

### 3.4.2. Other omics techniques

Pettai et al. (2016) found that the expression of *NLGN2* was higher in low-dose (10 mg) escitalopram responders compared to resistant patients at baseline and noted this to be the most interesting gene that distinguished between these two groups of individuals, since it's required for inhibitory post-synaptic differentiation and its deletion can result in the disruption of GABAergic synaptic transmission (Pouloupoulos et al., 2009). In addition, the *HTR7* gene, encoding the 5-HT7 serotonin receptor, was reported to be significantly differentially regulated between citalopram/escitalopram resistant and remitter individuals after whole transcriptome analysis of forebrain neurons from patient-derived iPSCs (Vadodaria et al., 2019b). Cathomas et al. (2022) observed that genes *GRM2* and *GRIN2D*, involved in glutamate signalling, were enriched in ketamine responders vs non-responders at baseline, before ketamine administration.

Metabolite profiling also revealed significant upregulation of glutamic acid levels (Rotroff et al., 2016), which has been linked to its antidepressant effects (Koike et al., 2011), as well as GABA and glutamate levels (Singh et al., 2022), following ketamine treatment. Furthermore, GABA receptor activation was identified as a significant pathway that was enriched with rare “damaging” variants in TRD patients that underwent whole-exome sequencing (McClain et al., 2020b).

## 3.5. Other pathways

Some evidence has gathered of the involvement of other pathways, whose relevance in TRD is less clear, including the cytoskeleton, blood coagulation and apoptosis/autophagy (Table 1).

### 3.5.1. GWAS

Studies found enrichment of regions comprising suggestive variants in cytoskeleton regulation (Fabbri et al., 2019b) and of duplications in genes related to actin cytoskeleton (O'Dushlaine et al., 2014). Li et al. (2020b) identified a genome-wide significant association between percentage change in depression severity score and *NME7*, which is involved in microtubule-nucleating activity, in TRD patients who received combined esketamine and antidepressant treatment. Furthermore, genes in regions containing variants with *p*-values suggestive of association with TRD showed enrichment in pathways including apoptosis (Fabbri et al., 2019b), and genes enriched in apoptotic signalling were nominally associated with esketamine remission status (Li et al., 2020b). Additionally, Fabbri et al. (2019b) found the chromatin silencing pathway to be associated with TRD compared with other patients (i.e. responders and non-responders) in a meta-analysis. This study, along with another (Fabbri et al., 2020), made a distinction between TRD and non-responders, which was based on the number of antidepressants that patients did not respond to; two or more corresponding to TRD and one corresponding to non-response.

Less validated and more exploratory findings have identified a genome-wide significant region in a meta-analysis with lead SNP rs150245813 (*ZNF37A-LINC00999*) in 10p11.1 (Li et al., 2020a), while O'Dushlaine et al. (2014) found the potential involvement of rare 100–200 kb duplications as well as deletions in *PABPC4L* gene and in the



9p23 region in TRD but with nominal significance. Maffioletti et al. (2020) reported a significant association between SNP rs78355601 A allele, located 170 Kb from the *VEGF* gene, with ECT non-response, particularly the AA vs AG and GG genotypes. Interestingly, Fabbri et al. (2018) utilised SNPs in the top identified pathway to create models that demonstrated good sensitivity but only modest specificity in predicting TRD.

Polygenic risk score (PRS) analyses were also conducted in some studies to investigate the genetic liability of psychiatric conditions to various TRD-related phenotypes. Only the PRS for attention-deficit-hyperactivity disorder (ADHD) was significantly associated with TRD vs non-TRD phenotype (Fabbri et al., 2021a). Antidepressant treatment resistance was reported to be nominally associated with neuroticism-PRS (Fanelli et al., 2021), as well as schizophrenia-PRS and MDD-PRS, with the latter also associated with stages of antidepressant resistance (Wigmore et al., 2020). Guo et al. (2018) found that genetic variants associated with ketamine response explained 6 % of the variance in scopolamine response in TRD. Moreover, Li et al. (2020b) identified suggestive correlations between esketamine response phenotypes and depressive symptoms-PRS; the same study also found that insomnia-PRS displayed a suggestive correlation with esketamine remission status.

### 3.5.2. Microarray/sequencing

McClain et al. (2020b) found enrichment of rare damaging variants in the cytoskeleton structural constituent pathway. Moreover, differentially expressed miRNA target genes were reported to be involved in coagulation cascades (Li et al., 2021).

Also, there is evidence of differential expression of genes involved in cell motility between escitalopram responders and resistant individuals at week 4 and 12 (Pettai et al., 2016).

*PCDHA6* and *PCDHA8* protocadherin alpha genes were found to be the most differentially expressed genes that were significantly down-regulated in resistant patients compared to citalopram/escitalopram remitters and controls (Vadodaria et al., 2019a). Furthermore, Fabbri et al. (2020) more recently found that rare variant pathway-based and rare plus common variant gene-based models significantly predicted TRD vs response, whose predictive performance was improved with the inclusion of clinical variables that was replicated in other samples, although models including only clinical predictors still performed better.

### 3.5.3. Protein/metabolite profiling

Protein profiling revealed significant changes in the levels of blood coagulation proteins anti-thrombin-III and histidine-rich glycoprotein 6 h after ECT, as well as platelet factor 4, which was significantly related to symptom improvement (Stelzhammer et al., 2013). The first two aforementioned proteins, along with others involved in blood coagulation, were also reported to be significantly different between TRD staging groups I and II by Ruland et al. (2016).

Furthermore, tryptophan metabolites indole-3-acetate and indole-3-lactate were found to be significantly altered following treatment with ketamine and/or esketamine in TRD patients, which was also observed for unknown metabolite-18,225 after both treatments (Rotroff et al., 2016). These indole derivatives result from tryptophan metabolism by gut microbiota and are reported to be involved in modulating immune function and neuronal differentiation (Gao et al., 2018; Wong et al., 2020). Furthermore, Singh et al. (2022) identified several metabolites implicated in lipid/energy metabolism and mitochondrial function to be significantly altered within 40 min and 24 h post-ketamine infusion including triacylglycerols, ceramides and sphingolipids/glycerophospholipids. They also reported significant changes in indole-3-acetate as well as other tryptophan metabolites indole-3-propionate and kynurenine following ketamine treatment. However the levels of these metabolites were found to be increased 24 h post-infusion, while in the aforementioned study (Rotroff et al., 2016) indole-3-acetate levels were decreased measured 2 h post-infusion.

### 3.5.4. Other omics techniques

Moschny et al. (2020) identified significant CpG sites located in genes linked to autophagy as well as additional implicated pathways like vascular remodelling and E3 ubiquitin-ligase activity, such as *TBC1D14*, *RNF213* and *RNF175*, that were differentially methylated between ECT responders and non-responders with TRD.

Moreover, zamifenacin, a muscarinic M3 and M5 receptor antagonist, was identified as the top candidate for drug repurposing in TRD (Fabbri et al., 2021c). In addition, among the compounds found to be enriched for genes in TRD-associated pathways, were those of known therapeutic effect in TRD, including tricyclic antidepressants, ketamine and lithium (Fabbri et al., 2021b).

### 3.6. Omics studies and ECT

Table 1 also includes results from studies examining MDD patients who received ECT.

Clements et al. (2021) conducted a GWAS of ECT-treated patients who presented with an MDE either solely in the context of MDD or in the context of other mood disorders. The rs142610580 SNP located on chromosome 7 was the only one reaching genome-wide significance in the MDD group. Foo et al. (2019) reported statistically significantly higher MDD-PRS in their ECT-treated patients compared to healthy controls. Furthermore, despite not reaching statistical significance, the authors noted a trend of an intermediate MDD-PRS for population-based individuals with self-reported depression compared to ECT-treated patients and controls, as well as a higher MDD-PRS in ECT non-responders than responders. MDD-PRS also statistically significantly correlated with the clinical variable “alcohol dependence/abuse” in their ECT sample. Interestingly, Ryan et al. (2017) identified 36 proteins to be significantly altered following ECT treatment in depressed individuals. Of note, this study included not only participants with MDD but also approximately 1/5 of individuals with bipolar depression. Several of these altered proteins were the same as, or belonged to, the same family as those reported in the other aforementioned proteomics studies, including serotransferrin and serum amyloid P-component (Ruland et al., 2016; Stelzhammer et al., 2013). The most common functional terms revealed through gene ontology analysis were those relating to actin cytoskeleton and immune response.

## 4. Discussion

### 4.1. Interpretation of findings

The findings of this systematic review confirm our hypothesis that individuals with TRD may possess an ‘omics’ profile that is different from both healthy controls and individuals with responsive MDD; there is also evidence of differences between ketamine, rTMS and ECT responders and non-responders. These distinctions are seemingly underpinned by the role of various SNPs, genes and molecules in multiple shared biological pathways considered to have established associations with TRD, including immune response, inflammation and the HPA axis, neuroplasticity/synaptic transmission, calcium signalling, and GABA/glutamate signalling. In addition, more novel pathways like cytoskeleton, blood coagulation, apoptosis/autophagy, tryptophan and lipid metabolism have also been revealed. These results also provide insight into the putative pathogenetic mechanisms contributing to TRD as well as response outcomes to treatments like ECT and ketamine in these patients, thus highlighting much needed neurobiological knowledge for better TRD characterisation.

Our findings further support results from studies indicating the dysregulation of these processes and their complex interaction in individuals with MDD/TRD and animal models of depression (Barnes et al., 2017; Cai et al., 2015; El-Hage et al., 2013; Hoirsch-Clapauch et al., 2014; Velbinger et al., 2000; Wong et al., 2013; Yang et al., 2019). Some of these mechanisms were found to be normalised or modulated by

ketamine (Liu et al., 2016a), ECT (Hestad et al., 2003) or antidepressant treatment (Paul, 2001; Piubelli et al., 2011). Although the evidence of the role of pathways like apoptosis/autophagy in TRD and ECT response in these patients is still preliminary (Gassen and Rein, 2019), it does warrant further study. In line with the results reported here, previous studies utilising omics approaches in models with relevance to depression have found that synthetic glucocorticoid pre-treatment before an inflammatory stimulus can lead to the upregulation of several innate and adaptive immune system genes, suggesting the potential immune potentiating properties of glucocorticoids (Horowitz et al., 2020), which at high concentrations was observed to inhibit the Hedgehog signalling pathway that subsequently resulted in reduced neurogenesis (Anacker et al., 2013). Moreover, patients who developed interferon- $\alpha$ -induced depression displayed differential expression of genes that were revealed to be involved in inflammation and neuroplasticity (Hepgul et al., 2016). In addition, Cattaneo et al. (2018) integrated transcriptomic and miR-Nomic data across species and demonstrated that a cluster of genes related to inflammatory and glucocorticoid receptor signalling displayed a significant interaction with childhood emotional stress in predicting depressive symptoms in adulthood.

The results were pooled based on the pathways involved and subgrouped according to the technique used, since the impact of genes identified from a GWAS is different from those identified with RNAseq or other omics analyses which are per definition more functional. Vadodaria et al. (2019a) further demonstrated that knocking down the expression of protocadherin alpha *PCDHA6/A8* genes, identified through RNA sequencing, led to longer iPSC-derived serotonergic neurite length in resistant MDD cases compared to remitters and controls, which has been reported to result in altered neuronal wiring in mice (Katori et al., 2009; Keeler et al., 2015). Additionally, the same group observed significantly higher forebrain neuronal activity in resistant MDD cases compared with remitters and controls following exogenous serotonin treatment; this hyperactivity was significantly reduced after pre-treatment with the 5-HT7 receptor antagonist Lurasidone (Vadodaria et al., 2019b). It was suggested that these changes may lead to abnormal neural circuitry that may contribute to SSRI resistance, thus providing insight into the putative cellular changes that may occur in TRD. As discussed above, Fabbri et al. (2020) found a greater predictive performance of models including only clinical factors over those including genetic factors. They postulated this was due to their sample consisting mainly of TRD patients with complex MDD that was more likely caused by clinical risk factors, hence denoting potential stratification of patients based on whether they have a predominantly genetic or environmental basis of TRD. These results provide a broad understanding and encouraging insights into the putative mechanisms underlying TRD pathogenesis as well as response to ketamine and ECT treatment in these patients, and therefore highlight the need for further investigation into the abovementioned pathways due to their collective implication at various molecular levels.

Promising candidates for future clinical validation, based on the above results, include the *PCDHA6/8*, *FAM19A4*, *NLGN2*, *NR2C2*, *ZNF641* and *FKBP1A* genes. These genes, in addition to others involved in immune-related processes, such as those among the 74 differentially expressed in Pettai et al. (2016), are potential biomarkers of citalopram/escitalopram resistance in MDD patients. Moreover, the rs78355601 A allele that is linked to lower VEGF levels, particularly the AA genotype, as well as significant differential DNA methylation at CpG sites in genes involved in vascular remodelling, immune system and autophagy, could serve as biomarkers of ECT non-response in TRD. Furthermore, proteins implicated in blood coagulation, complement activation and immune response could differentiate individuals with TRD in different staging groups. These findings emphasise the potential to enhance clinical staging as well as identify and treat high-risk individuals at an earlier stage who either have a biological/genetic predisposition to, or already have, TRD. This could prevent TRD development or progression to subsequent stages, which is linked to a decreased likelihood of response

to other treatments (Rush et al., 2003) and would exacerbate negative outcomes. Another application could be the classification of patients based on metabolomic sub-phenotypes, which could not only aid the establishment of a neurobiologically-based definition of TRD but also contribute to precision medicine in guiding clinicians to make better-informed treatment decisions and adopting more targeted therapeutic strategies rather than the current trial-and-error approach.

Many of the discussed pathways are known to affect antidepressant action (Cai et al., 2015), and have the potential to be targets for new antidepressants or treatment, including the chromatin silencing and cAMP signalling pathways, and related genes, which represent an alternative mechanism of action compared to the conventional targeting of monoamine neurotransmission of current antidepressants and thus may be promising as effective treatments for TRD. In fact, several studies have reported the ability of various compounds like RG-108 and vorinostat to target chromatin remodelling through inhibiting histone deacetylation and DNA methylation enzymes that lead to antidepressant-like effects in animal models of depression including those that are treatment-resistant (Misztak et al., 2018; Park et al., 2021; Sales et al., 2021; Uchida et al., 2018; Weaver et al., 2017). Additionally, inhibitors of the enzyme phosphodiesterase 4D, which degrades cAMP, like GEBR-7b and rolipram demonstrated antidepressant-like effects on behavior in animal models of depression and were shown to increase the expression of components of the cAMP signalling pathway such as phosphorylated CREB (Li et al., 2009; Liu et al., 2016b). Although similar findings have been reported in human MDD patients who received rolipram as a radioligand in addition to SSRI treatment, this was not correlated with symptom improvement (Fujita et al., 2017). Moreover, Fabbri et al. (2021c) suggested muscarinic receptor antagonism as the most promising pharmacological mechanism for treating TRD through drug repurposing analysis. This finding indicates the involvement of the cholinergic system, components of the TrkB signalling pathway, GABA interneurons and synaptogenesis in the mechanism of action of antimuscarinic drugs such as scopolamine, a drug that has a therapeutic effect in depressed patients, including those who are treatment-resistant (Drevets et al., 2013; Dulawa and Janowsky, 2019; Liu et al., 2021). This effect is mediated through M1 and M2 receptor antagonism (Dulawa and Janowsky, 2019; Liu et al., 2021), however the aforementioned results state the potential of M3 and M5 receptor blockade, whose impact on mood and depression has not been fully investigated (Dulawa and Janowsky, 2019).

#### 4.2. Limitations and strengths

Despite these favourable findings, they should be interpreted with caution. Most notably, nearly all studies reported to have a small sample size, which means that they may not possess sufficient statistical power to detect a small effect of a particular genetic variant or molecule on TRD, thus potentially failing to identify true significant associations. In addition, a quality assessment tool was not used to assess the risk of bias in each of the studies, due to the apparent heterogeneity in terms of their designs, cohorts/samples and methodologies; some included participants who were not necessarily diagnosed with MDD and factors like adjacent/differential medication use were not controlled for. Therefore the resulting associations may not be specific to the treatments being investigated or to treatment resistance in MDD, and confounders could explain some of the non-significant findings. Moreover, most participants were of European ancestry, which may reduce the representativeness and generalisability of the results to other ethnic populations.

Another conceptual limitation is the variability in defining treatment resistance between the studies; this lack of consistency can make it difficult to compare and replicate the results. Indeed, this topic has been extensively discussed before by Sforzini et al. (2021), who utilised a Delphi-method-based approach involving a group of experts to provide a consensus TRD definition for clinical studies. Still acknowledging that TRD exists on a continuum, the authors reached a strong consensus on

criteria for TRD of at least two antidepressant treatments, at an adequate dose and duration, with a <25 % reduction in MDD severity; a staging model was the agreed preferable method for defining TRD. In addition, they defined partially responsive depression (PRD) as a reduction between 25 and 50 % in MDD severity after one or more treatments. In the current review, 5 studies provided no TRD definition (Chen et al., 2021; Israel-Elgali et al., 2021; Moschny et al., 2020; Souza-Silva et al., 2020; Stelzhammer et al., 2013), 10 used a questionnaire or rating scale (Cathomas et al., 2022; Cole et al., 2021; Li et al., 2016; Li et al., 2020a; Li et al., 2020b; McClain et al., 2020b; Pettai et al., 2016; Rotroff et al., 2016; Vadodaria et al., 2019a; Vadodaria et al., 2019b), 4 employed a staging method (Bekhbat et al., 2021; Maffioletti et al., 2020; Pisanu et al., 2021; Ruland et al., 2016), 14 had utilised a categorical or generic definition with varying criteria relating to switching, drug prescriptions, failed medication trials, previous treatment strategies and presence of recurrent depressive episodes (Barakat et al., 2020; Fabbri et al., 2018, 2020; Fabbri et al., 2021a; Fabbri, Kasper, et al., 2019b, 2021b; Fabbri et al., 2021c; Fanelli et al., 2021; Guo et al., 2018; Gururajan et al., 2016; Li et al., 2021; McClain et al., 2020a; Singh et al., 2022; Wigmore et al., 2020), and one study had included two different samples where one had used a rating scale and the other used the generic definition (O'Dushlaine et al., 2014). Furthermore, 3 studies were included in this review that examined ECT-treated patients with MDD (Clements et al., 2021; Foo et al., 2019; Ryan et al., 2017). The results from Clements et al. (2021) and Foo et al. (2019) suggest that individuals who demonstrate ECT non-response possess a distinct genetic architecture compared to other MDD cases and ECT responders. In addition, the findings from all three studies are comparable to those from other omics papers. This further supports that non-responsive depression may be treated as a separate subgroup compared to responsive MDD. Finally, the number of papers focusing on untargeted metabolomics and microbiomics in human TRD patients was small, despite their implication in MDD aetiopathogenesis (Flux and Lowry, 2020; MacDonald et al., 2019) and TRD (Fontana et al., 2020; Humer et al., 2020).

In terms of our methodology, the current review may have suffered from publication bias due to the inclusion of papers published in peer-reviewed journals only, not performing grey literature searches or backward/forward citation tracking, thus potentially limiting the number of eligible articles to be included. However, a main strength was ensuring that the systematic search was less conservative by utilising an inclusive search strategy without applying any filters or limits, searching multiple relevant databases and using liberal inclusion/exclusion criteria that did not specify a TRD definition. Our search strategy included terms more recently being used as alternatives to TRD such as “difficult-to-treat depression”, and although articles only mentioning the term “non-response” were not considered, this was done to limit ambiguity and maintain consistency for easier replicability purposes as some studies categorise non-responders as a separate group to TRD. Moreover, prior to screening the studies, de-duplication was conducted using Rayyan, which is reported to have the highest sensitivity and be one of the most accurate methods for correctly identifying duplicates (McKeown and Mir, 2021). A manual check was also performed and the full-text screening stage was also carried out by a second reviewer with any inconsistencies discussed and resolved to minimise error and bias.

#### 4.3. Future recommendations and conclusions

To our knowledge, this is the first systematic and comprehensive appraisal of the literature on omics techniques in individuals with TRD. Omics techniques, mainly because of their broad perspective and hypothesis-free approach, appear to be the most promising to unravel the biological and molecular alterations that may contribute to TRD. The discussed findings provide a holistic understanding of these mechanisms of non-response as well as promising research avenues to disentangle the complex pathophysiology of TRD to ultimately better diagnose and treat this condition. The current review has several main implications

including the identification of potential genetic, transcriptomic and epigenetic biomarkers to indicate antidepressant resistance (and especially to citalopram/escitalopram) in MDD and ECT non-response in TRD patients. Furthermore, several pathways have been proposed as putative drug/treatment targets including muscarinic receptor antagonism, chromatin silencing and cAMP signalling, which may contribute to the much needed progression of drug development in this field. Additionally, a greater genetic burden for psychiatric disorders could serve as a diagnostic biomarker to differentiate between TRD and non-TRD or milder MDD cases, thus providing a biological basis to define TRD. Lastly, TRD patient stratification into different clinical staging groups through profiling proteins involved in blood coagulation, complement activation and immune response, could aid early intervention and prevention of disease progression. Despite the heterogeneity of the included studies, we believe the current findings may represent a promising starting point for future research and may be generalized to a more broadly-defined group of people with TRD who are at the severe end of the MDD spectrum.

However, these results require further replication and validation in large prospective studies that adopt standardised or well-established approaches for measuring molecules at multiple time points in different ethnic populations, which can also help determine causality over consequence. Furthermore, the obstacles preventing the establishment of definitive and operational criteria for TRD need to be addressed, which will involve further accurate and systematic evaluation of current assessment strategies and staging methods for predictive utility, as well as gaining a more holistic understanding of TRD pathophysiology (Berlim and Turecki, 2007; Ruhé et al., 2012; Sforzini et al., 2021). Additionally, examining resistance to specific classes of antidepressant drugs and non-response to certain types of treatment in TRD will all serve to advance tailored medicine by understanding which individuals are more likely to respond based on their omics profile. Finally, adopting a systems biology strategy by integrating multi-omics technologies with targeted approaches and clinical data, as well as other fields like imaging genetics, computational approaches and machine learning, is likely to increase the power to detect and replicate significant results that uncover possible causative mechanisms involved in TRD (Fabbri et al., 2019a; Hasin et al., 2017). While facilitating this large-scale research will require collaboration and cooperation between several research groups in order for eventual translation into the clinic, the foreseeable benefits would be substantial as reducing the significant financial burden on health care systems and ameliorating the quality of life of these individuals.

#### Conflict of interest

Research funded by the National Institute for Health Research (NIHR) Maudsley Biomedical Research Centre (BRC), London, United Kingdom. This work was also supported by the Wellcome Trust strategy award to the Neuroimmunology of Mood Disorders and Alzheimer's Disease (NIMA) Consortium (104025), which is also funded by Janssen, GlaxoSmithKline, Lundbeck and Pfizer.

Dr. Sforzini and Prof. Pariante have received research funding from the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement No 853966-2, as part of the EU-PEARL project. This Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation programme and EFPIA. Prof. Pariante is also funded by a Senior Investigator award from the National Institute for Health Research (NIHR); the Medical Research Council (grants MR/L014815/1, MR/J002739/1 and MR/N029488/1); the European Commission (EARLYCAUSE grant SC1-BHC-01-2019); the NARSAD; the Psychiatry Research Trust; and the Wellcome Trust (SHAPER, Scaling-up Health-Arts Programme to scale up arts interventions, grant 219425/Z/19/Z). <10 % of his support in the last 10 years derives from commercial collaborations, including consultation and speakers fees from Boehringer Ingelheim, Eli Lilly, Compass, Eleusis, GH Research,



Lundbeck, and Värde Partners.

### Acknowledgements

Dr. Sforzini and Prof. Pariante have received research funding from the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement No 853966-2, as part of the EU-PEARL project. This Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation programme and EFPIA. Prof. Pariante is also funded by a Senior Investigator award from the National Institute for Health Research (NIHR); the Medical Research Council (grants MR/L014815/1, MR/J002739/1 and MR/N029488/1); the European Commission (EARLYCAUSE grant SC1-BHC-01-2019); the NARSAD; the Psychiatry Research Trust; and the Wellcome Trust (SHAPER, Scaling-up Health-Arts Programme to scale up arts interventions, grant 219425/Z/19/Z). Less than 10 % of his support in the last 10 years derives from commercial collaborations, including consultation and speakers fees from Boehringer Ingelheim, Eli Lilly, Compass, Eleusis, GH Research, Lundbeck, and Värde Partners.

### Contributors

Ms. Nare Amasi-Hartoonian conducted the systematic literature searches, was the first reviewer for title/abstract and full-text screening and created the table and figure for data collection. She also took the lead in writing and editing the manuscript. Dr. Luca Sforzini and Prof. Carmine Pariante both provided guidance and supervision, in addition to proofreading and editing the manuscript. Dr. Annamaria Cattaneo was involved in proofreading the manuscript and provided suggestions regarding the subdivision of the results section. All authors contributed to and have approved the final manuscript.

### Role of funding source

Research funded by the National Institute for Health Research (NIHR) Maudsley Biomedical Research Centre (BRC), London, United Kingdom. This work was also supported by the Wellcome Trust strategy award to the Neuroimmunology of Mood Disorders and Alzheimer's Disease (NIMA) Consortium (104025), which is also funded by Janssen, GlaxoSmithKline, Lundbeck and Pfizer.

The funding sources had no involvement in study/research design, data collection and interpretation, the writing of the manuscript, or the decision to submit the article for publication.

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