Appendix 1 to Ciocan D, Cassard AM, Becquemont L et al. Blood microbiota and metabolomic signature of major depression before and after antidepressant treatment: a prospective case-control study. *J Psychiatry Neurosci* 2021. doi: 10.1503/jpn.200159

SUPPLEMENTARY DATA

Title: Blood microbiota and metabolomic signature of major depression before and after antidepressant treatment: a prospective case-control study

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Appendix 1 to Ciocan D, Cassard AM, Becquemont L et al. Blood microbiota and metabolomic signature of major depression before and after antidepressant treatment: a prospective case-control study. *J Psychiatry Neurosci* 2021. doi: 10.1503/jpn.200159

SUPPLEMENTAL METHODS AND MATERIALS

Study population

Standardized interviews documented the lifetime duration of MDD since the onset of the first MDE, the number of previous MDE, the lifetime duration of prior antidepressant treatment since the onset of MDD, and whether patients were antidepressant-free before inclusion (no antidepressant treatment for at least one year before inclusion). Medical records were also examined. If there were discrepancy between interviews and medical records, the latter were prioritized. The 17-item HAMD and the Clinical Global Impression (CGI) were rated at baseline, one month, three months and six months after initiation of current antidepressant treatment.

Blood microbiota and metabolomics

Microbiome composition was identified using the V3-V4 hypervariable regions of the 16S rDNA which were amplified and quantified by qPCR, sequenced with MiSeq technology (Vaiomer, Labège, France). Using EDTA tubes, 30 mL of blood samples were obtained between 8:00 and 10:00 a.m. after an overnight fast. Blood was afterwards centrifuged at 4°C immediately after sampling during 10 minutes at 2000 g. Plasma was immediately aliquoted (using a Bionek NX Beckman Coulter pipetting robot that limits cross and airway contaminations) into separate polypropylene tubes that were immediately stored at -80°C until analysis.

Bacterial DNA was extracted from 300 µL plasma from fasting specimens collected in the morning, as previously described (1,2). The concentration of 16S rRNA gene copies normalized to 1 mL of plasma in each sample was determined by real-time qPCR using primers EUBF 50-TCCTACGGGAGGCAGCAGT-30 and EUBR 50-GGACTACCAGGGTATCTAATCCTGTT-30 (3). As many reagents required in the qPCR and sequencing pipeline contain bacterial DNA which can be misinterpreted as present in the samples, numerous combinations of reagents were tested to minimize bacterial contaminants and adapted the protocol to increase the yield of amplification of the bacterial DNA present in the blood. Furthermore, numerous controls were performed both in vitro and in silico to ensure the absence of artefacts (such as bacterial DNA contaminants from reagents or nonspecific amplification of eukaryotic DNA) as previously described (2)). The V3-V4 hypervariable regions of the 16S rDNA were amplified and quantified by qPCR, sequenced with MiSeq technology (Vaiomer, Labège, France).

The sequences were processed using the quantitative insights into microbial ecology (QIIME v1.9.0) pipeline, using its default parameters (4). Sequences were then clustered into operational taxonomic units (OTUs) displaying at least 97.0% sequence similarity, by a closed reference-based picking approach in UCLUST software applied to the Greengenes 13.8 database of bacterial 16S rDNA sequences (5). The mean number of quality-controlled reads was 25,525 ± 13,126 (mean ± SD) per sample.

Appendix 1 to Ciocan D, Cassard AM, Becquemont L et al. Blood microbiota and metabolomic signature of major depression before and after antidepressant treatment: a prospective case-control study. *J Psychiatry Neurosci* 2021. doi: 10.1503/jpn.200159

After rarefaction at 8,000 reads per sample, the bacterial alpha diversity (species richness or number of taxa within a sample) was estimated based on the observed species, Faith's PD_Whole_Tree and Shannon's index. OTUs with a prevalence < 5% were removed from the analysis to decrease the probability of including OTUs generated by sequencing errors. The beta diversity (diversity of microbial communities between different categories) was assessed using weighted and unweighted UniFrac distances. The weighted Unifrac metric is weighted by the difference in the abundance of OTUs from each community, whereas unweighted UniFrac considers only the absence/presence of OTUs, providing different information. Both are phylogenetic beta diversity metrics. We investigated the OTUs not identified by QIIME further, using the Basic Local Alignment Search Tool (BLASTN program, vBLAST+ 2.6.0) from NCBI Blast, against the NCBI 16S Microbial database.

Inferred metagenomics

The functional composition of the intestinal metagenome was predicted using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt)(6) based on the Kyoto Encyclopedia of Genes and Genomes database. This is a computational approach that accurately predicts the abundance of gene families in the microbiota and thus provides information about the functional composition of the microbial community.

Metabolomic assay

Plasma samples were analyzed by a targeted metabolomics approach of combined direct flow injection and liquid chromatography MS/MS coupled to tandem mass spectrometry using the AbsoluteIDQ p180 kit (BIOCRATES Life Sciences AG, Innsbruck, Austria, https://www.biocrates.com/products/research-products/absoluteidq-p180-kit) according to the manufacturers' instructions. This metabolomic platform provides the simultaneous determination of 188 metabolites which includes 40 acylcarnitines, 42 aminoacids and biogenic amines, 90 glycerophospholipids (not included in this analysis), 15 sphingolipids (not included in this analysis), and sum of hexoses. Reported concentrations were within the quantification range validated for each metabolite. Concentrations of all metabolites were reported as µmol/L. This targeted metabolomics method has been validated in six testing laboratories to have a median inter-laboratory coefficient of variation of 7.6%, with 85% of metabolites with a median inter-laboratory variation of <20% (7).

Statistical analysis

The results are expressed as means ± SEM. Alpha diversity comparisons were performed with nonparametric Student's t-tests and Monte Carlo permutations in QIIME. Individual comparisons were performed at all the levels of classification or taxonomic rank (phylum, class, order, family and genus). Taxa were compared using Mann–Whitney U-tests and the ANOSIM test with 999 permutations was used to compare distance matrices (weighted and unweighted UniFrac) in QIIME. Homogeneity between groups was tested using betadisper function from the vegan

Appendix 1 to Ciocan D, Cassard AM, Becquemont L et al. Blood microbiota and metabolomic signature of major depression before and after antidepressant treatment: a prospective case-control study. *J Psychiatry Neurosci* 2021. doi: 10.1503/jpn.200159

package in R. The Benjamini–Hochberg false discovery rate (FDR) correction was used to correct for multiple hypothesis testing, when applicable.

Linear discriminative analysis (LDA) effect size (LEfSe) analysis was performed to identify the taxa displaying the largest differences in abundance in the microbiota between groups. Briefly it consists of a Kruskall-Wallis test that analyzes whether the values in different classes are differentially distributed and a pairwise Wilcoxon test that assesses whether all pairwise comparisons between subclasses within different classes significantly agree with the class level trend. Finally, the resulting subset of vectors is used to build a Linear Discriminant Analysis model, from which the relative difference among classes is used to rank the features according to the effect size with which they differentiate classes. The final output thus consists of a list of features that are discriminative with respect to the classes, consistent with the subclass grouping within classes, and ranked according to the effect size with which they differentiate classes(8). Only taxa with an LDA score >2 and a significance of $\alpha < 0.05$, as determined in Wilcoxon signed-rank tests, are reported. The size of the circles in the cladogram plot is proportional to bacterial abundance. LEfSe, and Picrust were accessed online (http://huttenhower.sph.harvard.edu/galaxy/).

The remaining comparisons were performed with R software v2.14.1 or GraphPad v7.01 (Graphpad Prism, Graphpad Software Inc, La Jolla, California, USA). Unpaired and paired t-tests or Mann–Whitney U-tests were used to compare continuous data between groups, as appropriate. Chi2 or Fisher's exact tests were used to compare discrete parameters between groups. Spearman correlations with FDR correction for multiple comparisons were performed between the relative abundance of taxa and clinical parameters. A Firth model analysis(9) using logistf package (version 1.21) was used to identify the taxa and metabolites that were independently associated to response to treatment. Adjustment was done for sex, age, BMI, diabetes, smoking, history of major depressive episodes and suicide attempt and HDRS at baseline. A p-value < 0.05 was considered to be statistically significant.

All the statistical analysis and pathway annotations for the metabolites were carried out using MetaboAnalyst web tool (www.metaboanalyst.ca). Data was normalized using log transformation and Pareto-scaling(10).

Appendix 1 to Ciocan D, Cassard AM, Becquemont L et al. Blood microbiota and metabolomic signature of major depression before and after antidepressant treatment: a prospective case-control study. *J Psychiatry Neurosci* 2021. doi: 10.1503/jpn.200159

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Appendix 1 to Ciocan D, Cassard AM, Becquemont L et al. Blood microbiota and metabolomic signature of major depression before and after antidepressant treatment: a prospective case-control study. *J Psychiatry Neurosci* 2021. doi: 10.1503/jpn.200159

Supplementary Table 1. Changes in the blood microbiome profile of depressed patients (MDE) and matched healthy controls (HC) using LDA Effective Size (LEfSe).

Phyla	Class	Ordre	Family	Genus	Increased in	LDA	p
Actinobacteria	Acidimicrobiia				HC	2,32	0,043
		Acidimicrobiales			HC	2,33	0,043
			Actinomycetaceae		HC	2,92	0,009
	Actinobacteria	Actinomycetales		Actinomyces	HC	2,91	0,017
			Micrococcaceae		HC	2,47	0,033
				Kocuria	MDE	2,24	0,029
		Acidimicrobiales			HC	2,30	0,043
Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae		HC	2,38	0,031
				Flavobacterium	HC	2,30	0,043
			Weeksellaceae		MDE	3,15	0,033
				;hryseobacteriur	MDE	3,09	0,009
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae		HC	2,38	0,043
				Enterococcus	HC	2,35	0,043
	Clostridia	Clostridiales	Tissierellaceae	Parvimonas	MDE	2,50	0,023
Fusobacteria	Fusobacteriia				HC	3,20	0,032
					HC	3,18	0,032
		Fusobacteriales			HC	3,18	0,032
			Fusobacteriaceae		HC	3,18	0,002
				Fusobacterium	HC	3,19	0,002
Proteobacteria		Rhodospirillales	Rhodospirillaceae		HC	2,75	0,049
	Alphaproteobacteria	Rhizobiales	Methylobacteriaceae		MDE	2,80	0,023
					MDE	2,22	0,023
		Rhodocyclales	Rhodocyclaceae		MDE	2,22	0,023
			Comamonadaceae	Curvibacter	HC	2,48	0,027
			Oxalobacteraceae		HC	2,85	0,027
			Comamonadaceae	Tepidimonas	HC	2,14	0,032
		Burkholderiales	Oxalobacteraceae	anthinobacteriur	MDE	2,92	0,014
	Betaproteobacteria	Neisseriales	Neisseriaceae	Neisseria	HC	2,96	0,007
	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Aggregatibacter	HC	3,07	0,001
Saccharibacteria					HC	2,37	0,015
	TM7_3				HC	2,29	0,015
		o._			HC	2,19	0,028
			f._		HC	2,21	0,028
				g._	HC	2,33	0,028

Appendix 1 to Ciocan D, Cassard AM, Becquemont L et al. Blood microbiota and metabolomic signature of major depression before and after antidepressant treatment: a prospective case-control study. *J Psychiatry Neurosci* 2021. doi: 10.1503/jpn.200159

Supplementary Table 2: Pathway Analysis based on metabolomic analysis in patients with major depression as compared to healthy controls.

Pathway Name	Match Status	p	FDR	Impact
Arginine and proline metabolism	12/77	<0.001	<0.001	0.57301
Tryptophan metabolism	3/79	<0.001	<0.001	0.20083
Taurine and hypotaurine metabolism	2/20	<0.001	<0.001	0.36331
Primary bile acid biosynthesis	2/47	<0.001	<0.001	0.01644
Biotin metabolism	1/11	<0.001	<0.001	0.0
Nitrogen metabolism	10/39	<0.001	<0.001	0.0083
Cysteine and methionine metabolism	4/56	<0.001	0.003	0.05003
Aminoacyl-tRNA biosynthesis	19/75	0.001	0.006	0.22536
Valine, leucine and isoleucine biosynthesis	4/27	0.002	0.007	0.03975
Glycine, serine and threonine metabolism	5/48	0.002	0.007	0.42039
Sphingolipid metabolism	1/25	0.002	0.007	0.0
Sulfur metabolism	1/18	0.002	0.007	0.0
Valine, leucine and isoleucine degradation	3/40	0.003	0.009	0.02232
Cyanoamino acid metabolism	4/16	0.003	0.009	0.0
Methane metabolism	2/34	0.010	0.024	0.01751
Pantothenate and CoA biosynthesis	2/27	0.016	0.036	0.0
Nicotinate and nicotinamide metabolism	1/44	0.017	0.038	0.0
beta-Alanine metabolism	3/28	0.031	0.064	0.06625
Lysine biosynthesis	3/32	0.042	0.081	0.16762
Histidine metabolism	3/44	0.068	0.123	0.14039

Appendix 1 to Ciocan D, Cassard AM, Becquemont L et al. Blood microbiota and metabolomic signature of major depression before and after antidepressant treatment: a prospective case-control study. *J Psychiatry Neurosci* 2021. doi: 10.1503/jpn.200159

Supplementary Table 3. Changes in the blood microbiome profile of depressed patients, before (M0) and after (M3) antidepressant treatment, using LDA Effective Size (LEfSe).

Phyla	Class	Ordre	Family	Genus	Increased in	LDA	p
Bacteroidetes	Cytophagia				M0	2,97	0,004
		Cytophagales			M0	3,05	0,004
			Cytophagaceae		M0	2,87	0,004
				Hymenobacter	M0	2,37	0,023
Firmicutes	Bacilli				M3	3,74	0,016
		Bacillales			M3	3,57	0,027
			taphylococcacea		M3	3,46	0,046
				Staphylococcus	M3	3,48	0,031
		Lactobacillales	Carnobacteriaceae		M3	2,62	0,004
			Carnobacteriac	Granulicatella	M3	2,65	0,007
			Aerococcaceae		M3	2,46	0,044
	Clostridia				M0	3,13	0,049
		Clostridiales			M0	3,13	0,049
Proteobacteria	Betaproteobacteria	Burkholderiales			M0	3,12	0,044
					M3	2,28	0,037
					M3	2,30	0,037
			)xalobacteracea		M0	3,17	0,002
					M0	2,85	0,002
				Janthinobacteriu	M0	2,95	0,002
		Neisseriales	Neisseriaceae	Neisseria	M3	2,69	0,033
	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae		M0	3,04	0,036
		Pseudomonadales	Moraxellaceae	Enhydrobacter	M3	3,02	0,016
	Epsilonproteobacteria	Campylobacterales	Campylobacter	Campylobacter	M0	2,86	0,043

Appendix 1 to Ciocan D, Cassard AM, Becquemont L et al. Blood microbiota and metabolomic signature of major depression before and after antidepressant treatment: a prospective case-control study. *J Psychiatry Neurosci* 2021. doi: 10.1503/jpn.200159

Supplementary Table 4: Changes in the blood microbiome predicted metabolic pathways of depressed patients, before (M0) and after treatment (M3), using LDA Effective Size (LEfSe).

Predicted metabolic pathways	Increased in	LDA	p
Glycosaminoglycan degradation	M3	2.00	0.04
Glycosphingolipid biosynthesis_lacto and neolacto series	M0	2.32	0.006
Penicillin and cephalosporin biosynthesis	M0	2.02	0.04
Carbon fixation pathways in prokaryotes	M3	2.44	0.02
Pyruvate metabolism	M3	2.53	0.03
Arginine and proline metabolism	M0	2.52	0.02
Cyanoaminoacid metabolism	M0	2.25	0.02

Appendix 1 to Ciocan D, Cassard AM, Becquemont L et al. Blood microbiota and metabolomic signature of major depression before and after antidepressant treatment: a prospective case-control study. *J Psychiatry Neurosci* 2021. doi: 10.1503/jpn.200159

Supplementary Table 5: Changes in the blood microbiome profile in depressed patients who respond to treatment, before and after treatment, using LDA Effective Size (LEfSe).

Phyla	Class	Ordre	Family	Genus	Increased in	LDA	p
Actinobacteria		Actinomycetales	Nocardiaceae		M0_REP	3,01	0,021
	Actinobacteria	Actinomycetales	Nocardiaceae	Rhodococcus	M0_REP	2,99	0,021
Bacteroidetes	Cytophagia				M0_REP	2,80	0,011
		Cytophagales			M0_REP	2,87	0,011
			Cytophagaceae		M0_REP	2,87	0,011
				Hymenobacter	M0_REP	2,23	0,041
	Flavobacteriia	Flavobacteriales	Weeksellaceae	Chryseobacterium	M0_REP	2,90	0,035
Firmicutes	Clostridia				M0_REP	3,40	0,030
		Clostridiales			M0_REP	3,40	0,030
	Bacilli	Lactobacillales	Aerococcaceae		M3_REP	2,34	0,011
Proteobacteria					M0_REP	3,44	0,039
					M0_REP	3,35	0,004
	Betaproteobacteria		Oxalobacteraceae		M0_REP	3,41	0,003
		Burkholderiales		g__	M0_REP	3,18	0,003
				Janthinobacterium	M0_REP	3,14	0,004
			Comamonadaceae	Tepidimonas	M0_REP	2,05	0,021
		Pseudomonadales	Moraxellaceae	Enhydrobacter	M3_REP	3,09	0,028
	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Aggregatibacter	M3_REP	2,40	0,041

Appendix 1 to Ciocan D, Cassard AM, Becquemont L et al. Blood microbiota and metabolomic signature of major depression before and after antidepressant treatment: a prospective case-control study. *J Psychiatry Neurosci* 2021. doi: 10.1503/jpn.200159

Supplementary Table 6: Changes in the blood microbiome profile in depressed patients who do not respond to treatment, before and after treatment, using LDA Effective Size (LEfSe).

Phyla	Class	Ordre	Family	Genus	Increased in	LDA	p
Actinobacteria	Rubrobacteria				M3_nonREP	2,70	0,039
		Rubrobacterales			M3_nonREP	2,60	0,039
			Rubrobacteraceae		M3_nonREP	2,80	0,039
				Rubrobacter	M3_nonREP	2,57	0,039
Firmicutes					M3_nonREP	3,93	0,034
	Bacilli				M3_nonREP	3,94	0,020
		Bacillales			M3_nonREP	3,78	0,017
			Planococcaceae	g__	M3_nonREP	2,22	0,027
			Staphylococcaceae		M3_nonREP	3,73	0,006
				Staphylococcus	M3_nonREP	3,73	0,006
			Carnobacteriaceae		M3_nonREP	3,03	0,010
		Lactobacillales	Carnobacteriaceae	Granulicatella	M3_nonREP	3,03	0,010
	Clostridia	Clostridiales	Ruminococcaceae		M3_nonREP	2,45	0,039
Proteobacteria					M0_nonREP	4,11	0,043
	Alphaproteobacteria				M0_nonREP	4,22	0,014
		Rhizobiales			M0_nonREP	4,19	0,029
	Betaproteobacteria	Neisseriales	Neisseriaceae	Neisseria	M3_nonREP	2,82	0,037

Appendix 1 to Ciocan D, Cassard AM, Becquemont L et al. Blood microbiota and metabolomic signature of major depression before and after antidepressant treatment: a prospective case-control study. *J Psychiatry Neurosci* 2021. doi: 10.1503/jpn.200159

Supplementary Table 7: Changes in the blood microbiome predicted metabolic pathways in depressed patients who do not respond to treatment, before and after treatment, using LDA Effective Size (LEfSe).

Predicted metabolic pathway	Increased in	LDA	p
D_Alanine metabolism	M3_nonREP	2,33	0,01
Glycolysis_Gluco neogenesis	M3_nonREP	2,91	0,01
Glycerolipid metabolism	M3_nonREP	2,63	0,00
Ubiquinone and other terpenoid_quinone biosynthesis	M3_nonREP	2,47	0,04
Vitamin B6 metabolism	M3_nonREP	2,21	0,04
beta_Alanine metabolism	M0_nonREP	2,72	0,03
Citratecycle_TCAcycle	M3_nonREP	2,76	0,00
Thiamine metabolism	M3_nonREP	2,45	0,04
Penicillin and cephalosporin biosynthesis	M0_nonREP	2,10	0,04
Metabolism of xenobiotics by cytochrome P450	M0_nonREP	2,83	0,00
Aminosugar and nucleotide sugar metabolism	M3_nonREP	3,03	0,01
Lipoicacid metabolism	M3_nonREP	2,23	0,01
Stilbenoid_diarylheptanoid and gingerol biosynthesis	M0_nonREP	2,30	0,01
Pyrimidine metabolism	M3_nonREP	3,15	0,02
Carbonfixation pathways in prokaryotes	M3_nonREP	2,70	0,02
Glutathione metabolism	M0_nonREP	2,90	0,01
Biosynthesis of vancomycin group antibiotics	M0_nonREP	2,14	0,05
Pyruvate metabolism	M3_nonREP	2,82	0,01
Retinol metabolism	M0_nonREP	2,14	0,05
Photosynthesis	M3_nonREP	2,51	0,01
Steroid hormone biosynthesis	M0_nonREP	2,16	0,04
Prenyltransferases	M3_nonREP	2,40	0,03
Cyanoaminoacid metabolism	M0_nonREP	2,46	0,00
Peptidoglycan biosynthesis	M3_nonREP	2,84	0,03
Lipid biosynthesis proteins	M0_nonREP	2,57	0,02
Nitrotoluene degradation	M0_nonREP	2,44	0,04
Terpenoid backbone biosynthesis	M3_nonREP	2,56	0,01
Xylene degradation	M3_nonREP	2,13	0,04
Arachidonicacid metabolism	M0_nonREP	2,28	0,02
Chloroalkane and chloroalkene degradation	M0_nonREP	2,67	0,03
Fructose and mannose metabolism	M3_nonREP	2,69	0,01
Limonene and pinene degradation	M0_nonREP	2,72	0,05
D_Glutamine and D_glutamate metabolism	M3_nonREP	2,20	0,04
Linoleicacid metabolism	M0_nonREP	2,26	0,02
Photosynthesis proteins	M3_nonREP	2,45	0,02
Phosphonate and phosphinate metabolism	M0_nonREP	2,01	0,03
Galactose metabolism	M3_nonREP	2,71	0,02
Zeatinbiosynthesis	M3_nonREP	2,39	0,02
f_1_1_1_Trichloro_2_2_bis_4_chlorophenyl_ethane_DDT_ degra	M0_nonREP	2,39	0,03
Riboflavin metabolism	M3_nonREP	2,35	0,02
Drug metabolism_cytochrome P450	M0_nonREP	2,86	0,01
One carbonpool by folate	M3_nonREP	2,67	0,03

Appendix 1 to Ciocan D, Cassard AM, Becquemont L et al. Blood microbiota and metabolomic signature of major depression before and after antidepressant treatment: a prospective case-control study. *J Psychiatry Neurosci* 2021. doi: 10.1503/jpn.200159

Supplementary Table 8: Baseline changes in the blood microbiome predicted metabolic pathways in depressed patients, associated with treatment response, using LDA Effective Size (LEfSe).

Predicted metabolic pathway	Increased in	LDA	p
Valine_leucine and isoleucine degradation	M0_nonREP	2,99	0,01
Fattyacid metabolism	M0_nonREP	2,90	0,02
Limonene and pinene degradation	M0_nonREP	2,82	0,01
Tryptophan metabolism	M0_nonREP	2,81	0,04
Geraniol degradation	M0_nonREP	2,80	0,01
Drug metabolism_cytochromeP450	M0_nonREP	2,77	0,02
Lysine degradation	M0_nonREP	2,75	0,01
Metabolism of xenobiotics by cytochrome P450	M0_nonREP	2,74	0,01
beta_Alanine metabolism	M0_nonREP	2,72	0,02
Chloroalkane and chloroalkene degradation	M0_nonREP	2,68	0,01
Naphthalene degradation	M0_nonREP	2,64	0,01
Caprolactam degradation	M0_nonREP	2,63	0,02
Bisphenol degradation	M0_nonREP	2,54	0,03
Lipid biosynthesis proteins	M0_nonREP	2,45	0,04
Linoleicacid metabolism	M0_nonREP	2,27	0,02
Retinol metabolism	M0_nonREP	2,20	0,00
D_Glutamine and D_glutamate metabolism	M0_REP	2,15	0,01
Betalain biosynthesis	M0_REP	2,18	0,01
Xylene degradation	M0_REP	2,20	0,03
Riboflavin metabolism	M0_REP	2,26	0,02
Indolealkaloid biosynthesis	M0_REP	2,29	0,02
Nicotinate and nicotinamide metabolism	M0_REP	2,34	0,03
Glycerophospholipid metabolism	M0_REP	2,39	0,02
Alanine_aspartate and glutamate metabolism	M0_REP	2,53	0,03
Pyruvate metabolism	M0_REP	2,56	0,04
Purine metabolism	M0_REP	2,95	0,03
Pyrimidine metabolism	M0_REP	3,09	0,04

Appendix 1 to Ciocan D, Cassard AM, Becquemont L et al. Blood microbiota and metabolomic signature of major depression before and after antidepressant treatment: a prospective case-control study. *J Psychiatry Neurosci* 2021. doi: 10.1503/jpn.200159

Supplementary Table 9: Baseline changes in the blood microbiome profile in depressed patients, associated with remission, using LDA Effective Size (LEfSe).

Phyla	Class	Ordre	Family	Genus	Increased in	LDA	p
Firmicutes	Clostridia				M0_REM	3,65	0,014
		Clostridiales			M0_REM	3,65	0,014
			Clostridiaceae		M0_REM	2,90	0,035
				Clostridium	M0_REM	2,94	0,035
			Lactobacillaceae		M0_nonREM	2,75	0,037
	Bacilli	Lactobacillales		Lactobacillus	M0_nonREM	2,76	0,037
Proteobacteria					M0_nonREM	4,10	0,026
		Sphingomonadales			M0_nonREM	2,92	0,018
			Sphingomonadaceae		M0_nonREM	3,01	0,004
				Sphingomonas	M0_nonREM	2,86	0,008
				Kaistobacter	M0_nonREM	2,76	0,043
		Caulobacterales			M0_nonREM	3,11	0,025
	Alphaproteobacteria		Caulobacteraceae		M0_nonREM	3,12	0,025
	Betaproteobacteria	Burkholderiales	Comamonadaceae	Tepidimonas	M0_REM	3,21	0,026

Appendix 1 to Ciocan D, Cassard AM, Becquemont L et al. Blood microbiota and metabolomic signature of major depression before and after antidepressant treatment: a prospective case-control study. *J Psychiatry Neurosci* 2021. doi: 10.1503/jpn.200159

Supplementary Table 10: Baseline changes in the blood microbiome predicted metabolic pathways in depressed patients, associated with remission, using LDA Effective Size (LEfSe).

Predicted metabolic pathway	Increased in	LDA	p
Bisphenol degradation	M0_nonREM	2.58	0.04
beta_Alanine metabolism	M0_nonREM	2.70	0.04
Propanoate metabolism	M0_nonREM	2.82	0.03
Naphthalene degradation	M0_nonREM	2.58	0.04
Metabolism of xenobiotics by cytochrome P450	M0_nonREM	2.81	0.006
Stilbenoid_diarylheptanoid and gingerol biosynthesis	M0_nonREM	2.42	0.03
beta_Lactam resistance	M0_nonREM	2.11	0.03
Pyrimidine metabolism	M0_REM	3.14	0.04
Aminobenzoate degradation	M0_nonREM	2.89	0.04
Glutathione metabolism	M0_nonREM	2.81	0.02
Fatty acid metabolism	M0_nonREM	2.91	0.03
D_Arginine and D_ornithine metabolism	M0_nonREM	2.37	0.03
Retinol metabolism	M0_nonREM	2.31	0.009
Purine metabolism	M0_REM	2.99	0.04
Drug metabolism_other enzymes	M0_REM	2.45	0.04
Xylene degradation	M0_REM	2.31	0.04
Valine_leucine and isoleucine degradation	M0_nonREM	2.99	0.02
Alanine_aspartate and glutamate metabolism	M0_REM	2.67	0.02
Chloroalkane and chloroalkene degradation	M0_nonREM	2.70	0.009
Limonene and pinene degradation	M0_nonREM	2.80	0.03
Tryptophan metabolism	M0_nonREM	2.86	0.04
Phosphonate and phosphinate metabolism	M0_nonREM	2.13	0.04
Nicotinate and nicotinamide metabolism	M0_REM	2.53	0.007
Lysine degradation	M0_nonREM	2.76	0.03
Drug metabolism_cytochrome P450	M0_nonREM	2.85	0.007
One carbon pool by folate	M0_REM	2.64	0.04

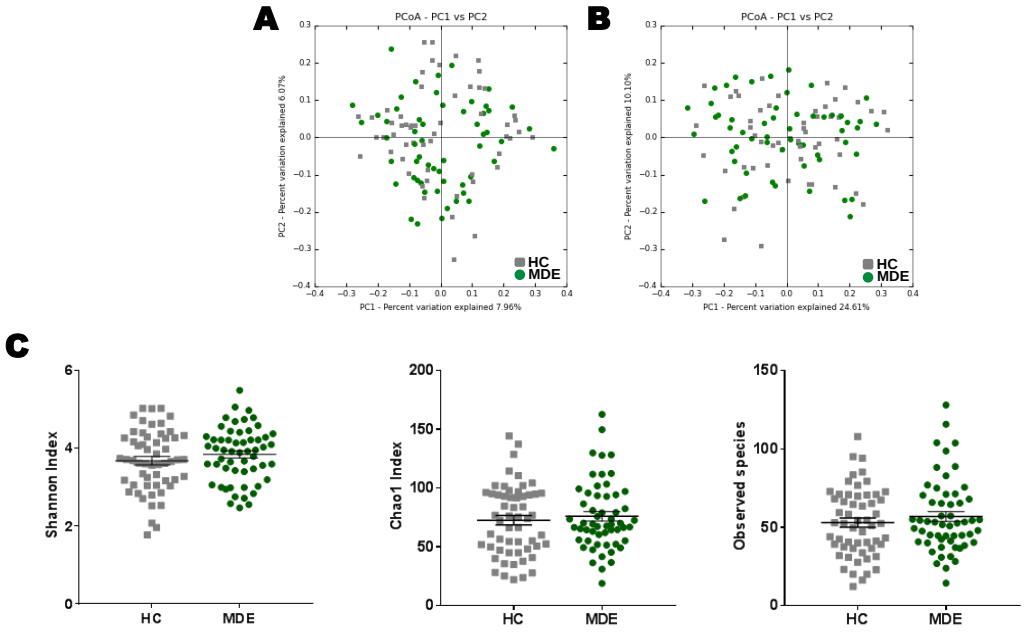
Appendix 1 to Ciocan D, Cassard AM, Becquemont L et al. Blood microbiota and metabolomic signature of major depression before and after antidepressant treatment: a prospective case-control study. *J Psychiatry Neurosci* 2021. doi: 10.1503/jpn.200159

Supplementary Table 11: Variables independently associated with response to antidepressant treatment in depressed patients**.**

Variables	Coefficient	95 % Confidence Interval		Chisq	P
Sex (female)	-0.26	-2.04	1.36	0.10	0.75
Age (years)	0.00	-0.05	0.06	0.00	0.96
Baseline HDRS	-4.35	-9.40	0.03	3.79	0.05
MDE history	1.36	-0.01	3.04	3.76	0.05
Suicide attempt history	0.10	-1.21	1.46	0.02	0.88
Diabetes (yes)	0.69	-2.89	4.11	0.17	0.68
Baseline BMI	-1.13	-5.41	2.80	0.32	0.57
Smoking (yes)	-0.87	-2.51	0.59	1.34	0.25
Firmicutes	1.39	-2.09	5.20	0.61	0.44
f_Intrasporangiaceae_Other (*Tetrasphaera*)	-5.42	-57.53	15.13	0.25	0.62
o_Rhizobiales_f_g (*Bosea*)	**-6.86**	**-13.52**	**-1.73**	**7.07**	**0.01**
Tryptophan	**0.08**	**0.01**	**0.17**	**5.55**	**0.02**
Octadecadienylcarnitine	-2.51	-5.90	0.28	3.06	0.08

Appendix 1 to Ciocan D, Cassard AM, Becquemont L et al. Blood microbiota and metabolomic signature of major depression before and after antidepressant treatment: a prospective case-control study. *J Psychiatry Neurosci* 2021. doi: 10.1503/jpn.200159

Supplementary fig 1



Supplementary Figure 1. Inter- (beta) and intra- (alpha) individually diversity of blood microbiota in depressed patients (MDE, n=56) and matched healthy controls (HC, n=56). (A) Unweighted distances and **(B)** weighted UniFrac distances showing a difference in the composition of the blood microbiota between MDE (green) patients and HC (blue) (p = 0.01 for unweighted UniFrac distances, ANOSIM test). Each point represents a subject and the distance between points is proportional to the similarity of the blood microbiota. **(C)** Alpha diversity assed by the Shannon Index (p=0.4), Chao1 Index (p=0.7) and Observed Species (p=0.5, unpaired Mann-Whitney test for all the alpha diversity comparisons).

Appendix 1 to Ciocan D, Cassard AM, Becquemont L et al. Blood microbiota and metabolomic signature of major depression before and after antidepressant treatment: a prospective case-control study. *J Psychiatry Neurosci* 2021. doi: 10.1503/jpn.200159

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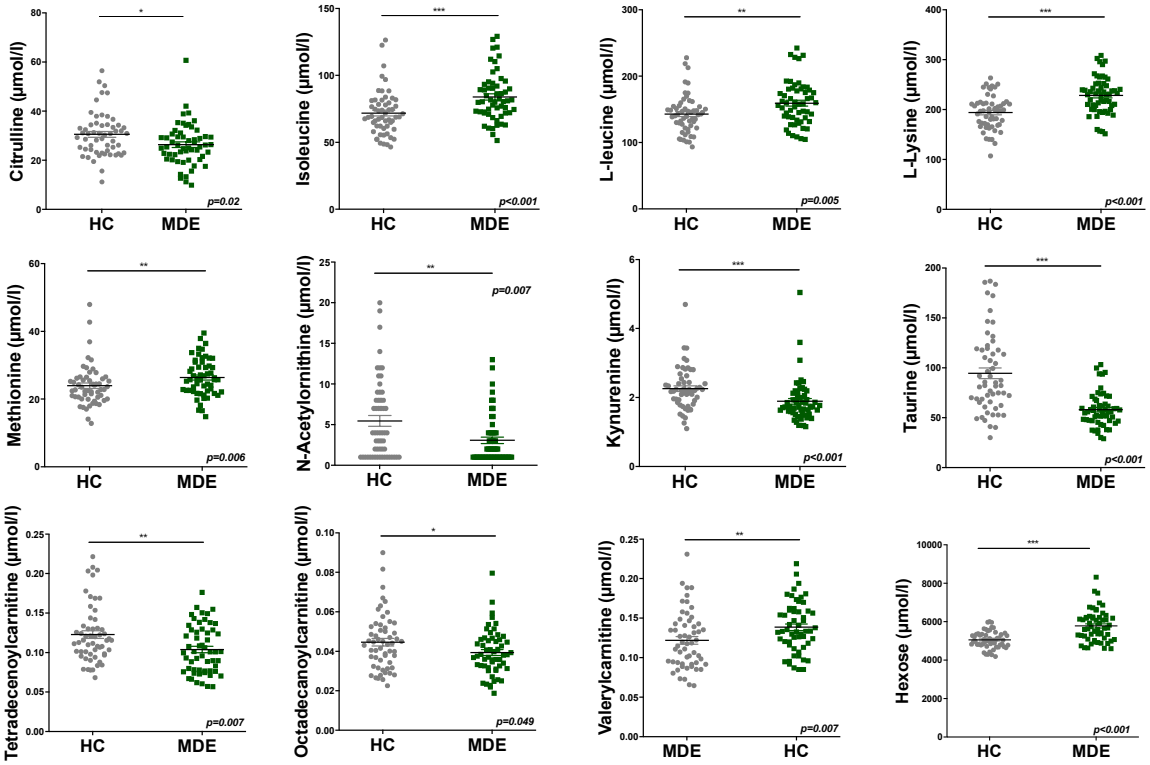
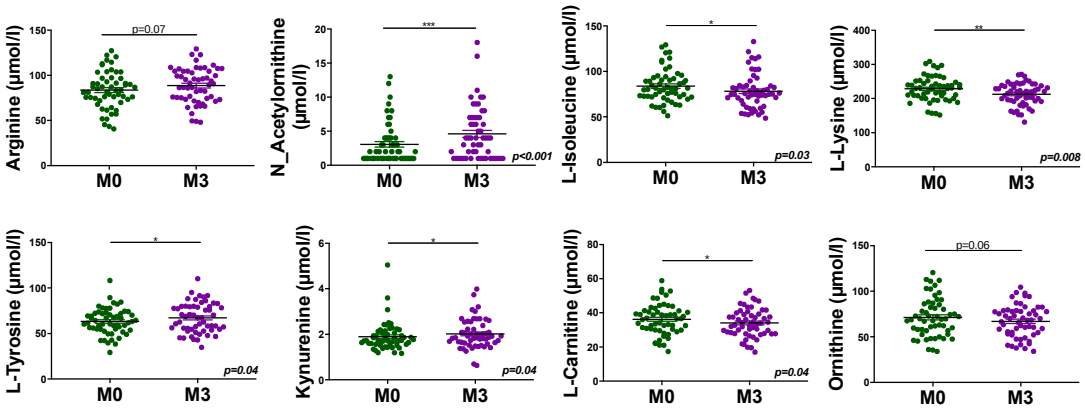
Supplementary fig 2



Supplementary Figure 2. Absolute plasma concentrations of the significant single metabolites that are different between depressed patients (MDE, n=56) and matched healthy controls (HC, n=56).

Appendix 1 to Ciocan D, Cassard AM, Becquemont L et al. Blood microbiota and metabolomic signature of major depression before and after antidepressant treatment: a prospective case-control study. *J Psychiatry Neurosci* 2021. doi: 10.1503/jpn.200159

Supplementary fig 3



episode before (n=56) and after (n=56) treatment.

Appendix 1 to Ciocan D, Cassard AM, Becquemont L et al. Blood microbiota and metabolomic signature of major depression before and after antidepressant treatment: a prospective case-control study. *J Psychiatry Neurosci* 2021. doi: 10.1503/jpn.200159

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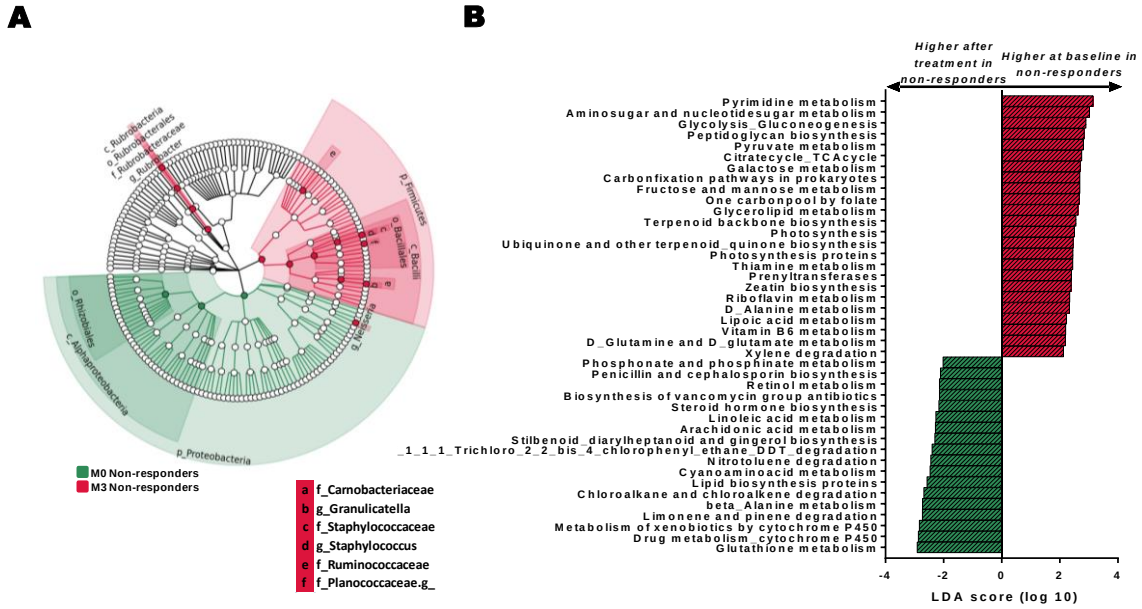
Supplementary fig 4



Supplementary Figure 4. Blood microbiota profiles before and after treatment in depressed patients (MDE), who do not respond to treatment (n=24). (A) Cladogram showing the taxa with the largest differences in abundance at according to the response to treatment: non-responders at baseline (green) and non-responders after treatment (red). From inside to outside, the circles represent the phylum, class, order, family, and genus. Only taxa with a LDA score > 2 and $p < 0.05$, determined by the Wilcoxon signed rank test, are shown. **(B)** LEfSe analysis for the predicted metagenome metabolic pathways (KEGG modules) in the baseline blood microbiota according to the response to treatment remitters (green) vs. non-remitters (pink). Only pathways with a LDA score > 2.0, $p < 0.05$ determined by the Wilcoxon signed rank test are shown.

Appendix 1 to Ciocan D, Cassard AM, Becquemont L et al. Blood microbiota and metabolomic signature of major depression before and after antidepressant treatment: a prospective case-control study. *J Psychiatry Neurosci* 2021. doi: 10.1503/jpn.200159

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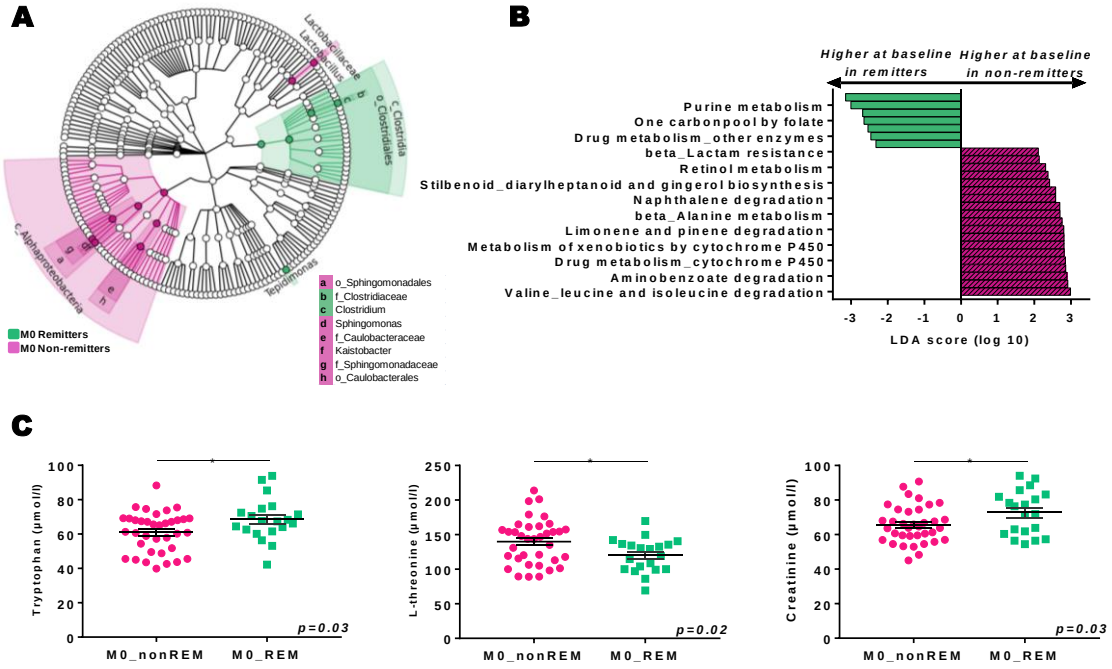
Supplementary fig 5



Supplementary Figure 5. Pre-treatment blood microbiota profile in depressed patients (MDE) who will be remitters after treatment (n=24). (A) Cladogram showing the taxa with the largest differences in abundance at baseline according to the response to treatment: remitters (green) and non-remitters (pink). From inside to outside, the circles represent the phylum, class, order, family, and genus. Only taxa with a LDA score > 2 and p < 0.05, determined by the Wilcoxon signed rank test, are shown. **(B)** LEfSe analysis for the predicted metagenome metabolic pathways (KEGG modules) in the baseline blood microbiota according to the response to treatment remitters (green) vs. non-remitters (pink). Only pathways with a LDA score > 2.0, p < 0.05 determined by the Wilcoxon signed rank test are shown. **(C)** Absolute plasma concentrations of the significant single metabolites that are different between depressed patients according to remission.