Relationship of Ketamine's Plasma Metabolites with Response, Diagnosis, and Side Effects in Major Depression

Supplemental Information

Table S1. Cytochrome P450 enzymes involved in the metabolism of ketamine and its metabolites.

Drug / Metabolite	Abbreviation	Cytochrome P450 (CYP) Enzymes Involved in Formation of Ketamine Metabolites
(R,S) Ketamine	Ketamine	
(R,S) Norketamine	NK	CYP2B6, CYP3A, CYP2A6, CYP2C8, CYP2D6, CYP2C9
(R,S) Dehydronorketamine	DHNK	CYP2B6, CYP2A6, CYP2C8
(2S,6S; 2R,6R)-hydroxynorketamine	HNK4a	CYP2B6, CYP2A6, CYP3A, CYP2C19, CYP2C8
(2S,6R; 2R,6S)-hydroxynorketamine	HNK4b	
(2S,5S; 2R,5R)-hydroxynorketamine	HNK4c	
(2S,4S; 2R,4R)-hydroxynorketamine	HNK4d	
(2S,4R; 2R,4S)-hydroxynorketamine	HNK4e	
(2S,5R; 2R,5S)-hydroxynorketamine	HNK4f	
(2S,6S; 2R,6R)-hydroxyketamine	HK5a	CYP2A6, CYP2C19, CYP3A
(2S,6R; 2R,6S)-hydroxyketamine	HK5b	

Table S2. List of CYPs, their corresponding alleles, SNP rs ID, phenotypic consequence and assay ID.

Gene	Allele	SNP rs ID	Nucleotide	Phenotype	Assay ID [1]
CYP2B6	2B6*2	rs8192709	64C>T	PM	C___2818162_20
	2B6*4	rs2279343	785A>G	EM	PCR-RFLP [2]
	2B6*5	rs3211371	1459C>T	EM	C__30634242_40
	2B6*6	rs2279343, rs3745274	785A>G, 516G>T	PM	PCR-RFLP [2], C___7817765_60
	2B6*9	rs3745274	516G>T	PM	C___7817765_60
	2B6*18	rs28399499	983T>C	PM	C__60732328_20
CYP2A6	2A6*2	rs1801272	479T>A	PM	C__27861808_60
	2A6*9	rs28399433	(-)48T>G	PM	C__30634332_10
CYP2C19	2C19*2	rs4244285	681G>A	PM	C__25986767_70
	2C19*3	rs4986893	636G>A	PM	C__27861809_10
	2C19*17	rs12248560	-806C>T	UM	C____469857_10
CYP3A5	3A5*3	rs776746	6986A>G	PM	C__26201809_30
	3A5*6	rs10264272	711G>A	PM	C__30203950_10
	3A5*7	rs41303343	280312T>TT	PM	C__32287188_10

CYP, cytochrome 450; PCR, polymerase chain reaction; SNP, single nucleotide polymorphism.

[1] Genotyping was performed for all the CYPs and their corresponding alleles (except *CYP2B6*4*) using the predeveloped TaqMan Genotyping Assays (Applied Biosystems, Foster City, CA) following the manufacturer's instructions.

[2] The *CYP2B6*4* allele was assayed by using PCR restriction length fragment polymorphism (PCR-RFLP) assay using a protocol modified from (1). Briefly, a 1474 bp PCR product for *CYP2B6*4* was generated using the following primers (IDT DNA, Coraville, IA): forward primer: 5'GTAGTCCTAACATGTCAGCAG and reverse primer: 5' AGAGCCTACAGTGCTCCCA. The PCR was carried out using JumpStart REDAccuTaq LA DNA polymerase (Sigma, St. Louis, MO) in a 16 µl reaction volume containing 30 ng of the genomic DNA. The initial denaturation was at 94ºC for 4 min, followed by 35 cycles of denaturation at 94ºC for 30s, annealing at 62ºC for 30s and extension at 68ºC for

2 min. A final extension was performed at 68ºC for 10 min. The PCR product (13 µl) was then digested using the restriction enzyme, Sty1 (New England Biolabs Inc., Beverly, MA) and was analyzed by 3% agarose gel electrophoresis. The wildtype *CYP2B6* allele (i.e., nucleotide A) resulted in 56/171/340/907 bp products and the variant allele (i.e., nucleotide G) resulted in 56/340/1078 bp product.

For each of the CYP gene, alleles that did not carry any of the variations. Based on the SNPs present, an individual can be classified as extensive metabolizers (with 'normal' enzyme activity; EM), poor metabolizers (reduced or no enzyme activity; PM), or ultra-rapid metabolizers (increased enzyme activity; UM).

Table S3. Average plasma concentrations (ng/mL) of ketamine and its major metabolites in the plasma of patients suffering from bipolar depression and major depressive disorder.

	40 m	**80 m**	**110 m**	**230 m**	**Day 1**
Bipolar Depression					
Ket	177.23 ± 53.8	83.21 ± 28.17	60.02 ± 25.01	27.63 ± 14.52	9.19 ± 10.92
NK	63 ± 24.82	69.96 ± 19.98	63.35 ± 20.55	43.49 ± 16.88	14.36 ± 9.27
DHNK	28.07 ± 18.72	48.07 ± 26.43	50.5 ± 27.44	43.08 ± 23.76	16.87 ± 13.51
4a	19.29 ± 7.99	29.56 ± 9.44	34.9 ± 10.62	37.59 ± 14.23	21.36 ± 9.39
4b	15.73 ± 5.74	9.09 ± 4.34	6.9 ± 3.57	4.51 ± 3.08	BQ
4c	6.12 ± 4.21	8.78 ± 5.27	7.96 ± 3.96	6.62 ± 3.75	BQ
4f	8.06 ± 6.31	10.63 ± 5.94	10.1 ± 6.14	7.38 ± 3.65	BQ
5a	BQ	BQ	BQ	ND	ND
5b	ND	ND	ND	ND	ND
Major Depressive Disorder					
Ket	204.13 ± 101.46	93.5 ± 31.06	65.03 ±23.17	33.86 ± 19.04	BQ
NK	55.52 ± 33.87	73.54 ± 31.86	62.74 ± 26.78	46 ± 22.97	12.39 ± 8.47
DHNK	7.52 ± 4.8	12.02 ± 6.19	13.27 ± 6.92	10.17 ± 6.65	BQ
4a	10.37 ± 6.26	18.56 ± 9.25	22.18 ± 8.06	23.19 ± 11.88	10.56 ± 6.76
4b	12.15 ± 6.56	6.12 ± 3.03	4.6 ± 2.26	BQ	BQ
4c	BQ	BQ	BQ	BQ	BQ
4f	4.38 ± 5.82	6.96 ± 7.29	6.57 ± 7.09	4.3 ± 5.09	BQ
5a	8.11 ± 4.99	9.24 ± 5.63	8.62 ± 5.94	6.64 ± 6.32	BQ
5b	ND	ND	ND	ND	ND

BQ, below quantitation; DHNK, dehydronorketamine; Ket, ketamine; ND, not detectable; NK, norketamine.

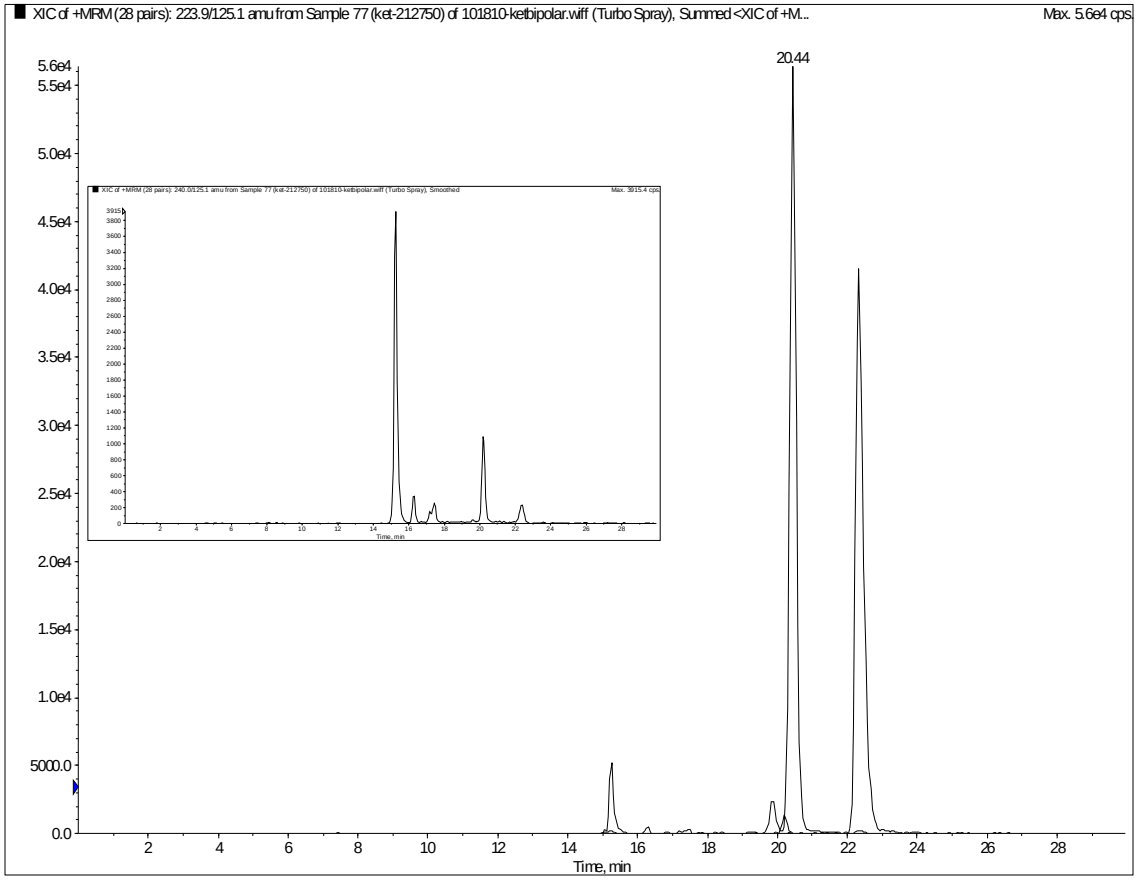

Figure S1. A representative chromatogram of ketamine and its major metabolites in the plasma samples obtained from patients with bipolar depression.

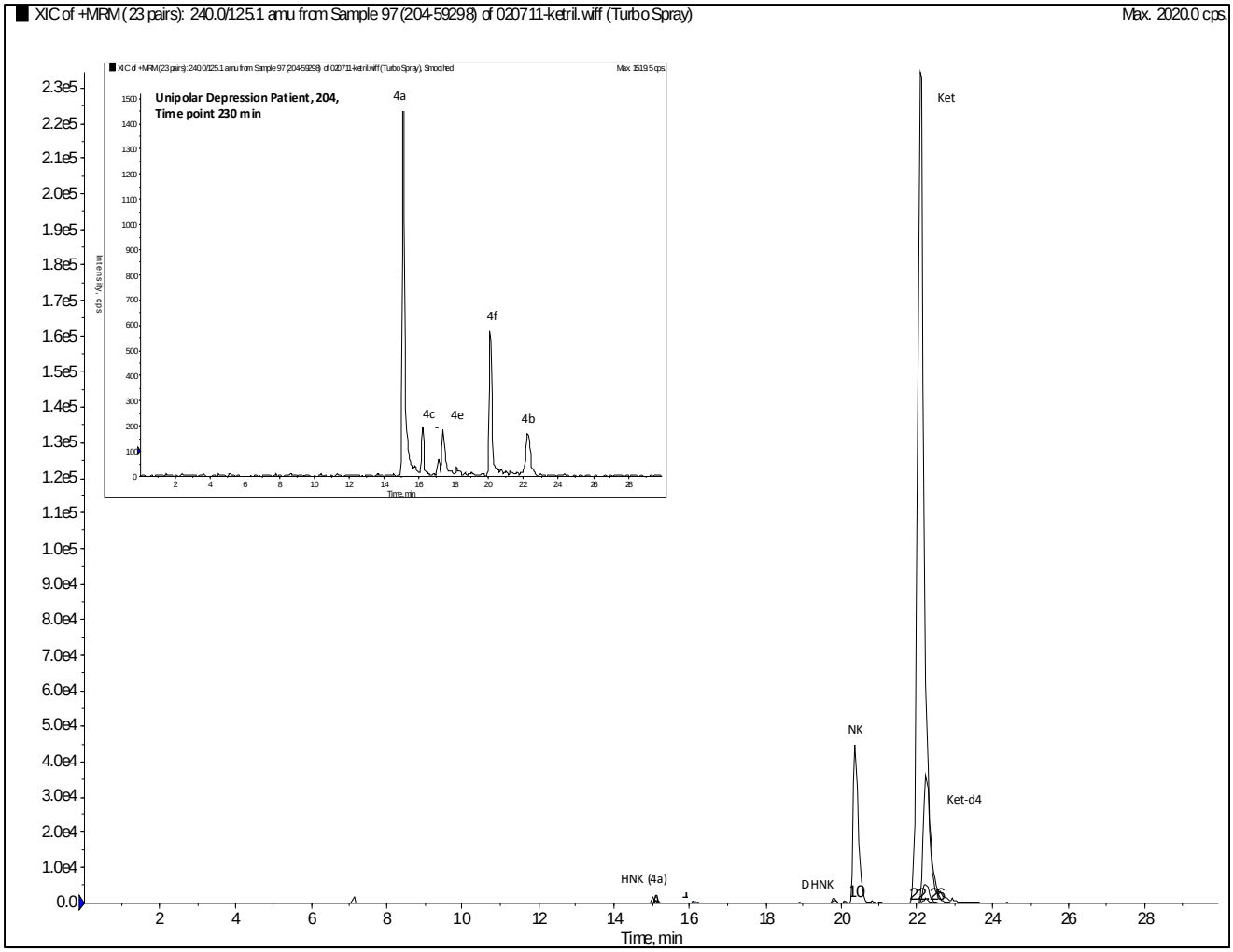

Figure S2. A representative chromatogram of ketamine and its major metabolites in the plasma samples obtained from patients with major depressive disorder.

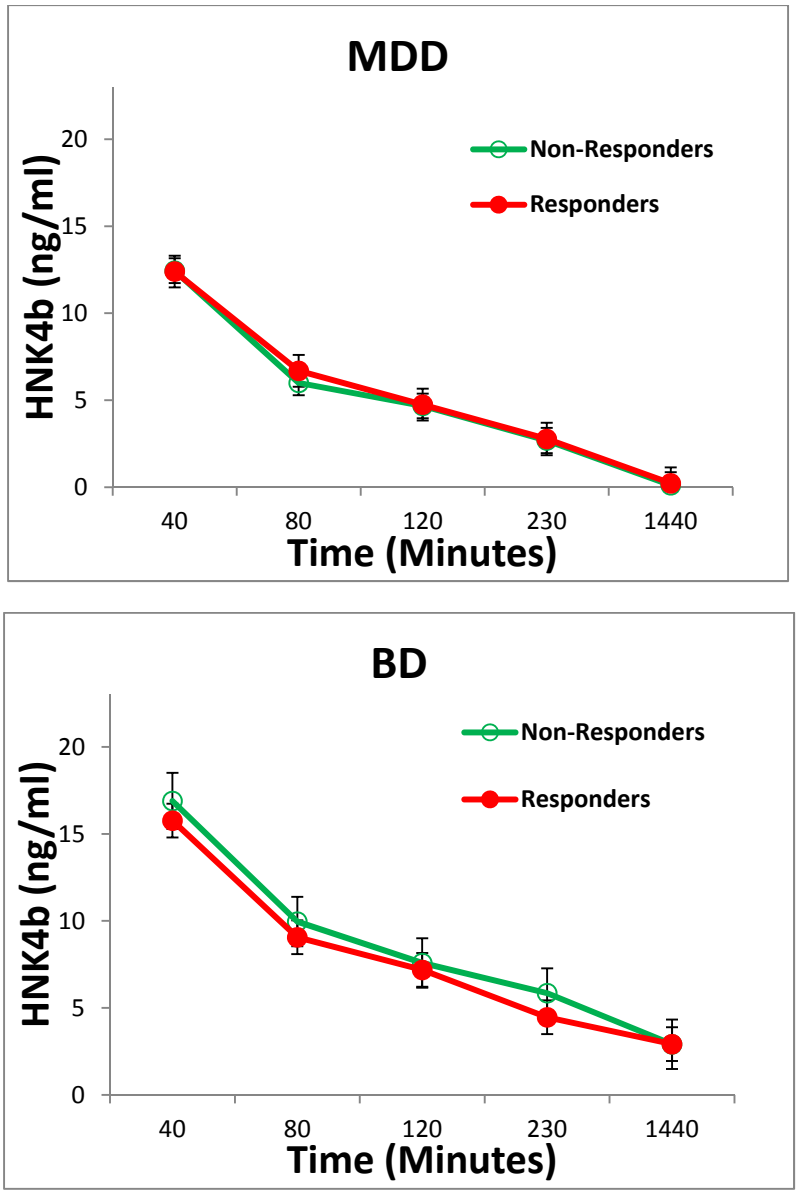

Figure S3. HNK4b plasma concentrations by response and diagnosis in patients with treatment-resistant depression. HNK4b plasma concentrations were significantly higher in patients with BD than MDD. BD, bipolar depression; MDD, major depressive disorder.

Supplemental References

1. Jacob RM, Johnstone EC, Neville MJ, Walton RT (2004): Identification of CYP2B6 sequence variants by use of multiplex PCR with allele-specific genotyping. *Clin Chem* 50:1372-1377.