Supplementary Information

Targeted metabolomics detects a putatively diagnostic signature in plasma and dried blood spots from head and neck paraganglioma patients

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***Supplementary Table S1*. Mass spectrometry (MS) parameters used for targeted flow injection analysis (FIA) coupled with tandem MS (MS/MS).** Multiple reaction monitoring (MRM) transition, cone voltage and collision energy (CE) are shown for each analyte. Internal standards (IS) are in brackets. *3-(5-methyl-1H-pyrazol-3-yl) propanoic acid (MPP) is used as IS for succinylacetone (SA).

Abbreviation (ISs)	Analyte	MRM transition	Cone (V)	CE (eV)
Arg ($^{2}H_4$, ^{13}C-Arg)	Arginine	175.1>70.1 180.1>75.1	34	21
Gly ($^{15}N_2$-^{13}C-Gly)	Glycine	76.0>30.0 78.0>32.0	22	7
Ala ($^{2}H_3$-Ala)	Alanine	90.1>44.0 93.1>47.1	22	8
Pro ($^{13}C_5$-Pro)	Proline	116.1>70.1 121.1>74.1	28	12
Orn ($^{2}H_6$-Orn)	Ornithine	133.1>70.1 139.1>76.1	22	16
Gln/Lys ($^{13}C_5$-Gln)	Glutamine/Lysine	147.1>84.0 152.1>88.1	22	16
Glu ($^{13}C_5$-Gln)	Glutamate	148.1>84.0 152.1>88.1	24	14
SA ($^{13}C_5$-MPP*)	Succinylacetone	155.1>109.1 160.1>114.1	24	22
ASA ($^{2}H_4$, ^{13}C-Arg)	Argininosuccinic acid	291.1>70.1 180.1>75.1	42	30
Ado ($^{2}H_5$-Ado)	Adenosine	268.1>136.1 273.1>136.1	32	18
dAdo ($^{2}H_5$-dAdo)	2-Deoxyadenosine	252.1>136.1 257.1>136.1	29	14
Cit ($^{2}H_2$-Cit)	Citrulline	176.1>113.1 178.1>115.1	24	16
Leu/Ile/Pro-OH ($^{2}H_3$-Leu)	Leucine/Isoleucine/Hydroxyproline	132.1>86.1 135.1>89.1	24	10
Met ($^{2}H_3$-Met)	Methionine	150.1>104.1 153.1>107.1	24	10
Phe ($^{13}C_6$-Phe)	Phenylalanine	166.1>120.1 172.1>126.1	25	12
Tyr ($^{13}C_6$-Tyr)	Tyrosine	182.1>136.1 188.1>142.1	26	12

Abbreviation (ISs)	Analyte	MRM transition	Cone (V)	CE (eV)
Val **($^{15}N_2$-$^{13}C_5$-Val)**	Valine	118.1>72.1 124.1>77.1	23	10
C0 **($^2H_9$-C0)**	Free carnitine	162.1>1030 171.2>103.0	38	16
C2 **($^2H_3$-C0)**	Acetylcarnitine	204.1>85.0 207.1>85.0	34	18
C3 **($^2H_3$-C3)**	Propionylcarnitine	218.1>85.0 221.2>85.0	32	18
C4 **C3DC/C4OH** **($^2H_3$-C4)**	Butyrylcarnitine Malonylcarnitine/3-hydroxybutyrylcarnitine	232.2>85.0 248.1>85.0 235.2>85.0	36	18
C5 **C5:1** **C4DC/C5OH** **($^2H_9$-C5)**	Valerylcarnitine Tiglylcarnitine Methylmalonylcarnitine/3-hydroxyisovalerylcarnitine	246.2>85.0 244.2>85.0 262.1>85.0 255.2>85.0	38	20
C6 **C6DC** **($^2H_3$-C6)**	Hexanoylcarnitine Methylglutarylcarnitine	260.2>85.0 290.2>85.0 263.2>85.0	37	20
C5DC/C6OH **($^2H_6$-C5DC)**	Glutarylcarnitine/3-Hydroxy-hexanoylcarnitine	276.2>85.0 282.2>85.0	40	24
C8 **C8:1** **($^2H_3$-C8)**	Octanoylcarnitine Octenoylcarnitine	288.2>85.0 286.2>85.0 291.2>85.0	42	22
C10 **C10:1** **C10:2** **($^2H_3$-C10)**	Decanoylcarnitine Decenoylcarnitine Decadienoylcarnitine	316.2>85.0 312.2>85.0 314.2>85.0 319.3>85.0	45	22
C12 **C12:1** **($^2H_3$-C12)**	Dodecanoylcarnitine Dodecenoylcarnitine	344.3>85.0 342.3>85.0 347.3>85.0	46	24
C14 **C14:1** **C14:2** **C14:OH** **($^2H_3$-C14)**	Tetradecanoylcarnitine (myristoylcarnitine) Tetradecenoylcarnitine Tetradecadienoylcarnitine Hydroxytetradecenoylcarnitine	372.3>85.0 370.3>85.0 368.3>85.0 388.3>85.0 375.3>85.0	52	25

Abbreviation (ISs)	Analyte	MRM transition	Cone (V)	CE (eV)
C16	Hexadecanoylcarnitine (palmitoylcarnitine)	400.3>85.0	55	26
C16:1	Hexadecenoylcarnitine	398.3>85.0		
C16:1OH/C17	Hydroxyhexadecenoylcarnitine	414.3>85.0		
C16:OH	Hydroxyhexadecanoylcarnitine	416.3>85.0		
($^2H_3$-C16)		403.4>85.0		
C18	Octadecanoylcarnitine	428.4>85.0	56	28
C18:1	Octadecenoylcarnitine	426.4>85.0		
C18:2	Octadecadienoylcarnitine	424.3>85.0		
C18:OH	Hydroxyoctadecanoylcarnitine	444.4>85.0		
C18:1OH	Hydroxyoctadecenoylcarnitine	442.4>85.0		
C18:2OH	Hydroxyoctadecadienoylcarnitine	440.3>85.0		
($^2H_3$-C18)		431.4>85.2		
C20	Arachidic-carnitine	456.4>85.0	69	34
C22	Behenic-carnitine	484.4>85.0		
C24	Tetracosanoic-carnitine	512.5>85.0		
C26	Hexacosanoic-carnitine	540.5>85.0		
($^2H_3$-C26)		543.5>85.0		
C20:0-LPC	1-arachidoyl-2-hydroxy-sn-glycero-3-phosphocholine	552.4>104.1	74	30
C22:0-LPC	1-behenoyl-2-hydroxy-sn-glycero-3-phosphocholine	580.4>104.1		
C24:0-LPC	1-lignoceroyl-2-hydroxy-sn-glycero-3-phosphocholine	608.5>104.1		
C26:0-LPC	1-hexacosanoyl-2-hydroxy-sn-glycero-3-phosphocholine	636.5>104.1		
($^2H_4$-C26:0-LPC)		640.5>104.1		

Supplementary Table 2. List of the 71 skull base tumors (59 paragangliomas, 10 acoustic neuromas and 2 cholesteatomas) and 24 healthy controls cases included in the study. Tumor site, gender. age at surgery and stage are reported for all cases of skull base tumors, mutational status only for paragangliomas. Gender and age are reported for healthy controls.

Case code	Tumor site	Gender	Age at surgery	Fisch stage* or Shamblin class**	*SDHx* mutational status
PTJ79	Left tympanojugular	M	40	C2 Di2	Noncarrier
PTJ86	Left tympanojugular	M	15	C1	*SDHD c.27delC*
PTJ115	Right tympanojugular	F	39	C3 De2	*Noncarrier*
PTJ116	Left tympanojugular	M	47	C2 Di1	*SDHC c.251T>C ete p.Leu84Pro*
PTJ117	Left tympanojugular	F	43	C2	*Noncarrier*
PTJ118	Right tympanojugular	F	16	C3 Di2	*SDHB c.287-1G>C*
PTJ119	Right tympanojugular	M	41	C2 Di2	*Noncarrier*
PTJ119^{R}	Right tympanojugular (intradural residual)	M	42	C2 Di2	*Noncarrier*
PC120	Left carotid body	F	44	Class II	*Noncarrier*
PTJ121	Left tympanojugular	F	12	C2	*Noncarrier*
PTJ122	Right tympanojugular	M	26	C1	*SDHD c.314+1G>C*
PTJ123	Left tympanojugular	F	51	C1	*Noncarrier*
PTJ125	Right tympanojugular	M	50	C2	*Noncarrier*
PTJ128	Left tympanojugular	F	41	C3 Di2	*SDHB c.72+10G>A; SDHB c.541-3C>G*
PTJ130	Right tympanojugular	M	49	C3 Ve1	*Noncarrier*
PTJ130^{R}	Right tympanojugular (intradural residual)	M	49	C3 Ve1	*Noncarrier*
PTJ131	Right tympanojugular	F	58	C3 De2	*SDHC c.224G>A p.(Gly75Asp)*
PT132	Left tympanojugular	F	45	A2	*Noncarrier*
PTJ133	Left tympanojugular	F	37	C4 Di2 Ve2	*SDHB c.286+1G>A*
PTJ133^{R}	Left tympanojugular (intradural residual)	F	37	C4 Di2 Ve2	*SDHB c.286+1G>A*
PC134	Left carotid body	F	39	Class III	*Noncarrier*
PTJ135	Right tympanojugular	M	38	C2	*Noncarrier*
PTJ136	Left tympanojugular	M	44	C3 De1	*Noncarrier*
PTJ137	Right tympanojugular	F	51	B1	*Noncarrier*
PTJ140	Right tympanojugular	F	47	C1 Di1	*Noncarrier*
PTJ141	Right tympanojugular	M	29	C2 De1	*SDHB c.303T>G*

PTJ142	Right tympanojugular	F	62	C3	*Noncarrier*
PTJ143	Left tympanojugular	M	21	C2	*SDHD c.305A>C p.His102Pro*
PTJ145	Left tympanojugular	F	34	C2/Class I	*SDHD c.52+2T>C*
PTJ146	Left tympanojugular	M	35	C1	*Noncarrier*
PTJ147	Left tympanojugular	F	53	C2 Di1	*Noncarrier*
PTJ148	Right tympanojugular	F	50	C1	*SDHB c.73-8A>G*
PC149	Right carotid body	F	55	Class II/III	*SDHAF2 c.233G>A p.(Gly78Glu)*
PTJ150	Left tympanojugular	M	50	C2	*Noncarrier*
PTJ151	Right tympanojugular	M	24	C2	*SDHA c.1304 T>A p.(Leu435Gln)*
PC152	Left carotid body	M	38	Class III	*Noncarrier*
PTJ154	Right tympanojugular	F	25	C2 De1	*SDHA c.1151C>G p.(Ser384*)*
PT155	Right tympanic	M	47	A1	*Noncarrier*
PTJ156	Right tympanojugular	F	32	C2	*SDHB c.546dup p.(Leu183Alafs*11)*
PTJ157	Right tympanojugular	M	64	C1	*Noncarrier*
PV158	Left vagal	F	63	Class III	*Noncarrier*
PTJ159	Left tympanojugular	F	46	C2	*Noncarrier*
PTJ161	Right tympanojugular	F	63	C2 De2	*Noncarrier*
PT168	Right tympanic	F	43	B3	*Noncarrier*
PC169	Left carotid body	F	56	NA	*SDHB c.778G>C p.(Gly260Arg)*
PV170	Left vagal	F	67	NA	*SDHA c.1766G>A p.(Arg589Gln)*
PTJ171	Right tympanojugular	F	27	C2 De1	*SDHD c.52+2T>C (variante non nota)*
PTJ173	Right tympanojugular	M	53	C4 Di1 Ve	*SDHC c.(20+1_21-1)_(77+1_78-1)del*
PTJ175	Left tympanojugular	F	58	C2 Di2	*Noncarrier*
PTJ176	Left tympanojugular	M	26	C1	NA
PTJ177	Right tympanojugular	M	26	C1	NA
PTJ178	Tympanojugular	F	58	NA	NA
PV179	Right vagal	F	30	Class IV	NA
PV180	Left vagal	M	53	NA	NA
PV181	Right vagal	F	41	Class IV	NA
PTJ182	Right tympanojugular	M	53	C2	NA
PTJ187	Left tympanojugular	F	68	C2	NA
PT188	Left tympanic	M	83	A1	*Noncarrier*
PTJ189	Right tympanojugular	M	49	C2	NA

Case code	Skull base tumors other than PGL	Gender	Age at surgery	Hannover classification***	
AN1	Acoustic neuroma	F	62	T4a	
AN2	Acoustic neuroma	F	28	T4a	
AN3	Acoustic neuroma	M	60	T2	
AN4	Acoustic neuroma	M	44	T3a	
AN5	Acoustic neuroma	M	43	T3b	
AN6	Acoustic neuroma	F	53	T1	
AN7	Acoustic neuroma	F	53	T3a	
AN8	Acoustic neuroma	M	58	T2	
AN9	Acoustic neuroma	M	75	T2	
AN10	Acoustic neuroma	M	66	T3a	
CH1	Cholesteatoma	M	25	-	
CH2	Cholesteatoma	M	45	-	
Case code	**Healthy controls**	**Gender**	**Age**		
HC1		F	45		
HC2		F	51		
HC3		M	27		
HC4		M	56		
HC5		F	53		
HC6		M	52		
HC7		F	59		
HC8		M	65		
HC9		M	36		
HC10		F	45		
HC11		M	70		
HC12		M	51		
HC13		F	69		
HC14		F	48		
HC15		M	37		
HC16		F	30		
HC17		F	30		
HC18		F	44		
HC19		F	32		
HC20		M	51		
HC21		M	33		
HC22		F	42		
HC23		M	52		
HC24		F	27		

*: Sanna's modified Fisch stage is reported in letters followed by arabic numbers according to Sanna M, Piazza P, Shin S-H, Flanagan S, Mancini F, Taibah A, et al. Microsurgery of Skull Base Paragangliomas. Microsurg Skull Base Paragangliomas. 2013 May 20; and Prasad SC, Paties CT, Schiavi F, Esposito DL, Lotti LV, Mariani-Costantini R, et al.

Tympanojugular Paragangliomas: Surgical Management and Clinicopathological Features. Paraganglioma A Multidiscip Approach [Internet]. 2019 Jun 13 [cited 2022 Aug 10];99–123. Available from: https://pubmed.ncbi.nlm.nih.gov/31294939/

**: Shamblin class is in roman numerals according to Prasad SC, Paties CT, Pantalone MR, Mariani-Costantini R, Sanna M. Carotid Body and Vagal Paragangliomas: Epidemiology, Genetics, Clinicopathological Features, Imaging, and Surgical Management. Paraganglioma A Multidiscip Approach [Internet]. 2019 Jun 13 [cited 2022 Aug 9];81–98. Available from: https://pubmed.ncbi.nlm.nih.gov/31294944/

***: Hannover classification is reported in letters followed by arabic numbers according to Samii M, Matthies C. Management of 1000 vestibular schwannomas (acoustic neuromas): surgical management and results with an emphasis on complications and how to avoid them. Neurosurgery [Internet]. 1997 Jan [cited 2022 Sep 26];40(1):11–23. Available from: https://pubmed.ncbi.nlm.nih.gov/8971819/

Supplementary Table S3. Age and sex distribution of patients and controls.

	HNPGL[1] n=56	HC[2] (n=25)	AN/CH[3] (n=12)	*p*-value HNPGL vs HC	*p*-value HNPGL vs AN/CH
Age (years)				0.5469	0.1164
Mean	43.84	45.88	51.00		
Median	44.50	45.00	53.00		
SD	14.58	12.50	14.81		
Sex [n (%)]				0.6670	0.1337
Male	24 (43)	12 (48)	8 (67)		
Female	32 (57)	13 (52)	4 (33)		

[1]HNPGL: head and neck paraganglioma patients, [2]HC: healthy controls, [3]AN/CH: acoustic neuroma/cholesteatoma patients

Supplementary Table S4. Clinicopathological and genetic characteristics of the tested head and neck paraganglioma (HNPGL) patients. Sanna's modified Fisch classification, which reflects anatomic location and tumor size, is given for 39 jugulotympanic paragangliomas, Shamblin classification, reflecting carotid vessels involvement, is provided for 7 carotid body paragangliomas.

HNPGL[1] characteristics	**n (%)**
Caroticum	5 (9)
Vagale	5 (9)
Jugulotympanicum	42 (75)
Tympanicum	4 (7)
Sanna's modified Fisch class	**n**
A1	2
A2	1
B1	1
B3	1
C1	9
C2	22
C3	7
C4	2
Shamblin class	**n**
I	0
II	1
II/III	1
III	3
IV	2
Unassigned[1]	4
***SDXx* mutations**	**n (%)**
SDHA	3 (5.4)
SDHB	7 (12.5)
SDHC	3 (5.4)
SDHD	5 (9.0)
SDHAF2	1 (1.8)
Noncarriers	27 (48)
NA[2]	10 (17.9)

([1]: unclassified; [2]: not evaluated).

Supplementary Table S5. Performances of the logistic regression model based on dAdo and C26:0-LPC.

	Estimate	Std. Error	95% CI	Odds ratio
Intercept	-4.296	1.105	-6,945 to -2,478	0,01362
dAdo	20.04	6.485	9,493 to 35,38	5,04E+08
C26:0-LPC	1.532	0.8729	-0,1166 to 3,420	4,628

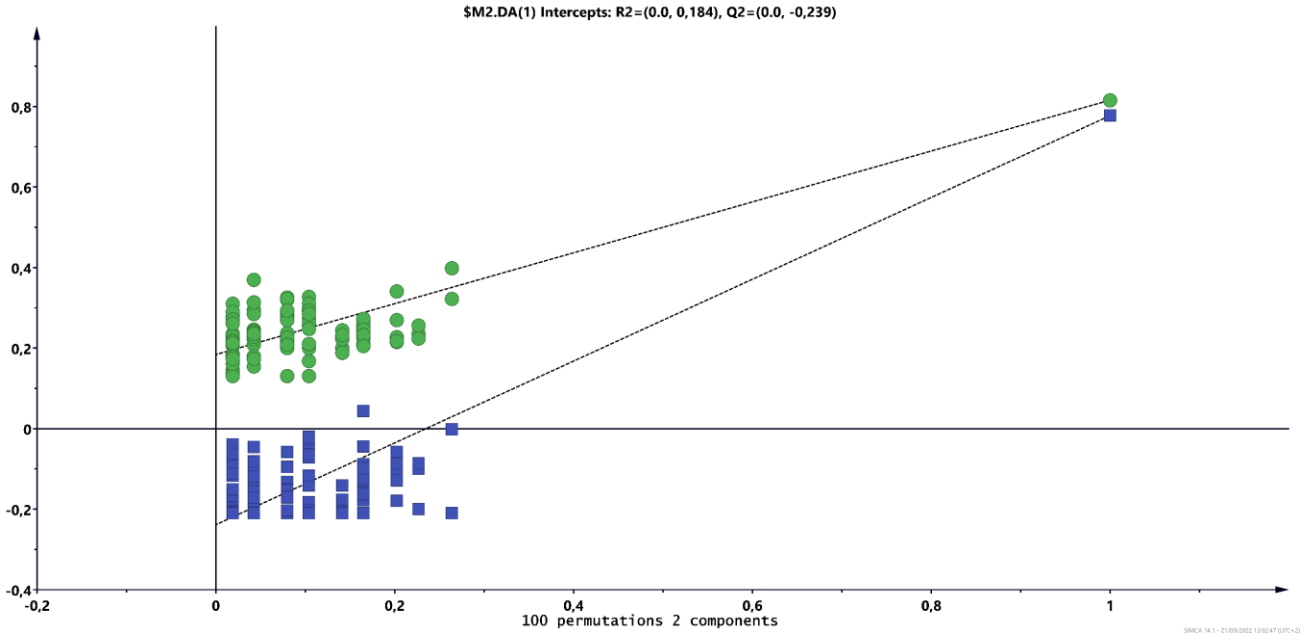

Figure S1. Cross-validation plot of the PLS-DA model obtained by 100 permutations, with R2 values in green and Q2 values in blue.

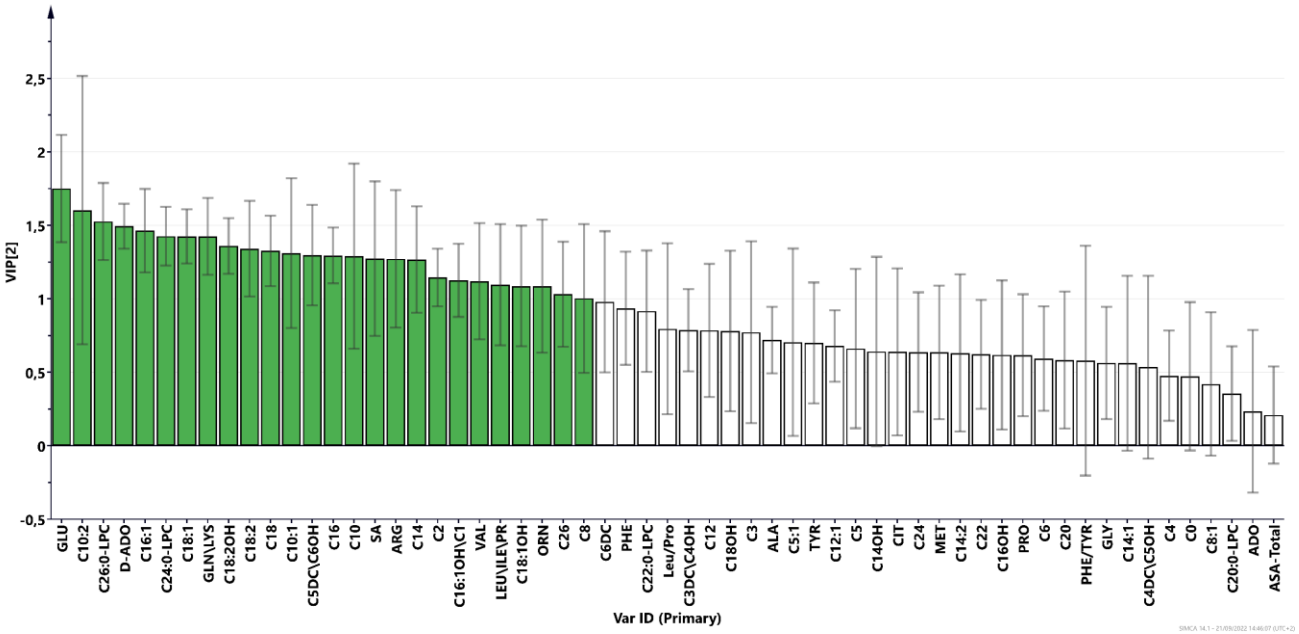

Figure S2. Variable importance in the projection (VIP) plot showing the association of the loadings with VIP values as a measure of their impact on the modeling.

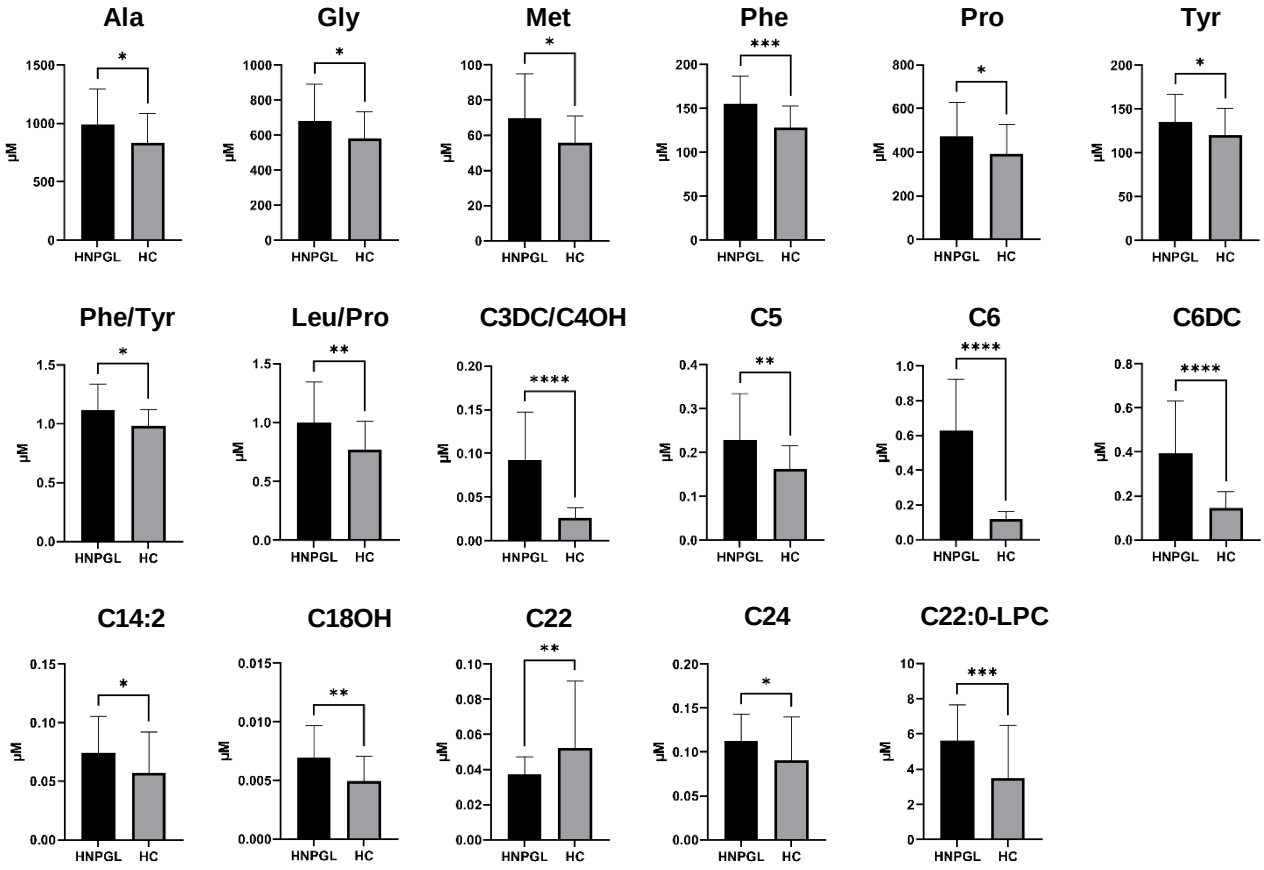

Figure S3. Bar charts for the metabolites with VIP values < 1 that showed significantly different plasma concentrations in the HNPGL patients (HNPGLs) versus the healthy controls (HCs), including amino acids (Ala, Gly, Met, Phe, Pro, Tyr, Phe/Tyr, Leu/Pro), acyl-carnitines (C3DC/C4OH, C5, C6, C6DC, C14:2, C24, C18OH, C22), and lysophosphatidylcholines (C22-0-LPC). Significance was established by t-test.