

Fearful dogs have increased plasma glutamine and γ -glutamyl glutamine

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Supplementary table S1. Detailed demographics of study participants. Information includes age, breed, sex, behavioral scores (human fear variable, situation fear variable and fear reaction variable), fasting status and diet of the dog. Dogs numbered from 1 to 20 are fearful dogs (case dogs) whereas dogs numbered from 21 to 41 are non-fearful dogs (control dogs).

	Age (years)	Breed	Sex	Human fear variable	Situation fear variable	Fear reaction variable	Fasting prior to blood sampling	Diet (Royal Canin Maxi Sensible)
1	7.3	German Shepherd	female	9	1	5	yes	yes
2	4.2	German Shepherd	female	14	2	8	yes	yes
3	3.9	German Shepherd	female	21	6	13.5	yes	yes
4	7.5	German Shepherd	female	21	0	10.5	yes	yes
5	2.1	German Shepherd	female	12	0	6	yes	yes
6	8.2	German Shepherd	female	3	6	4.5	no	yes
7	8.7	German Shepherd	male	2	6	4	yes	yes
8	4.1	German Shepherd	male	15	4	9.5	yes	yes
9	2.9	German Shepherd	male	10	9	8	no	yes
10	3.8	Great Dane	female	24	0	12	yes	yes
11	2.9	Great Dane	female	21	0	10.5	yes	yes
12	2.6	Great Dane	female	10	0	5	yes	yes
13	5.0	Great Dane	female	16	10	13	yes	yes
14	3.4	Great Dane	female	15	12	15.5	no	yes
15	1.6	Great Dane	female	6	0	6	no	yes
16	1.5	Great Dane	female	24	9	17	no	yes
17*	7.5	Great Dane	male	14	12	13	yes	yes
18	5.9	Great Dane	male	16	4	10	yes	yes
19*	6.7	Great Dane	male	30	9	19.5	no	yes
20	4.5	Great Dane	male	16	4	10	no	yes
21	1.6	German Shepherd	female	0	0	0	yes	yes
22	8.2	German Shepherd	female	0	0	0	yes	yes
23	5.6	German Shepherd	female	0	0	0	yes	yes
24	3.1	German Shepherd	female	0	0	0	yes	yes
25	8.6	German Shepherd	female	0	0	0	yes	yes
26	5.4	German Shepherd	female	0	0	0	no	yes
27	8.2	German Shepherd	female	0	0	0	no	yes
28	4.5	German Shepherd	male	0	0	0	yes	yes
29	3.6	German Shepherd	male	0	0	0	no	no
30	2.8	German Shepherd	male	0	0	0	no	yes
31	3.2	Great Dane	female	0	0	0	yes	yes
32	1.9	Great Dane	female	0	0	0	yes	yes
33	4.3	Great Dane	female	0	0	0	no	yes
34	3.0	Great Dane	female	0	0	0	no	yes
35	4.6	Great Dane	female	0	0	0	no	yes
36	2.7	Great Dane	female	0	0	0	no	yes
37	4.1	Great Dane	female	0	0	0	no	yes
38	4.3	Great Dane	male	0	0	0	yes	yes
39	6.4	Great Dane	male	0	0	0	yes	yes
40	5.4	Great Dane	male	0	0	0	yes	yes
41*	5.4	Great Dane	male	0	0	0	no	yes

* Dog was included also in previous metabolomics study cohort of canine fear (Puurunen, J., Tiira, K., Lehtonen, M., Hanhineva, K. & Lohi, H. Non-targeted metabolite profiling reveals changes in oxidative stress, tryptophan and lipid metabolisms in fearful dogs. *Behav. Brain Funct.* **12**, 7 (2016)).

Supplementary Table S2. Characteristics for the statistically significant metabolites in liquid chromatography-mass spectrometry analysis. Information includes molecular weight (MW), identified ion (*m/z*), retention time (rt), analytical mode and fragment ions in the tandem mass spectrometry (MS/MS fragmentation). Variable importance on projection (VIP) -values, fold change values and Mann-Whitney U FDR-corrected p-values (p_{FDR}) are listed as indicators of statistical significance.

Putative annotation	MW	<i>m/z</i>	rt	Mode	MS/MS fragmentation	VIP	Fold change ^a	p_{FDR} ^b
SDMA ¹	202.144	203.151	6.47	HILIC+	ESI(+) 203.150, 172.107, 116.072, 70.067	2.16	1.98	0.032
Glutamine ²	146.069	147.077	6.17	HILIC+	ESI(+) 147.077, 130.050, 84.045	2.14	1.23	0.005
γ -Glu Gln ³	275.113	276.119	7.37	HILIC+	ESI(+) 276.193, 242.065, 147.076, 130.049, 57.069	2.02	1.46	0.005
Unknown	106.024	105.017	0.98	HILIC-		1.61	1.33	0.047
Unknown	700.402	701.410	2.97	HILIC+		1.57	1.23	0.032
Unknown	134.019	133.012	0.98	HILIC-		1.45	1.22	0.047
Unknown	218.058	217.051	0.97	HILIC-		1.26	1.75	0.035
Unknown	173.055	172.047	0.97	HILIC-		1.20	1.47	0.036
threo-(Homo)2-isocitrate ⁴	220.057	219.050	0.97	HILIC-	ESI(-) 219.054, 173.043, 43.000	1.18	1.49	0.038
2-Oxopimelate ⁵	174.051	173.044	0.97	HILIC-	ESI(-) 173.050, 128.041, 43.000	1.16	1.41	0.035
Unknown	100.016	99.009	0.99	HILIC-		1.11	-1.77	0.036

^a Average fold change when compared against the control group. Fold changes $\geq \pm 1.2$ were considered as statistically significant. Positive values indicate increased plasma levels in case dogs vs. control dogs, whereas negative values indicate decreased plasma levels in case dogs vs. control dogs

^b Benjamini-Hochberg false discovery rate (FDR) corrected Mann Whitney U p-value (Benjamini, Y. & Hochberg, Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society. Series B (Methodological)* 57, 289–300 (1995))

SDMA = symmetric dimethylarginine, γ -Glu Gln = γ -glutamylglutamine

Upperscript numbers denote to identification references as follows: ¹ MID44873, Saigusa, D. et al. Determination of Asymmetric Dimethylarginine and Symmetric Dimethylarginine in Biological Samples of Mice Using LC/MS/MS. *Am. J. Anal. Chem.* Vol. 2, pp. 303–313 (2011); ² MID18, HMDB00641; ³ MT000128, Zhang, J. et al. A metabolomics approach for authentication of *Ophiocordyceps sinensis* by liquid chromatography coupled with quadrupole time-of-flight mass spectrometry. *Food Res. Int.* Vol. 76, pp. 489–497 (2015); ⁴ MID71254, KEGG C16597; ⁵ MID23, KEGG C16588

Supplementary Table S3. Human fear and situation fear related questions in the canine behavioral questionnaire, and the scoring of the answers. Questions concerning fearfulness towards strangers (A), and fearfulness in new situation or environment (B). Human fear variable and situation fear variable were derived from questions A and B, respectively.

A. Does your dog show shyness or fear when meeting a strange person?	Score
<i>Withdraws</i>	5
<i>Barks (does not go towards the person)</i>	1
<i>Growls (does not go towards the person)</i>	1
<i>Tail low / between the legs</i>	1
<i>Not willing to make contact</i>	1
<i>Stays close to the owner (even when not under a command)</i>	1
<i>Barks / growls and goes towards a stranger</i>	1
How often does the dog react fearfully?	Score
<i>Always, 100% of the times</i>	4
<i>Almost always, 60-100% of the times</i>	3
<i>Often, 40-60% of the times</i>	2
<i>Rarely, 0-40% of the times</i>	1

B. Does your dog show fear or stress in a new situation or in a new environment?	Score
<i>Wants out of the situation / new environment</i>	1
<i>Barks</i>	1
<i>Tail low / between the legs</i>	1
<i>Stays still, does not want to explore the new environment</i>	1
<i>Stays close to the owner (even when not under a command)</i>	1
<i>Walks low</i>	1
<i>Pants</i>	1
<i>Trembles</i>	1
How often does the dog react fearfully?	Score
<i>Always, 100% of the times</i>	4
<i>Almost always, 60-100% of the times</i>	3
<i>Often, 40-60% of the times</i>	2
<i>Rarely, 0-40% of the times</i>	1