On-Line Only Supplementary Material

Supplement to Pipino, Shah, Prudente, et al. "Association of the 1q25 diabetes-specific coronary heart disease locus with alterations of the γ-glutamyl cycle and increased methylglyoxal levels in endothelial cells."

Supplementary Table 1. Detection of metabolites in HUVEC extracts.

Metabolite	CHEMICAL_ID	Detection (%)*
gamma-glutamylthreonine	100001314	95.8
gamma-glutamylleucine	1268	100.0
gamma-glutamylvaline	100001126	98.4
beta-citrylglutamate	100003271	100.0
gamma-glutamylisoleucine*	100001485	98.4
S-lactoylglutathione	1343	85.2
gamma-glutamylcysteine	1036	99.2
ophthalmate	100001311	96.7
N-acetyl-aspartyl-glutamate (NAAG)	100001612	99.2
S-nitrosoglutathione (GSNO)	100004662	98.4
glutamate, gamma-methyl ester	100001103	99.2
gamma-glutamylglycine	100001294	25.4
N-acetylglutamate	100000282	100.0
S-1-pyrroline-5-carboxylate	35	76.0
pyroglutamine*	100001540	100.0
glutathione, reduced (GSH)	496	100.0
5-oxoproline	1021	100.0
2-hydroxybutyrate/2-hydroxyisobutyrate	100008928	97.5
4-hydroxy-nonenal-glutathione	100006240	88.3
glutamate	561	100.0
gamma-aminobutyrate (GABA)	141	100.0
gamma-glutamylglutamine	1140	100.0
glutamine	563	100.0
alpha-ketoglutarate	93	100.0
gamma-glutamylphenylalanine	100000491	79.6
gamma-glutamyl-epsilon-lysine	100001262	100.0
4-hydroxyglutamate	100002544	97.5
glycine	340	100.0
gamma-carboxyglutamate	100002679	100.0
cysteinylglycine	278	100.0
cysteine	800	100.0
carboxyethyl-GABA	100003260	30.1
gamma-glutamylglutamate	331	100.0
N-acetylglutamine	100001253	100.0
glutathione, oxidized (GSSG)	448	100.0

*Percent of HUVEC with metabolite levels above detection limit.

Supplementary Table 2. Association between rs10911021 C allele and metabolites related to glutamic acid metabolism and the γ-glutamyl cycle.

Metabolite	SNP effect in low glucose				SNP effect in high glucose				SNP effect in low+high glucose				SNP x glucose interaction
	Est*	95% CI		P	Est*	95% CI		P	Est*	95% CI		P	Inter_p
gamma-glutamylthreonine	64%	23%	118%	**0.001**	53%	14%	107%	**0.007**	58%	20%	109%	**0.002**	0.510
gamma-glutamylleucine	30%	6%	60%	**0.014**	36%	12%	65%	**0.004**	33%	9%	61%	**0.006**	0.522
gamma-glutamylvaline	35%	8%	69%	**0.012**	34%	9%	64%	**0.006**	35%	9%	66%	**0.007**	0.926
beta-citrylglutamate	15%	2%	30%	**0.023**	18%	5%	32%	**0.006**	17%	4%	30%	**0.009**	0.472
gamma-glutamylisoleucine*	26%	2%	56%	**0.034**	31%	7%	59%	**0.010**	28%	5%	56%	**0.018**	0.605
S-lactoylglutathione	-30%	-58%	16%	0.171	-54%	-74%	-18%	**0.011**	-41%	-62%	-10%	**0.019**	0.361
gamma-glutamylcysteine	17%	-4%	42%	0.124	27%	5%	52%	**0.014**	22%	3%	44%	**0.024**	0.441
ophthalmate	38%	3%	85%	**0.036**	34%	-2%	81%	0.069	36%	1%	82%	**0.044**	0.668
N-acetyl-aspartyl-glutamate (NAAG)	22%	2%	45%	**0.034**	16%	-2%	37%	0.083	19%	1%	40%	**0.046**	0.231
S-nitrosoglutathione (GSNO)	-13%	-24%	0%	0.051	-11%	-25%	5%	0.181	-12%	-23%	1%	0.081	0.957
glutamate, gamma-methyl ester	25%	-1%	58%	0.067	11%	-9%	36%	0.309	18%	-3%	43%	0.097	0.285
gamma-glutamylglycine	16%	-4%	41%	0.124	14%	-6%	38%	0.196	15%	-3%	37%	0.112	0.803
N-acetylglutamate	8%	-3%	20%	0.157	8%	-2%	18%	0.142	8%	-2%	18%	0.132	0.918
S-1-pyrroline-5-carboxylate	9%	-16%	41%	0.509	30%	-2%	73%	0.077	19%	-6%	51%	0.155	0.195
pyroglutamine*	-7%	-17%	3%	0.185	-5%	-13%	5%	0.307	-6%	-15%	4%	0.222	0.363
glutathione, reduced (GSH)	-5%	-14%	4%	0.277	-5%	-14%	4%	0.279	-5%	-14%	4%	0.253	0.920
5-oxoproline	5%	-5%	16%	0.385	6%	-4%	17%	0.249	5%	-4%	16%	0.266	0.873
2-hydroxybutyrate/2-hydroxyisobutyrate	-1%	-12%	11%	0.874	-9%	-18%	1%	0.081	-5%	-13%	4%	0.285	0.140
4-hydroxy-nonenal-glutathione	15%	-11%	50%	0.291	9%	-15%	39%	0.513	12%	-11%	40%	0.338	0.889
glutamate	3%	-4%	11%	0.385	3%	-4%	10%	0.475	3%	-4%	10%	0.397	0.753
gamma-aminobutyrate (GABA)	7%	-7%	23%	0.361	4%	-10%	19%	0.598	5%	-8%	20%	0.453	0.421
gamma-glutamylglutamine	-11%	-31%	14%	0.363	-3%	-23%	24%	0.832	-7%	-26%	18%	0.554	0.182
glutamine	2%	-16%	24%	0.862	9%	-8%	29%	0.318	5%	-11%	26%	0.556	0.158

alpha-ketoglutarate	-16%	-36%	9%	0.199	7%	-12%	28%	0.507	-6%	-22%	15%	0.567	0.062
gamma-glutamylphenylalanine	0%	-16%	19%	0.998	9%	-6%	26%	0.276	4%	-10%	20%	0.576	0.266
gamma-glutamyl-epsilon-lysine	-2%	-14%	13%	0.829	8%	-6%	24%	0.282	3%	-10%	17%	0.663	0.093
4-hydroxyglutamate	-4%	-18%	13%	0.641	-2%	-16%	15%	0.823	-3%	-16%	12%	0.706	0.759
glycine	-2%	-10%	7%	0.693	-1%	-8%	6%	0.779	-1%	-8%	6%	0.722	0.770
gamma-carboxyglutamate	-4%	-13%	5%	0.389	1%	-8%	12%	0.806	-2%	-10%	8%	0.734	0.140
cysteinylglycine	-3%	-15%	10%	0.614	-1%	-14%	15%	0.897	-2%	-14%	12%	0.793	0.755
cysteine	-8%	-21%	8%	0.311	5%	-12%	25%	0.597	-2%	-15%	14%	0.825	0.169
carboxyethyl-GABA	4%	-17%	29%	0.761	0%	-20%	25%	0.992	1%	-17%	25%	0.890	0.475
gamma-glutamylglutamate	1%	-10%	13%	0.857	-2%	-14%	10%	0.697	-1%	-10%	10%	0.890	0.555
N-acetylglutamine	-2%	-16%	13%	0.735	4%	-9%	20%	0.554	1%	-12%	16%	0.893	0.178
glutathione, oxidized (GSSG)	4%	-5%	13%	0.422	-3%	-11%	6%	0.455	0%	-8%	9%	0.984	**0.045**

*Percent change per copy of rs10911021 risk (‘C’) allele.

Supplementary Table 3. Pair-wise correlations among metabolites associated with rs10911021.

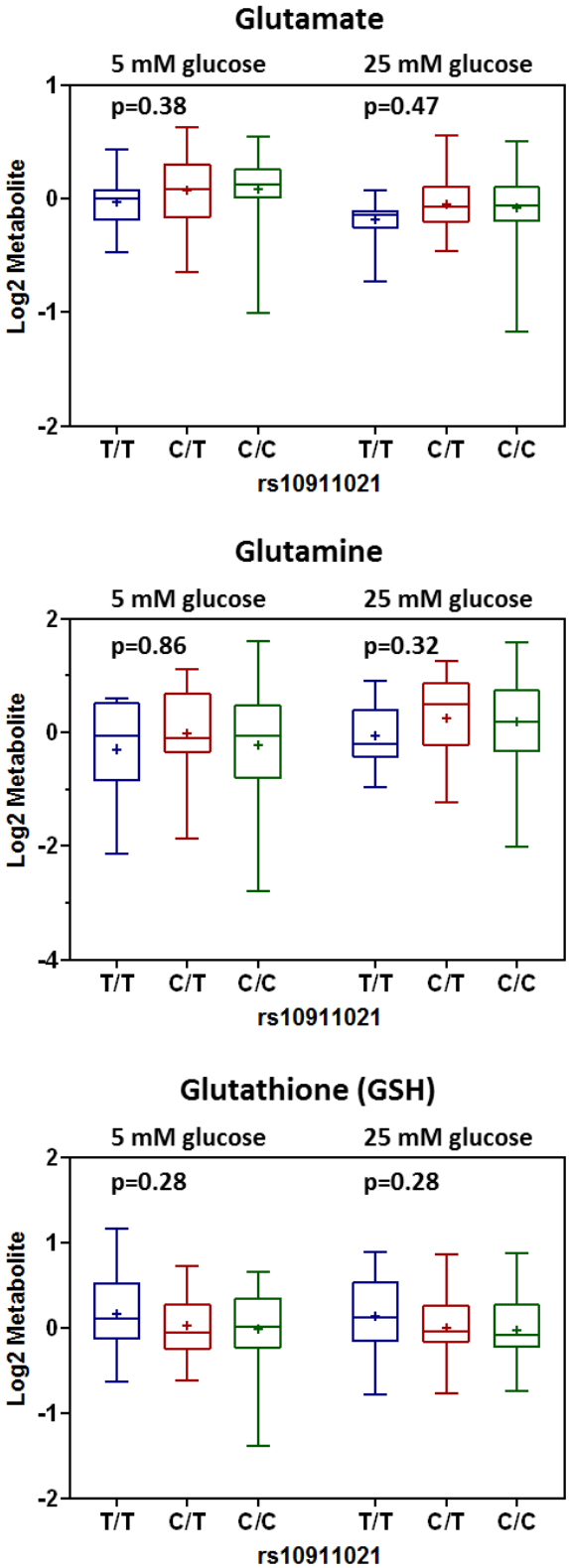
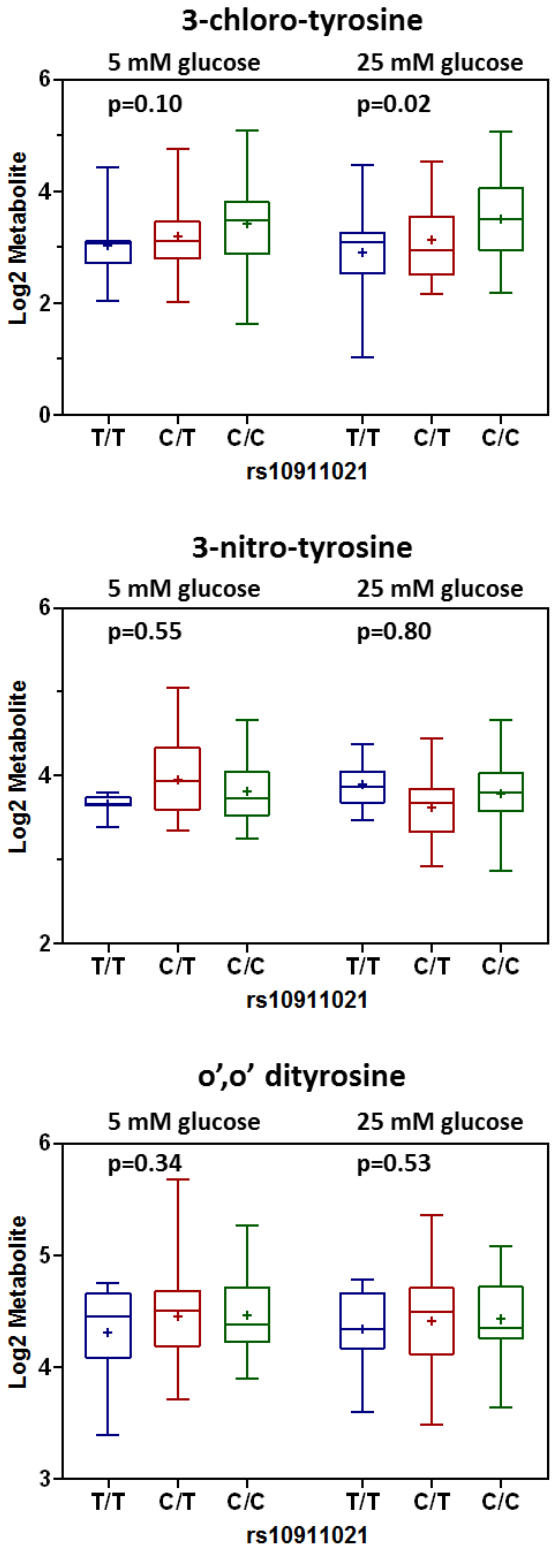
	γ-glutamyl threonine	γ-glutamyl leucine	γ-glutamyl valine	β-citryl glutamate	γ-glutamyl isoleucine	S-lactoyl glutathione	γ-glutamyl cysteine	Ophthalmate	NAAG
γ-glutamylthreonine		0.80 <0.0001	0.80 <0.0001	0.42 0.0007	0.75 <0.0001	-0.30 0.02	0.57 <0.0001	0.68 <0.0001	0.07 NS
γ-glutamylleucine	0.90 <0.0001		0.95 <0.0001	0.30 0.02	0.96 <0.0001	-0.16 NS	0.42 0.0008	0.75 <0.0001	0.07 NS
γ-glutamylvaline	0.89 <0.0001	0.96 <0.0001		0.29 0.02	0.92 <0.0001	-0.17 NS	0.42 0.0008	0.77 <0.0001	0.01 NS
β-citrylglutamate	0.39 0.002	0.36 0.005	0.35 0.006		0.26 0.04	-0.33 0.008	0.61 <0.0001	0.19 NS	0.40 0.001
γ-glutamylisoleucine	0.85 <0.0001	0.97 <0.0001	0.95 <0.0001	0.30 0.02		-0.14 NS	0.32 0.01	0.70 <0.0001	0.04 NS
S-lactoylglutathione	-0.15 NS	-0.19 NS	-0.14 NS	-0.33 0.009	-0.13 NS		-0.39 0.002	-0.15 NS	-0.26 0.04
γ-glutamylcysteine	0.65 <0.0001	0.59 <0.0001	0.54 <0.0001	0.58 <0.0001	0.47 <0.0001	-0.49 <0.0001		0.39 0.002	0.29 0.02
ophthalmate	0.78 <0.0001	0.80 <0.0001	0.84 <0.0001	0.22 NS	0.80 <0.0001	0.04 NS	0.32 0.01		0.04 NS
N-acetyl-aspartyl-glutamate (NAAG)	0.08 NS	0.05 NS	-0.02 NS	0.51 <0.0001	0.01 NS	-0.28 0.03	0.26 0.05	0.01 NS	

Values are Pearson correlation coefficients (upper line) and the corresponding p values (lower line).
Correlations above and below the diagonal refer to low and high glucose, respectively.

Supplementary Table 4. Correlation between metabolites associated with rs10911021 and glutamate, glutamine, GSH, and GSH/glutamate ratio.

	5 mM glucose					25 mM glucose			
	Glutamate	Glutamine	GSH	GSH/ glutamate		Glutamate	Glutamine	GSH	GSH/ glutamate
γ-glutamylthreonine	0.46 0.0002	0.15 NS	-0.08 NS	-0.50 P<0.0001		0.25 0.048	0.19 NS	-0.29 0.02	-0.61 <0.0001
γ-glutamylleucine	0.38 0.002	0.11 NS	-0.25 0.05	-0.62 P<0.0001		0.26 0.0438	0.22 0.09	-0.30 0.02	-0.63 <0.0001
γ-glutamylvaline	0.36 0.004	0.09 NS	-0.26 0.04	-0.62 P<0.0001		0.25 0.0532	0.25 0.05	-0.29 0.02	-0.61 <0.0001
β-citrylglutamate	0.66 <0.0001	0.30 0.02	0.43 0.0004	-0.06 NS		0.64 <0.0001	0.36 0.004	0.38 0.002	-0.11 NS
γ-glutamylisoleucine	0.36 0.004	0.16 NS	-0.27 0.03	-0.63 <0.0001		0.20 0.1155	0.24 0.06	-0.34 0.006	-0.63 <0.0001
S-lactoylglutathione	-0.20 NS	-0.06 NS	-0.04 NS	0.13 NS		-0.32 0.01	-0.20 NS	-0.17 NS	0.07 NS
γ-glutamylcysteine	0.62 <0.0001	0.08 NS	0.42 0.0006	-0.04 NS		0.53 <.00001	0.11 NS	0.26 0.04	-0.17 NS
ophthalmate	0.20 NS	0.11 NS	-0.25 0.05	-0.47 0.0001		0.04 NS	0.18 NS	-0.38 0.002	-0.53 <0.0001
N-acetyl-aspartyl-glutamate (NAAG)	0.20 NS	-0.08 NS	0.1 NS	-0.05 NS		0.19 NS	0.02 NS	0.12 NS	-0.02 NS

Supplementary Figure 1. Intracellular levels of glutamate, glutamine, and reduced glutathione in relation to rs10911021 genotype. No association between these metabolites and rs1091102 was observed in either low or high glucose.



Supplementary Figure 2. Intracellular levels of oxidized tyrosines. 3-chloro-tyrosine levels were significantly associated with the rs10911021 genotype in 25 mM glucose (p=0.02). No association with the genotype was detected for 3-nitro-tyrosine and o',o'dityrosine in either low or high glucose.