

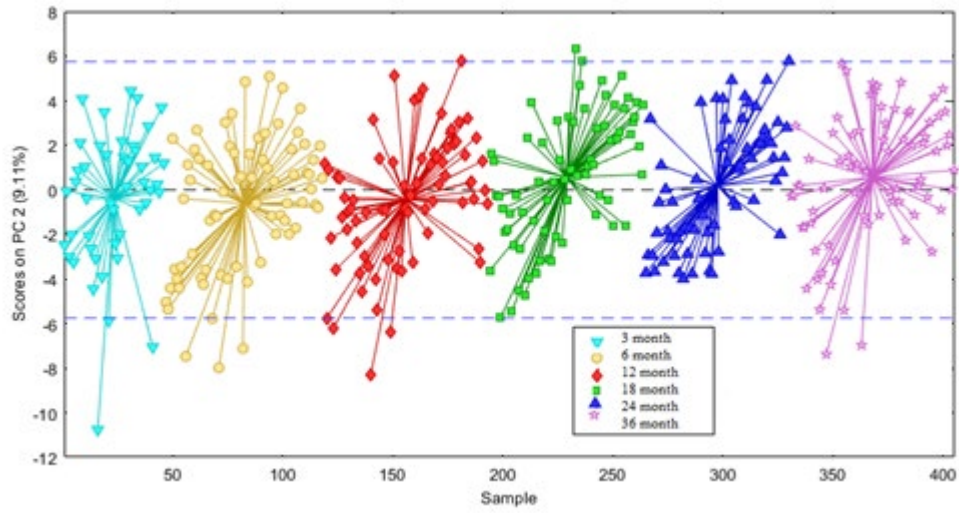
Circulating Metabolites in Progression to Islet Autoimmunity and Type 1 Diabetes

Electronic supplementary material

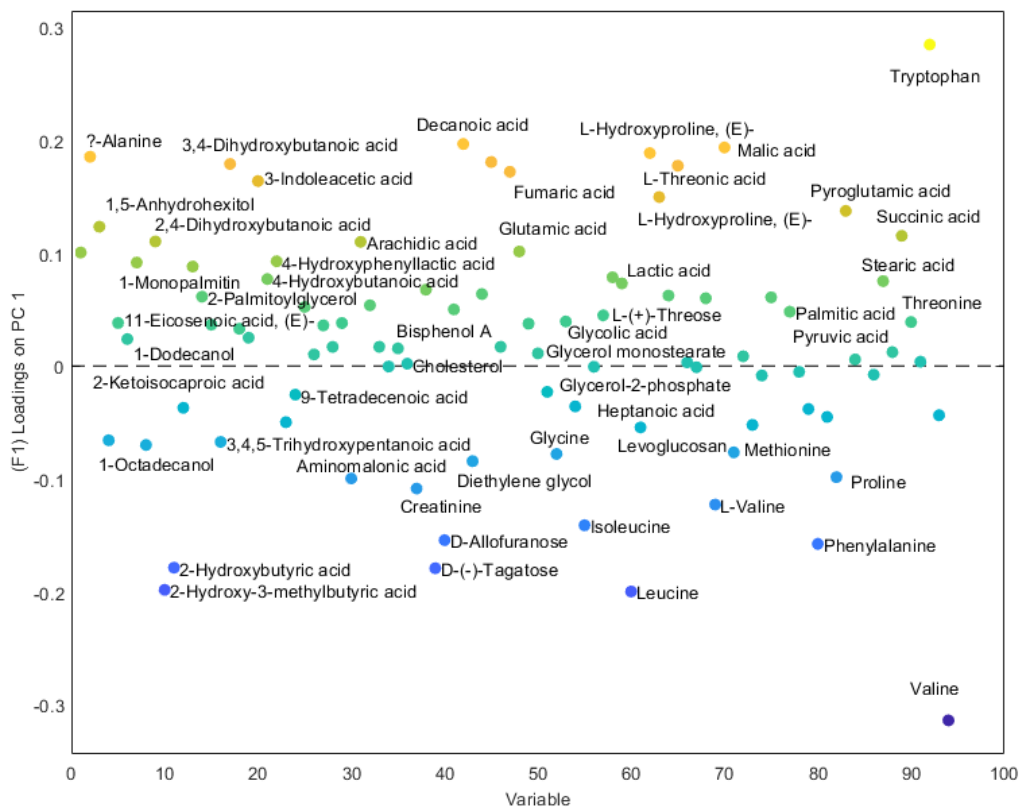
ESM Methods

Two step derivatization:

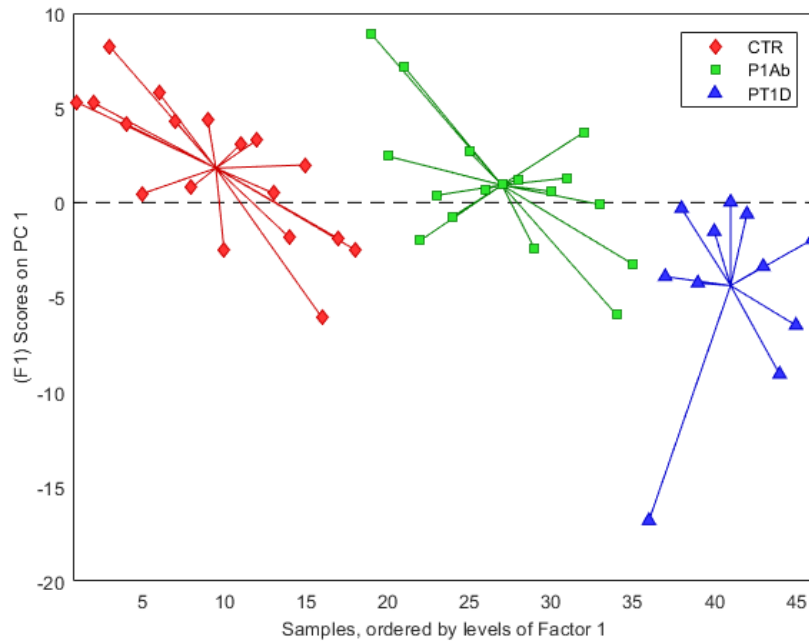
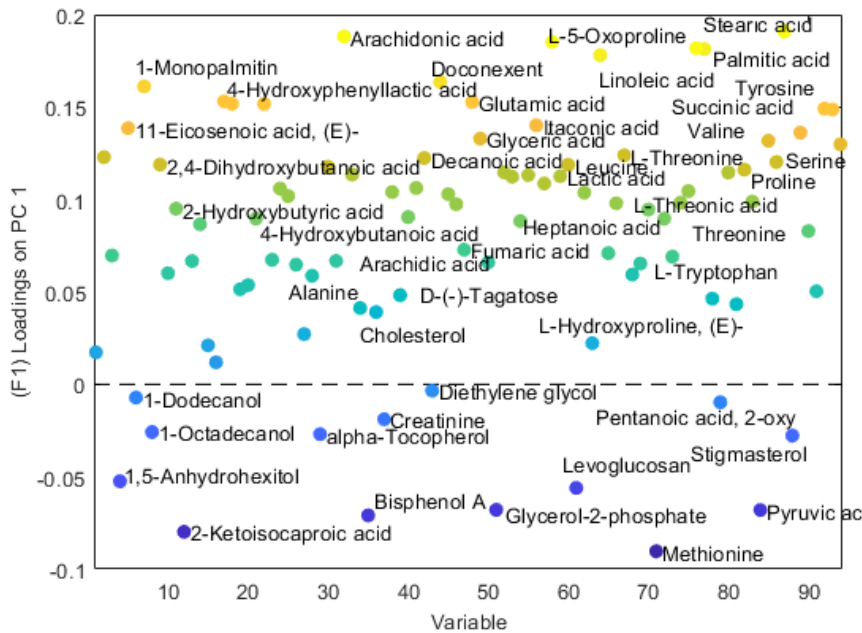
In the first step 25 μ l of methoxyamine hydrochloride (TS-45950; Thermo Scientific: USA) was added to the sample. While mixing, the solution was incubated for one hour at 45 °C. In the second step, 25 μ l of N-methyl-N-trimethylsilyltrifluoroacetamide (Sigma-Aldrich; Steinheim, Germany) was added. Incubation was again performed for one hour at 45 °C. Before injection 50 μ l of hexane was added to increase the volatility of the solvent. Additional standards here added during derivatization. n-alkanes (c = 8 mg/l in MSTFA) were used for calculation of retention indexes and 4,4'-dibromooctafluorobiphenyl (c = 9.8 mg/l in hexane) were used as syringe standard to control the quality of injection. 1 μ l of derivatized sample was injected after derivatization program was completed.



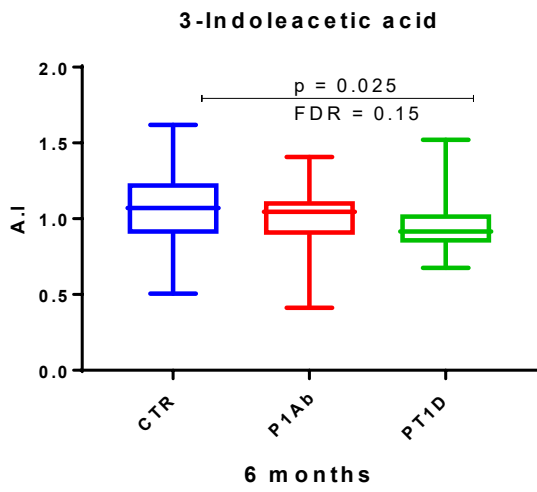
ESM Figure 1. PCA score plot of the second principal component obtained from 405 plasma samples.



ESM Figure 2. ASCA PC₁ loading plot for Fig 1. The loading explains the pattern seen in the score plot that provides the means to interpret the class specific metabolic alteration related to age. x-axis is the variable order and y-axis represents the metabolic pattern corresponding to the score plot. Here, colour of the loadings indicates the loading scores in PC₁.

a**b**

ESM Figure 3. PCA score plots and loadings based on ANOVA-simultaneous component analysis (ASCA). **(a)** PC₁ score plot obtained based on the factor study cases score in ASCA analysis at age of 3 months. Here, each sample is represented by a point and coloured according to the case (red diamond: subjects who remained islet autoantibody negative during the follow-up age (CTR), green square: subjects who tested positive for at least one antibody in a minimum of two consecutive samples but did not progress to clinical T1D during the follow-up (P1Ab), blue triangle up: progressors to type-1-diabetes (PT1D)). Samples with similar score are clustered together. **(b)** The corresponding PC₁ loading plot. The loadings explain the pattern seen in the score plot which provides the means to interpret the metabolic alteration related to case. X-axis is the variable order and y-axis represents the metabolic pattern corresponding to the score plot. Here, colour of the loadings indicates the loading scores in PC₁.



ESM Figure 4. Concentrations of 3-Indole acetic acid at 6 months of age.

ESM Table 1. Anthropometric characteristics of study population.

	PT1D	P1Ab	CTR
Sex(female, male)	(14, 26)	(14, 26)	(14, 26)
Age at time of diagnosis (mean ± SD)	4.75 ± 2.94	NA	NA
Age at time of first seroconversion (mean ± SD)	1.34 ± 0.58	3.05± 2.50	NA
HLA information			
High risk (DR3 - DQA1*05 - DQB1*02) /DRB1*0401/2/4/5 - DQA1*03 - DQB1*0302)	9	4	7
Moderately increased risk*	21	22	14
Slightly increased risk*	10	9	7
Neutral*		3	4
Decreased risk and/or not possible to form haplotype*		2	8

*other than DR3 - DQA1*05 - DQB1*02) /DRB1*0401/2/4/5 - DQA1*03 - DQB1*0302. This table has been adapted from [10]

ESM Table 2. Coefficient of variation across the analysis (415 samples).

Metabolite	% Coefficient of variation (CV)
Nonadecanoic acid	32.61
Diethylene glycol	32.7
Myo-Inositolphosphate	34.86
Fumaric acid	37.55
2-Phenylisopropanol	39.87
Valine	41.32
p-Hydroxydiisopropylbenzene	41.49
L-5-Oxoproline	43.44
á-D-(+)-Mannopyranose	44.42
Cholesterol	44.52
Serine	44.64
Aminomalonic acid	45.53
Lactic acid	45.69
Alanine	46.6
2-Palmitoylglycerol	46.89
Methylmaleic acid	47.02
3,4-Dihydroxybutanoic acid	47.68
Glycerol-2-phosphate	47.95
Itaconic acid	48.22
á-D-Glucopyranose	48.31
Octanoic acid	49.4
Threonine	49.93
Malic acid	50.06
Stearic acid	50.63
L-Threonic acid	50.88
Palmitic acid	51.35
Isoleucine	52.58
1-Dodecanol	53.48
4-Hydroxyphenyllactic acid	53.51
Linoleic acid	53.82
2-Hydroxybutyric acid	53.89
Leucine	54.34
Arachidic acid	54.44
Arachidonic acid	54.51
L-Threonine	54.52
4-Hydroxybutanoic acid	55.59
Pentadecanoic acid	56.62
Stigmasterol	57.5
Doconexent	58.25
Creatinine	59.25
1-Monopalmitin	59.73
Succinic acid	59.97

Phenylalanine	60.71
Proline	61.17
9-Hexadecenoic acid	61.55
Oleic acid	61.65
(R*,R*)-2,3-Dihydroxybutanoic acid	61.81
9-Tetradecenoic acid	61.84
Aconitic acid, (Z)-	63.7
Pentanoic acid, 2-oxy	64.1
L-Threonic acid	64.54
L-Valine	64.73
Decanoic acid	64.79
D-Allofuranose	64.9
Glycine	65.03
Triethylene glycol	65.28
Pyroglutamic acid	66.76
1-Octadecanol	67.35
Heptanoic acid	69.93
2-Ketoisocaproic acid	71.48
D-(-)-Lyxofuranose	72.28
Glycerol monostearate	72.45
11-Eicosenoic acid, (E)-	72.57
L-Hydroxyproline, (E)-	73.85
L-Hydroxyproline, (E)-	74.66
3-Indoleacetic acid	74.9
Tyrosine	75.16
2,4-Dihydroxybutanoic acid	76.21
3,4,5-Trihydroxypentanoic acid	77.85
2-Oleoylglycerol	80.69
Glyceric acid	81.65
alpha-Tocopherol	83.87
L-(+)-Threose	84.3
D-Arabinose	84.91
1,5-Anhydrohexitol	85.65
L-Tryptophan	89.45
Dodecanoic acid	91.4
Tryptophan	92.05
3-Hydroxyisovaleric acid	94.33
Glutamic acid	100.48
3-Hydroxybutyric acid	114.25
Pyruvic acid	114.32
Methionine	116.18
?-Alanine	117.19
D-(-)-Tagatose	120.25
Aspartic acid	124.85
Azelaic acid	127.08

Bisphenol A	131.44
Levoglucofan	133.58
Ribonic acid	148.97
1,5-Anhydrohexitol	171.48
Ethanolamine	194.86
Glycolic acid	225.34
2-Hydroxy-3-methylbutyric acid	245.9

Here, the median % CV (50th centile % CV) is 61.82 and the 90th centile % CV is 120.71.

ESM Table 3. Plasma metabolites different between CTR and PT1D at age 3 months.

S:N	Metabolite	Nominal p-values	Adjusted-pvalues	Log2F
1	11-Eicosenoic acid, (E)-	0.0013	0.057	-0.341
2	L-5-Oxoproline	0.0013	0.057	-0.143
3	Stearic acid	0.0021	0.057	-0.102
4	Palmitic acid	0.0024	0.057	-0.084
5	Oleic acid	0.0032	0.0616	-0.097
6	Arachidonic acid	0.0043	0.067	-0.132
7	1-Monopalmitin	0.0057	0.067	-0.186
8	Linoleic acid	0.0057	0.067	-0.095
9	Glutamic acid	0.0065	0.068	-0.312
10	3-Hydroxybutyric acid	0.023	0.22	-0.171
11	Doconexent	0.033	0.26	-0.19
12	D-Arabinose	0.037	0.26	-0.216
13	Ribonic acid	0.041	0.26	-0.755
14	L-Threonic acid	0.041	0.26	-0.162
15	Glyceric acid	0.041	0.26	-0.438

ESM Table 4. Plasma metabolites different between CTR and PT1D at age 6 months.

S:N	Metabolite	Nominal P-values	Adjusted-pvalues	Log ₂ F
1	Ribonic acid	7.4E-05	0.0070	-0.919
2	D-Arabinose	0.00063	0.020	-0.23
3	L-Threonic acid	0.00063	0.020	-0.198
4	Methionine	0.0015	0.036	2.049
5	Pentadecanoic acid	0.0019	0.036	-0.177
6	Glyceric acid	0.0025	0.036	-0.402
7	L-5-Oxoproline	0.0027	0.036	-0.093
8	Succinic acid	0.0040	0.045	-0.187
9	1,5-Anhydrohexitol	0.00431	0.045	-0.125
10	Glutamic acid	0.0056	0.052	-0.291
11	L-Hydroxyproline, (E)-	0.0077	0.0655	-0.221
12	4-Hydroxyphenyllactic acid	0.0098	0.075	-0.334
13	Aspartic acid	0.010	0.075	-0.732
14	D-(-)-Lyxofuranose	0.014	0.093	-0.482
15	Tryptophan	0.015	0.093	-0.295
16	3-Indoleacetic acid	0.026	0.15	-0.166
17	alpha-Tocopherol	0.027	0.15	0.405
18	Malic acid	0.046	0.22	-0.085
19	9-Tetradecenoic acid	0.048	0.21	-0.223
20	Creatinine	0.048	0.26	0.153

ESM Table 5. The detailed results of potential metabolic pathways that altered between CTRL and PTiD at age 3 months

Metabolic pathway	Total Cpd	Hits	Nominal p	-log(p)	Holm adjust	FDR	Impact
Alanine, aspartate and glutamate metabolism	24	2	0.0007	7.1429	0.0166	0.002	0.441
Aminoacyl-tRNA biosynthesis	75	2	0.0007	7.14	0.016	0.002	0.112
D-Glutamine and D-glutamate metabolism	11	1	0.001	6.44	0.025	0.002	0.112
Tryptophan metabolism	79	1	0.052	2.93	0.106	0.055	0.047
Arginine and proline metabolism	77	2	0.0007	7.14	0.016	0.002	0.035
Glyoxylate and dicarboxylate metabolism	50	1	0.017	4.02	0.106	0.020	0.032
Pentose phosphate pathway	32	1	0.017	4.02	0.106	0.020	0.021
Glycerolipid metabolism	32	1	0.017	4.02	0.106	0.020	0.020
Glutathione metabolism	38	1	0.001	6.44	0.025	0.002	0.010
Butanoate metabolism	40	2	0.0004	7.67	0.0101	0.002	0.004
Ubiquinone and other terpenoid-quinone biosynthesis	36	1	0.065	2.73	0.106	0.065	0.0006
Nitrogen metabolism	39	2	0.0007	7.14	0.016	0.002	0.0006
Histidine metabolism	44	2	0.0007	7.14	0.016	0.002	0.0005
Glycine, serine and threonine metabolism	48	2	0.003	5.72	0.025	0.004	0.0004
Porphyrin and chlorophyll metabolism	104	1	0.0015	6.44	0.025	0.002	0
Cysteine and methionine metabolism	56	1	0.001	6.39	0.025	0.002	0
Lysine biosynthesis	32	1	0.001	6.39	0.025	0.002	0
beta-Alanine metabolism	28	1	0.001	6.39	0.025	0.002	0
Cyanoamino acid metabolism	16	1	0.001	6.39	0.025	0.002	0
Nicotinate and nicotinamide metabolism	44	1	0.001	6.39	0.025	0.002	0
Pantothenate and CoA biosynthesis	27	1	0.001	6.39	0.025	0.002	0
Synthesis and degradation of ketone bodies	6	1	0.030	3.48	0.106	0.033	0

ESM Table 6. The detailed results of potential metabolic pathways that altered between CTRL and PT1D at age 6 months

Metabolic pathway	Total Cpd	Hits	Nominal p	-log(p)	Holm adjust	FDR	Impact
Alanine, aspartate and glutamate metabolism	24	3	0.00086	7.06	0.019	0.002568	0.4416
Tryptophan metabolism	79	2	0.0084	4.78	0.046	0.008711	0.15633
Arginine and proline metabolism	77	4	0.00016	8.74	0.004	0.001581	0.11325
Aminoacyl-tRNA biosynthesis	75	4	9.72E-05	9.24	0.002	0.001581	0.11268
D-Glutamine and D-glutamate metabolism	11	1	0.0011	6.85	0.021	0.002568	0.1123
Cysteine and methionine metabolism	56	2	0.00024	8.33	0.006256	0.001581	0.03806
Ubiquinone and other terpenoid-quinone biosynthesis	36	2	0.0044	5.43	0.035311	0.005776	0.03749
Glyoxylate and dicarboxylate metabolism	50	2	0.0018	6.30	0.031202	0.00321	0.03291
Pentose phosphate pathway	32	1	0.0022	6.12	0.035311	0.00321	0.02181
Glycerolipid metabolism	32	1	0.0022	6.12	0.035311	0.00321	0.0206
Butanoate metabolism	40	2	0.0010	6.87	0.021825	0.002568	0.01774
Citrate cycle (TCA cycle)	20	1	0.0071	4.95	0.045828	0.007628	0.01446
Glutathione metabolism	38	2	0.000382	7.87	0.009171	0.00159	0.01285
Ascorbate and aldarate metabolism	45	2	0.00018	8.61	0.004902	0.001581	0.00802
Propanoate metabolism	35	1	0.0071	4.95	0.045828	0.007628	0.00134
Nitrogen metabolism	39	3	0.00027	8.21	0.006815	0.001581	0.00067
Histidine metabolism	44	2	0.0010	6.86	0.021825	0.002568	0.00051
Glycine, serine and threonine metabolism	48	3	0.00038	7.87	0.009171	0.00159	0.00047
Amino sugar and nucleotide sugar metabolism	88	1	0.0065	5.03	0.045828	0.007628	7.00E-05
Porphyrin and chlorophyll metabolism	104	1	0.0011	6.85	0.021825	0.002568	0
Lysine biosynthesis	32	1	0.0022	6.11	0.035311	0.00321	0
beta-Alanine metabolism	28	1	0.0022	6.11	0.035311	0.00321	0
Cyanoamino acid metabolism	16	1	0.0022	6.11	0.035311	0.00321	0
Nicotinate and nicotinamide metabolism	44	1	0.0022	6.11	0.035311	0.00321	0
Pantothenate and CoA biosynthesis	27	1	0.0022	6.11	0.035311	0.00321	0
Tyrosine metabolism	76	2	0.0028	5.87	0.035311	0.003895	0
Pentose and glucuronate interconversions	53	1	0.0065	5.03	0.045828	0.007628	0
Phenylalanine metabolism	45	1	0.0071	4.95	0.045828	0.007628	0
Phenylalanine, tyrosine and tryptophan biosynthesis	27	1	0.011	4.47	0.045828	0.011493	0

ESM Table 7. Plasma metabolites different between B-P₁Ab and A-P₁Ab.

S:N	Metabolite	Nominal p-values	Adjusted-p-values
1	Glutamic acid	0.00017	0.016
2	Aspartic acid	0.0015	0.047
3	Malic acid	0.0015	0.047
4	3,4-Dihydroxybutanoic acid	0.0022	0.052
5	Glyceric acid	0.010	0.19
6	3-Hydroxybutyric acid	0.016	0.26
7	(R*,R*)-2,3-Dihydroxybutanoic acid	0.024	0.29
8	Ribonic acid	0.025	0.29
9	D-Arabinose	0.040	0.38
10	3-Indoleacetic acid	0.048	0.38
11	alpha-Tocopherol	0.049	0.38

Abbreviations: Before seroconversion in P₁Ab (B-P₁Ab), after seroconversion in P₁Ab (A-P₁Ab).

ESM Table 8. Plasma metabolites different between B-PT₁D and A-PT₁D.

S:N	Metabolite	Nominal-p-values	Adjusted p-values
1	Malic acid	0.0017	0.16
2	Tyrosine	0.0037	0.17
3	Glycolic acid	0.0074	0.19
4	L-Threonine	0.0080	0.19
5	3,4,5-Trihydroxypentanoic acid	0.023	0.40
6	Glutamic acid	0.026	0.40
7	2-Phenylisopropanol	0.044	0.60

Abbreviations: Before seroconversion in progressors (B-PT₁D), after seroconversion in progressors (A-PT₁D).

ESM Table 9. The detailed results of potential metabolic pathways that altered between B-P₁Ab and A-P₁Ab.

Metabolic pathway	Total Cpd	Hits	Nominal p	-log(p)	Holm adjust	FDR	Impact
Alanine, aspartate and glutamate metabolism	24	2	0.00079	7.1429	0.0166	0.002455	0.4416
Aminoacyl-tRNA biosynthesis	75	2	0.00079	7.1429	0.0166	0.002455	0.11268
D-Glutamine and D-glutamate metabolism	11	1	0.001595	6.4409	0.025519	0.002455	0.1123
Tryptophan metabolism	79	1	0.052983	2.9378	0.10694	0.055506	0.0478
Arginine and proline metabolism	77	2	0.00079	7.1429	0.0166	0.002455	0.03582
Glyoxylate and dicarboxylate metabolism	50	1	0.017823	4.0273	0.10694	0.020637	0.03291
Pentose phosphate pathway	32	1	0.017823	4.0273	0.10694	0.020637	0.02181
Glycerolipid metabolism	32	1	0.017823	4.0273	0.10694	0.020637	0.0206
Glutathione metabolism	38	1	0.001595	6.4409	0.025519	0.002455	0.01095
Butanoate metabolism	40	2	0.000462	7.6796	0.010168	0.002455	0.0048
Ubiquinone and other terpenoid-quinone biosynthesis	36	1	0.065137	2.7313	0.10694	0.065137	0.00069
Nitrogen metabolism	39	2	0.00079	7.1429	0.0166	0.002455	0.00067
Histidine metabolism	44	2	0.00079	7.1429	0.0166	0.002455	0.00051
Glycine, serine and threonine metabolism	48	2	0.003253	5.7281	0.025519	0.004473	0.00047
Porphyrin and chlorophyll metabolism	104	1	0.001595	6.4409	0.025519	0.002455	0
Cysteine and methionine metabolism	56	1	0.001674	6.3928	0.025519	0.002455	0
Lysine biosynthesis	32	1	0.001674	6.3928	0.025519	0.002455	0
beta-Alanine metabolism	28	1	0.001674	6.3928	0.025519	0.002455	0
Cyanoamino acid metabolism	16	1	0.001674	6.3928	0.025519	0.002455	0
Nicotinate and nicotinamide metabolism	44	1	0.001674	6.3928	0.025519	0.002455	0
Pantothenate and CoA biosynthesis	27	1	0.001674	6.3928	0.025519	0.002455	0
Synthesis and degradation of ketone bodies	6	1	0.030629	3.4858	0.10694	0.033692	0

ESM Table 10. The detailed results of potential metabolic pathways that altered between B-PT1D and A-PT1D.

Metabolic pathway	Total Cpd	Hits	Nominal p	-log(p)	Holm adjust	FDR	Impact
Alanine, aspartate and glutamate metabolism	24	1	0.077339	2.5596	0.46403	0.077339	0.17664
D-Glutamine and D-glutamate metabolism	11	1	0.077339	2.5596	0.46403	0.077339	0.1123
Glycine, serine and threonine metabolism	48	1	0.037713	3.2778	0.3017	0.058283	0.09661
Aminoacyl-tRNA biosynthesis	75	3	0.00216	6.1376	0.026103	0.005246	0.05634
Tyrosine metabolism	76	1	0.001631	6.4183	0.026103	0.004622	0.04724
Arginine and proline metabolism	77	1	0.077339	2.5596	0.46403	0.077339	0.03582
Glutathione metabolism	38	1	0.077339	2.5596	0.46403	0.077339	0.01095
Phenylalanine, tyrosine and tryptophan biosynthesis	27	1	0.001631	6.4183	0.026103	0.004622	0.00738
Glyoxylate and dicarboxylate metabolism	50	1	0.011235	4.4887	0.11235	0.023874	0.00686
Histidine metabolism	44	1	0.077339	2.5596	0.46403	0.077339	0.00051
Nitrogen metabolism	39	2	0.001151	6.7676	0.019558	0.004622	0
Ubiquinone and other terpenoid-quinone biosynthesis	36	1	0.001631	6.4183	0.026103	0.004622	0
Phenylalanine metabolism	45	1	0.001631	6.4183	0.026103	0.004622	0
Thiamine metabolism	24	1	0.001631	6.4183	0.026103	0.004622	0
Porphyrin and chlorophyll metabolism	104	2	0.026077	3.6467	0.23469	0.049257	0
Valine, leucine and isoleucine biosynthesis	27	1	0.037713	3.2778	0.3017	0.058283	0
Butanoate metabolism	40	1	0.077339	2.5596	0.46403	0.077339	0