

Mendelian Randomization study on amino acid metabolism suggests tyrosine as causal trait for type 2 diabetes

Susanne Jäger^{1,2}, Rafael Cuadrat^{1,2}, Clemens Wittenbecher^{1,2,3}, Anna Floegel⁴,
Per Hoffmann^{5,6}, Cornelia Prehn⁷, Jerzy Adamski^{2,7,8,9}, Tobias Pischon^{10,11,12}, Matthias B.
Schulze^{1,2,13*}

¹ Department of Molecular Epidemiology, German Institute of Human Nutrition Potsdam-Rehbruecke, Nuthetal, Germany.

² German Center for Diabetes Research (DZD), Germany.

³ Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, MA, United States.

⁴ Leibniz Institute for Prevention Research and Epidemiology-BIPS, Bremen, Germany.

⁵ Human Genomics Research Group, Department of Biomedicine, University of Basel, Basel, Switzerland.

⁶ Institute of Human Genetics, Division of Genomics, Life & Brain Research Centre, University Hospital of Bonn, Bonn, Germany.

⁷ Research Unit Molecular Endocrinology and Metabolism, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany.

⁸ Chair of Experimental Genetics, Center of Life and Food Sciences Weihenstephan, Technische Universität München, Freising-Weihenstephan, Germany.

⁹ Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, 8 Medical Drive, Singapore 117597, Singapore

¹⁰ Molecular Epidemiology Research Group, Max Delbrueck Center for Molecular Medicine in the Helmholtz Association (MDC), Berlin, Germany.

¹¹ Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin and Berlin Institute of Health (BIH), Berlin, Germany

¹² MDC/BIH Biobank, Max Delbrueck Center for Molecular Medicine in the Helmholtz Association (MDC) and Berlin Institute of Health (BIH), Berlin, Germany

¹³ Institute of Nutritional Science, University of Potsdam, Potsdam, Germany.

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Table S1 Characteristics of studies included within the DIAGRAM consortium

See Excel file in Supplement B

Previously published in Mahajan et al. 2018 [1]

Table S2 Associations of single genetic instruments used for MR of amino acids traits from EPIC-Potsdam and type 2 diabetes

See Excel file in Supplement B

Table S3 Total causal effects of amino acid traits from EPIC-Potsdam and of type 2 diabetes using independent instruments ($R \geq 0.001$)

Amino acid trait	Instruments	N (SNPs)*	Beta (SE) from IVW	P- Value	Heterogeneity between SNPs; Q- statistic, p-value	Directional horizontal pleiotropy **; Egger- intercept (SE), p-value	Outlier detected
Glycine	suggestive	2/2	-0.006 (0.011)	0.621	no; 0.16, 0.68	n.a.	no
	genome-wide	1/1	-0.007 (0.011)	0.547	n.a	n.a	n.a
Glycine/Serine	suggestive	4/4	0.018 (0.041)	0.663	yes; 37.0, <0.001	no; 0.04 (0.02), 0.16	n.a
	genome-wide	2/2	-0.007 (0.013)	0.588	no; 0.07, 0.79	n.a.	n.a.
Phenylalanine	suggestive	1/1	0.053 (0.045)	0.237	n.a.	n.a.	n.a.
Phenylalanine/Arginine	suggestive	2/2	0.043 (0.029)	0.131	no; 0.39, 0.53	n.a.	no
Serine/Phenylalanine	suggestive	3/4	0.000 (0.023)	0.995	no; 0.61, 0.74	no; 0.08 (0.16), 0.62	n.a.
Tryptophan/Glutamine	suggestive	2/2	0.003 (0.029)	0.931	no; 0.17, 0.68	n.a.	n.a.
Tyrosine/Methionine	suggestive	3/3	-0.024 (0.023)	0.296	yes; 26.5, <0.001	no; 0.10 (0.1), 0.33	n.a.
	genome-wide	1/1	-0.141 (0.033)	<0.001	n.a.	n.a.	n.a.
xLeucine/Methionine	suggestive	1/2	-0.031 (0.043)	0.437	n.a.	n.a.	n.a.
Valine/xLeucine	suggestive	1/1	-0.012 (0.044)	0.777	n.a.	n.a.	n.a.

IVW, inverse variance weighted method; n.a., not applicable; SE, standard error; T2DM, type 2 diabetes

* used instruments/suitable instruments available in the GWAS of T2DM [2]

** assessed by MR-Egger (>2 variants needed)

Table S4 Total causal effects of amino acid traits from Shin et al. and type 2 diabetes and sensitivity analyses

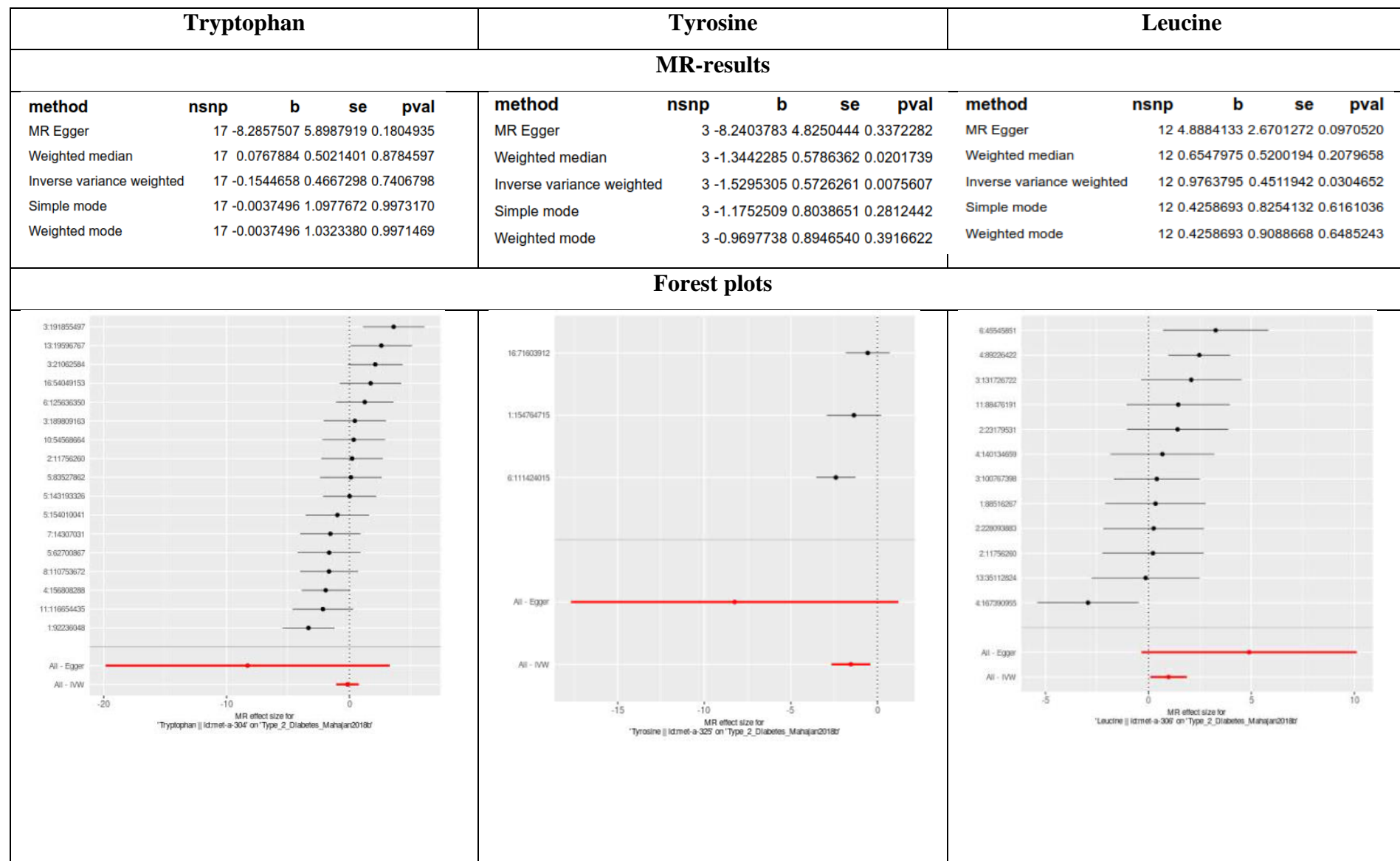
Amino acid trait*	N (SNPs)**	Beta (SE) from IVW/Wald ratio method for T2DM*	P-Value	Heterogeneity Q-statistic, p-value	Directional horizontal pleiotropy*** Egger-intercept (SE), p-value
Glycine	1	-0.05 (0.09)	0.61	n.a.	n.a.
Phenylalanine	1	1.28 (0.87)	0.14	n.a.	n.a.
Tryptophan	17	-0.15 (0.47)	0.74	yes 40.1, 0.0008	0.04 (0.03), 0.19
Tyrosine	3	-1.53 (0.57)	0.008	no 4.5, 0.10	0.07 (0.05), 0.40
Methionine	1	-1.17 (1.00)	0.24	n.a.	n.a.
Valine	1	2.47 (0.77)	0.001	n.a.	n.a.
Leucine	12	0.98 (0.45)	0.03	No 19.7, 0.05	-0.02 (0.01), 0.17

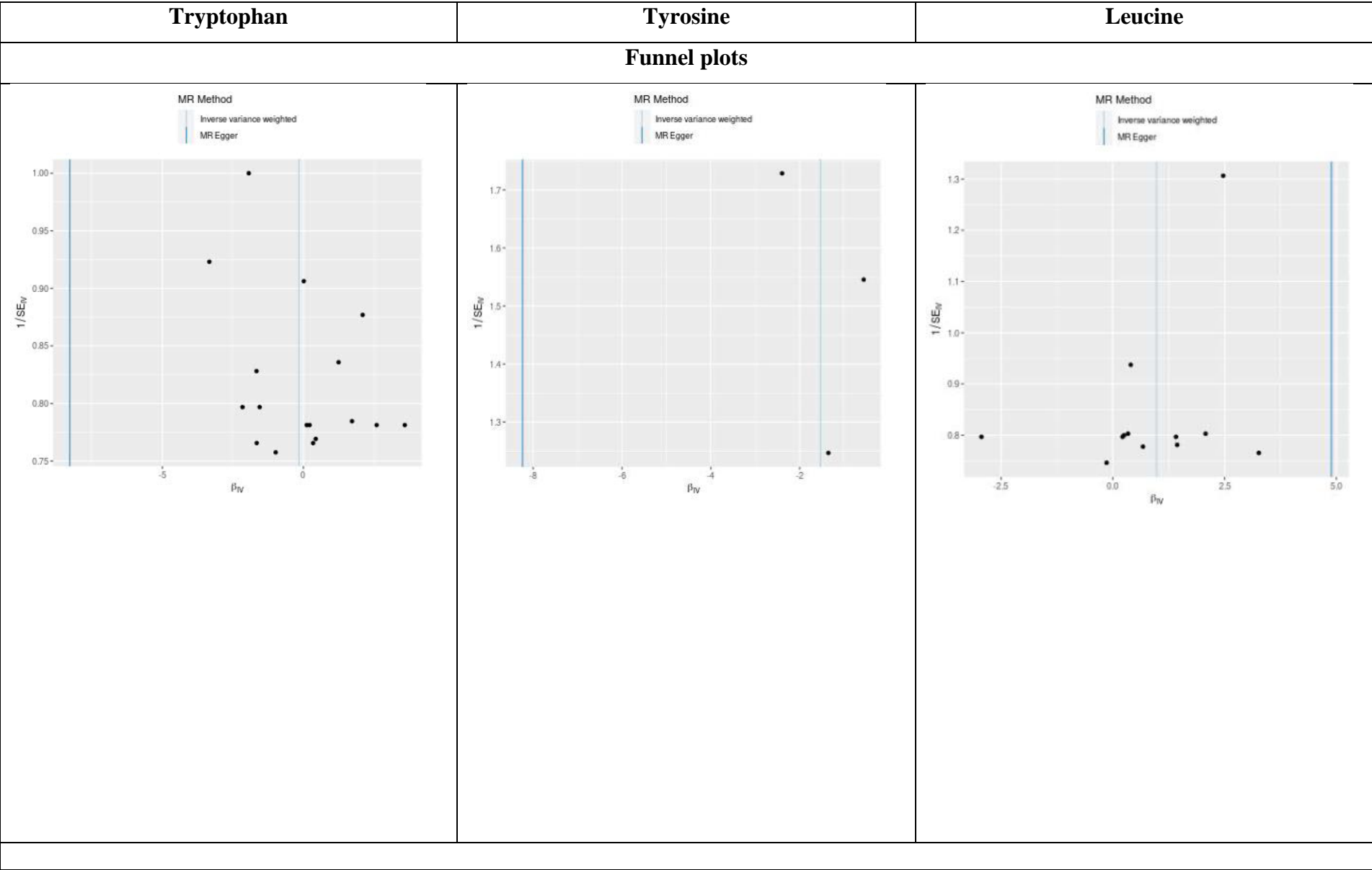
IVW, inverse variance weighted method; n.a., not applicable; SE, standard error; T2DM, type 2 diabetes

* assessed using public data by Shin et al. [3] and Mahajan et al. [2]

** significance threshold = 5×10^{-8} ; clumping threshold $R^2 \geq 0.001$

*** assessed by MR-Egger (>2 variants needed)





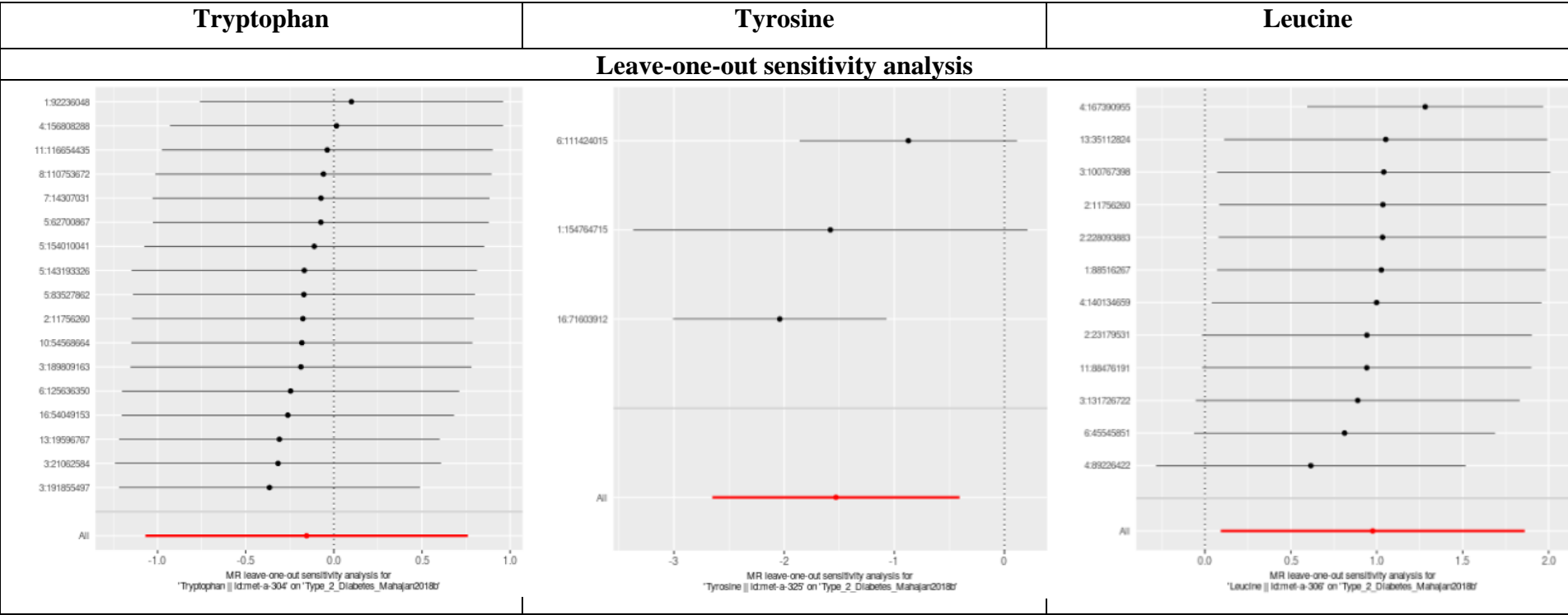


Table S5 Associations of single genetic instruments used for MR of amino acids traits from Shin et al. and type 2 diabetes

See Excel file in Supplement B

Table S6 Direct causal effects of diabetes associated amino acid traits from Shin et al. and type 2 diabetes

Amino acid trait*	N(SNPs)**	Beta (SE) from multivariable IVW method for T2DM*	P-Value	Conditional F-statistic***	Beta (95% CI) **** accounting for weak instruments
Glycine	1	0.05 (0.16)	0.77	0.37	0.04 (-0.79-1.97)
Phenylalanine	1	1.07 (1.30)	0.41	0.53	1.19 (-2.31-4.46)
Tryptophan	17	-0.20 (0.70)	0.77	2.02	-0.17 (-1.81-1.51)
Tyrosine	3	-1.66 (0.78)	0.03	0.36	-1.76 (-3.68--0.03)
Leucine	11	-0.70 (1.56)	0.65	0.31	-0.66 (-4.62-2.40)
Isoleucine	0	1.21 (2.27)	0.59	0.08	1.23 (-2.51-5.07)
Valine	1	1.33 (2.54)	0.60	0.10	1.19 (-2.60-6.96)

* assessed using public data by Shin et al. [3] and Mahajan et al. [2]

** significance threshold = 5×10^{-8} ; clumping threshold $R^2 \geq 0.001$

*** using covariance matrix from EPIC-Potsdam phenotypes

**** Confidence intervals are calculated using a non-parametric bootstrap with 1000 iterations

Table S7Associations of single genetic instruments used for MVMR of amino acids traits from Shin et al. and type 2 diabetes

See Excel file in Supplement B

Table S8 Total causal effects of tyrosine risk of type 2 diabetes using other public data sources

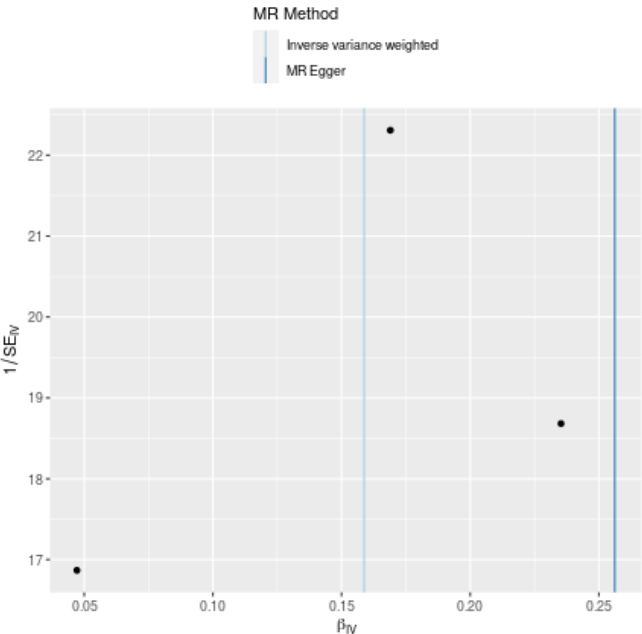
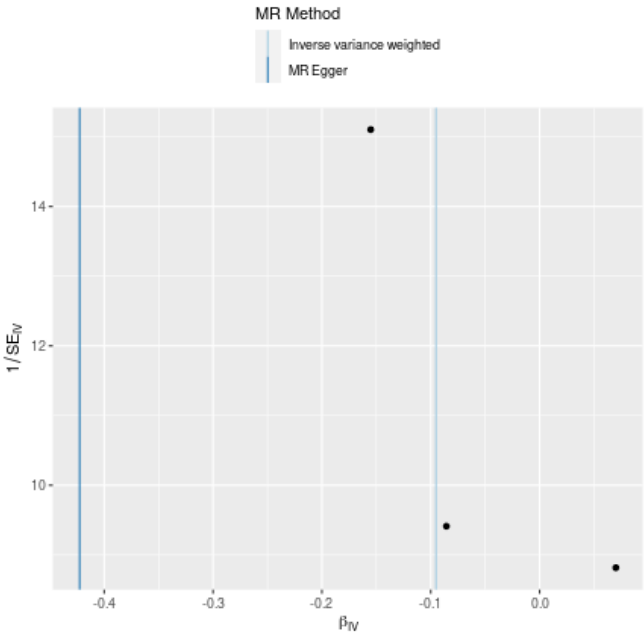
Tyrosine from Kettunen et al. 2016; n=24,925					Tyrosine from Locke et al. 2019; n=8,754				
method	nsnp	b	se	pval	method	nsnp	b	se	pval
MR Egger	3	-0.4225385	0.2266672	0.3134551	MR Egger	3	0.2561767	0.2200496	0.4517975
Weighted median	3	-0.1176278	0.0586697	0.0449720	Weighted median	3	0.1617589	0.0420126	0.0001180
Inverse variance weighted	3	-0.0951712	0.0611713	0.1197518	Inverse variance weighted	3	0.1587883	0.0499333	0.0014727
Simple mode	3	-0.1142686	0.0897026	0.3307251	Simple mode	3	0.1900944	0.0591888	0.0847988
Weighted mode	3	-0.1366342	0.0614610	0.1562546	Weighted mode	3	0.1783170	0.0552536	0.0840818

Forest plots	
<p>MR effect size for 'Tyrosine Idmet-c-938' on 'Type_2_Diabetes_Mahajan2018b'</p>	
<p>MR effect size for 'Tyrosine_Locke_2019' on 'Type_2_Diabetes_Mahajan2018b'</p>	

Tyrosine from Kettunen et al. 2016; n=24,925

Tyrosine from Locke et al. 2019; n=8,754

Funnel plots



Tyrosine from Kettunen et al. 2016; n=24,925

Tyrosine from Locke et al. 2019; n=8,754

Leave-one-out sensitivity analysis

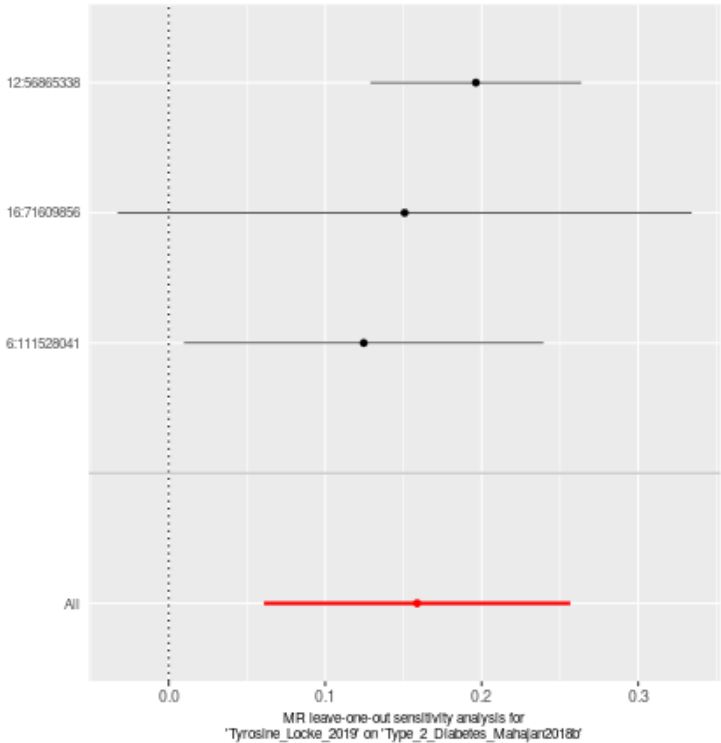
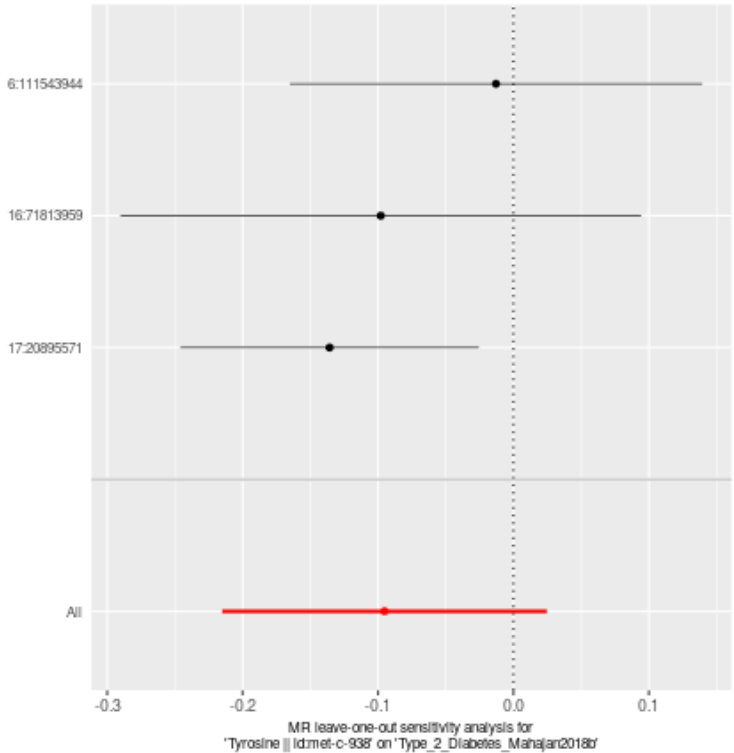


Table S9 Associations of single genetic instruments used for MR of tyrosine from Kettunen et al. and Locke et al. and type 2 diabetes

See Excel file in Supplement B

Table S10 Total causal effects of tyrosine from Shin et al. and other risk factors for type 2 diabetes

Outcome	Data source*	MR-Base-ID/Author/PMID	Beta (SE) from IVW	P-Value	Heterogeneity Q-statistic, p-value	Directional horizontal pleiotropy** Egger-intercept (SE), p-value
BMI	UK Biobank	ukb-b-19953/ Ben Elsworth	-0.26 (0.33)	0.43	15.13, 0.001	-0.01 (0.05), 0.91
Waist circumference	UK Biobank	ukb-b-9405/ Ben Elsworth	-0.03 (0.25)	0.91	10.76, 0.005	0.0003 (0.04), 0.995
Fasting blood glucose	MAGIC	ebi-a-GCST000568/ Dupuis 2010/ 20081858	-0.39 (0.22)	0.08	1.58, 0.45	0.02 (0.03), 0.66
Fasting blood glucose adjusted for BMI	MAGIC	ebi-a-GCST005186/ Manning 2012/ 22581228	-0.68 (0.26)	0.008	3.91, 0.14	-0.02 (0.03), 0.58
Blood glucose	UK Biobank	ukb-d-30740_irnt/ Neale Lab	-0.43 (0.15)	0.004	0.06, 0.97	0.0007 (0.02), 0.97
Fasting blood insulin	MAGIC	ebi-a-GCST000571/ Dupuis J 2010/ 20081858	-0.16 (0.23)	0.50	1.14, 0.57	0.02 (0.03), 0.63
Fasting blood insulin adjusted for BMI	MAGIC	ebi-a-GCST005185/ Manning 2012/ 22581228	-0.01 (0.19)	0.95	0.73, 0.70	0.02 (0.02), 0.56

IVW, inverse variance weighted method; PMID, Pubmed identifier; SE, standard error;
Tyrosine was instrumented by three variants from Shin et al. [3] (see Table S3/S4)

*accessed via MR-Base platform [4]

** assessed by MR-Egger (>2 variants needed)

Table S11 Instruments from genetic risk score for insulin resistance*

SNP*	Chr:Position	Gene	Effect allele	Other allele	EAF**	Beta**	SE**	P-Value**
rs4846565	1:219722104	<i>LYPLAL1</i>	G	A	0.690	0.015	0.0026	2.01E-08
rs10195252	2:165513091	<i>GRB14</i>	C	T	0.442	-0.016	0.0026	4.87E-10
rs2943645	2:227099180	<i>IRS1</i>	C	T	0.372	-0.013	0.0025	1.37E-07
rs17036328	3:12390484	<i>PPARG</i>	T	C	0.903	0.015	0.0036	1.92E-05
rs6822892	4:157734675	<i>PDGFC</i>	A	C/G	0.646	0.011	0.0025	3.16E-05
rs3822072	4:89741269	<i>FAM13A1</i>	G	A	0.455	-0.0092	0.0024	1.19E-04
rs4865796	5:53272664	<i>ARL15</i>	A	G	0.708	0.015	0.0026	2.09E-08
rs459193	5:55806751	<i>ANKRD55/ MAP3K1</i>	G	A	0.783	0.014	0.0027	6.57E-08
rs2745353	6:127452935	<i>RSPO3</i>	T	C	0.550	0.014	0.0025	5.48E-09
rs731839	19:33899065	<i>PEPD</i>	A	G	0.659	-0.014	0.0026	1.72E-08

EAF, effect allele frequency; SE, standard error; T2DM, type 2 diabetes

* GRS for Insulin resistance [5]

**GWAS data from MAGIC on fasting insulin [6]

Table S12 Total causal effects of insulin resistance on amino acid traits from EPIC-Potsdam

Amino acid trait	N (SNPs) ^a	Beta (SE) from IVW	P-Value	Heterogeneity between SNPs; Q-statistic, p- value	Directional horizontal pleiotropy ^b ; Egger-intercept (SE), p-value	Outlier detected
Glycine	9/10	-0.354 (0.756)	0.640	no; 7.57, 0.48	no; 0.04 (0.08), 0.65	no
Glycine/Serine	9/10	1.167 (0.756)	0.123	no; 5.06, 0.75	no; 0.12 (0.08), 0.12	no
Phenylalanine	9/10	0.155 (0.855)	0.856	no; 10.13, 0.26	no; -0.04 (0.09), 0.70	no
Phenylalanine/Arginine	9/10	1.592 (0.927)	0.086	no; 11.95, 0.15	no; 0.10 (0.09), 0.30	no
Serine/Phenylalanine	9/10	-1.895 (0.862)	0.028	no; 10.85, 0.21	no; -0.06 (0.09), 0.50	no
Tryptophan/Glutamine	9/10	0.080 (0.750)	0.915	no; 7.85, 0.45	no; -0.01 (0.08), 0.90	no
Tyrosine/Tryptophan	9/10	0.515 (0.748)	0.491	no; 4.66, 0.79	no; 0.03 (0.07), 0.71	no
Tyrosine/Methionine	9/10	1.486 (0.744)	0.046	no; 3.63, 0.89	no; -0.01 (0.07), 0.93	no
xLeucine/Methionine	9/10	1.626 (0.864)	0.060	no; 10.90, 0.21	no; -0.01 (0.09), 0.89	Yes (rs2943645)

	8/9	0.974 (0.783)	0.213	no; 4.31, 0.74	no; -0.052 (0.08), 0.49	no
Valine/xLeucine	9/10	-0.975 (0.916)	0.287	no; 11.84, 0.16	no; 0.04 (0.10), 0.72	no

IVW, inverse variance weighted method; SE, standard error; xLeu = Isoleucine + Leucine

^a used instruments/suitable instruments available in the GWAS of amino acid trait; rs6822892 excluded as triallelic variant;

^b assessed by MR-Egger (>2 variants needed)

Table S13 Associations of single genetic instruments used for MR of insulin resistance and amino acids traits from EPIC-Potsdam

See Excel file in Supplement B

Table S14 Total causal effects of insulin resistance and single amino acids

Amino acid trait*	N (SNPs)**	Beta (SE) from IVW method	P-Value	Heterogeneity; Q-statistic, p-value	Directional horizontal pleiotropy*** Egger-intercept (SE), p- value
EPIC-Potsdam					
Serine	9/10	-1.75 (0.75)	0.02	no; 7.54, 0.48	-0.091 (0.075), 0.22
Tyrosine	9/10	0.29 (0.81)	0.72	no; 9.38, 0.31	-0.016 (0.086), 0.85
Methionine	9/10	-0.96 (0.76)	0.21	no; 6.49, 0.59	-0.015 (0.075), 0.84
Shin et al [3]					
Serine	9/10	-0.07 (0.06)	0.21	no; 2.64, 0.95	-0.002 (0.006), 0.73
Phenylalanine	9/10	0.003 (0.03)	0.92	no; 4.64, 0.80	0.001 (0.003), 0.87
Tyrosine	9/10	0.05 (0.04)	0.20	no; 8.78, 0.36	0.001 (0.005), 0.88
Methionine	9/10	-0.02 (0.04)	0.65	no; 9.92, 0.27	0.003 (0.004), 0.53

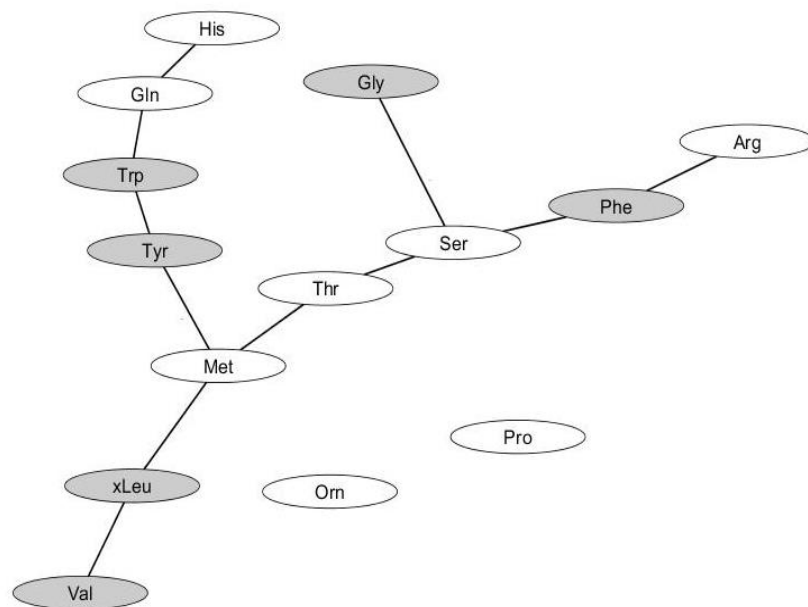
IVW, inverse variance weighted method; SE, standard error

* single amino acids from ratios, showing causal influence by insulin resistance in EPIC-Potsdam

** used instruments/suitable instruments available in the GWAS of single amino acids; rs6822892 excluded as triallelic variant

Table S15 Associations of single genetic instruments used for MR of insulin resistance and single amino acids

See Excel file in Supplement B



Amino acid traits tested within this study:

Glycine	Gly
Phenylalanine	Phe
Glycine/Serine	Gly/Ser
Serine/Phenylalanine	Ser/Phe
Phenylalanine/Arginine	Phe/Arg
Valine/xLeucine	Val/xLeu
xLeucine/Methionine	xLeu/Met
Tyrosine/Methionine	Tyr/Met
Tyrosine/Tryptophan	Tyr/Trp
Tryptophan/Glutamine	Trp/Glu

Figure S1 Network structure of amino acids within EPIC-Potsdam

Diabetes-associated amino acids are depicted in grey ellipses; solid line indicates direct association between amino acids, GGM network was adapted from the publication by A. Floegel *et al.* [7] and drawn by using Cytoscape Software v3.2.1 [8]

xLeucine = Isoleucine + Leucine

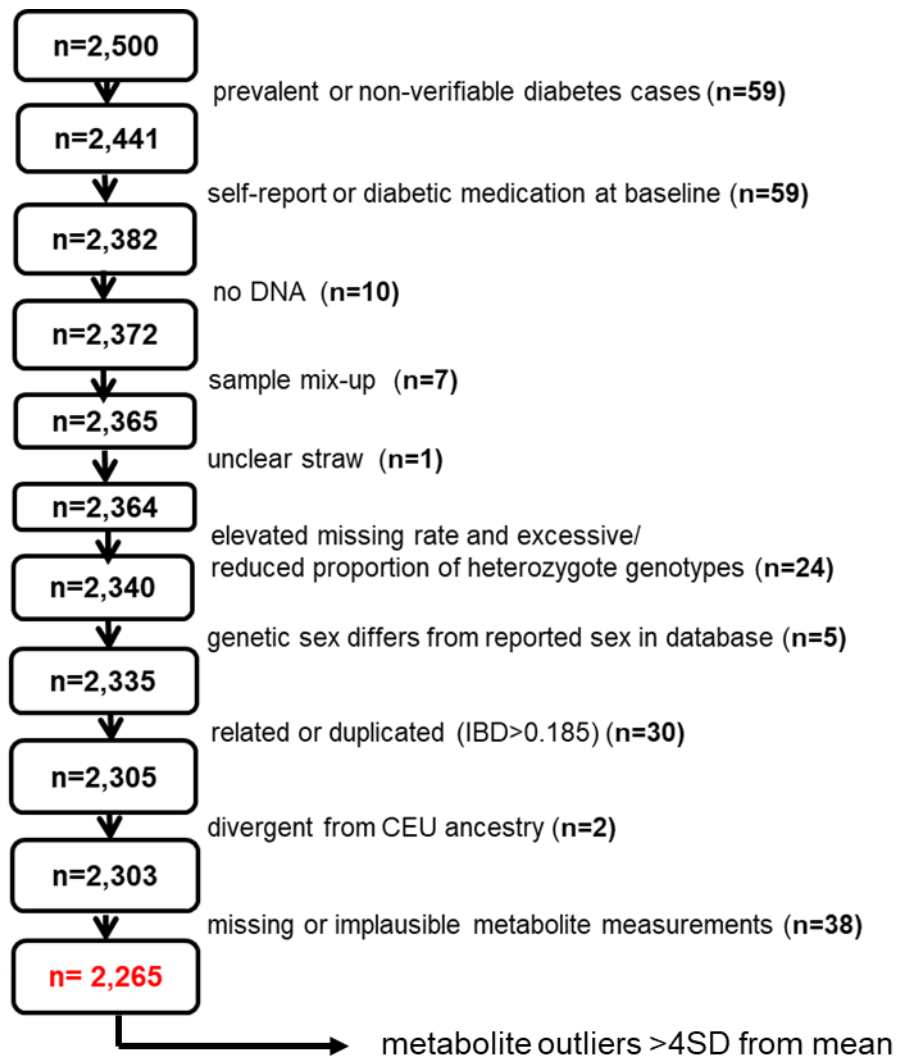


Figure S2 Flow-chart of final study population in EPIC-Potsdam

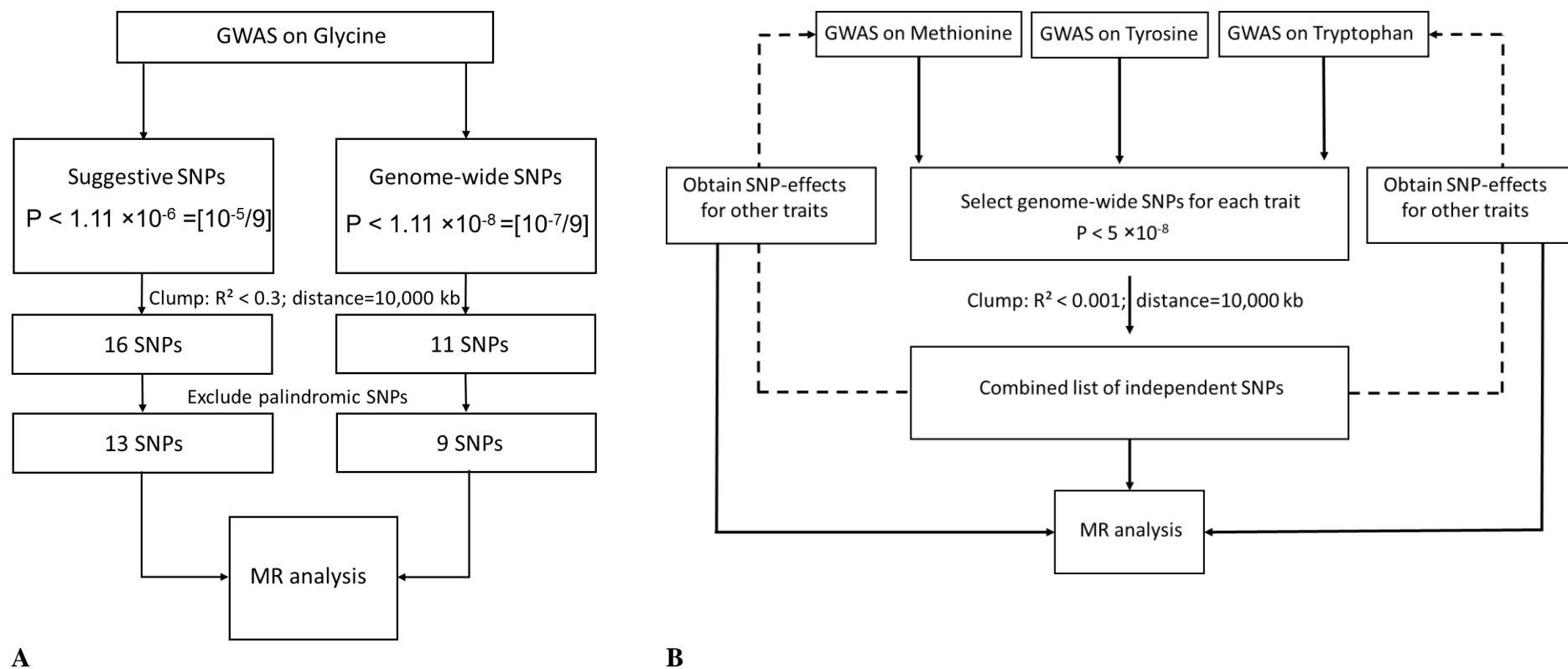
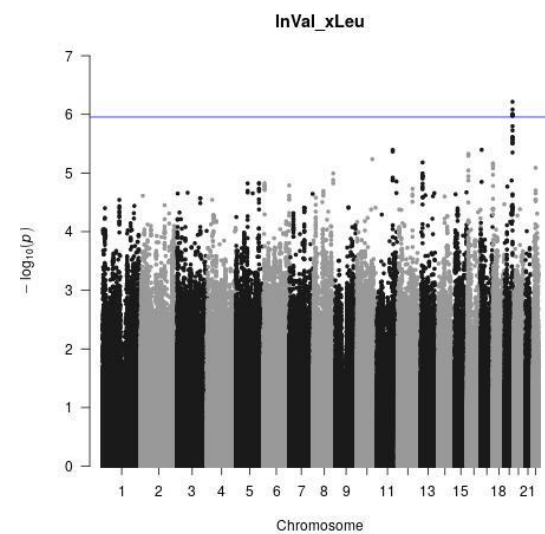
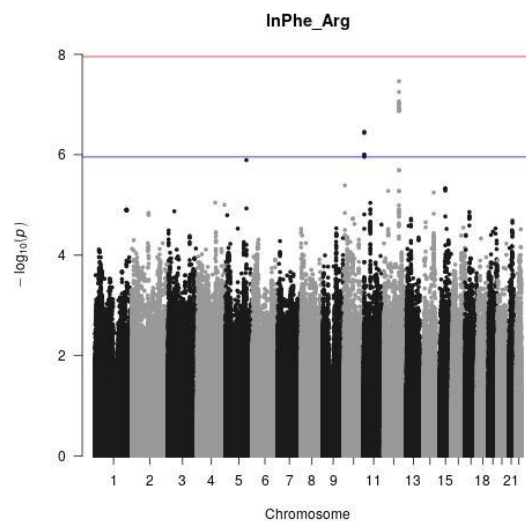
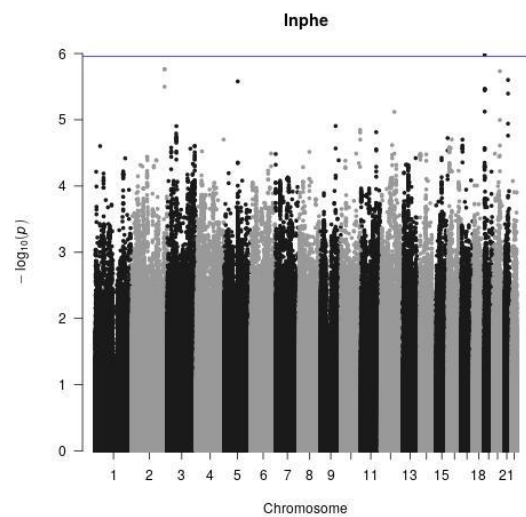
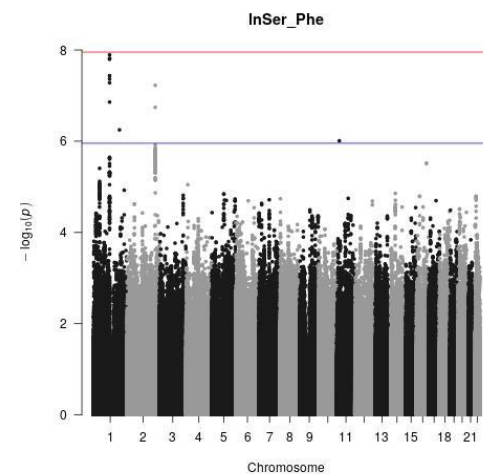
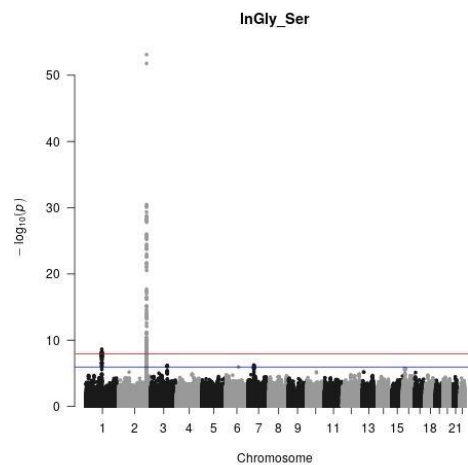
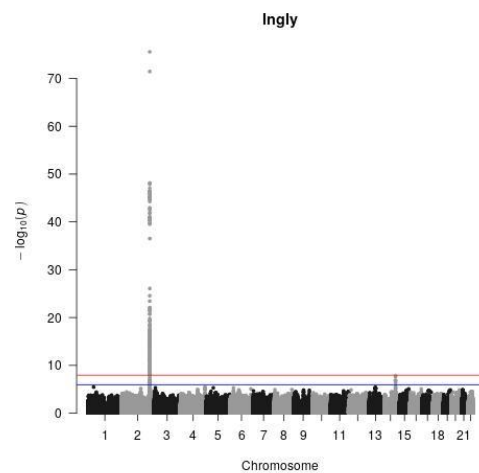


Figure S3 Example flow charts of inclusion and exclusion of SNPs into the analysis

A univariable MR: First, we restricted to the set of SNPs according to the suggestive or genome-wide significance threshold. Next, we clumped the SNP list with thresholds $R^2 \leq 0.3$ or $R^2 \leq 0.001$ to receive independent instruments. **B** multivariable MR: First, we restricted to the set of SNPs according to the genome-wide significance threshold for each exposure. Next, we clumped the SNP list with thresholds $R^2 \leq 0.001$ to receive independent instruments and obtained associations with the other exposures. In MR analysis, palindromic SNPs were excluded.



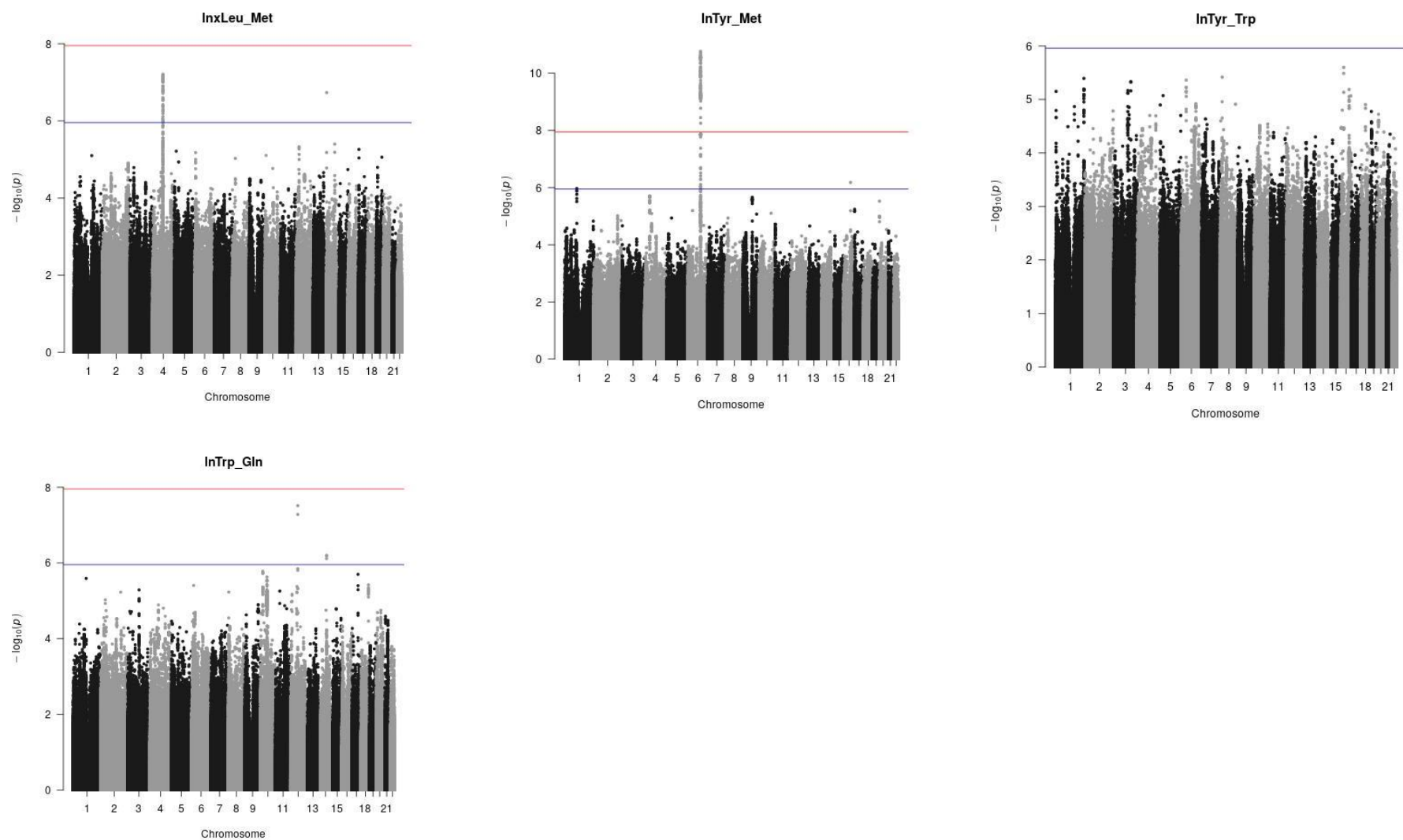


Figure S4 Manhattan plots visualizing GWAS results for amino acid traits in EPIC-Potsdam

Blue line indicates suggestive significance threshold ($P < 1.11 \times 10^{-6}$), orange line indicates genome-wide significance threshold ($P < 1.11 \times 10^{-8}$)

References

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