Association between circulating alanine and cancer susceptibility using Mendelian randomization

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Abstract

Background

Circulating alanine concentrations are associated with several cancers, but little is known about the causal direction of the associations. This study aims to explore whether there is a relationship between circulating alanine and ten common cancers.

Methods

We conducted two-sample Mendelian randomization (MR) analysis to assess the causal effects of circulating alanine on ten common cancers. According to published genome-wide association studies (GWASs), we obtained 36 alanine-related single nucleotide polymorphisms used as instrumental variables. For exposure data, genetic association data of lung, breast, pancreatic, liver, colorectal, esophageal, stomach, thyroid, prostate and ovarian cancer from GWAS Consortia were used, including up to 1,213,351 participants of European origin and 196,187 participants of East Asian. The inverse variance weighting (IVW) method was used for MR analysis, and MR-Egger and the weighted median method further evaluated the pleiotropic effect.

Results

Specific to cancer GWAS, we found that circulating alanine was significantly associated with increased squamous cell lung cancer (odds ratio [OR]: 1.37, 95% CI = 1.00-1.87; P = 0.048), pancreatic cancer (OR 3.02, 95% CI = 1.35 to 6.76; P = 0.007), low grade serous ovarian cancer (OR 1.81, 95% CI = 1.01 to 3.25; P = 0.047). We have no evidence of a convincing causal effect of circulating alanine concentrations predicted by genetics on other cancer risks.

Conclusion

We observed the possible causal relationship between circulating alanine and lung squamous cell carcinoma, pancreatic cancer, low-grade serial ovarian cancer. Further research is needed to verify this causal relationship.

Introduction

Metabolic reprogramming has been widely elucidated in tumor cells since the discovery of the Warburg effect in the 1920s(Koppenol 2011). Metabolic reprogramming is a typical marker of tumor cells, including glucose fatty acid and amino acid metabolism(Martínez-Reyes 2021). Amino acids are among the most suitable candidates for focused metabolomics because they are either ingested or synthesized endogenous and play important physiological roles as basic metabolites and metabolic regulators. L-alanine is a non-essential amino acid and one of the amino acids that make up human protein. It is produced by pyruvate catalyzed by alanine transaminase. Because L-alanine can participate in protein synthesis and gluconeogenesis, activate the AMPK signal pathway and regulate cell, tissue and systemic metabolism(Adachi 2018), it has been widely used in daily medical and healthcare products. Furthermore, the growth of tumors also needs nutrition. The

accumulated evidence of basic research shows that serine(Muthusamy 2020), glycine(Tajan 2021) and other non-essential amino acids(LeBoeuf 2020) are essential for tumor progression. Since alanine is the second richest amino acid in protein located at the central hub of carbon metabolism, it is likely to be absorbed by cancer cells(Caiola 2020), so evaluating the causal relationship between alanine and various cancers is urgent.

Most existing studies show that alanine can cause the proliferation of Kelch-like ECH-associated protein 1 (Keap1) mutant cells(LeBoeuf 2020), breast cells and inhibit the proliferation of gastric cancer cells(Gu 2015). It can also affect the growth of non-adherent tumor cells(Muthusamy 2020). In observational research, S Leij-Halfwerk suggests abnormal alanine metabolism in lung cancer patients with weight loss. These changes may be related to weight loss but not lung cancer itself(Leij-Halfwerk 2000). Interestingly, alanine levels in the plasma metabolic spectrum of breast cancer patients and healthy people decrease or increase(Huang 2016). Increased alanine concentration was also observed in patients with prostate cancer(Swanson 2006). In a metabolic analysis of ovarian cancer, benign ovarian tumors and normal people, the alanine level of ovarian cancer was higher than that of the other two groups(Zhong 2022).Meanwhile, due to the limitations of the experimental design, observational studies may be affected by potential confounders, leading to different conclusions. Moreover, alanine has been considered a biomarker for detecting certain cancers(Kumar 2015). Therefore, it remains unclear whether there is a true causal relationship between alanine itself and lung cancer.

MR is a new approach that could provide evidence about the putative causal relationship between modifiable risk factors and disease[6]. Mendelian randomization relies on the natural assortment of genetic variants during meiosis yielding a random distribution of genetic variants in a population[7]. Individuals are assigned genetic variations or not at birth, regardless of environmental factors. With genetic variants used as instrumental variables for risk factors, MR can be regarded as a natural analog of classical randomized controlled trials (RCTs), which could determine whether the risk factors are causal for the disease conveniently[8].

This study conducted a two-sample MR analysis to comprehensively evaluate the causal association between alanine and lung cancer, using the summary data from the published GWASs.

Method

Data Source

The current MR analysis was comprehensively performed by leveraging information from ten GWASs totaling 1,213,351 participants of European origin and 196,187 participants of East Asian, including 247,271 cancer cases and 996,080 controls across the lung (including lung adenocarcinoma and lung squamous cell carcinoma), breast, pancreatic (including ER-positive and ER-negative), liver, colorectal, esophagus, stomach, thyroid, prostate and ovarian cancer (including high-grade serous ovarian cancer cases and low-grade serous ovarian cancer). The characteristics of each cancer-specific GWAS, including sample sizes and data sources, are illustrated in Table 2.

Instrumental variable (IV) selection

Circulating alanine was the main exposure of interest. We collected GWAS-identified circulating alanine-associated single-nucleotide polymorphisms (Table 1) from a large GWAS available to date, which met the

following criteria as instruments for MR analysis: (i) reported P-value < 5.00×10^{-8}, (ii) minor allele frequency (MAF) ≥ 0.05. We use the F statistic (F = beta2/se2) to assess the strength of IV to avoid potential weak tool bias as much as possible. If F > 10, the correlation between IV and exposure is considered to be strong enough that the results of the MR analysis can be protected from weak instrumental bias(Burgess 2011).

Sensitivity analyses

To ensure that our findings were robust MR results, we performed heterogeneity analysis and MR-Egger regression analysis. The heterogeneity test was used to assess whether a genetic variant's effect on cancer risk was proportional to its effect on circulating alanine. A fixed-effects model was utilized when no statistically significant heterogeneity was presented. Otherwise, the random-effects model was used to provide more conservative estimates(Greenland1987). MR-Egger regression (MR-Egger intercept test) was fitted to evaluate the presence of horizontal pleiotropy(Sproviero 2021). Therefore, IVW analysis should be performed after the removal of genetic variants in the sensitivity analysis.

Our research used MR-base (http://www.mrbase.org/) for data analysis and three R packages (R version 4.20), MRInstruments, TwoSampleMR, and googleAuthR, for statistical analysis.

Statistical Analysis

Our MR studies identified and extracted exposure-associated SNP information at the genome-wide significance level. In addition, traits associated with alanine-related SNPs were searched on the PhenoScanner website (http://www.phenoscanner.medschl.cam.ac.uk/) to determine whether the selected instrumental variables were associated with any confounding factors in the potential association between alanine levels and cancer. To ensure genetic instruments were independent, a pairwise-linkage disequilibrium (LD) clumping method was used with an r2 cutoff of 0.001. The unqualified SNP is excluded from MR analysis.

Two-sample MR analysis evaluated the association between circulating alanine intake at baseline and the cancer risk. Estimates of genetic causality were obtained by applying fixed effects IVW analysis. This was done to determine the overall effect of alanine concentration on each outcome. In the MR analysis, the statistical significance threshold was P < 0.05. For each single nucleotide polymorphism (SNP), the ratio of the causal effect of exposure on the outcome was estimated as the ratio of the effect of the SNP on the outcome to the effect of the SNP on the exposure. In IVW, the overall estimate was derived from an IVW randomized analysis of the ratio estimates for all variables in a set of instrumental variables. Considering that IVW methods can be affected by directional pleiotropy (genetic variation affecting the outcome through pathways other than exposure), we used MR-Egger and weighted median methods to check the robustness of IVW method estimates(Hartwig 2017).

Results

Descriptives

For alanine-specific GWAS, the smallest F-statistics of the SNPs was 30 (larger than 10), indicating a strong instrumental strength. List the genetic variation characteristics used to represent alanine, and estimates of R^2 and F-statistics for each target are presented in Table 1. The sample sizes for each site-specific cancer in each

participating study are listed in Table 2. Table 1 summarizes the characteristics of selected SNPs related to alanine.

Table 1
Summary of 36 traits related to circulating alanine-associated SNPs.

SNP	Exposure	Effect allele	EAF	*P* value	Beta	R^2	F-statistic
rs2808454	Alanine	T	0.543	1.7×10^{-25}	-0.043	0.001	109
rs79687284	Alanine	C	0.035	4.7×10^{-33}	0.134	0.001	143
rs2160387	Alanine	C	0.428	5.9×10^{-28}	-0.045	0.001	120
rs6752053	Alanine	C	0.409	5.7×10^{-10}	-0.026	0.000	38
rs1047891	Alanine	A	0.315	1.6×10^{-10}	-0.028	0.000	41
rs1260326	Alanine	C	0.604	1.9×10^{-40}	-0.056	0.001	177
rs11715633	Alanine	A	0.271	3.3×10^{-10}	-0.029	0.000	39
rs7624902	Alanine	G	0.538	4.1×10^{-21}	-0.039	0.001	89
rs4693210	Alanine	G	0.440	1.8×10^{-15}	-0.033	0.001	63
s138373837	Alanine	T	0.024	1.3×10^{-12}	-0.096	0.000	50
rs6931514	Alanine	G	0.262	1.4×10^{-15}	0.037	0.001	64
rs144262262	Alanine	C	0.004	2.1×10^{-08}	-0.194	0.000	31
rs2385127	Alanine	A	0.617	1.8×10^{-11}	-0.028	0.000	45
s11558471	Alanine	G	0.315	3.1×10^{-12}	-0.031	0.000	49
rs13277542	Alanine	G	0.489	4.9×10^{-15}	-0.033	0.001	61
rs56335308	Alanine	A	0.027	3.7×10^{-14}	0.095	0.000	57
rs12379111	Alanine	G	0.095	2.5×10^{-09}	-0.042	0.000	36
rs4237150	Alanine	C	0.403	9.1×10^{-12}	0.028	0.000	47
rs2168101	Alanine	A	0.308	9.0×10^{-31}	-0.053	0.001	133
rs7925445	Alanine	G	0.556	5.6×10^{-13}	0.030	0.000	52
rs74093304	Alanine	A	0.119	2.3×10^{-15}	0.050	0.001	63
s2933243	Alanine	A	0.183	3.3×10^{-56}	0.084	0.002	250
rs7978353	Alanine	G	0.405	2.0×10^{-11}	0.028	0.000	45

Note: SNP, single nucleotide polymorphism; EAF, effect allele frequency; MAF, minor allele frequency; SE, standard error. R^2 was calculated as follows: $2*beta^2*EAF*(1-EAF)$. The *F*-statistic for each SNP was calculated as follows: $F = beta^2/se^2$.

SNP	Exposure	Effect allele	EAF	*P* value	Beta	R^2	F-statistic
rs11183581	Alanine	A	0.213	1.3×10^{-31}	0.058	0.001	137
rs4554975	Alanine	G	0.563	3.3×10^{-27}	-0.045	0.001	117
rs4766214	Alanine	G	0.457	8.3×10^{-10}	-0.025	0.000	38
rs5023078	Alanine	C	0.531	2.8×10^{-09}	-0.024	0.000	35
rs58116791	Alanine	C	0.235	4.2×10^{-08}	0.027	0.000	30
rs4903264	Alanine	T	0.559	2.6×10^{-08}	0.023	0.000	31
rs339969	Alanine	A	0.616	7.3×10^{-09}	0.024	0.000	33
rs8056893	Alanine	A	0.733	2.8×10^{-08}	-0.026	0.000	31
rs34495668	Alanine	C	0.096	1.1×10^{-14}	0.054	0.001	60
rs8061221	Alanine	A	0.746	4.0×10^{-35}	0.058	0.001	153
rs7503139	Alanine	T	0.167	1.3×10^{-13}	-0.041	0.000	55
rs73201506	Alanine	G	0.046	8.6×10^{-09}	0.056	0.000	33
rs807672	Alanine	G	0.531	3.9×10^{-10}	0.026	0.000	39

Note: SNP, single nucleotide polymorphism; EAF, effect allele frequency; MAF, minor allele frequency; SE, standard error. R^2 was calculated as follows: $2*beta^2*EAF*(1-EAF)$. The *F*-statistic for each SNP was calculated as follows: $F = beta^2/se^2$.

Table 2
Summary of cancer-specific GWAS data included in Mendelian randomization (MR) analysis.

Trait	Number of cases	Number of controls	Sample size	Population	PubMed ID	Year	Source[a]
Lung cancer overall	11,348	15,861	27,209	European	24880342	2014	ILCCO
Adenocarcinoma	3,442	14,894	18,336	European	24880342	2014	ILCCO
Squamous cell carcinoma	3,275	15,038	18,313	European	24880342	2014	ILCCO
Breast cancer	122,977	105,974	228,951	European	29059683	2017	BCAC
ER-positive	69,501	105,974	175,475	European	29059683	2017	BCAC
ER-negative	21,468	105,947	127,442	European	29059683	2017	BCAC
Pancreatic cancer	442	195,745	196,187	East Asian	32581250	2019	Biobank Japan
Liver cancer	168	372,016	372,184	European		2021	UK Biobank
Colorectal cancer	5,657	372,016	377,673	European		2021	UK Biobank
Esophageal Cancer	740	372,016	372,756	European		2021	UK Biobank
Stomach Cancer	633	174,006	174,639	European		2021	GWAS
Thyroid cancer	649	431	1,080	European	23894154	2013	Italian
Prostate cancer	79,148	61,106	140,254	European	29892016	2018	PRACTICAL
Ovarian Cancer	25,509	40,941	66,450	European	28346442	2021	OCAC
High grade serous ovarian cancer	13,037	40,941	53,978	European	28346442	2021	OCAC
Low grade serous ovarian cancer	1,012	40,941	41,953	European	28346442	2021	OCAC

A dbGaP, the database of Genotypes and Phenotypes; ILCCO, the International Lung Cancer Consortium Consortium (https://ilcco.iarc.fr/); BCAC, the Breast Cancer Association Consortium (http://bcac.ccge.medschl.cam.ac.uk/); Biobank Japan, (http://biobankjp.org); PRACTICAL, the Prostate Cancer Association Group to Investigate Cancer Associated Alterations in the Genome (http://practical.icr.ac.uk/blog/); OCAC, the Ovarian Cancer Association Consortium (http://ocac.ccge.medschl.cam.ac.uk/).

A causal association between circulating alanine and cancer risk

To investigate the causal effect of alanine on lung cancer, we conducted the conventional Mendelian randomization analysis (IVW method). Table 3 shows MR estimates of circulating alanine and each cancer risk. Notably, genetically predicted higher circulating alanine was causally associated with an increased risk of lung

squamous cell carcinoma (OR 1.37, 95% CI = 1.01-1.87; P = 0.048) (*Supplementary Fig. 1*), pancreatic cancer (OR 3.02, 95% CI = 1.35 to 6.76; P = 0.007) (*Supplementary Fig. 2*) and low grade serous ovarian cancer (OR 1.81, 95% CI = 1.01 to 3.25; P = 0.047) (*Supplementary Fig. 3*). Our two-sample MR analysis indicated that higher circulating alanine was not associated with risks of the following cancers: lung adenocarcinoma, liver cancer, colorectal cancer, esophageal cancer, stomach cancer, thyroid cancer, high-grade serous ovarian cancer, where all P-values were above 0.05 ($P_{IVW} > 0.05$).

Table 3
Associations of circulating alanine instrumental variants (IVs) with multiple cancer risk in cancer-specific GWAS.

Cancer site	IVW method		MR-Egger		Weighted median method	
	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value
Lung cancer overall	1.0435(0.8574-1.2700)	0.6712	0.9794(0.5647–1.6985)	0.9415	0.9924(0.7591–1.2973)	0.9553
Adenocarcinoma	0.9284(0.6891–1.2508)	0.6253	0.8864(0.3968-1.9800)	0.7710	0.9975(0.6390–1.5569)	0.9910
Squamous cell carcinoma	1.3705(1.0026–1.8735)	0.0481	0.8605(0.3648-2.0300)	0.7341	1.1280(0.7234–1.7589)	0.5951
Breast cancer	1.0628(0.9348–1.2083)	0.3525	0.9986(0.6959–1.4327)	0.9938	1.0600(0.9245–1.2155)	0.4037
ER-positive	1.0628(0.9748–1.2347)	0.1242	0.9894(0.7119–1.3750)	0.9499	1.1321(0.9893–1.3027)	0.0830
ER-negative	0.9671(0.8178–1.1436)	0.0855	0.9392(0.5843–1.5098)	0.7891	0.9496(0.7681–1.1740)	0.6327
Pancreatic cancer	3.0175(1.3476–6.7568)	0.0072	10.5136(1.0706–103.2430)	0.0553	1.5418(0.4812–4.9404)	0.4662
Liver cancer	1.0001(0.9995–1.0008)	0.7255	1.0005(0.9986–1.0024)	0.5947	1.0001(0.9993–1.0010)	0.7563
Colorectal cancer	1.0002(0.9974–1.0030)	0.8937	0.9936(0.9866–1.0006)	0.0821	0.9997(0.9956–1.0037)	0.8760
Esophageal Cancer	1.0003(0.9992–1.0013)	0.6432	1.0000(0.9973–1.0028)	0.9766	1.0001(0.9985–1.0016)	0.9398
Stomach Cancer	0.8862(0.4933–1.5919)	0.6861	0.8198(0.1604–4.1888)	0.8129	0.8071(0.3496–1.8631)	0.6156
Thyroid cancer	1.7829(0.6218–5.1124)	0.2820	1.0795(0.0672–17.3468)	0.9576	1.7649(0.3991–7.8049)	0.4538
Prostate cancer	1.0627(0.9334–1.2099)	0.3581	1.4505(1.0609–1.9832)	0.0272	1.1656(1.0184–1.3341)	0.0261
Ovarian Cancer	0.9628(0.8136–1.1392)	0.6585	1.2298(0.7792–1.9410)	0.3835	0.9029(0.7104–1.1476)	0.4038
High grade serous ovarian cancer	0.8892(0.7097–1.1142)	0.3075	1.4385(0.7941–2.6060)	0.2426	0.9740(0.7384–1.2848)	0.8520
Low grade serous ovarian cancer	1.8084(1.0077–3.2453)	0.0471	4.3559(0.8616–22.0216)	0.0883	2.2330(0.9205–5.4168)	0.0756

OR: odds ratio; 95% CI: 95% confidence interval; IVW = Inverse-variance weighted.

Sensitivity analysis

Implementing more SNP instruments would usually translate to better trait prediction and hence better power for MR. It also has the potential to introduce higher levels of heterogeneity amongst the effects of the genetic instruments. Except that breast cancer has heterogeneity ($P_{heterogeneity} < 0.05$) and prostate cancer shows heterogeneity and diversity ($P_{heterogeneity} < 0.05$; $P_{MR-Egger\ intercept} < 0.05$), other causal estimates have no heterogeneity or directional pleiotropy ($P_{heterogeneity} > 0.05$; $P_{MR-Egger\ intercept} > 0.05$) (Table 4).

Table 4
MR-Egger pleiotropy test of the associations between circulating alanine and risk of cancer-specific GWAS.

Outcome	Heterogeneity test	MR-Egger	
	P-value	intercept	P-value
Lung cancer overall	0.9133	0.0027	0.8110
Adenocarcinoma	0.6086	0.0020	0.9041
Squamous cell carcinoma	0.3338	0.0201	0.2641
Breast cancer	0.000008	0.0027	0.7199
ER-positive	0.0226	0.0044	0.5154
ER-negative	0.0832	0.0012	0.8982
Pancreatic cancer	0.4422	-0.0558	0.2646
Liver cancer	0.0791	-0.00002	0.6587
Colorectal cancer	0.5206	0.0003	0.0515
Esophageal Cancer	0.405	0.00001	0.8701
Stomach Cancer	0.9389	0.0035	0.9207
Thyroid cancer	0.8778	0.0215	0.7069
Prostate cancer	0.0001	-0.0142	0.0431
Ovarian Cancer	0.3440	-0.0106	0.2701
High grade serous ovarian cancer	0.1004	-0.0207	0.1018
Low grade serous ovarian cancer	0.6434	-0.0377	0.2660

Discussion

In this large-scale genetic association study, we evaluated the causal relationship of circulating alanine with the risk of common cancers capitalizing on the largest available cancer-specific GWAS data, the UK Biobank cohort of European ancestry, and the part of the East Asian population. Our MR analysis demonstrated that genetically predicted alanine level was causally associated with an increased risk of lung squamous cell carcinoma, pancreatic cancer, prostate cancer and low-grade serous ovarian cancer, while no causal association was observed for other cancers.

The main function of amino acids in protein synthesis. Meanwhile, amino acid metabolism is an important metabolic pathway for regulating tumor cell growth, reproduction(Vettore 2020) and supporting immunity(Kelly 2020). A growing body of evidence has suggested that amino acids are potential biomarkers that could be used in cancer screening(Mandarano 2021; Shingyoji 2013). Our research provides evidence for the potential causal relationship between alanine and cancer. Previous observational studies and routine epidemiological analysis reports are only sometimes consistent. In a case-control study involving 564 researchers, it was found that the plasma concentration of Ala in NSCLC patients was significantly higher than that in the control group. The model was constructed in combination with other amino acids and its stability was verified in 4079 sample species(Maeda 2010). In a prospective nested case-control study, Wang S conducted an untargeted metabolomics study in subjects with incident pancreatic cancer (n = 68) and matched controls (n = 136) and the results showed that alanine was significantly associated with an increased risk of pancreatic cancer(Wang 2022). Moreover, in another study, the concentration of alanine in plasma and urine of the control group was lower than that of ovarian cancer(Zhong 2022). In a case-control study, statistically significant differences in serum alanine levels were found in patients with prostate cancer compared to the control group(Dereziński 2017). Yang Q et al. also found that the concentration of alanine in malignant ascites of patients with ovarian cancer was significantly higher than that of normal and was related to the stage of the disease(Yang 2022). However, in another clinical and laboratory study, alanine has a proliferative effect on breast cancer cells and an inhibitory effect on gastric cancer cells(Gu 2015).

Alanine is located at the center of carbon metabolism and is synthesized by alanine aminotransferase (ALAT). It is thought to be mainly carried out in mitochondria. It competes with pyruvate dehydrogenase (PDH) for mitochondrial pyruvate and then forms acetyl coenzyme A. Despite these inconsistencies in observational studies, there is still some new evidence indicating the importance of alanine metabolism in cancer. For example, the biosynthesis of alanine has been proven to be related to cell proliferation(Coloff 2016). Caiola E et al. determined that the catabolism of alanine-derived pyruvate plays an important role in driving NSCLC cell survival after glutaminase inhibition(Caiola 2020). Recent evidence shows that ALAT activity is associated with α- Ketoglutaric acid and is crucial to driving ECM formation(Elia 2019). More sufficient evidence shows that pancreatic stellate cells secrete alanine through autophagy to support the TCA cycle and promote the proliferation and survival of pancreatic cancer cells(Davidson 2017). Pancreatic cancer and pancreatic stellate cells have used the differential expression of SLC38A2 and SLC1A4 to form an alanine exchange niche. Cells lacking SLC38A2 cannot concentrate alanine in the cells, resulting in significant tumor growth restriction. It is a promising method for treating pancreatic cancer by inhibiting SLC38A2-targeted alanine uptake and utilization(Parker 2020).

In our study, IVW results did not show a causal relationship between circulating alanine and prostate cancer, but due to its heterogeneity ($P_{heterogeneity} < 0.05$) (Table 4), we need to consider the impact of heterogeneity, and in the case of horizontal pleiotropy ($P_{MR-Egger\ intercept} < 0.05$) (Table 4), we use the results of the MR-Egger method(Sproviero 2021) ($OR_{MR-Egger}$ 1.45, 95% CI = 1.06 to 1.98; $P_{MR-Egger} = 0.027$) (Table 3). The same result was also shown in another gene predicting the risk relationship between alanine and prostate cancer(Yang 2022).

Our study has several strengths. This was the first large-scale MR analysis that systemically evaluated a causal association between circulating alanine and the risk of multiple cancers, leveraging cancer-specific GWAS data

of 247,271 solid cancer cases and controls. This may enrich our understanding of various potential risk factors for cancer.

This study has several limitations. First, because the sample size of some cancers is not enough, it may not represent the overall population. Second, our results are mainly based on participants of European ancestry and may not be generalizable to other ethnic populations.

Conclusion

In summary, based on this MR study making causal inferences between circulating alanine concentrations and the risk of multiple cancers. Our MR shows that circulating high alanine has a causal effect on the development risk of lung squamous cell carcinoma, pancreatic cancer, prostate cancer and low-grade serous ovarian cancer, which conveys an important public health message that taking alanine supplements may promote the occurrence and development of some cancers. In addition, because of the limited statistical capacity of some of the cancers in our studies, large-scale studies of these cancers will be needed in the future to confirm our results. At the same time, further studies are warranted to validate the circulating alanine and the potential mechanisms of the effects on different cancers.

Declarations

Author contributions

Qi Cai, Xiwen Liu, Lixuan Lin and Miao He designed the whole article. Shuting Zhan performed data analysis and made the graphes. Huiting Liu and Linchong Huang collated the results of the data. Qi Cai, Xiwen Liu and Lixuan Lin wrote the first draft of the manuscript. Qi Cai and Miao He revised the primary version. Wenhua Liang and Jianxing He revised the final version of the manuscript. All authors contributed to revisions of the manuscript. Qi Cai is the guarantor. All authors approved the final manuscript.

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Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of interest

The authors have no conflict of interest regarding this study.

References

1. Adachi Y, De Sousa-Coelho A L, Harata I, Aoun C, Weimer S, Shi X, Gonzalez Herrera K N, Takahashi H, Doherty C, Noguchi Y, Goodyear L J, Haigis M C, Gerszten R E Patti M E (2018) l-Alanine activates hepatic

AMP-activated protein kinase and modulates systemic glucose metabolism. Molecular metabolism 17, 61-70.http://dx.doi.org/10.1016/j.molmet.2018.08.002

2. Burgess S Thompson S G (2011) Avoiding bias from weak instruments in Mendelian randomization studies. International journal of epidemiology 40, 755-764.http://dx.doi.org/10.1093/ije/dyr036
3. Caiola E, Colombo M, Sestito G, Lupi M, Marabese M, Pastorelli R, Broggini M Brunelli L (2020) Glutaminase Inhibition on NSCLC Depends on Extracellular Alanine Exploitation. Cells 9.http://dx.doi.org/10.3390/cells9081766
4. Coloff J L, Murphy J P, Braun C R, Harris I S, Shelton L M, Kami K, Gygi S P, Selfors L M Brugge J S (2016) Differential Glutamate Metabolism in Proliferating and Quiescent Mammary Epithelial Cells. Cell metabolism 23, 867-880.http://dx.doi.org/10.1016/j.cmet.2016.03.016
5. Davidson S M, Jonas O, Keibler M A, Hou H W, Luengo A, Mayers J R, Wyckoff J, Del Rosario A M, Whitman M, Chin C R, Condon K J, Lammers A, Kellersberger K A, Stall B K, Stephanopoulos G, Bar-Sagi D, Han J, Rabinowitz J D, Cima M J, Langer R Vander Heiden M G (2017) Direct evidence for cancer-cell-autonomous extracellular protein catabolism in pancreatic tumors. Nature medicine 23, 235-241.http://dx.doi.org/10.1038/nm.4256
6. Dereziński P, Klupczynska A, Sawicki W, Pałka J A Kokot Z J (2017) Amino Acid Profiles of Serum and Urine in Search for Prostate Cancer Biomarkers: a Pilot Study. International journal of medical sciences 14, 1-12.http://dx.doi.org/10.7150/ijms.15783
7. Elia I, Rossi M, Stegen S, Broekaert D, Doglioni G, van Gorsel M, Boon R, Escalona-Noguero C, Torrekens S, Verfaillie C, Verbeken E, Carmeliet G Fendt S M (2019) Breast cancer cells rely on environmental pyruvate to shape the metastatic niche. Nature 568, 117-121.http://dx.doi.org/10.1038/s41586-019-0977-x
8. Greenland S (1987) Quantitative methods in the review of epidemiologic literature. Epidemiologic reviews 9, 1-30.http://dx.doi.org/10.1093/oxfordjournals.epirev.a036298
9. Gu Y, Chen T, Fu S, Sun X, Wang L, Wang J, Lu Y, Ding S, Ruan G, Teng L Wang M (2015) Perioperative dynamics and significance of amino acid profiles in patients with cancer. Journal of translational medicine 13, 35.http://dx.doi.org/10.1186/s12967-015-0408-1
10. Hartwig F P, Davey Smith G Bowden J (2017) Robust inference in summary data Mendelian randomization via the zero modal pleiotropy assumption. International journal of epidemiology 46, 1985-1998.http://dx.doi.org/10.1093/ije/dyx102
11. Huang S, Chong N, Lewis N E, Jia W, Xie G Garmire L X (2016) Novel personalized pathway-based metabolomics models reveal key metabolic pathways for breast cancer diagnosis. Genome medicine 8, 34.http://dx.doi.org/10.1186/s13073-016-0289-9
12. Kelly B Pearce E L (2020) Amino Assets: How Amino Acids Support Immunity. Cell metabolism 32, 154-175.http://dx.doi.org/10.1016/j.cmet.2020.06.010
13. Koppenol W H, Bounds P L Dang C V (2011) Otto Warburg's contributions to current concepts of cancer metabolism. Nature reviews. Cancer 11, 325-337.http://dx.doi.org/10.1038/nrc3038
14. Kumar D, Gupta A, Mandhani A Sankhwar S N (2015) Metabolomics-derived prostate cancer biomarkers: fact or fiction? Journal of proteome research 14, 1455-1464.http://dx.doi.org/10.1021/pr5011108
15. LeBoeuf S E, Wu W L, Karakousi T R, Karadal B, Jackson S R, Davidson S M, Wong K K, Koralov S B, Sayin V I Papagiannakopoulos T (2020) Activation of Oxidative Stress Response in Cancer Generates a Druggable

Dependency on Exogenous Non-essential Amino Acids. Cell metabolism 31, 339-350.e334.http://dx.doi.org/10.1016/j.cmet.2019.11.012

16. Leij-Halfwerk S, Dagnelie P C, van Den Berg J W, Wattimena J D, Hordijk-Luijk C H Wilson J P (2000) Weight loss and elevated gluconeogenesis from alanine in lung cancer patients. The American journal of clinical nutrition 71, 583-589.http://dx.doi.org/10.1093/ajcn/71.2.583
17. Maeda J, Higashiyama M, Imaizumi A, Nakayama T, Yamamoto H, Daimon T, Yamakado M, Imamura F Kodama K (2010) Possibility of multivariate function composed of plasma amino acid profiles as a novel screening index for non-small cell lung cancer: a case control study. BMC cancer 10, 690.http://dx.doi.org/10.1186/1471-2407-10-690
18. Mandarano M, Orecchini E, Bellezza G, Vannucci J, Ludovini V, Baglivo S, Tofanetti F R, Chiari R, Loreti E, Puma F, Sidoni A Belladonna M L (2021) Kynurenine/Tryptophan Ratio as a Potential Blood-Based Biomarker in Non-Small Cell Lung Cancer. International journal of molecular sciences 22.http://dx.doi.org/10.3390/ijms22094403
19. Martínez-Reyes I Chandel N S (2021) Cancer metabolism: looking forward. Nature reviews. Cancer 21, 669-680.http://dx.doi.org/10.1038/s41568-021-00378-6
20. Muthusamy T, Cordes T, Handzlik M K, You L, Lim E W, Gengatharan J, Pinto A F M, Badur M G, Kolar M J, Wallace M, Saghatelian A Metallo C M (2020) Serine restriction alters sphingolipid diversity to constrain tumour growth. Nature 586, 790-795.http://dx.doi.org/10.1038/s41586-020-2609-x
21. Parker S J, Amendola C R, Hollinshead K E R, Yu Q, Yamamoto K, Encarnación-Rosado J, Rose R E, LaRue M M, Sohn A S W, Biancur D E, Paulo J A, Gygi S P, Jones D R, Wang H, Philips M R, Bar-Sagi D, Mancias J D Kimmelman A C (2020) Selective Alanine Transporter Utilization Creates a Targetable Metabolic Niche in Pancreatic Cancer. Cancer discovery 10, 1018-1037.http://dx.doi.org/10.1158/2159-8290.Cd-19-0959
22. Shingyoji M, Iizasa T, Higashiyama M, Imamura F, Saruki N, Imaizumi A, Yamamoto H, Daimon T, Tochikubo O, Mitsushima T, Yamakado M Kimura H (2013) The significance and robustness of a plasma free amino acid (PFAA) profile-based multiplex function for detecting lung cancer. BMC cancer 13, 77.http://dx.doi.org/10.1186/1471-2407-13-77
23. Sproviero W, Winchester L, Newby D, Fernandes M, Shi L, Goodday S M, Prats-Uribe A, Alhambra D P, Buckley N J Nevado-Holgado A J (2021) High Blood Pressure and Risk of Dementia: A Two-Sample Mendelian Randomization Study in the UK Biobank. Biological psychiatry 89, 817-824.http://dx.doi.org/10.1016/j.biopsych.2020.12.015
24. Swanson M G, Zektzer A S, Tabatabai Z L, Simko J, Jarso S, Keshari K R, Schmitt L, Carroll P R, Shinohara K, Vigneron D B Kurhanewicz J (2006) Quantitative analysis of prostate metabolites using 1H HR-MAS spectroscopy. Magnetic resonance in medicine 55, 1257-1264.http://dx.doi.org/10.1002/mrm.20909
25. Tajan M, Hennequart M, Cheung E C, Zani F, Hock A K, Legrave N, Maddocks O D K, Ridgway R A, Athineos D, Suárez-Bonnet A, Ludwig R L, Novellasdemunt L, Angelis N, Li V S W, Vlachogiannis G, Valeri N, Mainolfi N, Suri V, Friedman A, Manfredi M, Blyth K, Sansom O J Vousden K H (2021) Serine synthesis pathway inhibition cooperates with dietary serine and glycine limitation for cancer therapy. Nature communications 12, 366.http://dx.doi.org/10.1038/s41467-020-20223-y
26. Vettore L, Westbrook R L Tennant D A (2020) New aspects of amino acid metabolism in cancer. British journal of cancer 122, 150-156.http://dx.doi.org/10.1038/s41416-019-0620-5

27. Wang S, Li M, Yan L, He M, Lin H, Xu Y, Wan Q, Qin G, Chen G, Xu M, Wang G, Qin Y, Luo Z, Tang X, Wang T, Zhao Z, Xu Y, Chen Y, Huo Y, Hu R, Ye Z, Dai M, Shi L, Gao Z, Su Q, Mu Y, Zhao J, Chen L, Zeng T, Yu X, Li Q, Shen F, Chen L, Zhang Y, Wang Y, Deng H, Liu C, Wu S, Yang T, Li D, Ning G, Wu T, Wang W, Bi Y Lu J (2022) Metabolomics study reveals systematic metabolic dysregulation and early detection markers associated with incident pancreatic cancer. International journal of cancer 150, 1091-1100.http://dx.doi.org/10.1002/ijc.33877
28. Yang Q, Bae G, Nadiradze G, Castagna A, Berezhnoy G, Zizmare L, Kulkarni A, Singh Y, Weinreich F J, Kommoss S, Reymond M A Trautwein C (2022) Acidic ascites inhibits ovarian cancer cell proliferation and correlates with the metabolomic, lipidomic and inflammatory phenotype of human patients. Journal of translational medicine 20, 581.http://dx.doi.org/10.1186/s12967-022-03763-3
29. Yang S, Song J, Yang H, Liu W, Jiang Y, Sun X, Ye D, Xu S Mao Y (2022) Genetically Predicted Circulating Concentrations of Alanine and Alanine Aminotransferase Were Associated with Prostate Cancer Risk. Clinical epidemiology 14, 1255-1264.http://dx.doi.org/10.2147/clep.S382116
30. Zhong X, Ran R, Gao S, Shi M, Shi X, Long F, Zhou Y, Yang Y, Tang X, Lin A, He W, Yu T Han T L (2022) Complex metabolic interactions between ovary, plasma, urine, and hair in ovarian cancer. Frontiers in oncology 12, 916375.http://dx.doi.org/10.3389/fonc.2022.916375

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