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Research Article

Circulating Biomarkers of Tryptophan and the Kynurenine Pathway and Lung Cancer Risk

Shu-Chun Chuang[1], Anouar Fanidi[6], Per Magne Ueland[10,12], Caroline Relton[3], Øivind Midttun[13], Stein Emil Vollset[11,14], Marc J. Gunter[1], Michael J. Seckl[2], Ruth C. Travis[4], Nicholas Wareham[5], Antonia Trichopoulou[16,19], Pagona Lagiou[16,17,19], Dimitrios Trichopoulos[17,18,19], Petra H.M. Peeters[1,20], H. Bas Bueno-de-Mesquita[21,22], Heiner Boeing[23], Angelika Wientzek[23], Tilman Kuehn[24], Rudolf Kaaks[24], Rosario Tumino[25], Claudia Agnoli[26], Domenico Palli[27], Alessio Naccarati[28], Eva Ardanaz Aicua[29], María-José Sánchez[30,31], José Ramón Quirós[32], María-Dolores Chirlaque[31,33], Antonio Agudo[34], Mikael Johansson[35], Kjell Grankvist[36], Marie-Christine Boutron-Ruault[7], Françoise Clavel-Chapelon[8], Guy Fagherazzi[9], Elisabete Weiderpass[15], Elio Riboli[1], Paul J. Brennan[6], Paolo Vineis[1,28], and Mattias Johansson[6]

Abstract

Background: Imbalances in tryptophan metabolism have been linked to cancer-related immune escape and implicated in several cancers, including lung cancer.

Methods: We conducted a nested case–control study within the European Prospective Investigation into Cancer and Nutrition (EPIC) that included 893 incident lung cancer cases and 1,748 matched controls. Circulating levels of tryptophan and six of its metabolites were measured and evaluated in relation to lung cancer risk.

Results: Tryptophan ($P_{trend} = 2 \times 10^{-5}$) and the kynurenine/tryptophan ratio (KTR; $P_{trend} = 4 \times 10^{-5}$) were associated with lung cancer risk overall after adjusting for established risk factors. The ORs comparing the fifth and first quintiles ($OR_{5th\ vs.\ 1st}$) were 0.52 [95% confidence interval (CI), 0.37–0.74] for tryptophan and 1.74 (95% CI, 1.24–2.45) for KTR. After adjusting for plasma methionine (available from previous work, which was strongly correlated with tryptophan), the associations of tryptophan (adjusted $P_{trend} = 0.13$) and KTR ($P_{trend} = 0.009$) were substantially attenuated. KTR was positively associated with squamous cell carcinoma, the $OR_{5th\ vs.\ 1st}$ being 2.83 (95% CI, 1.62–4.94, $P_{trend} = 3 \times 10^{-5}$) that was only marginally affected by adjusting for methionine.

Conclusions: This study indicates that biomarkers of tryptophan metabolism are associated with subsequent lung cancer risk. Although this result would seem consistent with the immune system having a role in lung cancer development, the overall associations were dependent on methionine, and further studies are warranted to further elucidate the importance of these metabolites in lung cancer etiology.

Impact: This is the first prospective study investigating the tryptophan pathway in relation to lung cancer risk. *Cancer Epidemiol Biomarkers Prev; 23(3); 461–8. ©2013 AACR.*

Authors' Affiliations: [1]School of Public Health; [2]Department of Surgery and Cancer, Faculty of Medicine, Imperial College London, London; [3]Institute for Ageing and Health, Newcastle University, Newcastle; [4]Cancer Epidemiology Unit, Nuffield Department of Clinical Medicine, University of Oxford, Oxford; [5]Department of Public Health and Primary Care, University of Cambridge, Cambridge, United Kingdom; [6]International Agency for Research on Cancer, Lyon; [7]Inserm U1018, Centre for Research in Epidemiology and Population Health (CESP); [8]University Paris Sud, UMRS 1018; [9]IGR, Villejuif, France; [10]Section of Pharmacology, Institute of Medicine; [11]Department of Public Health and Primary Health Care, University of Bergen; [12]Laboratory of Clinical Biochemistry, Haukeland University Hospital; [13]Bevital AS; [14]Division of Epidemiology, Norwegian Institute of Public Health, Bergen; [15]Department of Community Medicine, Faculty of Health Sciences, University of Tromsø, Tromsø, Norway; [16]WHO Collaborating Center for Food and Nutrition Policies, Department of Hygiene, Epidemiology and Medical Statistics, University of Athens Medical School; [17]Bureau of Epidemiologic Research, Academy of Athens; [18]Hellenic Health Foundation, Athens, Greece; [19]Department of Epidemiology, Harvard School of Public Health, Boston, Massachusetts; [20]Department of Epidemiology, Julius Center for Health Sciences and Primary Care, University Medical Center; [21]Department of Gastroenterology and Hepatology, University Medical Centre, Utrecht; [22]National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands; [23]Department of Epidemiology, German Institute of Human Nutrition Potsdam-Rehbruecke, Nuthetal; [24]Division of Cancer Epidemiology, German Cancer Research Center (DKFZ), Heidelberg, Germany; [25]Cancer Registry and Histopathology Unit, "Civile M.P.Arezzo" Hospital, ASP Ragusa; [26]Nutritional Epidemiology Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan; [27]Molecular and Nutritional Epidemiology Unit, Cancer Research and Prevention Institute–ISPO, Florence; [28]Human Genetics Foundation (HuGeF), Torino, Italy; [29]Navarre Public Health Institute, Pamplona; [30]Andalusian School of Public Health, Granada; [31]CIBER Epidemiología y Salud Pública (CIBERESP), Madrid; [32]Public Health Directorate Asturias, Oviedo; [33]Department of Epidemiology, Murcia Regional Health Authority, Murcia; [34]Unit of Nutrition, Environment, and Cancer, Catalan Institute of Oncology, Barcelona, Spain [35]Department of Radiation Sciences & Oncology, University Hospital; and [36]Department of Medical Biosciences, Clinical Chemistry, Umeå University, Umeå, Sweden

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S.-C. Chuang and A. Fanidi contributed equally to this work.

Current affiliation for Shu-Chun Chuang: Division of Health Policy Translation, Institutes of Population Health Sciences, National Health Research Institutes, Miaoli, Taiwan.

Corresponding Author: Mattias Johansson, International Agency for Research on Cancer (IARC), 150 cours Albert Thomas, 69008 Lyon, France. Phone: 33-4-7273-8023; Fax: 33-4-7273-8342; E-mail: johanssonm@iarc.fr

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Introduction

Lung cancer is annually responsible for 1.4 million deaths and remains the most lethal cancer worldwide (1). In Europe, lung cancer was the third most common cancer (12% of all new cancers) and the most common cause of death from cancer (20% of total cancer deaths) in 2008 (2), the age- and area-adjusted 5-year survival rate being 12% (3). There are four major histologic types of lung cancer, which comprise more than 90% of all cases: squamous cell carcinoma (SCC), large cell carcinoma (LCC), adenocarcinoma, and small-cell lung carcinoma (SCLC; ref. 4). Cigarette smoking is the single most important risk factor for all types of lung cancer; however, adenocarcinoma typically shows a weaker association with smoking than do the other histologic types (5, 6).

There is a massive body of evidence on the role of the immune system in cancer development and progression (7, 8). Cancer cells that escape immune response/surveillance have important growth advantages, often leading to tumor progression (8, 9). The kynurenine pathway (Fig. 1), and in particular, the key enzymes involved, 2,3-dioxygenase (IDO) and 2,3-tryptophan dioxygenase (TDO), have been implicated in cancer-related immune escape (8, 10–13). High IDO activity causes an increased conversion of tryptophan to its primary catabolite, kynurenine. Kynurenine can be further metabolized into anthranilic acid, xanthurenic acid, 3-hydroxykynurenine, kynurenic acid, and 3-hydroxyanthranilic acid (HAA) via vitamin B2- and B6-dependent enzymes (ref. 14; Fig. 1). High levels of kynurenine inhibit T-cell proliferation, induce T-cell apoptosis, and lead to immune tolerance, thus providing a microenvironment for tumor cells to grow (11, 14).

Another potential mechanism for the role of tryptophan metabolism is related to cigarette smoking. Tobacco smoke contains a wide array of known carcinogens, including polycyclic aromatic hydrocarbon (PAHs), nitrosamines, and aromatic amines (15). In particular benzo[a]pyrene, a strong PAH carcinogen in the lung, is mediated by the aryl hydrocarbon receptor (AHR), and recent data from Opitz and colleagues linked TDO expression to AHR activation (9, 16). This provides an additional mechanism by which enzymes related to metabolism of tryptophan through the kynurenine pathway may play a role in lung cancer etiology. It is methodologically complex to measure cellular IDO and TDO activity directly in an epidemiologic setting, but the ratio between circulating

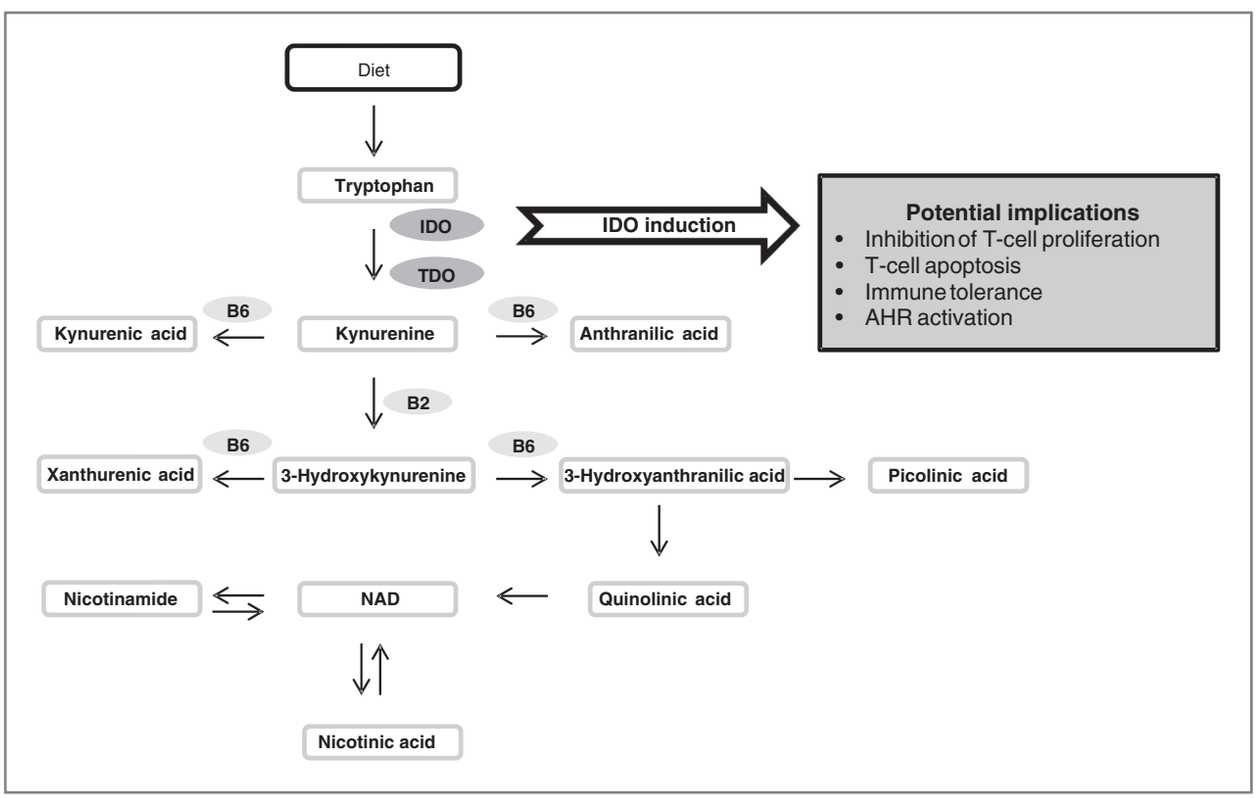

Figure 1. The tryptophan degradation pathway and potential implications of IDO induction. Tryptophan is an essential amino acid and is obtained by external sources. IDO transforms tryptophan into kynurenine, which is then metabolized into other catabolites within the kynurenine pathway. IDO induction decreases the local concentration of tryptophan, while increasing the concentration of downstream metabolites. IDO, indolamine 2,3-dioxygenase; TDO, tryptophan 2,3-dioxygenase; NAD, nicotinamide adenine dinucleotide; AHR, aryl hydrocarbon receptor; B2, riboflamin; B6, pyridoxal 5′-phosphate.

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kynurenine and tryptophan (KTR) is widely accepted as an indirect measure of IDO activity (11, 13). Circulating tryptophan and kynurenine concentrations have been associated with poor outcome among patients with lung cancer (17, 18), but have not yet been evaluated in relation to lung cancer incidence in a prospective setting.

The aim of the current study was to prospectively investigate the associations between circulating tryptophan, kynurenine pathway metabolites, and lung cancer risk in a large cohort.

Materials and Methods

The European Prospective Investigation into Cancer and Nutrition study

European Prospective Investigation into Cancer and Nutrition (EPIC) is a multicenter prospective cohort study, which recruited subjects from 23 centers in 10 countries between 1992 and 2000. EPIC recruitment procedures and collection of questionnaire data and blood samples have been described in detail elsewhere (19). In brief, standardized questionnaire data were collected from 521,330 individuals across Europe, of whom 385,747 provided a blood sample. Blood samples were fractionated (serum, plasma, red cells, and buffy coat), aliquoted into 0.5 mL straws, and the majority were stored in liquid nitrogen tanks at the International Agency for Research on Cancer (IARC) in Lyon, France at −196°C, and four centers (Umeå, Malmö, Copenhagen, and Aarhus) stored their samples in local biobanks. Informed consent forms were completed at each local center and the study was approved by the Institutional Review Board at the IARC and by the local ethical committees.

Ascertainment of cases and control selection

Incident cancer cases were identified through population cancer registries (Denmark, Italy except Naples, the Netherlands, Norway, Spain, Sweden, and the United Kingdom) or by active follow-up (France, Germany, Greece, and Naples). Active follow-up involved a combination of methods, including review of health insurance records, cancer and pathology registries, as well as direct contact with participants and their next of kin. Subjects were followed up from study entry until cancer diagnosis (except nonmelanoma skin cancer), death, emigration, or the end of the follow-up period for the relevant study center. End of follow-up was defined as the latest date of complete follow-up for both cancer incidence and vital status and varied between study centers from 2002 to 2006.

The selection criteria of the EPIC lung cancer study have been described previously (20). In brief, the study included eight of the 10 participating countries: the Netherlands, the United Kingdom, France, Germany, Spain, Italy, Greece, and Sweden (except for Malmö center). For each incident case, two controls were selected by incidence density sampling and matched by center, gender, date of blood collection (±1 month, relaxed to ±5 months for sets without available controls), and date of birth (±1 year, relaxed to ±5 years for sets without available controls). Overall, 904 cases and 1,825 controls were included in the study. We further excluded subjects without kynurenine and tryptophan measurements (nine cases and 23 controls), two additional cases without matched controls, and 54 controls without matched cases. The final dataset for analysis was composed of 893 cases and 1,748 controls.

Biologic samples and laboratory analyses

Concentrations of tryptophan and kynurenine were determined simultaneously by gas chromatography/tandem mass spectrometry (21). Concentrations of anthranilic acid, xanthurenic acid, 3-hydroxykynurenine, kynurenic acid and HAA, neopterin, and cotinine were determined by liquid chromatography/tandem mass spectrometry (22). Measurements of methionine and vitamin B6 (pyridoxal 5′-phosphate; PLP) were available from a previous investigation of the same study population (20). All laboratory analyses were performed at Bevital AS (23).

Samples were analyzed in batches of 86 and quality control included six calibration samples, two control samples, and one blank sample in each batch. Samples from case and control participants were kept at −80°C and analyzed in random order. The within and between day coefficients of variation for the assays were 3% to 16% (22). The staffs at the Bevital laboratory were blinded to the case–control and the demographic status of the blood samples.

Statistical analysis

Quintile cutoff values for each biomarker were defined on the basis of the distributions among controls. The correlation between biomarkers was assessed by Spearman ranked partial correlation coefficients (ρ) adjusted for age, sex, country, cotinine concentrations (nmol/L), and body mass index (BMI; kg/m^2).

Conditional logistic regression was used to estimate OR and 95% confidence intervals (CI) for lung cancer, with the lowest quintile serving as the reference category. All models included adjustments by tobacco smoking (never, former, current, unknown, and cotinine concentrations). Adjusting for additional smoking variables (duration of smoking, average cigarettes smoked per day) did not alter the results notably. Further adjustments were conducted for education (no degree/primary school, technical or professional school, secondary school, university degree, and not specified/missing), BMI (kg/m^2), as well as quintiles of PLP and methionine that were strongly associated with lung cancer risk in a previous EPIC study (20).

Stratified risk analyses were conducted by histology, smoking status, and time from blood draw to diagnosis by unconditional logistic regression adjusted for the matching variables (age, in 5-year categories, sex, and country). The overall trend for each biomarker (P_{trend}) and stratified analyses were calculated by including the base 2 logarithm ($\log_2$) of the biomarker concentrations as a continuous variable in separate logistic regression models. The OR trend estimate from this model may be interpreted as the relative risk associated with a doubling in circulating concentrations of the biomarker of interest.

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The association between lifestyle factors with biomarker levels was investigated using linear regression models, adjusting for age, sex, and country, and further adjusting for cotinine (in quintiles) when appropriate.

All statistical tests were two sided and performed using SAS 9.1.

Results

Detailed baseline characteristics of the study population were presented previously (20). Briefly (Table 1), 893 lung cancer cases and 1,748 controls in total were included in the current study, out of whom 62% were men and 38% were women. The proportion of current smokers was 59% among cases and 23% among controls, whereas 29% of cases and 37% of controls were former smokers. The overall concentrations of tryptophan and kynurenines in cases and controls are shown in Table 1. The Spearman ranked correlation coefficients between biomarkers are presented in Supplementary Table S1.

Table 2 shows the associations between tryptophan, kynurenine, KTR, and lung cancer risks overall. After controlling for tobacco smoking and cotinine concentration, tryptophan displayed an inverse association with lung cancer risk ($P_{trend} = 1 \times 10^{-5}$) and KTR displayed a corresponding positive association with risk ($P_{trend} = 3 \times 10^{-5}$). The OR comparing the fifth versus first quintiles ($OR_{5th\ vs.\ 1st}$) were 0.52 (95% CI, 0.37–0.72) for tryptophan and 1.72 (95% CI, 1.23–2.40) for KTR. Further adjustment for educational attainment and BMI did not notably affect the associations of tryptophan and KTR with lung cancer risk, but controlling for PLP and methionine levels considerably attenuated both the association of tryptophan (adjusted $P_{trend} = 0.13$) and KTR (adjusted $P_{trend} = 0.009$), this being primarily driven by a strong correlation between methionine and tryptophan ($\rho = 0.51$; Supplementary Tables S1 and S2). Further adjusting for combined intake of dairy and total fruit, nut and seed did not materially affect the overall association of circulating tryptophan or KTR with lung cancer risk (adjusted $P_{trend} = 0.17$ and $P_{trend} = 0.009$, respectively). As a secondary analysis, we also investigated other catabolites in the kynurenine pathway (Supplementary Table S3). In particular, plasma levels of kynurenic acid and xanthurenic acid were inversely associated with lung cancer risk ($P_{trend} = 0.009$ and 1×10^{-4}, respectively), but the associations were attenuated and no longer statistically significant after further adjustment by methionine and PLP ($P_{trend} = 0.39$ and 0.07, respectively).

Stratified risk analysis for KTR by smoking status did not reveal large differences in its association with lung cancer risk [$P_{heterogeneity}$ (P_{het}) = 0.31], but the association differed substantially by histology ($P_{het} = 0.01$), being more prominent for cancers classified as SCCs and others (Fig. 2 and Supplementary Table S4). The $OR_{5th\ vs.\ 1st}$ for SCC was 2.83 (95% CI, 1.62–4.94, $P_{trend} = 3 \times 10^{-5}$), and adjusting for PLP and methionine only moderately attenuated this association ($OR_{5th\ vs.\ 1st} = 2.32$, 95% CI, 1.30–4.15, $P_{trend} = 4 \times 10^{-4}$). The association between KTR and lung cancer risk was only apparent in former smokers ($P_{trend} = 0.002$ and Supplementary Table S5), but testing for heterogeneity did not indicate notable differences in OR when compared with never- and current smokers ($P_{het} = 0.33$; Fig. 2)

Discussion

This is the first study exploring the association between tryptophan metabolites and lung cancer risk in a prospective study design. We found that the KTR was positively associated with overall risk, an association that was largely explained by a strong correlation between methionine and tryptophan. The association of KTR with risk was particularly prominent for SCC.

The key enzymes in degradation of tryptophan through the kynurenine pathway, IDO and TDO, as well as downstream catabolites may influence the immune system (11, 24), and thus affect cancer development and progression (7, 8, 10, 12, 17, 18, 25). KTR is considered an indirect marker of IDO activity and was in the current study associated with increased risk of lung cancer. Although no previous prospective studies have investigated KTR in relation to lung cancer, our data would seem consistent with studies conducted in case series in which tryptophan depletion was associated with worse clinical outcome (17, 18). However, the association of KTR with lung cancer risk was primarily driven by an inverse association of tryptophan, to a large extent explained by its strong correlation with methionine, which was previously found associated with lung cancer risk in the same study population (20). The association of KTR with lung cancer risk was particularly prominent for cancers classified as SCC, and controlling for methionine in this subgroup had little impact on the OR estimates. This observation is intriguing because kynurenine is an endogenous ligand of AHR (16), which in turn mediates the metabolism of PAHs, including potent carcinogens that are specifically relevant in SCC development (26, 27). The association of KTR with SCC cancers may therefore indicate activation of a pathway related to detoxification of environmental chemicals.

There is limited evidence for the other kynurenines, anthranilic acid, kynurenic acid, xanthurenic acid, and HAA, in relation to cancer development or progression (Supplementary Table S5). For xanthurenic acid, we observed an inverse association with overall risk that was independent of KTR. Immunologic studies suggest that the downstream kynurenines may serve as a feedback of TH1 cell suppression and enhance TH2 response (24). Indeed, inflammation biomarkers have previously been strongly associated with lung cancer risk, indicating that inflammatory processes may be important in early lung cancer pathogenesis (28, 29). It would be useful to obtain measurement of inflammation biomarkers, such as C-reactive protein, to further evaluate whether these associations are linked to inflammation. Alternatively, the associations may reflect the PLP-dependent catabolism of

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Table 1. Baseline and clinical characteristics of study participants

	No. (%) of participants in group	
	Cases (n = 893)	**Controls (n = 1,748)**
Discrete variables		
Participating countries		
France	24 (3)	48 (3)
Italy	139 (16)	276 (16)
Spain	130 (14)	259 (15)
United Kingdom	175 (20)	347 (20)
The Netherlands	120 (13)	239 (13)
Greece	90 (10)	180 (10)
Germany	159 (18)	308 (18)
Sweden	56 (6)	93 (5)
Sex		
Men	556 (62)	1,086 (62)
Women	337 (38)	662 (38)
Smoking status		
Never	96 (11)	674 (39)
Former	257 (29)	648 (37)
Current	526 (59)	396 (23)
Unknown	14 (1)	30 (1)
Education		
None or primary school completed	458 (51)	751 (43)
Technical/professional school	192 (22)	369 (21)
Secondary school	109 (12)	238 (14)
Higher education	95 (11)	311 (18)
Not specified or missing	39 (4)	78 (4)
Body mass index[a]		
<20	40 (4)	42 (2)
(20–25)	343 (38)	568 (33)
(25–30)	382 (43)	836 (48)
(30–35)	105 (12)	250 (14)
≥35	23 (3)	52 (3)
Continuous variables, median (5th–95th percentile)		
Age at blood draw, y	59 (42–72)	59 (42–72)
Circulating concentrations for components of the one-carbon metabolism		
Cotinine (nmol/L)	929 (0–2354)	2.8 (0–1483)
Pyridoxal 5′-phosphate (nmol/L)	31.7 (13.2–87.9)	40.4 (17.0–115.7)
Methionine (μmol/L)	27.38 (19.11–42.58)	29.23 (20.92–44.25)
Neopterin (nmol/L)	16.9 (10.10–31.12)	17.11 (10.16–30.45)
Tryptophan (μmol/L)	72.2 (51.86–94.84)	74.37 (56.87–99.12)
Kynurenine (μmol/L)	1.51 (1.02–2.29)	1.56 (1.11–2.19)
Kynurenine (nmol/L)/tryptophan (μmol/L)	20.97 (14.60–32.66)	20.86 (14.67–30.45)
3-Hydroxykynurenine (nmol/L)	32.82 (18.53–61.66)	33.84 (19.06–59.82)
Kynurenic acid (nmol/L)	43.05 (22.45–79.42)	46.79 (25.65–86.86)
Xanthurenic acid (nmol/L)	11.93 (4.27–26.36)	12.95 (5.51–30.00)
Anthranilic acid (nmol/L)	13.77 (7.37–27.11)	14.95 (8.23–27.49)
3-hydroxyanthranilic acid (nmol/L)	34.03 (18.75–67.18)	37.52 (20.46–67.38)
Clinical characteristics, case participants only		
Age at diagnosis, median (range), y	64 (48–78)	
Months from blood draw to diagnosis, median (range)	62 (10–118)	
Histology, no. (%)		
Small cell lung carcinoma	140 (16)	
Adenocarcinoma	284 (32)	
Large cell carcinoma	63 (7)	
Squamous cell carcinoma	198 (22)	
Other carcinoma[b]	208 (23)	

[a]BMI is calculated as weight in kilograms divided by height in meters squared.

[b]Includes carcinoma Not Otherwise Specified [(NOS), n = 163], carcinoid tumor, NOS (n = 12), sarcomatoid carcinoma (n = 7), salivary gland tumors (n = 1), mesenchymal tumors (n = 1), lymphoproliferative tumors (n = 1), missing histology for the French study (n = 23).

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Table 2. ORs of lung cancer for circulating levels of tryptophan, kynurenine, and KTR

Quintile (range)	Case/control participants[a]	OR (95% CI) Model 1[b] (n = 891/1,748)[b]	Model 2[c] (n = 891/1,748)[c]	Model 3[d] (n = 891/1,748)[d]
Tryptophan (μmol/L)				
1 ≤65.09	239/349	1 (reference)	1 (reference)	1 (reference)
2 (65.09–71.26)	183/351	0.68 (0.50–0.91)	0.67 (0.50–0.91)	0.76 (0.56–1.03)
3 (71.26–77.51)	164/348	0.65 (0.48–0.88)	0.67 (0.49–0.91)	0.80 (0.58–1.11)
4 (77.51–85.67)	177/351	0.74 (0.54–1.01)	0.75 (0.54–1.03)	1.04 (0.74–1.47)
5 >85.67	130/349	0.52 (0.37–0.72)	0.52 (0.37–0.74)	0.88 (0.59–1.30)
P_{trend}[e]		1×10^{-5}	2×10^{-5}	0.13
Kynurenine (μmol/L)				
1 ≤1.30	229/344	1 (reference)	1 (reference)	1 (reference)
2 (1.30–1.48)	188/349	0.97 (0.71–1.32)	0.97 (0.71–1.32)	1.02 (0.74–1.40)
3 (1.48–1.65)	169/358	0.95 (0.69–1.30)	0.94 (0.68–1.30)	1.06 (0.77–1.48)
4 (1.65–1.87)	140/347	0.88 (0.64–1.23)	0.91 (0.65–1.26)	1.02 (0.72–1.43)
5 >1.87	167/350	1.12 (0.80–1.56)	1.11 (0.79–1.56)	1.30 (0.92–1.84)
P for trend[e]		0.41	0.41	0.09
Kynurenine (nmol/L)/tryptophan (μmol/L)				
1 ≤17.59	185/349	1 (reference)	1 (reference)	1 (reference)
2 (17.59–19.77)	162/351	1.08 (0.78–1.50)	1.12 (0.81–1.55)	1.01 (0.72–1.42)
3 (19.77–22.03)	187/353	1.40 (1.01–1.94)	1.43 (1.03–1.98)	1.19 (0.85–1.66)
4 (22.03–25.09)	165/347	1.30 (0.93–1.80)	1.33 (0.95–1.86)	1.07 (0.76–1.50)
5 >25.09	194/348	1.72 (1.23–2.40)	1.74 (1.24–2.45)	1.36 (0.96–1.92)
P_{trend}[e]		3×10^{-5}	4×10^{-5}	0.009

[a]Numbers include all case and control participants for whom laboratory measurements were available.
[b]Model 1 assessed by conditional logistic regression adjusted by smoking status (in three groups) and cotinine (continuous).
[c]Model 2 included variables from Model 1 and further adjustment for educational attainment (in five groups) and BMI (continuous).
[d]Model 3 included variables from Model 1 and further adjustment for pyridoxal 5'-phosphate (in quintiles) and methionine (in quintiles).
[e]P_{trend} assessed by the base 2 logarithm of the circulating levels.

kynurenine (Fig. 1; ref. 14) and is consistent with the positive correlations observed between PLP and kynurenic acid, xanthurenic acid and HAA (Supplementary Table S1). As previously reported (20), PLP is inversely associated with lung cancer risk in this population, but adjusting for PLP did not affect the association with xanthurenic acid (data not shown).

The concentrations of tryptophan and kynurenines measured in the current study are similar to those published for other cohorts (30, 31). This suggests adequate preanalytical samples handling because hydroxylated kynurenines are degraded in serum and plasma at room temperature or during prolonged storage at −25°C (30, 32). KTR showed positive associations with age and BMI as previously shown (33–37), and with neopterin, which can be explained by the fact that both neopterin and IDO are stimulated by INF-γ (38–40). Both KTR and neopterin were negatively associated with PLP, which is in accordance with multiple observations of inflammation or immune activation in subject with low PLP status (41). We also observed an inverse association of KTR, and several kynurenines with cotinine, in line with published results (42), which may reflect the immunosuppressive effect from smoking (43).

An unexpected observation of this study was the strong correlation between tryptophan and methionine (Supplementary Table S1 and S2). To our knowledge, there is currently little evidence implicating methionine in metabolism of tryptophan, but it is possible that intake, uptake, and metabolism of methionine and tryptophan are coregulated (44). The fact that methionine accounted for the overall association with risk of tryptophan, and consequently, to a large extent, the association of KTR, suggests that these associations with overall lung cancer risk are dependent of methionine. Whether they reflect common and correlated sources of these amino acids, a common pathway in which they act in relation to risk, or whether tryptophan is confounded by methionine, are unresolved questions that will require further research.

In conclusion, this study indicates that biomarkers of tryptophan metabolism are associated with subsequent lung cancer risk. Although this result would seem consistent with the immune system being important in lung cancer development, the associations were dependent on

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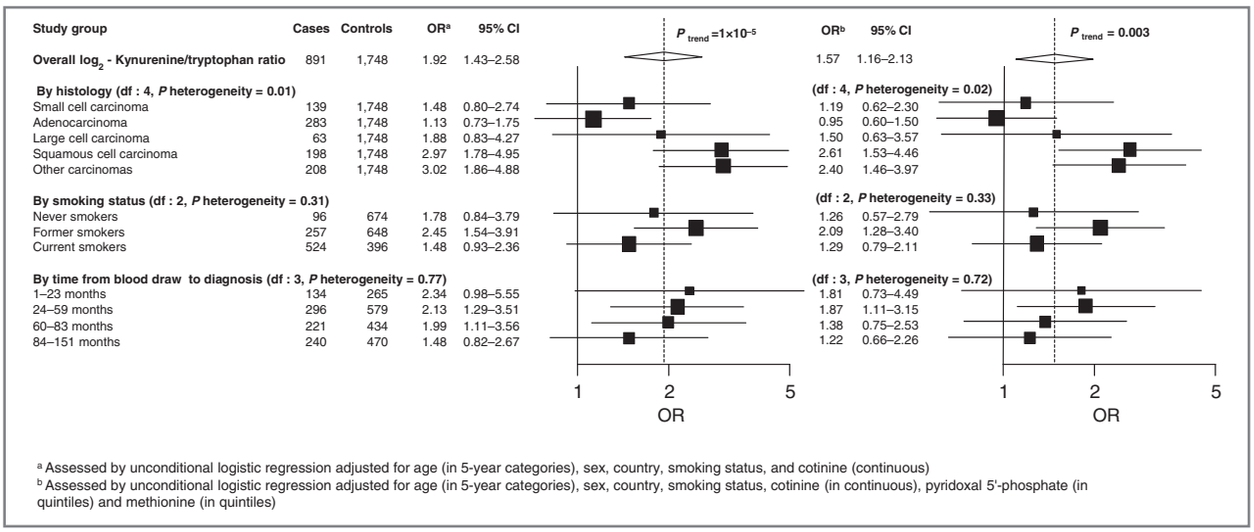

[a] Assessed by unconditional logistic regression adjusted for age (in 5-year categories), sex, country, smoking status, and cotinine (continuous)
[b] Assessed by unconditional logistic regression adjusted for age (in 5-year categories), sex, country, smoking status, cotinine (in continuous), pyridoxal 5'-phosphate (in quintiles) and methionine (in quintiles)

Figure 2. Forest plot shows stratified ORs of lung cancer of $\log_2$- of circulating KTR.

methionine, and further studies are warranted to further elucidate the importance and mechanisms of these complex pathways in lung cancer etiology.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: S.-C. Chuang, A. Fanidi, P. M. Ueland, P.H.M. Peeters, H.B. Bueno-de-Mesquita, R. Tumino, E. Ardanaz Aicua, E. Weiderpass, P.J. Brennan, P. Vineis, M. Johansson

Development of methodology: S.-C. Chuang, P. M. Ueland, Ø. Midttun, H.B. Bueno-de-Mesquita, E. Ardanaz Aicua, E. Weiderpass

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A. Fanidi, P.H.M. Peeters Ø. Midttun, R.C. Travis, N. Wareham, A. Trichopoulou, P. Lagiou, D. Trichopoulos, H.B. Bueno-de-Mesquita, H. Boeing, R. Kaaks, R. Tumino, D. Palli, A. Naccarati, E. Ardanaz Aicua, M.-J. Sanchez, J.R. Quiros, M.-D. Chirlaque, A. Agudo, K. Grankvist, M.-C. Boutron-Ruault, F. Clavel-Chapelon, E. Weiderpass, P.J. Brennan, M. Johansson

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): S.-C. Chuang, A. Fanidi, C. Relton, S.E. Vollset, M. J. Gunter, M. J. Seckl, E. Ardanaz Aicua, A. Agudo, E. Weiderpass, P.J. Brennan, M. Johansson

Writing, review, and/or revision of the manuscript: S.-C. Chuang, A. Fanidi, P. M. Ueland, C. Relton, Ø. Midttun, S. E. Vollset, M. J. Gunter, M. J. Seckl, R.C. Travis, N. Wareham, A. Trichopoulou, P. Lagiou, D. Trichopoulos, P.H.M. Peeters, H.B. Bueno-de-Mesquita, H. Boeing, A. Wientzek, T. Kuehn, R. Kaaks, C. Agnoli, D. Palli, A. Naccarati, E. Ardanaz Aicua, M.-J. Sanchez, J.R. Quiros, M.-D. Chirlaque, A. Agudo, M. Johansson, K. Grankvist, M.-C. Boutron-Ruault, G. Fagherazzi, E. Weiderpass, E. Riboli, P.J. Brennan, P. Vineis, M. Johansson

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): P.M. Ueland, P.H.M. Peeters, H.B. Bueno-de-Mesquita, R. Tumino, E. Ardanaz Aicua, M.-J. Sanchez, J.R. Quiros, M.-D. Chirlaque, E. Weiderpass, M. Johansson

Study supervision: A. Fanidi, P.H.M. Peeters, H.B. Bueno-de-Mesquita, R. Tumino, E. Ardanaz Aicua, E. Weiderpass, P.J. Brennan, M. Johansson

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References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA: Cancer J Clin 2011;61:69–90.
2. Ferlay J, Parkin DM, Steliarova-Foucher E. Estimates of cancer incidence and mortality in Europe in 2008. Eur J Cancer 2010;46:765–81.
3. Sant M, Allemani C, Santaquilani M, Knijn A, Marchesi F, Capocaccia R, et al. EUROCARE-4. Survival of cancer patients diagnosed in 1995–1999. Results and commentary. Eur J Cancer 2009;45:931–91.
4. Spitz MR, Wu X, Wilkinson A, Wei Q. Cancer of the lung. Cancer Epidemiology and Prevention. 3rd ed; 2006. pp. 638–58.
5. Barbone F, Bovenzi M, Cavallieri F, Stanta G. Cigarette smoking and histologic type of lung cancer in men. Chest 1997;112:1474–9.
6. Pesch B, Kendzia B, Gustavsson P, Jockel KH, Johnen G, Pohlabeln H, et al. Cigarette smoking and lung cancer-relative risk estimates for the

Published OnlineFirst December 19, 2013; DOI: 10.1158/1055-9965.EPI-13-0770

major histological types from a pooled analysis of case-control studies. Int J Cancer 2012;131:1210–9.
7. Lyon DE, Walter JM, Starkweather AR, Schubert CM, McCain NL. Tryptophan degradation in women with breast cancer: a pilot study. BMC Res Notes 2011;4:156.
8. Topalian SL, Weiner GJ, Pardoll DM. Cancer immunotherapy comes of age. J Clin Oncol: official journal of the American Society of Clinical Oncology 2011;29:4828–36.
9. Prendergast GC. Cancer: Why tumours eat tryptophan. Nature 2011;478:192–4.
10. Engin AB, Ozkan Y, Fuchs D, Yardim-Akaydin S. Increased tryptophan degradation in patients with bronchus carcinoma. Eur J Cancer Care 2010;19:803–8.
11. Le Floc'h N, Otten W, Merlot E. Tryptophan metabolism, from nutrition to potential therapeutic applications. Amino Acids 2011;41:1195–205.
12. Prendergast GC. Immune escape as a fundamental trait of cancer: focus on IDO. Oncogene 2008;27:3889–900.
13. Schrocksnadel K, Wirleitner B, Winkler C, Fuchs D. Monitoring tryptophan metabolism in chronic immune activation. Clin Chim Acta 2006;364:82–90.
14. Kolodziej LR, Paleolog EM, Williams RO. Kynurenine metabolism in health and disease. Amino Acids 2011;41:1173–83.
15. Hecht SS. Tobacco carcinogens, their biomarkers and tobacco-induced cancer. Nat Rev Cancer 2003;3:733–44.
16. Opitz CA, Litzenburger UM, Sahm F, Ott M, Tritschler I, Trump S, et al. An endogenous tumour-promoting ligand of the human aryl hydrocarbon receptor. Nature 2011;478:197–203.
17. Karanikas V, Zamanakou M, Kerenidi T, Dahabreh J, Hevas A, Nakou M, et al. Indoleamine 2,3-dioxygenase (IDO) expression in lung cancer. Cancer Biol Ther 2007;6:1258–62.
18. Suzuki Y, Suda T, Furuhashi K, Suzuki M, Fujie M, Hahimoto D, et al. Increased serum kynurenine/tryptophan ratio correlates with disease progression in lung cancer. Lung Cancer 2010;67:361–5.
19. Riboli E, Hunt KJ, Slimani N, Ferrari P, Norat T, Fahey M, et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. Public Health Nutr 2002;5:1113–24.
20. Johansson M, Relton C, Ueland PM, Vollset SE, Midttun O, Nygård O, et al. Serum B vitamin levels and risk of lung cancer. JAMA 2010;303:2377–85.
21. Ueland PM, Midttun O, Windelberg A, Svardal A, Skalevik R, Hustad S. Quantitative profiling of folate and one-carbon metabolism in large-scale epidemiological studies by mass spectrometry. Clin Chem Lab Med 2007;45:1737–45.
22. Midttun O, Hustad S, Ueland PM. Quantitative profiling of biomarkers related to B-vitamin status, tryptophan metabolism and inflammation in human plasma by liquid chromatography/tandem mass spectrometry. Rapid Commun Mass Spectrom 2009;23:1371–9.
23. Bevital AS. Assessment of nutritional status by vitamin markers [accessed 2014 Jan 6]. Available from: http://www.bevital.no/.
24. Mandi Y, Vecsei L. The kynurenine system and immunoregulation. J Neural Transm 2012;119:197–209.
25. Schroecksnadel K, Winkler C, Fuith LC, Fuchs D. Tryptophan degradation in patients with gynecological cancer correlates with immune activation. Cancer Letters 2005;223:323–9.
26. Burns DM, Anderson CM, Gray N. Do changes in cigarette design influence the rise in adenocarcinoma of the lung? Cancer Causes & Control: CCC 2011;22:13–22.
27. Thun MJ, Lally CA, Flannery JT, Calle EE, Flanders WD, Heath CW Jr. Cigarette smoking and changes in the histopathology of lung cancer. J Natl Cancer Inst 1997;89:1580–6.
28. Chaturvedi AK, Caporaso NE, Katki HA, Wong HL, Chatterjee N, Pine SR, et al. C-reactive protein and risk of lung cancer. J Clin Oncol 2010;28:2719–26.
29. Pine SR, Mechanic LE, Enewold L, Chaturvedi AK, Katki HA, Zheng YL, et al. Increased levels of circulating interleukin 6, interleukin 8, C-reactive protein, and risk of lung cancer. J Natl Cancer Inst 2011;103:1112–22.
30. Hustad S, Eussen S, Midttun O, Ulvik A, van de Kant PM, Mørkrid L, et al. Kinetic modeling of storage effects on biomarkers related to B vitamin status and one-carbon metabolism. Clin Chem 2012;58:402–10.
31. Midttun O, Ulvik A, Ringdal Pedersen E, Ebbing M, Bleie O, Schartum-Hansen H, et al. Low plasma vitamin B-6 status affects metabolism through the kynurenine pathway in cardiovascular patients with systemic inflammation. J Nutr 2011;141:611–7.
32. Darlington LG, Forrest CM, Mackay GM, Smith RA, Smith AJ, Stoy N, et al. On the biological importance of the 3-hydroxyanthranilic acid: anthranilic acid ratio. Int J Tryptophan Res 2010;3:51–9.
33. Frick B, Schroecksnadel K, Neurauter G, Leblhuber F, Fuchs D. Increasing production of homocysteine and neopterin and degradation of tryptophan with older age. Clin Biochem 2004;37:684–7.
34. Ledochowski M, Murr C, Widner B, Fuchs D. Association between insulin resistance, body mass and neopterin concentrations. Clin Chim Acta 1999;282:115–23.
35. Oxenkrug GF. Metabolic syndrome, age-associated neuroendocrine disorders, and dysregulation of tryptophan-kynurenine metabolism. Ann N Y Acad Sci 2010;1199:1–14.
36. Ozkan Y, Mete G, Sepici-Dincel A, Sepici V, Simsek B. Tryptophan degradation and neopterin levels in treated rheumatoid arthritis patients. Clin Rheumatol 2012;31:29–34.
37. Spencer ME, Jain A, Matteini A, Beamer BA, Wang NY, Leng SX, et al. Serum levels of the immune activation marker neopterin change with age and gender and are modified by race, BMI, and percentage of body fat. J Gerontol A Biol Sci Med Sci 2010;65:858–65.
38. Oxenkrug G. Interferon-gamma - inducible inflammation: contribution to aging and aging-associated psychiatric disorders. Aging and Dis 2011;2:474–86.
39. Oxenkrug GF. Interferon-gamma-inducible kynurenines/pteridines inflammation cascade: implications for aging and aging-associated psychiatric and medical disorders. J Neural Transm 2011;118:75–85.
40. Taylor MW, Feng GS. Relationship between interferon-gamma, indoleamine 2,3-dioxygenase, and tryptophan catabolism. FASEB J 1991;5:2516–22.
41. Sakakeeny L, Roubenoff R, Obin M, Fontes JD, Benjamin EJ, Bujanover Y, et al. Plasma pyridoxal-5-phosphate is inversely associated with systemic markers of inflammation in a population of U.S. adults. J Nutr 2012;142:1280–5.
42. Pertovaara M, Heliovaara M, Raitala A, Oja SS, Knekt P, Hurme M. The activity of the immunoregulatory enzyme indoleamine 2,3-dioxygenase is decreased in smokers. Clin Exp Immunol 2006;145:469–73.
43. Stampfli MR, Anderson GP. How cigarette smoke skews immune responses to promote infection, lung disease and cancer. Nat Rev Immunol 2009;9:377–384.
44. Nawashiro H, Otani N, Shinomiya N, Fukui S, Ooigawa H, Shima K, et al. L-type amino acid transporter 1 as a potential molecular target in human astrocytic tumors. Int J Cancer 2006;119:484–92.