Ther Adv Drug Saf
2021, Vol. 12: 1–25
DOI: 10.1177/2042098621997727

Aristolochic acid-associated urinary tract cancers: an updated meta-analysis of risk and oncologic outcomes after surgery and systematic review of molecular alterations observed in human studies

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Abstract

Background: The risk of primary aristolochic acid (AA)-associated urothelial carcinoma (AA-UC) has been summarized by a 2013-published meta-analysis. Given that additional evidence has been continuously reported by original studies, an updated meta-analysis is needed. Meanwhile, to complete the whole picture, a systematic review of molecular alterations observed in AA-urinary tract cancers (AA-UTC) was also performed.

Methods: We searched PubMed, Embase and four Chinese databases up to October 2020. Observational studies comparing risk or oncologic outcomes of UTC between patients with and without AA exposure were eligible for systematic review and meta-analysis. Studies investigating molecular alterations in AA-UTC using human tissue samples were eligible for systematic review.

Results: In total, 38 and 20 studies were included in the systematic review and meta-analysis, respectively. Exposure to AA led to an overall increased risks of primary UTC [UC and renal cell carcinoma (RCC)] (OR 6.085, 95% CI 3.045–12.160) and postoperatively recurrent UC (RR 1.831, 95% CI 1.528–2.194). Subgroup analysis of postoperative primary AA-upper tract UC (AA-UTUC) showed increased risks of bladder recurrence (adjusted RR 1.949, 95% CI 1.462–2.597) and contralateral UTUC recurrence (crude RR 3.760, 95% CI 2.225–6.353), worse overall survival (adjusted HR 2.025, 95% CI 1.432–2.865) and worse disease-specific survival (adjusted HR 3.061, 95% CI 1.190–7.872), but no effect on cancer-specific survival (adjusted HR 0.772, 95% CI 0.269–2.215). High mutation load with AA mutational signature presenting largely in the putative driver genes was observed in AA-UTUC. In contrast, AA mutational signature is rarely found in the mutated RCC driver genes and the mutation load in AA-RCC is low. Therefore, AA has different roles in the genesis of UTUC and RCC.

Conclusions: Implementing effective strategies to completely protect people from exposure to AA is urgently needed. Additionally, more effort should be made in identifying the precise carcinogenic mechanisms of AA to determine the future treatment strategies.

Plain language summary

Risk, recurrence and survival outcomes after surgery and molecular changes possibly involved in the genesis of aristolochic acid-associated urinary tract cancers

Background: The association between aristolochic acid (AA) and primary urothelial carcinoma (UC) has been summarized by a 2013-published meta-analysis. Given that additional evidence has been reported in the past 7 years, an updated meta-analysis is

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needed. Meanwhile, to complete the whole picture, a systematic review of molecular changes possibly involved in AA-mediated urinary tract carcinogenesis was also performed.
Methods: We searched PubMed, Embase and four Chinese databases for human studies up to October 2020. Studies comparing the risk of urinary tract cancer (UTC) between patients with and without AA exposure and studies investigating the molecular changes in AA-associated UTC (AA-UTC) using human tissue samples were eligible for inclusion. Thirty-eight studies were finally included.
Results: The results showed that exposure to AA was associated with a 6-fold increased risk of primary UTC (UC and renal cell carcinoma, RCC) and a 1.8-fold increased risk of postoperatively recurrent UC. After studies reporting primary AA-upper tract UC (AA-UTUC) were analyzed, a 1.9-fold increased risk of bladder recurrence and a 3.8-fold increased risk of contralateral UTUC recurrence was observed. Additionally, exposure to AA worsened the postoperative survival of patients with UTUC by a 2-fold increased risk of overall death and a 3-fold increased risk of death from other diseases and recurrences. However, there was no effect on death due to cancer. Lastly, AA seemed to play different roles in the etiology of UTUC and RCC based on the observations of different mutation loads and different distributions of AA-induced mutations in AA-UTUC and AA-RCC samples.
Conclusions: Implementing effective strategies to completely protect people from exposure to AA is urgently needed. Moreover, more effort should be made in identifying the precise carcinogenic mechanisms of AA-UTC to determine the future treatment strategies.

Keywords: aristolochic acid, bladder recurrence, contralateral upper tract urothelial carcinoma recurrence, molecular alterations, oncologic outcomes, updated meta-analysis, updated systematic review, urothelial carcinoma, upper tract urothelial carcinoma

Received: 22 November 2020; revised manuscript accepted: 3 February 2021.

Introduction

Aristolochic acid (AA), a toxic compound existing in plants of genera *Aristolochia* and *Asarum*, is mainly composed of a mixture of AAI and AAII.[1,2] It seems that the nephrotoxic effect of AA is induced by AAI, while the genotoxic and carcinogenic effects were attributed to both AAI and AAII.[2] The amount of AA content varies with genera and species of plants.[1] Aristolochic acid nephropathy (AAN) occurring after intake of AA-containing herbal medicines is characterized by chronic tubulointerstitial fibrosis with progression to end-stage renal disease (ESRD) and is accompanied by a high risk of upper tract urothelial carcinoma (UTUC)[2,3] and subsequent onset of bladder urothelial carcinoma.[4] Balkan endemic nephropathy (BEN), predominantly observed in Balkan countries, is an environmental form of AAN resulting from chronic dietary consumption of wheat flour contaminated by *Aristolochia clematitis*.[2,3,5] The mechanism of carcinogenesis of AA has been extensively studied. After metabolic activation, AA binds covalently to dA and dG residues in DNA to form aristolactam-DNA (AL-DNA) adducts which are concentrated in the renal cortex and causally related to the initiation phase of tumorigenesis.[6,7] Both dG and dA adducts block DNA replication and give rise to misincorporation of dA.[8,9] The dA-AL adducts are more mutagenic and persistent because, when paired with thymidine (dA-AL:dT), they are repaired by transcription-coupled repair but resistant to global genome nucleotide excision repair (GG-NER).[6,9] Such a selective repair results in a mutational pattern of marked nontranscribed strand bias and the persistence of dA-AL adducts in tissues even after stopping exposure to AA for decades.[6,9] However, when dAMP is inserted opposite the dA-AL adduct owing to misincorporation,[8,9] the resultant dA-AL:dA pair is susceptible to GG-NER by which dA-AL is excised and replaced with dTMP leading to permanent A-to-T transversion.[6,9] This distinct single base substitution (SBS) signature, characterized by predominant A:T-to-T:A transversions occurring most commonly in the 5′-Py_A_G-3′ trinucleotide context and enriched on the nontranscribed strand,[9] is labeled as SBS22 in the Catalogue Of Somatic Mutations In Cancer (COSMIC) database (v3.1, https://cancer.sanger.ac.uk/cosmic/signatures/).

AL-DNA adducts coupled with the AA mutational signature serve as the robust biomarkers of AA-associated UTUC (AA-UTUC).[10–12] UTUC tumorigenesis driven by AA-induced mutations was previously considered *via* affecting the *TP53* tumor suppressor gene[10–12] but subsequently confirmed also involving many other oncogenic driver genes throughout the genome by whole-genome/exome sequencing (WGS/WES) using next-generation sequencing (NGS) methods.[13,14] Due to its potent toxicity, AA was classified as a class I human carcinogen in 2002,[15] leading to the official bans of AA-containing herbs and products in many regions/areas.[1] However, people still can purchase certain AA-containing herbal products through different methods without prescription (e.g. internet, local markets, or pharmacies).[1,2,16] Additionally, AA-containing herbs are still allowed to be used in some areas, such as mainland China,[17] Taiwan,[18] and Romania.[19] As a result, cases of AAN are constantly reported worldwide.[1,2,20]

Wu and Wang[21] conducted a meta-analysis in 2013 to estimate the risk of primary AA-urothelial carcinoma (AA-UC) with the result of pooled odds ratio (OR) 5.97 [95% confidence interval (CI) 2.78–12.84]. However, their literature search was conducted solely using PubMed and they might miss relevant articles collected in mainland China and Taiwan databases. Moreover, the association between AA and another type of urinary tract cancer (UTC), renal cell carcinoma (RCC), has been shown in the past 7 years by the detection of AL-DNA adducts[10,19] and genome-wide present A:T-to-T:A transversions[22,23] in the RCC patients. Therefore, clinicians need to have the state-of-the-art information to make the most appropriate clinical decision. We aimed to fill the literature gap *via* performing an updated meta-analysis to summarize the evidence on AA-UTC, and provide the latest estimation. Meanwhile, except for the well-known AA-induced *TP53* mutations, other molecular alterations involved in AA-mediated carcinogenesis remain incompletely understood and under investigation. Bara *et al.*[24] performed a systematic review to identify the possible carcinogenic role of various AA-associated cancers (including AA-UTUC and AA-RCC) in 2017. Later, Hassler *et al.*[25] performed another systematic review to investigate the molecular characterization of all causes of UTUC (including AA-UTUC) in 2020. To complete the whole picture of the present systematic review, we updated the information of the two systematic reviews on the molecular alterations observed in AA-UTC, including AA-UTUC, AA-RCC, and AA-bladder cancer (AA-BC), to October 2020.

Materials and methods

Search strategy

We followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline for the present systematic review and meta-analysis.[26] The electronic database search included PubMed, Embase, and four Chinese databases: Airiti Library, China National Knowledge Infrastructure (CNKI), VIP information/Chinese Scientific Journals database (CSJD-VIP) and Wanfang Data. The search period was between the inception date of every database and 31 October 2020, except for Wanfang Data with the search period up to 31 July 2020. PubMed and Embase were searched with the following search strategy: (Aristolochic acid OR Balkan endemic nephropathy) AND (urothelial carcinoma OR urothelial cancer OR transitional cell carcinoma OR transitional cell cancer OR renal pelvis cancer OR renal pelvic neoplasms OR bladder cancer OR urinary bladder neoplasms OR ureteral cancer OR ureteral neoplasms OR renal cell carcinoma OR renal cell cancer). Except for "Balkan endemic nephropathy," all of the search key words in Chinese characters were also used in the search of Chinese databases (see Supplemental material Appendix A). The reference lists from relevant studies were surveyed as well to identify additional eligible studies for inclusion.

Selection criteria

We attempted to include original human studies with no restriction on publication dates, and languages. Moreover, the name of AA-containing herbs or herbal products, or the term "aristolochic acid" should be explicitly stated in those studies assessing AA exposure *via* intake of AA-containing herbs when the information was obtained without objective evidence (i.e. information only obtained from the medical records or patients' histories). Those studies only used "Chinese herbal medicines" or other similar terms that we could not assess the study participants' AA exposure were not considered to be included. Studies that compared the risk or oncologic outcomes of UTC between patients with and without a history of

AA exposure were evaluated using the following inclusion criteria: (1) the study design was observational; (2) data were reported as OR, relative risks (RR) or hazard ratio (HR) with 95% CI, or number of events with sample sizes; (3) case reports, case series, single-arm descriptive observational studies, and studies without sufficient information for our analysis were excluded. Studies that aimed to identify the molecular alterations possibly involved in AA-mediated urinary tract carcinogenesis were eligible for inclusion if tissue samples from patients with AA-UTC were used. All study designs were acceptable except for case reports. When searching for studies investigating the molecular alterations in AA-UTUC, we focused on those not just identifying AA-induced *TP53* A:T-to-T:A transversions.

Data extraction and validity assessment

YCK and MHC independently extracted the data using an extraction form with consensus on all extracted items. Extracted data of studies reporting risk of AA-UTC were publication year and type, study location, design, and period, baseline renal function of patients, study outcome, exposed AA-containing herbs and exposure time, methods of AA exposure assessment, classification and diagnostic criteria of AAN/BEN/AA-UC, follow-up time, outcome data for each included study and adjuvant/neoadjuvant therapy in studies reporting recurrent UC. The methodological quality of the included studies was assessed using the Newcastle–Ottawa scale (NOS)[27] with slight modifications adapted for this study (see Supplemental material Appendix B). A study with a total quality score of 5 or less was deemed at high risk of bias and was not included in the meta-analysis. Extracted data of studies investigating the molecular alterations in AA-UTC were publication year and type, country where tissue samples obtained, cancer type of AA-exposed samples, method of AA exposure assessment, type of non-AA-exposed control samples, tissue, analytical method, and molecular alterations identified in the AA-exposed samples.

Statistical analysis

Studies reporting the risk or oncologic outcomes of AA-UTC were analyzed in the meta-analysis. The outcome data of these studies were presented with either number of events with sample sizes or point estimates with 95% CIs. Comprehensive Meta-Analysis version 3 (CMA 3; Biostat Inc., Englewood, NJ, USA) was used to combine the two different formats of data. Because studies reporting primary AA-UTC included cohort and case-control studies, summary ORs were calculated for the meta-analysis. Pooled HRs were calculated for meta-analysis of survival outcomes. Because HR was not available in some studies in studies reporting recurrent AA-UC, RRs were used in these studies and combined with HRs to obtain the summary RRs. All meta-analyses were done by the DerSimonian and Laird (inverse variance) random-effects model.[28] A few studies reporting recurrent AA-UC had zero events in control groups and imbalanced sample sizes between AA and control groups; applying inverse variance method with the default constant continuity correction of 0.5 in the CMA may bias the result toward no effect and generate an underestimated summary estimate,[29] especially when the proportion of zero-event studies in a meta-analysis is over 50%.[30] Under this circumstance, Mantel–Haenszel (M-H) method without zero-cell correction was applied because it provides less biased summary estimate.[29] Only M-H pooled OR but not M-H pooled RR has been evaluated in the methodological research of meta-analysis for zero-event studies. However, because the performance of RRs is very similar to their corresponding OR measurements in rare events,[31] we used M-H pooled RR without zero-cell correction. The M-H method has two assumptions: (1) studies with little between-study heterogeneity and available data of event numbers and sample sizes are combined; (2) fix-effects model is used.[29] When any of the two assumptions was not met, we would still use inverse variance method with 0.5 continuity correction, or did not meta-analyze the data when the proportion of zero-event studies in a meta-analysis was over 50%. For a study presenting different consumption levels of AA, the highest one would be chosen. For a study presenting data of AL-DNA adducts and *TP53* A:T-to-T:A mutations separately, the data of AL-DNA adducts would be analyzed in the meta-analysis based on the diagnostic criteria of AAN proposed by Gökmen *et al.*[3] For a study including both patients with and without AAN diagnosis, only the data of cases with AAN diagnosis were analyzed in the meta-analysis. For a study presenting both unadjusted and adjusted estimates, only adjusted estimates were used in the meta-analysis. For a study presenting several adjusted estimates, the one adjusting the largest number of potential confounding factors was used to determine the pooled estimates. Between-study

heterogeneity was assessed by the chi-squared (χ^2)-based Q-statistic (significance level at $p < 0.1$) and quantified by I^2-measure (25%: low heterogeneity, 50%: moderate heterogeneity, and 75%: high heterogeneity).[32] Potential sources of heterogeneity were explored by subgroup analyses. Possible publication bias was assessed by the funnel plot method[33] and the Egger's linear regression test.[34] Sensitivity analyses were also conducted to test the robustness of our findings. The meta package in R version 4.0.0 (R-4.0.0, www.r-project.org/) was used to calculate the M-H summary estimates without zero-cell correction and CMA was used to performed all of the other statistical analyses in the present study and generate forest plots.

Results

Comparison of the included studies between the present and previous systematic reviews

The flow diagram for the study selection process is presented in Figure 1. In total, 38 studies, including 22 studies reporting the risk or oncologic outcomes of AA-UTC,[35–56] 13 studies identifying the molecular alterations in AA-UTC,[10,13,14,22,57–65] and three studies investigating both the risk/oncologic outcomes and molecular alterations in AA-UTC,[66–68] were included in the present systematic review. Among the eight studies[10,12,36,38,39,66,69,70] included in Wu and Wang's meta-analysis,[21] nine studies[14,23,49,52,59,61,62,66,67] included in the systematic review of Bara *et al.*,[24] and five studies[13,14,58,61,62] included in the systematic review of Hassler *et al.*,[25] five studies reporting the risk[36,38,39] or oncologic outcomes[49,52] of AA-UC, seven studies reporting the molecular alterations in AA-UTUC[10,13,14,58,61,62] or AA-BC,[59] and two studies reporting both the risk and molecular alterations in AA-UTUC[66] or AA-RCC[67] were also included in the present review. Two studies reporting the risk of AA-UC[69,70] were excluded because only "Chinese herbs" were reported, and the history of AA exposure could not be confirmed. One study investigating only *TP53* mutation in AA-UTUC[12] was excluded. One study investigating AA-RCC[23] was excluded because

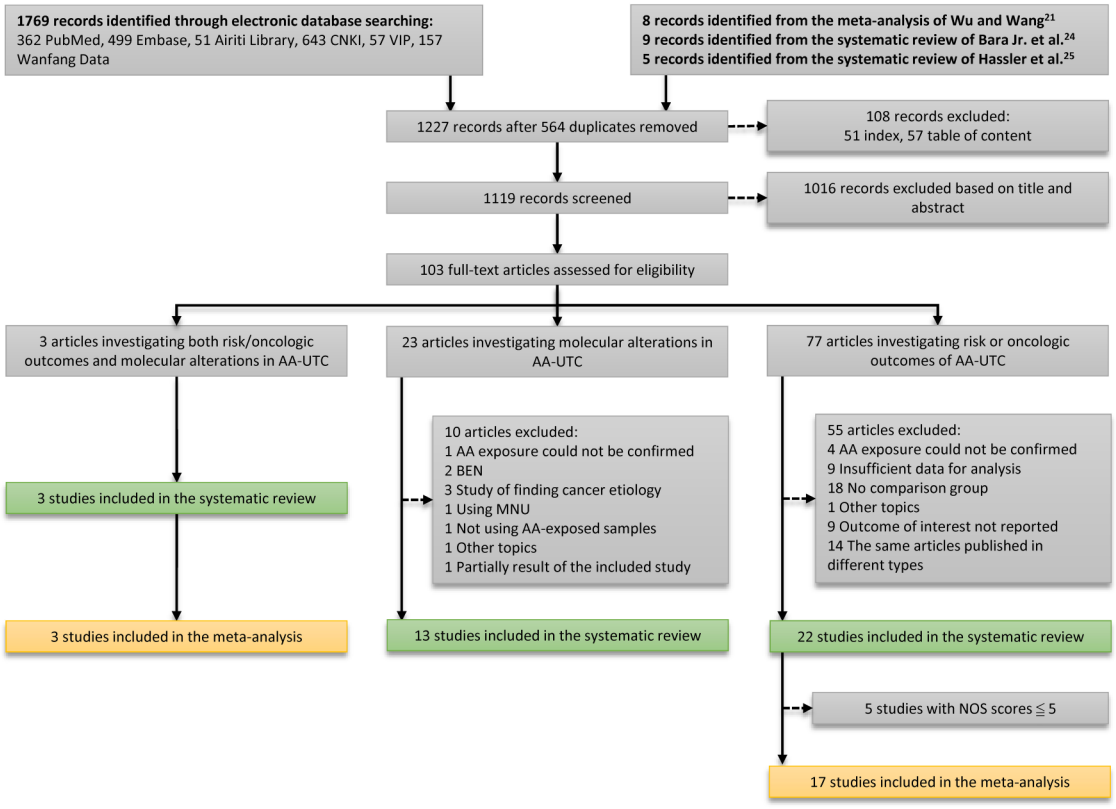

Figure 1. Flow diagram for the selection process of eligible studies.
AA, aristolochic acid; AA-UTC, aristolochic acid-associated urinary tract cancer; BEN, Balkan endemic nephropathy; MNU, morphologically normal human urothelium, NOS, Newcastle–Ottawa scale.

the aim of the study was to find the etiology of RCC in BEN regions. Seventeen studies reporting the risk[35,37,40–47] or oncologic outcomes[44,48,50,51,53–56] of AA-UTC, six studies reporting the molecular alterations in AA-UTUC[57,63–65] or AA-RCC,[22,60] and one study reporting both the risk and molecular alterations in AA-UTUC[68] were newly retrieved in the present systematic review.

Characteristics of the included studies reporting risk or oncologic outcomes of AA-UTC

Tables 1 and 2 summarize the characteristics of the 25 studies reporting risk or oncologic outcomes of AA-UTC.[35–56,66–68] Twenty-four were peer-reviewed articles published from 1991 to 2020,[35–42,44–56,66–68] and one was a master's thesis completed in 2011.[43] Among them, 18 were cohort studies[37,38,40–44,46,48–55,56,68] and seven were case-control studies.[35,36,39,66,45,67,47] Study locations included Taiwan,[39,45–46,49,52,67] mainland China,[36,38,40–44,48,51,53–56,68] Croatia,[35,37] and Serbia.[47,50] Baseline renal functions of study participants included chronic kidney disease (CKD) stages 0–5,[35,39,45,47–50,52–56,67,68] chronic renal failure (CRF),[36,40,43,46] and renal transplant recipients (RTRs).[37,38,41,42,44,51,66] The outcome of interest in 15 studies was risk of primary UTC, including UC,[35–46,66] RCC,[37,41,43,67] and BC.[47] In RTRs, post-transplant malignancies were reported. Ten[48–56,68] studies reported oncological outcomes of primary AA-UC after surgery; nine[48–54,56,68] of them reported recurrence, and five[50,54–56,68] of them reported survival outcomes. One study reported both the risk of primary and postoperatively recurrent AA-UC.[44] Fourteen studies assessed AA exposure according to the prescription history of AA-containing herbal medicines,[39,46,67] medical records,[38,41,48,53,55,66] results of the questionnaire survey,[45] self-reported data from patients[54] or residence in BEN areas.[35,47,50] By contrast, in addition to AA exposure history, eight studies assessed AA exposure based on the diagnosis of AAN[36,40,42–44,51,56]/BEN.[37] Three molecular epidemiological studies assessed AA exposure based on the detection of AL-DAN adducts and *TP53* gene A:T-to-T:A mutations,[49,52] or the genome-wide present AA signature.[68] The NOS scores of the 25 included studies were ranged from 4 to 9 (see Supplemental material Appendix B). After excluding four studies reporting primary UTC[37,40,41,43] and one study reporting recurrent UC[48] with NOS scores of 5 or less, a total of 20 studies[35,36,38,39,42,44–47,49–55,66–69] were ultimately identified for inclusion in the meta-analysis.

Partially overlapping study participants were noted in several studies. Two population-based cohort studies reporting primary UC were conducted in Taiwan using the National Health Insurance Research Database (NHIRD).[39,46] Lai *et al.*[39] analyzed patients with all stages of CKD from 1997–2002. Wang *et al.*[46] analyzed patients with ESRD from 1998 to 2002. Conducted over almost the same period of time, the patients with ESRD in the studies of Lai *et al.*[39] and Wang *et al.*[46] were overlapped. As a result, the data from the study of Lai *et al.*[39] were selected for the main meta-analysis, and the data from the study of Wang *et al.*[46] was used when subgroup analysis among patients with ESRD was performed. The two molecular epidemiological cohort studies of Chen *et al.*,[49,52] published in 2013[49] and 2016,[52] were conducted in the same hospital with study periods from 1999 to 2011[49] and from 1999 to 2012,[52] respectively. Their study participants were mostly overlapped. Both studies reported bladder and contralateral UTUC recurrences. The data from the study published in 2013[49] were selected for the main meta-analysis of UC recurrence and the subgroup analysis of contralateral UTUC recurrence because more complete data were presented in the study. The data from the study published in 2016[52] were selected for the subgroup analysis of bladder recurrence because adjusted estimate was available. Although Ji *et al.*[53] and Zhong *et al.*[54] analyzed on the same 942 UTUC patients, data of both studies were used for meta-analyses due to different reported outcomes (i.e. contralateral UTUC recurrence and bladder recurrence). Ji *et al.*[53] reported AA as an independent risk factor of contralateral UTUC recurrence; however, the adjusted HR was unreasonably less than 1 (HR 0.290, 95%CI 0.097–0.866). Therefore, we calculated the crude risk ratio for the meta-analysis instead. The cohort study of Zhong *et al.*[54] and the molecular epidemiological cohort study of Lu *et al.*[68] were conducted in the same hospital with the same reported outcome of bladder recurrence. Zhong *et al.*[54] analyzed the clinical data of 942 patients from 1999 to 2014 using patients' self-reported data to assess the AA exposure. Lu *et al.*[68] performed WGS on 47 UTUC patients from 2005 to 2013 and 43 patients from 2015 to 2017 to compare the oncologic outcomes between patients with and without AA mutational signature. Both studies[54,68]

Table 1. Characteristics of included studies exploring aristolochic acid exposure and risk of primary urinary tract cancers.

Author, type	Study location, design, period	Baseline renal function of patients	AA-containing herbs exposed/exposure time (range)	Method of AA exposure assessment	Classification of AAN/BEN diagnosis[a] (criteria[b])	Follow-up time after AA exposure or RT (range)	Study outcome	Primary UTC events/OR, HR (95% CI)			
								AA		Non-AA	
								UTC	No UTC	UTC	No UTC
Sostarić and Vukelić[35], PRA	Croatia, retrospective study, 1974–1989	CKD stages 0–5	*Aristolochia clematitis*/permanent exposure	Permanent residence in BEN regions	No diagnosis	NR	UC	67	10027	126	96180
Li *et al.*[36], PRA	China, HBCC, 2004	Uremia on dialysis	Guan Mu Tong, Qing Mu Xiang, Xi xin/median: UC: 5 (0.15–40) years, non-UC: 4.5 (0.08–40) years	Medical records; questionnaire	Possible AAN (1, 2, 3, 4)	AA group: median: UC: 10 (3–40) years; non-UC: 7 (2–44) years	UC	9[c]	20[c]	2	196
Zivcić-cosić *et al.*[37], PRA	Croatia, HBC, 1985–2006	RTRs	*Aristolochia clematitis*/NR	Residence in BEN regions	Possible AAN (6)	AA group: median 6.7 (5.2–8.1) years	UC, RCC	3	3	3	546
Li *et al.*[38], PRA	China, HBC, 1996–2005	RTRs	NR/⩾2 months	Medical records	No diagnosis	Mean 71.2 (18–132) months	UC	16	279	11	1123
Lai *et al.*[39], PRA	Taiwan, PBCC, 1997–2002	CKD stages 0–5	Mu-Tong, Fangchi, Xi-xin/NR	Prescription records	No diagnosis	4–6 years	UC	36 AA >500 mg: aOR 2.0 (1.4–2.9)[c,f] Mu-Tong >200 g: aOR 2.1 (1.3–3.4)[c] Fangchi >100 g: aOR 1.3 (0.9–2.0)[c] Xi-xin >300 g: aOR 1.3 (0.9–2.0)[c]	577	3274	121820
Wang *et al.*[40], PRA	China, HBC, 2001–2009	CRF	Qing Mu Xiang, Guan Mu tong/NR	Medical records	Probable AAN (1, 3, 4, 5)	NR	UC	14	58	1	461
Zhou *et al.*[41], PRA	China, HBC, 2000–2007	RTRs	Guan Mu tong/ median UTC: 2.5 (1–29) years; non-UTC: 1 (0.1–10) years; total: 2.75 (0.33–29) years	Medical records	No diagnosis	NR	UC, RCC	14	9	1	255
Xiao *et al.*[42], PRA	China, HBC, 2000–2009	RTRs	NR/⩾6 months	Medical records	Possible AAN (1, 2, 3, 4)	Mean 31 (11–72) months	UC	8	20	4	614
Gao[43], master's thesis	China, multi-hospital cohort, 1998–2009	CRF	NR/NR	Medical records	Made diagnosis of AAN (NR)	NR	UC, RCC	3	8	4	594
Xiao *et al.*[66], PRA	China, HBCC, 1974–2011	RTRs	NR/NR	Clinical data	No diagnosis	Median 34.5 (2–273) months	UC	53	327	47	3363

(Continued)

Table 1. (Continued)

Author, type	Study location, design, period	Baseline renal function of patients	AA-containing herbs exposed/exposure time (range)	Method of AA exposure assessment	Classification of AAN/BEN diagnosis[a] (criteria[b])	Follow-up time after AA exposure or RT (range)	Study outcome	Primary UTC events/OR, HR (95% CI)			
								AA		Non-AA	
								UTC	No UTC	UTC	No UTC
Yang and Liu[44], PRA	China, HBC, 2001–2005	RTRs (528)	NR/NR	Medical records	Possible AAN (1, 4)	AA group: 1–5 years, non-AA group: 5–7 years	UC, RCC	5	34	2	487
Yang *et al.*[45], PRA	Taiwan, CC, 1985–2001	Control group excluded uremia patients	Fangchi/NR	Questionnaire	No diagnosis	4–17 years	UC	AA group: $n = 24$, control group: $n = 140$ aHR 2.7 (0.6–11.4)[d]			
Wang *et al.*[46], PRA	Taiwan, PBC, 1998–2002	ESRD	Mu-Tong, Fangchi, Xi-xin/NR	Prescription records	No diagnosis	4–6 years	UC	9	101	270	32550
								AA >300 mg: aHR 5.18 (2.86–9.40)[c,f] Mu-Tong >200 g: aHR 3.46 (1.70–7.03)[c] Fangchi >100 g: aHR 1.89 (0.76–4.68)[c] Xi-xin >200 g: aHR 0.78 (0.34–1.81)[c]			
Hoang *et al.*[67], PRA	Taiwan, PBCC, 1997–2003	Excluded RTRs	NR/NR	Prescription records	No diagnosis	3–12 years	ccRCC	118	347	3520	14281
								AA consumption >250 mg: aOR 1.246 (1.004–1.547)[e]			
Matic *et al.*[47], PRA	Serbia, HBCC, NR	CKD stages 0–5	*Aristolochia clematitis*/NR	Residence in BEN regions	No diagnosis	NR	BC	67	26	134	96
								OR 1.84 (1.08–3.14)			

[a]Classification of AAN diagnosis was based on the criteria proposed by Gökmen *et al.*[3]
[b]Diagnostic criteria: (1) history of long-term AA-containing herbs intake before renal impairment, (2) without long-term (⩾3 months) use of antibiotics, antipyretic analgesics or antineoplastic drugs before renal failure, (3) clinical tubulointerstitial nephropathy, (4) ruling out other causes of renal disease, (5) characteristic renal histopathology, (6) WHO criteria for BEN: (*Bull World Health Organ* 1965; 32: 431–448): medical history, clinical findings, and laboratory results in the familial, geographical, and epidemiological context, ruling out other causes of renal diseases.
[c]Adjusted for age, sex, residence in a township with endemic black foot disease, and history of chronic urinary tract infection.
[d]Adjusted for cigarette smoking.
[e]Adjusted for monthly income, urbanization level, hypertension, diabetes, hyperlipidemia, chronic obstructive pulmonary disease, chronic hepatitis C infection, chronic kidney disease, cystic kidney disease, kidney stones, sickle cell disease, aspirin, non-steroidal anti-inflammatory drugs, and acetaminophen.
[f]Data used in the present meta-analyses.
AA, Aristolochic acid; AAN, Aristolochic acid nephropathy; aHR, adjusted hazard ratio; aOR, adjusted odds ratio; BC, bladder cancer; BEN, Balkan endemic nephropathy; CC, case-control study; ccRCC, clear cell renal cell carcinoma; CKD, chronic kidney disease; CRF, chronic renal failure; ESRD, end-stage renal disease; HBC, hospital-based cohort study; HBCC, hospital-based case-control study; HR, hazard ratio; NR, not reported, OR, odds ratio; PBC, population-based cohort study; PBCC, population-based case-control study; PRA, peer-reviewed article; RCC, renal cell carcinoma; RT, renal transplantation; RTR, renal transplant recipient; UTC, urinary tract cancer; UC, urothelial carcinoma; UTUC, upper tract urothelial carcinoma.

Table 2. Characteristics of the included studies exploring the oncologic outcomes of aristolochic acid-associated urothelial carcinoma after surgery.

Author, type	Study location, design, period	Baseline renal function of patients	AA-containing herbs exposed/exposure time (range)	Method of AA exposure assessment	Classification of AAN/BEN/AA-UC diagnosis[a] (criteria[b])	Type of primary UC	(1) Neoadjuvant therapy; (2) Adjuvant therapy	Follow-up time after surgery (range)	Study outcome
Li *et al.*[48], PRA	China, HBC, 2000–2006	CKD, including RTRs	Guan Mu Tong, Qing Mu Xiang, Guang Fangchi/mean: 1.8 (3–10) years	Medical records; face-to-face or telephone surveys	Only two patients diagnosed with AAN (NR)	UC	(1) NR; (2) NR	NR	Recurrence
Yang and Liu[44], PRA	China, HBC, 2001–2005	RTRs	NR/NR	Medical records	Possible AAN (1, 4)	UC	(1) NR; (2) NR	Mean 39 months	Recurrence
Chen *et al.*[49], PRA	Taiwan, molecular epidemiological cohort, 1999–2011	CKD stages 0–5	NR/NR	AL-DNA adducts and *TP53* gene A:T to T:A transversions	AA-UTUC (6, 7)	UTUC	(1) NR; (2) Postoperatively intravesical instillation of chemotherapy or BCG in patients with synchronous bladder tumors	Median 46 (3–144) months	Recurrence
Milenkovic-Petronic *et al.*[50], PRA	Serbia, HBC, 1999–2011	CKD stages 0–5	*Aristolochia clematitis*/ permanent exposure	Permanent residence in BEN regions	No diagnosis	UTUC	(1) No; (2) Cisplatin-based combination chemotherapy in patients with disease pT3 or pT4 and/or nodal involvement	Median 36 (1–154) months	Recurrence, survival
Liu *et al.*[51], PRA	China, HBC, 2006–2013	RTRs	NR/NR	Medical records	Made diagnosis of AAN (NR)	UTUC	(1) NR; (2) Regularly intravesical instillation of pirarubicin or epirubicin after surgery for one year	Median 38 (12–104) months	Recurrence
Chen *et al.*[52], PRA	Taiwan, molecular epidemiological cohort, 1999–2012	CKD stages 0–5	NR/NR	AL-DNA adducts	Possible AA-UTUC (6)	UTUC	(1) NR; (2) No	Median 59 (4–208) months	Recurrence
Ji *et al.*[53], PRA	China, HBC, 2000–2014	CKD, including RTRs[c]	NR/>3 months	Medical records	No diagnosis	UTUC	(1) No; (2) Yes, but treatment details not reported	Mean 70.2 (4–193) months	Recurrence
Zhong *et al.*[54], PRA	China, HBC, 1999–2014	CKD, including RTRs[c]	NR/>6 years	Self-reported data from patients	No diagnosis	UTUC	(1) No; (2) Yes, but treatment details not reported	Median 60 (IQR 36–100) months	Recurrence, survival
Wang *et al.*[55], PRA	China, HBC, 2011–2017	CKD stages 0–5	Guan Mu Tong continuous use >15 days or discontinuous use >2 months or other AA-containing herbs >6 months	Clinical data	No diagnosis	UTUC	(1) NR; (2) Yes, 242 patients received postoperative bladder perfusion chemotherapy	Mean 62.5 (18–84) months	Survival

(Continued)

Table 2. (Continued)

Author, type	Study location, design, period	Baseline renal function of patients	AA-containing herbs exposed/exposure time (range)	Method of AA exposure assessment	Classification of AAN/BEN/AA-UC diagnosis[a] (criteria[b])	Type of primary UC	(1) Neoadjuvant therapy; (2) Adjuvant therapy	Follow-up time after surgery (range)	Study outcome
Lu *et al.*[68], PRA	China, molecular epidemiological cohort, 2005–2013, 2015–2017	CKD stages 1–5	NR/median: patient with recurrence: 60 (0–120) months; patients without recurrence: 12 (0–360) months; total: 12 (0–360) months	Genome-wide present AA signature	AA-UTUC	UTUC	(1) NR; (2) AA group: no; non-AA group: patient with recurrence: 7.7% (1/13) received chemotherapy; patient without recurrence: 12% (6/50) received chemotherapy, 6% (3/50) received radiotherapy, 4% (2/50) received both therapies	Median 31.5 (3–168) months	Recurrence, survival, metastasis
Shan *et al.*[56], PRA	China, HBC, 2010–2017	CKD stages 0–5	NR/NR	Medical records	Probable AAN (1, 2, 3, 4, 5)	UTUC	(1) Patients with advanced disease received gemcitabine and cisplatin; (2) all patients received postoperative single dose of intravesical mitomycin C and patients with advanced disease received gemcitabine and cisplatin	Mean 43.2 (6–72) months	Recurrence, survival

	Type of recurrence	Recurrent UC events				HR (95% CI)			
		AA	Total	Non-AA	Total	Recurrence	Cancer-specific survival	Overall survival	Disease-specific survival
Li *et al.*[48]	UC	6	18	22	94				
Yang and Liu[44]	UC	3	5	0	2				
Chen *et al.*[49]	UC	23	40	22	52				
	Contralateral UTUC	10		0					
	BC	13		22					
Milenkovic-Petronic *et al.*[50]	BC	NR	64	NR	139	aHR 2.01 (1.04–4.22)[d]	aHR 1.28 (0.79–2.06)[d]		
Liu *et al.*[51]	BC	7	8	9	29	aHR 2.179 (1.085–8.093)[e]			
Chen *et al.*[52]	Metachronous BC	26	79	13	42	aHR 0.88 (0.33–2.31)[f]			

(Continued)

Table 2. (Continued)

	Type of recurrence	Recurrent UC events				HR (95% CI)			
		AA	Total	Non-AA	Total	Recurrence	Cancer-specific survival	Overall survival	Disease-specific survival
Ji *et al.*[53]	Contralateral UTUC	11	80	44	862				
Zhong *et al.*[54]	BC	NR	86	NR	856	aHR 2.117 (1.488–3.013)[g]	aHR 0.436 (0.214–0.888)[g]		
Wang *et al.*[55]	BC	NR	173	NR	266			aHR 1.883 (1.238–2.865)[h]	
Lu *et al.*[68]	BC	6	27	13	63	1.036 (0.393–2.730)			
Shan *et al.*[56]	UC	25	42	84	238			aHR 2.370 (1.428–4.902)[i]	aHR 3.061 (1.190–7.872)[i]
	BC	14		47					
	Contralateral UTUC	6		12					
	Local	5		25					

[a]Classification of AAN and AA-UTUC diagnosis was based on the criteria proposed by Gökmen *et al.*[3] and Chen *et al.*,[49] respectively.
[b]Diagnostic criteria: (1) history of long-term AA-containing herbs intake before renal impairment, (2) without long-term (⩾3 months) use of antibiotics, non-steroidal anti-inflammatory drugs, diuretics or Chinese traditional medicines containing minerals or metals, (3) clinical tubulointerstitial nephropathy, (4) ruling out other causes of renal disease, (5) characteristic renal histopathology, (6) AL-DNA adducts, (7) A:T-to-T:A transversions in *TP53* gene.
[c]Information available from another study of Zhong *et al.*: *Chin J Urol* 2017; 38: 901–904 (in Chinese).
[d]Adjusted for sex, age, concomitant bladder cancer, history of bladder cancer, tumor location, tumor focality, tumor size, tumor grade, tumor stage, lymphovascular invasion, lymph node metastasis, and mode of operation (nephroureterectomy *versus* conservative).
[e]Adjusted for tumor focality, and distal ureter invasion.
[f]Adjusted for *TP 53* mutation other than A > T, diabetes mellitus, and classical prognostic factors.
[g]Adjusted for gender, age, tobacco consumption, tumor side, main tumor location, main tumor size, multifocality, concomitant carcinoma *in situ*, tumor stage, tumor grade, lymph node status.
[h]Adjusted for smoking, age, sex, number of tumors, history of BC, lymph node metastasis, tumor size, tumor location, tumor stage, tumor grade, operation mode, diabetes mellitus.
[i]Adjusted for age, tumor stage, lymph node status, tumor grade.
AA, Aristolochic acid; AAN, Aristolochic acid nephropathy; AA-UTUC, aristolochic acid-associated upper tract urothelial carcinoma; aHR, adjusted hazard ratio; BC, bladder cancer; BCG, Bacillus Calmette-Guérin; BEN, Balkan endemic nephropathy; CKD, chronic kidney disease; HBC, hospital-based cohort study; HR, hazard ratio; IQR, interquartile range, NR, not reported; pT, primary tumor; PRA, peer-reviewed article; RTR, renal transplant recipient; UC, urothelial carcinoma; UTUC, upper tract urothelial carcinoma.

reported bladder recurrence. Specifically, the multivariate-adjusted estimates calculated by Zhong *et al.*[54] were used in the main meta-analysis of recurrent UC and subgroup analysis of bladder recurrence for studies with multivariate-adjusted data. The AA mutational signature data investigated by Lu *et al.*[68] were used in subgroup analysis of bladder recurrence for studies in which patients with AAN/BEN or AA-UTUC diagnosis were included.

Characteristics of the included studies identifying molecular alterations in AA-UTC

Characteristics and summary findings of the 16 studies reporting the molecular alterations in AA-UTC[10,13,14,22,57–68] are presented in Table 3. All were peer-reviewed articles published from 2009 to 2020. Countries where the AA-exposed samples were obtained included Taiwan,[10,13,14,59,67] mainland China,[60,61,66,68] Belgium,[57] Singapore,[59] Croatia,[58] Bosnia and Herzegovina,[58] Serbia,[62–65] and Romania.[22] Types of AA-exposed tumor samples included UC,[10,57,66] UTUC,[13,14,58,61–65,68] clear RCC (ccRCC),[22,60,67] and BC.[59] For comparison, 12 studies[10,14,22,58,60,61–66,68] used non-AA-exposed tissue samples; four studies[13,67,57,59] compared their findings with the publicly available data. Molecular alterations identified were classified as somatic mutations,[10,13,14,22,57–60,66,67] altered microRNA (miRNA) expression[61,62] and altered protein expression.[63–65] AA exposure assessment among 11 studies identifying somatic mutations[10,13,14,22,57–60,66–68] were in one study based on medical records,[66] in one study[57] based on meeting the definite diagnostic criteria of AAN proposed by Gökmen *et al.*,[3] in one study based on the presence of AL-DNA adducts or *TP53* gene A:T-to-T:A transversions,[10] in two studies based on the genome-wide present AA signature,[60,68] and in six studies,[13,14,22,58,59,67] besides the genome-wide present AA signature, two studies also based on patients' (medical) history,[13,59] one study[58] also based on meeting the BEN diagnosis criteria proposed by Jelaković *et al.*,[12,71] and the other three studies also based on the presence of AL-DAN adducts[14,22,67] or *TP53* A:T-to-T:A transversions.[14,67] AA exposure assessment among two studies identifying altered miRNA expression[61,62] was in one study based on AAN diagnosis[61] and in one study based on residence in BEN regions.[62] Assessment of AA exposure in three studies identifying altered protein expression was all based on residence in BEN regions.[63–65] Although Aydin *et al.*[57] investigated AA-induced *TP53* mutation in UTUC, they however focused on the detection of C-to-T mutations and were thus included.

Meta-analysis: risk of primary AA-UTC

Figure 2 is the forest plot for the 10 studies[35,36,38,39,42,44,45,47,66,67] exploring the association between AA exposure and risk of primary AA-UTC. Meta-analysis comprising eight unadjusted[35,36,38,39,42,44,47,66] and two adjusted estimates[45,67] showed an overall increased risk of UTC with substantial heterogeneity across studies (OR 6.085, 95% CI 3.045–12.160, $I^2 = 94.632\%$). Sensitivity analysis conducted by sequentially removing each study led to changes in estimates between 4.827 (95% CI 2.434–9.575) and 7.304 (95% CI 3.773–14.140). Subgroup analyses were undertaken. The pooled OR of UC was 7.304 (95% CI 3.773–14.140, $I^2 = 90.190\%$). The pooled OR of RCC was not calculated for two studies reporting RCC[44,67] because one[44] of them (50%) had zero event in the control group and the between-study heterogeneity was very high ($I^2 = 75.439\%$). The pooled ORs was 7.846 (95% CI 3.101–19.850, $I^2 = 95.197\%$) when AA exposure was *via* intake of AA-containing herbal medicines and 3.141 (95% CI 1.158–8.522, $I^2 = 90.659\%$) *via* consumption of AA-contaminated food. The pooled ORs was 2.252 (95% CI 1.169–4.338, $I^2 = 92.959\%$) for patients with CKD stages 0–5, 13.218 (95% CI 1.648–106.047, $I^2 = 83.466\%$) for patients with CRF and 16.046 (95% CI 6.725–38.290, $I^2 = 73.142\%$) for RTRs. After we divided the 10 studies[35,36,38,39,42,44,45,47,66,67] into two groups based on the diagnosis of AAN/BEN in the AA-exposed groups, seven studies[35,38,39,45,47,66,67] in which patients without diagnosis were responsible for the high heterogeneity ($I^2 = 95.136\%$) with a pooled OR of 3.301 (95% CI 1.637–6.657). In contrast, the I^2 between the other three studies[36,42,44] in which patients were diagnosed with AAN/BEN decreased to 0.000% with a pooled OR of 48.456 (95% CI 20.536–114.339). No asymmetry in the funnel plot was detected by the Egger's test for assessing publication bias ($p = 0.07$).

Meta-analysis: oncologic outcomes of primary AA-UC after surgery

Figure 3 is the forest plot showing the pooled RR of risk of postoperative recurrent UC 1.831 (95% CI 1.528–2.194, $I^2 = 0.000\%$) from the seven

Table 3. Characteristics and summary of findings of the included studies identifying molecular alterations in aristolochic acid-associated urinary tract cancers.

Author, type	Country where sample obtained	Cancer type of AA-exposed samples (no. of patients)	Method of AA exposure assessment	Non-AA-exposed control samples (no. of patients)	Tissue	Analytical method	Molecular alterations identified in the AA-exposed samples
Somatic mutations							
Xiao *et al.*[66], PRA	China	UC (48)	Clinical data	Non-AA-UC from China (42)	FFPE	PCR and Sanger sequencing	Mutated genes: *TP53* (4/48, 8.3%), *HRAS* (3/48, 6.3%)
Chen *et al.*[10], PRA	Taiwan	UC (151)	AL-DNA adducts or *TP53* gene A:T-to-T:A transversions	RCC from Taiwan (25)	FF	1. *TP53*: microarray; 2. *FGFR3, HRAS*: pyrosequencing	A:T-to-T:A mutations: *TP53* (38/148, 26%), *HRAS* (7/150, 4.7%), *FGFR3* (6/150, 4.0%)
Aydin *et al.*[57], PRA	Belgium	UC (5)	Definite diagnosis of AAN	Compared to publicly available data	FF and FFPE	1. ICH staining; 2. LCM; 3. Nested-PCR and Sanger sequencing	1. A>T transversion was not only within the *TP53* hotspot region and but in p53-negative dysplastic urothelial cells. 2. In addition to A>T, C>T transversions were highly prevalent in *TP53*
Hoang *et al.*[14], PRA	Taiwan	UTUC (19)	1. AL-DNA adducts or *TP53* gene A:T-to-T:A transversions; 2. Genome-wide present AA signature	SA-UTUC from Taiwan (7)	FF	WES	Known driver genes identified[a]: *TP53* (12/18, 66.7%)[b], *MLL2* (11/18, 61.1%), *CREBBP* (8/18, 44.4%), *STAG2* (5/18, 27.8%), *BRCA2* (4/18, 22.2%), *NRAS* (4/18, 22.2%), *KDM6A* (3/18, 16.7%), *FGFR3* (1/18, 5.6%)
Poon *et al.*[13], PRA	Taiwan	UTUC (9)	1. Medical record and case histories 2. Genome-wide present AA signature	Compared to publicly available data	NR	WES, WGS	Frequently mutated genes: *KDM6A* (8/9, 88.9%), *LRRK2* (7/9, 77.8%), *DCHS2* (7/9, 77.8%), *USH2A* (7/9, 77.8%), *SCN1A* (6/9, 66.7%), *ADAMTSL1* (5/9, 55.6%), *ATRX* (5/9, 55.6%), *DNAH9* (5/9, 55.6%), *MYO5C* (5/9, 55.6%), *TP53* (5/9, 55.6%), *CHD6* (4/9, 44.4%), *CDH10* (4/9, 44.4%), *CREBBP* (4/9, 44.4%), *SETX* (4/9, 44.4%), *ARID1A* (3/9, 33.3%)
Castells *et al.*[58], PRA	Croatia, Bosnia and Herzegovina	UTUC (10)	1. Diagnosis of BEN; 2. Genome-wide present AA signature/ COSMIC Signature 22	Non-AA-UTUC from a metropolitan area of United States (2)	FFPE	LC-WES	1. Known driver genes carrying nonsynonymous A>T mutations and frequently mutated: *XIRP2* (6/10, 60%), *ATRX* (5/10, 50%), *NEB* (5/10, 50%), *AHNAK* (4/10, 40%), *SMCHD1* (4/10, 40%), *STAG2* (4/10, 40%), *TRRAP* (4/10, 40%), *ARID1B* (3/10, 30%), *ASH1L* (3/10, 30%), *CHD5* (3/10, 30%), *HDAC9* (3/10, 30%), *HUWE1* (3/10, 30%), *KDM6A* (3/10, 30%), *MLL2* (3/10, 30%), *NAV3* (3/10, 30%), *SYNE1* (3/10, 30%), *TRIO* (3/10, 30%) 2. APOBEC mutational signature
Lu *et al.*[68], PRA	China	UTUC (27)	Genome-wide present AA signature/COSMIC Signature 22	No-AA signature UTUC from China (63)	FFPE and FF	WGS	Frequently mutated genes: *KMT2A,C* and *D* (14/27, 51.9%), *MUC16* (13/27, 48.1%), *TP53**(13/27, 48.1%), *MUC4* (10/27, 37.0%), *FRG2C**(7/27, 25.9%), *FAT1* (6/27, 22.2%), *ARID1A* (5/27, 18.5%), *COL2A1* (5/27, 18.5%), *FBLN2* (5/27, 18.5%), *PCM1* (5/27, 18.5%), *SPEN* (2/27, 7.4%), *TERT* promoter mutations (C228T and C250T) (6/27, 22.2%)
Poon *et al.*[59], PRA	Taiwan, Singapore, China	BC (3)	1. Know AA exposure history; 2. Genome-wide present AA signature	Compared to and analyzed publicly available data	NR	WES	1. CpG>TpG signature 2. APOBEC mutational signature
Scelo *et al.*[22], PRA	Romania	ccRCC (14)	1. Genome-wide present AA signature; 2. AL-DNA adducts[c]	Non-AA-ccRCC from Czech Republic (38), Russia (38), and the UK (31)	FF	WGS	1. Know driver genes carrying nonsynonymous A>T mutations: *PBRM1* (3/14, 21.4%), *TP53* (1/14, 7.1%) 2. Know driver genes carrying other nonsynonymous mutations: *PBRM1* (7/14, 50%), *VHL* (7/14, 50%), *KDM5C* (4/14, 28.6%), *SETD2* (4/14, 28.6%), *BAP1* (2/14, 14.3%)

(Continued)

Table 3. (Continued)

Author, type	Country where sample obtained	Cancer type of AA-exposed samples (no. of patients)	Method of AA exposure assessment	Non-AA-exposed control samples (no. of patients)	Tissue	Analytical method	Molecular alterations identified in the AA-exposed samples
Hoang *et al.*[67], PRA	Taiwan	ccRCC (10)	1. AL-DNA adducts; 2. Genome-wide present AA signature	Compared to publicly available data	FF	WES	1. Know driver genes carrying nonsynonymous A > T mutations: *PBRM1* (1/10, 10%), *SETD2* (1/10, 10%) 2. Know driver genes carrying other nonsynonymous mutation ns: *VHL* (7/10, 70%), *PBRM1* (2/10, 20%), *BAP1* (1/10, 10%), *EPAS1* (1/10, 10%), *GNB2L1* (1/10, 10%), *PIK3CA* (1/10, 10%) 3. Loss of chromosome 3p (6/10, 60%)
Wang *et al.*[60], PRA	China	ccRCC (43)	Genome-wide present AA signature/COSMIC SBS22	Non-AA-ccRCC from China (109)	FF	WES	Significantly mutated genes: *CSMD3** (22.5% AA-exposed samples *versus* 6.25% non-AA-exposed samples, $p = 0.012723$)
Altered miRNA expression							
Tao *et al.*[61], PRA	China	UTUC (5)	AAN diagnosis	Non-AAN-UTUC (5)	FFPE	miRNA microarray	Differentially expressed miRNA: The most upregulated: has-miR-488-3p, has-miR-4434, has-miR-4274, has-miR-224-3p The most downregulated: has-miR-4795-5p, has-miR-4784, has-miR-330-3p, has-miR-181c-5p, has-miR-15a-5p, has-miR-10a-5p, has-miR-200c-3p, has-miR-3916
Popovska-Jankovic *et al.*[62], PRA	Serbia	UTUC (7)	Residence in BEN regions	Non-tumor kidney sample (4)	FFPE	miRNA microarray	Differentially expressed miRNA: Upregulated: hsa-miR-205-5p, hsa-miR-4322, hsa-miR-99b-3p, hsa-miR-3620-3p, hsa-miR-373-5p, hsa-miR-3656, hsa-miR-1290 Downregulated: hsa-miR-30a-5p, hsa-miR-127-3p, hsa-miR-154-5p
Altered protein expression							
Jankovic-Velickovic *et al.*[63], PRA	Serbia	UTUC (40)	1. Residence in BEN regions; 2. Seven patients with tubulointerstitial lesions similar to BEN	Non-BEN-UTUC from non-endemic regions in Serbia (45)	FFPE	IHC staining	E-cadherin: 1. More frequent aberrant expression in BEN UTUC than control ($p < 0.01$); 2. Decreased expression in BEN UTUC was linked to solid growth pattern
Jankovic-Velickovic *et al.*[64], PRA	Serbia	UTUC (44)	Residence in BEN regions	Non-BEN-UTUC from non-endemic regions in Serbia (61)	FFPE	IHC staining	Apoptosis-related biomarkers: 1. Higher expression of antiapoptotic marker Survivin in BEN UTUC with high grade ($p < 0.005$) and solid growth ($p < 0.05$) than control; 2. Lower expression of proapoptotic marker Bax in BEN UTUC with high grade ($p < 0.05$), high stage ($p < 0.05$), necrosis ($p < 0.05$), and without metaplastic change ($p < 0.05$) than control
Jankovic-Velickovic *et al.*[65], PRA	Serbia	UTUC (50)	Residence in BEN regions	Non-BEN-UTUC from non-endemic regions in Serbia (60)	FFPE	IHC staining	Angiogenesis-related biomarkers: 1. Lower expression of VEGFR1 in BEN UTUC than control ($p < 0.005$); 2. Higher expression of MVD CD31 than control was seen in BEN UTUC with papillary growth ($p < 0.05$)

[a]Identified from 18 samples after excluding two samples in which the percentages of A:T-to-T:A transversions were not consistent with the AA signature and including a control sample in which AL-DNA adducts and AA signature was found during this study.
[b]Frequency may be skewed because selection of AA-UTUCs was based on A-to-T mutation in *TP53*.
[c]AL-DNA adducts were later detected in the study of Turesky *et al.*[19]
*Identified by MutSigCV.
AA, Aristolochic acid; AAN, Aristolochic acid nephropathy; AA-UTUC, AA-associated UTUC; AA-UTC, AA-associated urinary tract cancer; BEN, Balkan endemic nephropathy; ccRCC, clear cell renal cell carcinoma; FF, fresh-frozen; FFPE, formalin-fixed paraffin-embedded; IHC immunohistochemistry staining; LCM, laser capture microdissection; LC-WES, low-coverage whole-exome sequencing; miRNA, microRNA; MVD, microvessel density; NR, not reported; PCR, polymerase chain reaction; PRA, peer-reviewed article; RCC, renal cell carcinoma; SA, smoking-associated; SBS, single base substitution; UC, urothelial carcinoma; UTUC, upper tract urothelial carcinoma; WES, whole-exome sequencing.

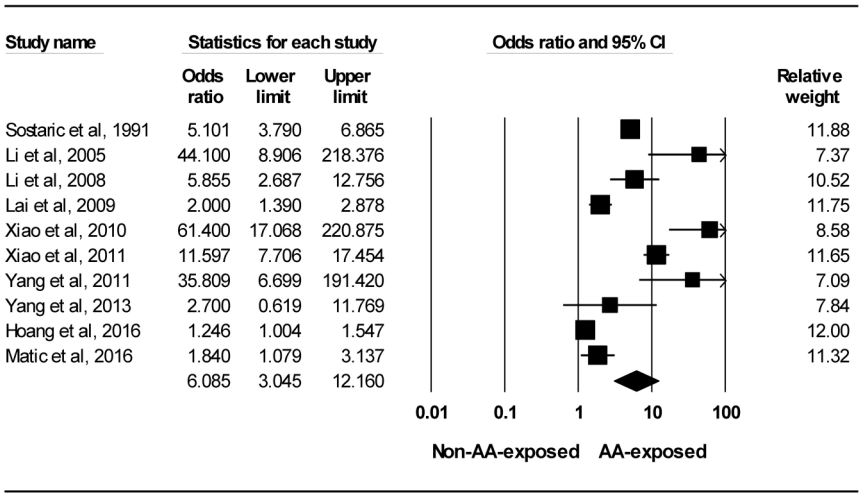

Figure 2. Forest plot for studies exploring the association between aristolochic acid exposure and risk of primary urinary tract cancer.
AA, aristolochic acid; CI, confidence interval.

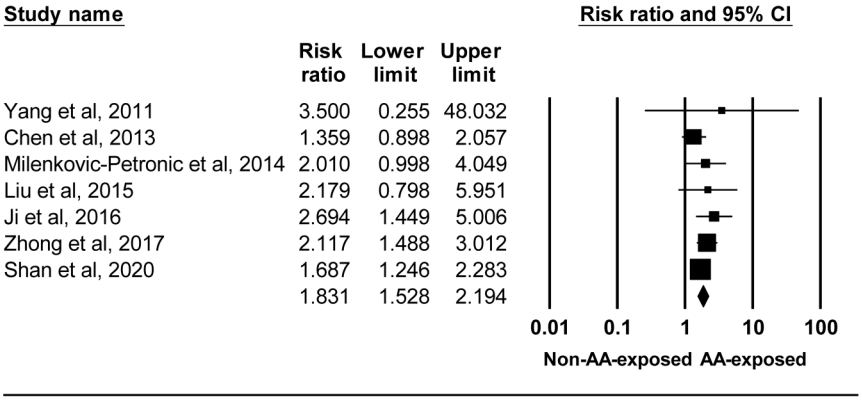

Figure 3. Forest plot for studies exploring the association between aristolochic acid exposure and risk of postoperative recurrent urothelial carcinoma.
AA, aristolochic acid; CI, confidence interval.

studies reporting recurrent UC following surgical resection for primary AA-UC.[44,49,50,51,53,54,56] No funnel plot asymmetry was detected by the Egger's test ($p = 0.327$). Although one[44] of these seven studies had zero event, the pooled RR was calculated by inverse variance method with 0.5 continuity correction because event numbers and sample sizes were unavailable in two studies[50,54] for calculating the pooled M-H RR without zero-cell correction. Sensitivity analysis revealed no change in the statistical significance of the combined RR, ranging from 1.738 (95% CI 1.408–2.146) to 1.964 (95% CI 1.606–2.402). Subgroup analysis by route of exposure to AA showed a pooled RR of 1.819 (95% CI 1.508–2.193, $I^2 = 0.000\%$) when exposure was *via* intake of AA-containing herbal medicines and 2.010 (95% CI 1.040–4.220) *via* consumption of AA-contaminated food. The pooled RR for patients with and without AAN/BEN diagnosis was 1.684 (95% CI 1.337–2.121, $I^2 = 21.9\%$) and 2.206 (95%CI 1.666–2.921, $I^2 = 0.000\%$), respectively. Subgroup analyses by sites of post-operative recurrence were conducted for patients

with primary AA-UTUC from eight studies.[49–54,56,68] The standard surgical treatment, radical nephroureterectomy (RNU) with bladder cuff excision, was performed in seven[49,51–54,56,68] of the eight studies.[49–54,56,68] Although 86.2% of patients received RNU and 13.8% of selected patients including those with BEN received conservative surgery in the other one study,[50] the different modes of operations, however, did not result in significantly different risk of bladder recurrence in the multivariate analysis.[50] The pooled RR of bladder recurrence was 1.949 (95% CI 1.462–2.597, $I^2 = 0.000\%$) for four studies where RR adjusted for common clinicopathological risk factors were provided,[50–52,54] and 1.477 (95%CI 1.015–2.147, $I^2 = 0.000\%$) for four studies where patients diagnosed with AAN were included.[51,52,56,68] Only unadjusted data were available for studies reporting contralateral UTUC recurrence and local recurrence with a pooled crude RR of 3.760 (95% CI 2.225–6.353, $I^2 = 0.0\%$) for three studies[49,53,56] and a crude RR of 1.151 (95% CI 0.414–3.198) from one study,[56] respectively. Contralateral UTUC recurrence in AAN patients was not further analyzed because one[49] of two studies[49,56] (50%) had zero event and there was moderate between-study heterogeneity ($I^2 = 55.470\%$). For meta-analysis of survival outcomes, after adjustment for common clinicopathological factors in the multivariate analysis, AA exposure in patients with surgically treated primary UTUC showed worse overall survival (HR 2.025, 95% CI 1.432–2.865, $I^2 = 0.000\%$) and disease-specific survival (HR 3.061, 95% CI 1.190–7.872), but had no effect on cancer-specific survival (HR 0.772, 95% CI 0.269–2.215, $I^2 = 83.484\%$). Table 4 displays the results of meta-analyses and subgroup analyses in the present study.

Discussion

The present systematic review and meta-analysis provided updated evidence on the risk, oncologic outcomes and molecular alterations observed in AA-UTC *via* searching PubMed, Embase and four Chinese databases, Airiti Library, CNKI, CSJD-VIP and Wanfang Data, with time extending up to 31 October 2020, except for Wanfang Data with the search period up to 31 July 2020. In the meta-analysis of the risk of primary AA-UC, the updated OR was 7.304. Moreover, the impact of AA exposure on patients with various degrees of renal dysfunction and diagnosis of AAN on the changes of the risk of primary UTC was investigated. The risk of primary AA-UTC increased to 13.218-fold for patients with CRF and 16.046-fold for RTRs, and further increased to over 48.456-fold if both such patients were diagnosed with AAN. These results were in accordance with a previous consensus statement highlighting the importance and necessity of diagnosis of AAN/BEN among people with history of AA exposure to identify the higher risk ones for close follow-up, especially for CRF patients and RTRs.[71] The meta-analysis also showed that AA exposure was associated with an overall 1.831-fold increased risk of UC recurrence following surgical resection for primary AA-UC. In Western countries, bladder tumors account for 90–95% of primary UC while UTUC are account for only 5–10% of UC.[72] Postoperative recurrences of UTUCs after RNU also commonly occur at bladder (30%) and locoregional (20%), but only 2–6% at contralateral upper tract.[73] Our subgroup analysis showed that AA exposure was associated with 1.949-fold increased risk of intravesical recurrence of UTUC, and 1.477-fold increased risk in patients diagnosed with AAN. It is worth noting that among the three studies reporting contralateral UTUC recurrence,[49,53,56] the recurrence rates of 0–5% in the control groups were similar to that of 2–6% in the general population. However, the rates in the AA groups were very high (13.75–25%),[49,53,56] which contributed to an overall 3.760-fold increased risk. Two hypotheses (the field cancerization hypothesis and the intraluminal seeding and implantation hypothesis) have been proposed to explain the multifocal nature of urothelial tumors.[74] The field cancerization hypothesis describes that the whole uroepithelium is exposed to common carcinogenic insults in each patient and multifocal urothelial tumors arise from independent clones of transformed transitional cells.[74] The intraluminal seeding and implantation hypothesis describes that multifocal urothelial tumors are derived from a single progenitor cell and develop by the seeding or implantation of intraluminal dispersed viable cancer cells or by intraepithelial spread.[74] The WGS analysis conducted by Lu *et al.*[68] showed that the urothelial tumor that occurred earlier in the renal pelvis of a patient with AA signature and multifocal UTUC shared no genetic alterations with the subsequent renal pelvis tumor or bladder tumor 8 years later. The two subsequently occurring

Table 4. Results of meta-analyses and subgroups analyses.

Meta-analyses	Number of studies	Number of patients in analysis	Pooled OR/RR/HR (95% CI)	*p* Value	I^2 (%)	*p* for χ^2 test
Primary UTC (UC and RCC)	10[35,36,38,39,42,44,45,47,66,67]	257,480	OR 6.085 (3.045–12.160)	<0.001	94.632	<0.001
Subgroup analysis by route of AA exposure						
AA-containing herbal medicines	8[36,38,39,42,44,45,66,67]	150,757	OR 7.846 (3.101–19.850)	<0.001	95.197	<0.001
AA-contaminated food	2[35,47]	106,723	OR 3.141 (1.158–8.522)	0.025	90.659	0.001
Subgroup analysis by renal function						
CKD stages 0–5	5[35,39,45,47,67]	250,860	OR 2.252 (1.169–4.338)	0.015	92.959	<0.001
CRF (including dialysis)	2[36,46]	33,157	OR 13.218 (1.648–106.047)	0.015	83.466	0.014
RTRs	4[38,42,44,66]	6393	OR 16.046 (6.725–38.290)	<0.001	73.142	0.011
Subgroup analysis by AAN diagnosis						
No	7[35,38,39,45,47,66,67]	256,079	OR 3.301 (1.637–6.657)	0.001	95.136	<0.001
Yes	3[36,42,44]	1401	OR 48.456 (20.536–114.339)	<0.001	0.000	0.874
Primary UC	9[35,36,38,39,42,44,45,47,66]	239,214	OR 7.304 (3.773–14.140)	<0.001	90.190	<0.001
Postoperative recurrent UC	7[44,49,50,51,53,54,56]	2503	RR 1.831 (1.528–2.194)[a]	<0.001	0.000	0.566
Subgroup analysis by route of AA exposure						
AA-containing herbal medicines	6[44,49,51,53,54,56]	2300	RR 1.819 (1.508–2.193)	<0.001	0.000	0.446
AA-contaminated food	1[50]	203	RR 2.010 (1.040–4.220)	0.037	–	–
Subgroup analysis by AAN diagnosis						
No	3[50,53,54]	2087	RR 2.206 (1.666–2.921)	<0.001	0.000	0.771
Yes	4[44,49,51,56]	416	RR 1.684 (1.337–2.121)[b]	<0.0001	21.9	0.279
Subgroup analysis of postoperative UTUC by sites of recurrence						
All studies						
Contralateral UTUC recurrence	3[49,53,56]	1314	RR 3.760 (2.225–6.353)[b]	<0.0001	0.0	0.479
Bladder recurrence	5[50–52,54,56]	1583	RR 1.880 (1.466–2.411)	<0.001	0.000	0.546
Local recurrence	1[56]	280	RR 1.151 (0.414–3.198)	0.787	–	–
Studies with multivariate-adjusted data only						
Bladder recurrence	4[50–52,54]	1303	RR 1.949 (1.462–2.597)	<0.001	0.000	0.418
Studies included patients with AAN/AA-UTUC diagnosis						
Bladder recurrence	4[51,52,56,68]	528	RR 1.477 (1.015–2.147)	0.041	0.000	0.484
Local recurrence	1[56]	280	RR 1.151 (0.414–3.198)	0.787	–	–
Survival outcomes of postoperative UTUC						
Cancer-specific survival	2[50,54]	1145	HR 0.772 (0.269–2.215)	0.631	83.484	0.014
Overall survival	2[55,56]	719	HR 2.025 (1.432–2.865)	<0.001	0.000	0.546
Disease-specific survival	1[56]	280	HR 3.061 (1.190–7.872)	0.020	–	–

[a]Inverse variance random-effects RR with 0.5 continuity correction.
[b]Mantel–Haenszel fixed-effects RR without zero-cell correction.
AA, aristolochic acid; AAN, aristolochic acid nephropathy; CI, confidence interval; CKD, chronic kidney disease; CRF, chronic renal failure; OR, odds ratio; RR, risk ratio; RCC, renal cell carcinoma; RTRs, renal transplant recipients; UC, urothelial carcinoma; UTC, urinary tract cancer; UTUC, upper tract urothelial carcinoma.

tumors, however, were genetically related. Hence, multifocality and intravesical recurrence of primary AA-UTUC was considered the co-contribution of field cancerization and intraluminal seeding.[68] In the study of Ji *et al.*,[53] few patients had vesicoureteral reflux and there were no correlations between ureteroscopy and new contralateral UTUC, the field cancerization thus served as the hypothesis explaining the contralateral recurrence pattern of AA-UTUC. Because the pooled estimate of contralateral UTUC recurrence in the present meta-analysis was calculated from crude RRs,[49,53,56] further research designed for adjusting potential confounding factors is needed to corroborate these findings to develop the postoperative monitoring guidelines of primary AA-UTUC. Moreover, due to the lack of sufficient data and limited number of studies, subgroup analyses by sites of recurrence were not further analyzed in patients with advanced CKD. Chen *et al.*[49] reported that all AA-UTUC patients developing metachronous contralateral UTUC recurrence had poor renal function of CKD stage 3 or worse. In the study of Liu *et al.*[51] conducted on RTRs, an increased risk of bladder recurrence was observed in the native AAN group (adjusted HR 2.179, 95%CI 1.085–8.093). However, due to small sample size of the study,[51] many potential risk factors for bladder recurrence were unable to be adjusted. More studies are thus required to investigate the recurrence pattern of AA-UTUC in patients with advanced CKD and RTRs. Meta-analyses of survival outcomes showed that patients with surgically treated primary AA-UTUC had worse overall survival and disease-specific survival, but had no effect on cancer-specific survival. In addition to the high rate of postoperative recurrence, the high risk of death from other diseases was considered to be owing to the various cardiovascular and cerebrovascular complications of AAN, especially in patients receiving maintenance dialysis.[56]

Among the 16 studies identifying the molecular alterations in AA-UTC,[10,13,14,22,57–68] eight studies assessed AA exposure by WGS/WES through NGS approaches.[13,14,22,58–60,67,68] WGS was performed on AA-UTUC in one study[68] and AA-RCC in one study.[22] WES or low-coverage WES (LC-WES) was performed on AA-UTUC in one study,[58] AA-BC in one study,[59] and AA-RCC in three studies.[14,60,67] One study performed both WGS and WES on AA-UTUC.[13] By sequencing the entire genome/exome, numerous putative driver genes carrying nonsynonymous A-to-T mutations other than *TP53* were identified in AA-UTUC (Table 3). The recurrently mutated genes of AA-UTUC varied in different geographic areas. *TP53*, *CREBBP* and *LRRK2* are mutated mostly in the Taiwanese samples in contrast to *AHNAK*, *ATRX*, *SMCHD1* and *XIRP2* in the BEN samples.[58] The possible contributing factors resulting in the difference include modes of AA exposure (short-term high-dose intake of AA-containing herbs in Asia *versus* long-term low-dose exposure to contaminated food in BEN regions) and disease susceptibility due to different genetic background.[58] In addition to identifying the potential mutated driver genes of AA-UTUC from mainland Chinese patients, survival outcomes of the patients analyzed in the study of Lu *et al.*[68] were also based on the result of the WGS analysis. Kaplan–Meier analysis showed that cancer-specific survival and metastasis-free survival were both significantly better in patients with COSMIC Signature 22 than those without COSMIC Signature 22.[68] Such a favorable outcome of cancer-specific survival was considered to be related to the lower tumor stage of AA signature-positive UTUC.[54,68,75] Thus, the authors[68] concluded that AA signature-positive UTUC is a low-risk subtype which can be treated with kidney-sparing surgical management.[68] The low tumor stage of AA signature-positive UTUC was similar to the AAN-UTUC in another study from mainland China[56] but contrary to the AA signature-positive UTUC from Taiwan.[49,52] The reason why AA exposure is associated with the development of the lower-stage UTUC still needs to be investigated.[75] In general, the worsening renal function after radical surgery prevents most AA-UTUC patients from receiving chemotherapy. However, previous systematic review[25] implied that immune checkpoint inhibitor therapy may have effects on AA-UTUC. Specifically, studies included in the present review indicate that somatic mutations in AA-UTUC are characterized by high mutation load,[13,14,58] high *TP53* but rare *FGFR3* mutation rates[10,13,14,58,68] and presence of APOBEC mutational signature[58] (Table 3). When considering the aforementioned results together with the findings from Lu *et al.*[68] (i.e. high numbers of predicted neoantigens and tumor-infiltrating lymphocytes), immune checkpoint inhibitor could be an alternative for treating AA-UTUC.[25,68] Other molecular alterations observed in AA-UTUC were summarized as follows. The studies of Tao *et al.*[61] and Popovska-Jankovic *et al.*[62] identified two totally

different sets of differentially expressed miRNA in AAN-UTUC samples from mainland China and BEN-UTUC samples from Serbia, respectively. These different results were supposed to be due to the small sample size in the two studies and different types of control samples (i.e. non-AA-UTUC samples in the former study[61] in contrast to non-tumor kidney samples in the latter study[62]). Lastly, altered expressions of E-cadherin, apoptosis-related biomarkers, and angiogenesis-related biomarkers have also been observed in BEN-UTUC samples.

However, different mutation loads were observed between AA-UTUC and AA-RCC. In Taiwanese tissue samples, higher mutation load of A:T-to-T:A transversions in patients with AA-UTUC[14] (median 188 per exome) than that in patients with AA-RCC[67] (median 46 per exome) was found. These observations can be mutually confirmed in two similarly conducted Taiwanese population-based case-control studies of AA-UC[39] and AA-RCC,[67] where the risk of AA-UC (crude OR 1.9)[39] was higher than that of AA-RCC (crude OR 1.4)[67] at a similar AA cumulative dosage of more than 250 mg. Such result was presumed that the sensitivity of the renal tissue to the carcinogenic effects of AA is lower than the urothelium, or renal tumorigenesis only occurs in people who are sensitive to the nephrotoxicity of AA.[67] Moreover, different distributions of the AA mutational signature were also observed between AA-UTUC and AA-RCC. The AA mutational signature in UTUC is present largely in the putative driver genes, implying that AA is the causative factor of UTUC.[9,76] Castells *et al.*[58] meta-analyzed the data of 37 AA signature-positive UTUC samples from their study ($n=10$) and the studies of Poon *et al.*[13] ($n=9$) and Hoang *et al.*[14] ($n=18$). Eighty-three recurrently mutated cancer driver genes carrying nonsynonymous A:T-to-T:A transversions were identified, including many known drivers and chromatin-associated factors such as *TP53* (40.5% of samples), *MLL2* (40.5%), *CREBBP* (35.1%), *KDM6A* (35.1%), *ATRX* (32.4%), *CHD5* (24.3%), *ARID1B* (18.9%), *TRRAP* (18.9%), *FAT1* (16.2%), *SETBP1* (16.2%), *CHD8* (10.8%), and *CHD2* (8.1%). In contrast, the AA mutational signature is rarely found in the mutated RCC driver genes. We also meta-analyzed the data of both AL-DNA adducts- and AA signature-positive clear cell RCC samples from the studies of Scelo *et al.*[22] ($n=14$) and Hoang *et al.*[67] ($n=6$). The mutation patterns of the frequently mutated RCC driver genes in the 20 AA-RCC samples were as follows: *VHL* (0% of samples with A-to-T mutations *versus* 60% of samples with other mutations), *PBRM1* (20% *versus* 40%), *KDM5C* (0% *versus* 20%), *SETD2* (5% *versus* 20%), and *BAP1* (0% *versus* 15%). The different distributions of AA-signature mutations in UTUC and RCC driver genes may indicate the different roles of AA in the etiology of the two types of tumors.[9,76] However, it is still possible that these different distributions may just reflect the time of the occurrence of AA exposure (e.g. coincident with or prior to tumor initiation, or at some following time).[9,76] Due to the limited number of AA-RCC cases in the published literature, further studies are warranted to clarify the underlying mechanisms of these observations.

In comparison with the relatively low-dose dietary consumption of AA in BEN areas, our results revealed that AA exposure *via* ingestion of larger dose in the herbal products had greater increased risk of primary UTC (7.846-fold *versus* 3.141-fold), although the increased risks of UC recurrence were similar (1.819-fold *versus* 2.010-fold). However, over 20 kinds of AA-containing herbs, including those with high amount of AA, are currently allowed in mainland China.[17] Replacing of AA-containing herbs by non-AA-containing herbs has been proposed.[77] Unfortunately, some AA-containing herbs are hard to replace because of the unequal efficacy of the alternatives.[78] Some studies reported that the amount of AA in raw herbs may be reduced *via* several detoxification methods, including the pretreatment processes called Paozhi, compatibility of AA-containing herbs with other herbs, or extraction proces ses.[17,78–80] Disappointingly, none of these aforementioned methods can completely avoid the toxicity of AA.[17,78,80] Due to different individuals' susceptibility and detoxification capabilities to AA[1,7] and the impact of various degrees of renal impairment on AA excretion,[18] it is unclear whether cumulative toxicity would still occur after prolonged use of these products with "attenuated toxicity." Containing relatively low amount of AA,[1,17,81] Xi xin (*Herba Asari*) is still allowed to be used in many areas in Asia, including Taiwan,[82] Hong Kong,[83] mainland China,[17] Japan,[82] and Korea.[82] Increased risks of primary UC after intake of herbal products containing Xi xin were

not observed in the two studies on a nationwide Taiwanese population.[39,46] Nevertheless, these results were obtained only from the prescription records of NHIRD without calculating the risk induced by the herbal products purchased by patients themselves, and were thus unable to fully reflect the real-world condition. The amounts of AA in Xi xin were found to vary between plant parts, species, origins, processing methods and extraction processes.[17,84] In general, the underground parts (rhizome and root), especially root, have a lower level of AA than the aboveground parts (leaves, flowers, and stems).[79] The medicinal part of Xi xin has thus been stipulated to be switched from the whole plant to the root in Taiwan[85] and the root and rhizome in mainland China.[17] The extraction process of herbal products containing Xi xin in Taiwan should be done by water decoction to make sure AA is undetectable in the final products.[85] However, AA in raw herbs of Xi xin may exceed the standards[17,82] and should be used with caution. Researchers found that the level of AA in the underground parts of some species of Xi xin were similar to the whole plants or even higher than the aboveground parts.[86,87] Furthermore, it was observed that the level of AAIVa, a less studied AA analog, in Xi xin was higher in the root and stem than in the leaf, and the impact on human health needs to be investigated.[17] When herbal products containing Xi xin are generally considered to be less toxic, people more easily ignore its toxicity. Inadvertent use of Xi xin products with considerably high toxicity may put people in dangerous situations.[17,79]

The evidence shown in the present meta-analysis may raise awareness among healthcare professionals and public concerns regarding the long-term impact of AA on human health. Regulatory authorities across countries may use our findings to implement effective safety strategies to protect people from exposure to AA. In countries where AA-containing herbs have been banned, the government agencies should enforce strict laws and regulations to prevent AA-containing herbs and products from being sold or purchased privately. In countries where AA-containing herbs are not entirely banned, medical personnel should use these herbs with caution. There is also an urgent need to seek out effective alternative medicinal herbs to minimize the use of AA-containing herbs. The public should be educated that taking herbal medicines must be under the instruction of Chinese medicine practitioners or pharmacists, rather than purchasing them privately. Furthermore, prudent reassessment of total prohibition of AA-containing herbs is required. Recently, sporadic forms of BEN were found in patients residing outside of the established endemic regions of Croatia and Bosnia.[88] The broader growth of *Aristolochia* plants in different geographic environments deserves our attention.[88] Moreover, due to free AA released from the decayed seeds of *Aristolochia clematitis*, AAs has been identified as a new contaminant in soil, which will then contaminate food crops through root uptake.[89] In the endemic areas of the Balkan countries, developing methods for remediating AA-contaminated farmland is suggested and the residents should be informed of the existence of AAs in their cultivated fields and food.[89]

Limitations

The present meta-analysis had several limitations. Firstly, using the diagnostic criteria of AAN proposed by Gökmen *et al.*,[3] most AAN patients included in the present meta-analysis were classified as possible[36,42,44] or probable[56] AAN rather than definite diagnosis. The risk of primary UC in AAN patients might be underestimated. Moreover, because the estimated risk of primary UC in AAN patients was derived from CRF and RTR populations, it may not be generalizable to patients with other degrees of renal impairments. Secondly, through the literature review, we were unable to gauge the level of AA transformation of the cancers (e.g. how the oncogenes are affected by AA in terms of level of mutational signatures), which may also underestimate the true impact of AA-mediated urothelial carcinogenesis in the meta-analysis. Thirdly, only surgically treated patients were included in studies reporting oncologic outcomes of AA-UTUC, and we could not evaluate the impact of AA exposure on the outcomes of patients who did not require surgical treatment. Moreover, neoadjuvant and/or adjuvant therapies were in some studies administered in selected patients without adjustment in the multivariate analysis[50,54] and in some studies not clearly reported.[44,49,51–55,58] Some studies included RTRs as a subset of the study participants; however, limited information on these patients was provided.[53,54] We thus could not analyze the possible effects of neoadjuvant and/or adjuvant therapies and immunosuppression on the postoperative recurrence rate and survival outcomes of AA-UTUC. Fourthly, a few wide

CIs were noted when conducting the subgroup analysis of primary UC. These wide CIs were considered as owing to meta-analysis of studies with very rare events in the control groups, severely imbalanced sample sizes between AA and the control groups, and the very large overall effect sizes.[31] Fifthly, one[44] of the seven studies reporting recurrent UC[44,49–51,53,54,56] had zero event in the control group; applying the inverse variance method with 0.5 continuity correction for the meta-analysis might generate an underestimated estimate. Although the low proportion of zero-event study (14.3%) may not lead to serious bias in the overall effect,[30] the result should still be interpreted with caution. Lastly, although we have thoroughly searched Chinese databases from Taiwan and mainland China, we did not search other language databases (e.g. Romanian and Korean) and might have missed relevant studies.

Conclusions

AA remains a global public health hazard. Given that new evidence on the impact of AA exposure on humans has been constantly reported, developing and implementing effective safety strategies to completely protect people from both iatrogenic and environmental exposure to AA is urgently needed. Additionally, more effort should be made in identifying the precise carcinogenic mechanisms of AA-UTC to determine the future treatment strategies.

Acknowledgements

The authors would like to thank the Biostatistics Center, Kaohsiung Chang Gung Memorial Hospital for statistics work and providing CMA 3 software.

Conflict of interest statement

Yu-Chan Kang, Ming-Hong Chen, Chung-Ying Lin, Chih-Yun Lin and Yen-Ta Chen declare that they have no conflict of interest.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: The study was financially supported by grants from the Chang Gung Memorial Hospital, Kaohsiung, Taiwan (CMRPG8B1051).

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Supplemental material

Supplemental material for this article is available online.

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