

Association of *N-acetyltransferases 1 and 2* Polymorphisms with Susceptibility to Head and Neck Cancers—A Meta-Analysis, Meta-Regression, and Trial Sequential Analysis

Hady Mohammadi ¹, Mehrnoush Momeni Roochi ², Masoud Sadeghi ³, Ata Garajei ^{2,4}, Hosein Heidar ², Bayazid Ghaderi ⁵, Jyothi Tadakamadla ⁶, Ali Aghaie Meybodi ⁴, Mohsen Dallband ⁷, Sarton Mostafavi ⁸, Melina Mostafavi ⁹, Mojtaba Salehi ², Dena Sadeghi-Bahmani ^{10,11,12,13} and Serge Brand ^{10,11,12,14,15,*}

- ¹ Department of Oral and Maxillofacial Surgery, Fellowship in Maxillofacial Trauma, Health Services, Kurdistan University of Medical Sciences, Sanandaj 6617713446, Iran; hadi.mohammadi@muk.ac.ir
 - ² Department of Oral and Maxillofacial Surgery, Fellowship in Maxillofacial Trauma, School of Dentistry, Tehran University of Medical Sciences, Tehran 1439955991, Iran; mehrnoushmomeni@yahoo.com (M.M.R.); atagarajei@tums.ac.ir (A.G.); drheidar@yahoo.com (H.H.); mojtabasalehi9241@gmail.com (M.S.)
 - ³ Department of Biology, Science and Research Branch, Islamic Azad University, Tehran 1477893855, Iran; sadeghi_mbrc@yahoo.com
 - ⁴ Department of Head and Neck Surgical Oncology and Reconstructive Surgery, The Cancer Institute, School of Medicine, Tehran University of Medical Sciences, Tehran 1439955991, Iran; ali.aghaimyebodi@gmail.com
 - ⁵ Cancer and Immunology Research Center, Department of Internal Medicine, School of Medicine, Kurdistan University of Medical Sciences, Sanandaj 6617913446, Iran; Bayazidg@yahoo.com
 - ⁶ School of Medicine and Dentistry, Griffith University, Brisbane, QLD 4222, Australia; j.tadakamadla@griffith.edu.au
 - ⁷ Department of Oral and Maxillofacial Surgery, Dental School, Taleghani Hospital, Shahid Beheshti University of Medical Sciences, Tehran 1983963113, Iran; m-dalband@smbu.ac.ir
 - ⁸ English Department, Baneh Branch, Islamic Azad University, Baneh 6691133845, Iran; sartonmostafave@yahoo.com
 - ⁹ Tehran Medical Branch, Islamic Azad University, Tehran 1419733171, Iran; mostafavi.melina@yahoo.com
 - ¹⁰ Sleep Disorders Research Center, Kermanshah University of Medical Sciences, Kermanshah 6719851115, Iran; bahmanid@stanford.edu
 - ¹¹ Center for Affective, Stress and Sleep Disorders, University of Basel, Psychiatric Clinics, 4001 Basel, Switzerland
 - ¹² Substance Abuse Prevention Research Center, Kermanshah University of Medical Sciences, Kermanshah 6715847141, Iran
 - ¹³ Department of Psychology, Stanford University, Stanford, CA 94305, USA
 - ¹⁴ Division of Sport Science and Psychosocial Health, Department of Sport, Exercise and Health, University of Basel, 4052 Basel, Switzerland
 - ¹⁵ School of Medicine, Tehran University of Medical Sciences, Tehran 1416753955, Iran
- * Correspondence: Serge.Brand@upk.ch

Citation: Mohammadi, H.; Roochi, M.M.; Sadeghi, M.; Garajei, A.; Heidar, H.; Ghaderi, B.; Tadakamadla, J.; Meybodi, A.A.; Dallband, M.; Mostafavi, S.; et al. Association of *N-acetyltransferases 1 and 2* Polymorphisms with Susceptibility to Head and Neck Cancers—A Meta-Analysis, Meta-Regression, and Trial Sequential Analysis. *Medicina* **2021**, *57*, 1095. <https://doi.org/10.3390/medicina57101095>

Academic Editor: Mario Pérez-Sayáns

Received: 23 August 2021

Accepted: 09 October 2021

Published: 13 October 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2021 by the author. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).

Abstract: *Background and objective:* *N-acetyltransferases 1 and 2* (*NAT1* and *NAT2*) genes have polymorphisms in accordance with slow and rapid acetylator phenotypes with a role in the development of head and neck cancers (HNCs). Herein, we aimed to evaluate the association of *NAT1* and *NAT2* polymorphisms with susceptibility to HNCs in an updated meta-analysis. *Materials and methods:* A search was comprehensively performed in four databases (Web of Science, Scopus, PubMed/Medline, and Cochrane Library until 8 July 2021). The effect sizes, odds ratio (OR) along with 95% confidence interval (CI) were computed. Trial sequential analysis (TSA), publication bias and sensitivity analysis were conducted. *Results:* Twenty-eight articles including eight studies reporting *NAT1* polymorphism and twenty-five studies reporting *NAT2* polymorphism were involved in the meta-analysis. The results showed that individuals with slow acetylators of *NAT2* polymorphism are at higher risk for HNC OR: 1.22 (95% CI: 1.02, 1.46; $p = 0.03$). On subgroup analysis, ethnicity, control source, and genotyping methods were found to be significant factors in the association of *NAT2* polymorphism with the HNC risk. TSA identified that the amount of information was not large enough and that more studies are needed to establish associations. *Conclusions:* Slow acetylators in *NAT2* polymorphism were related to a high risk of HNC. However, there was no relationship between *NAT1* polymorphism and the risk of HNC.

Keywords: head and neck carcinoma; oral carcinoma; polymorphism; N-acetyltransferases; meta-analysis

1. Introduction

Cellular inflammation and immunity can play a significant role in various stages of carcinogenesis [1] such as head and neck cancers (HNCs). HNC mortality rates are elevating and disproportionately affect people in low- and middle-income countries and areas with restricted resources [2]. Global Burden of Disease Study (GBD) in 2016 estimated 512,492 deaths due to HNC (a minimum of 15,018 deaths in North Africa and the Middle East to a maximum of 199,280 in South Asia) and predicted the death count to reach 705,901 in 2030 [3,4]. HNC involves a series of tumors originating in the oropharynx, hypopharynx, oral cavity, lip, larynx, or nasopharynx [5]. Smoking, alcohol consumption, and high-risk human papillomaviruses have been related to HNC [5–7]. In connection with the role of genetics in HNC, several recent meta-analyses have reported the association of polymorphisms with the risk of HNCs [8–11].

A number of heterocyclic and aromatic amines are the main carcinogenic compounds of tobacco smoke [12,13] that their metabolism in humans is complex and includes acetylation as a main pathway for DNA mutation and the onset of carcinogenesis [14]. In particular, two N-acetyltransferases, *NAT1* and *NAT2* perform a role in catalyzing the deactivation and activation of several carcinogenic amines through N- and O-acetylation, respectively [14,15]. Both *NAT* genes (*NAT1* and *NAT2*) have polymorphisms in humans and in accordance with slow and rapid acetylator phenotypes [16]. The *NAT2* metabolized gene is located in region 10 of chromosome 8p21, which contains two exons with a long intron of about 8.6 kb [17]. Exon 1 is very short (100 bp) and the entire protein-coding region in Exon 2 is 870 bp [18]. Also, the *NAT1* gene is located on the short arm of chromosome 8 (8p21) [19,20]. *NAT1* accelerates acetylation specifically for arylamine receptor structures such as p-aminosalicylic and p-aminobenzoic acids [21] and *NAT2* acetylates other arylamine-acceptor structures, such as isoniazid, sulfasalazine, procainamide, and caffeine [19].

Evidence from the published articles on the relationship between *NAT1* and *NAT2* polymorphisms and HNC susceptibility is conflicting [22,23]. The association between the polymorphisms (*NAT1* and *NAT2*) and the HNC risk has been evaluated by one [24] and four [25–28] meta-analyses, respectively. However, these studies were published several years ago with the most recent one being published in 2015. Therefore, through this meta-analysis, we intend to update the evidence on the association between the polymorphisms and the HNC risk by including more studies. In addition, we aim to conduct trial sequential analysis (TSA) and meta-regression.

2. Materials and Methods

2.1. Study Design

The present meta-analysis follows the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) protocols [29]. The PI/ECO (population, intervention/exposure, comparison, and outcome) question was: Are polymorphisms of *NAT1* and *NAT2* associated with the risk of HNC?

2.2. Identification of Articles

A search was comprehensively performed by one author (M.S.) in four databases of Web of Science, Scopus, PubMed/Medline, and Cochrane Library until 8 July 2021, without any restrictions in language, publication year, age, and sex to retrieve the relevant articles (Figure 1). The titles and abstracts of the relevant articles were assessed by the same author (M.S.); subsequently, the full-texts of the articles found to be relevant based

on the eligibility criteria were downloaded. The search strategy included: (“N-acetyl transferases” or “N-acetyltransferase” or “NAT2” or “NAT1”) and (“mouth” or “OSCC” or “oral” or “tongue” or “head and neck” or “HNSCC” or “nasopharyngeal” or “nasopharynx” or “oropharyngeal” or “salivary gland” or “laryngeal” or “larynx” or “hypopharyngeal” or “pharyngeal” or “pharynx” or “oral cavity” or “hypopharynx”) and (“tumor” or “carcinoma” or “cancer” or “neoplasm”) and (“allele” or “variant” or “polymorphism” or “genotype” or “gene”). The reference lists of the retrieved articles were reviewed to ensure that no important study was missed. Another author (H.M.) re-checked the process of searching and article selection. A lack of agreement between both authors was resolved by another author (J.T.).

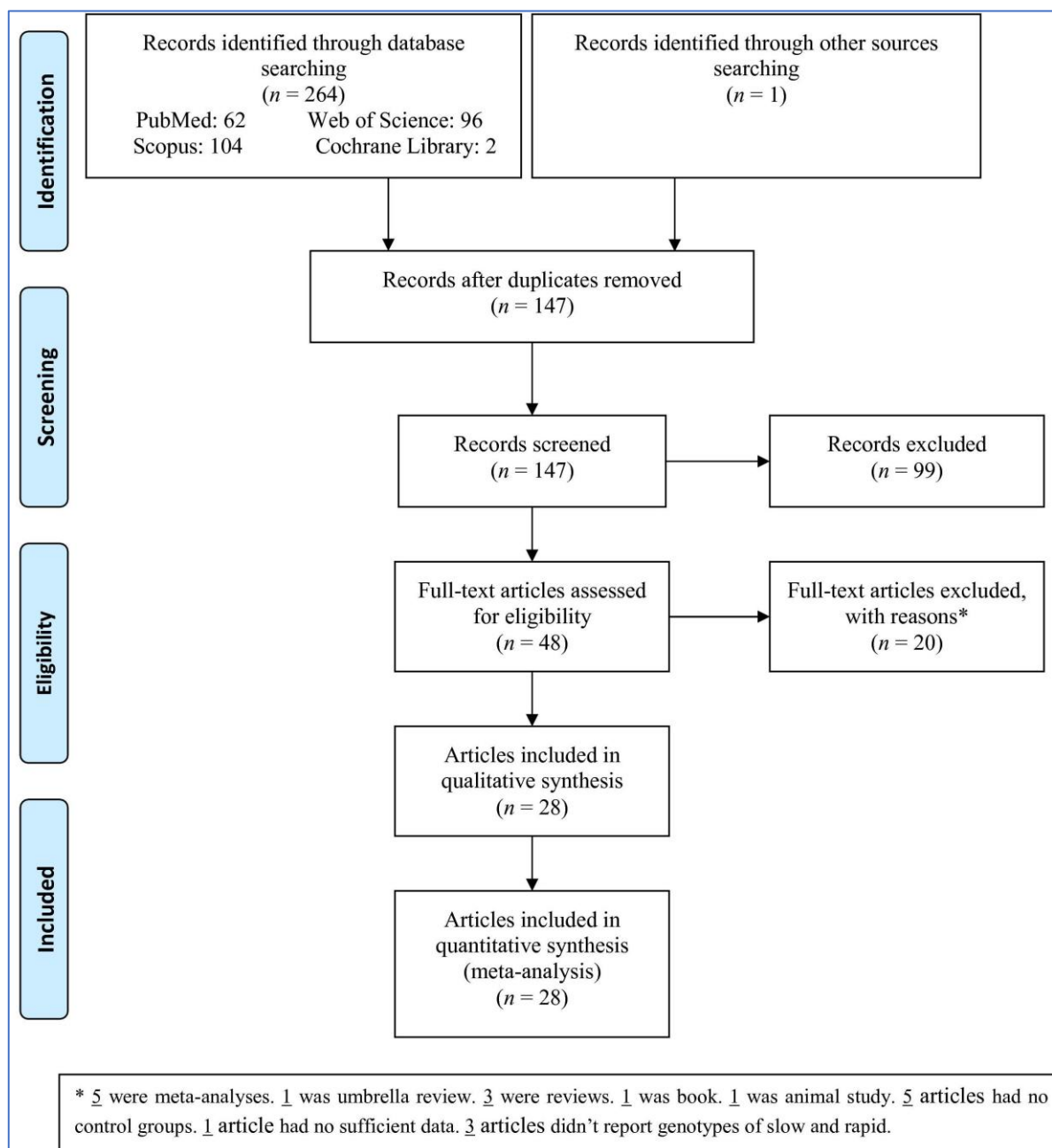


Figure 1. Flowchart of the study selection.

2.3. Eligibility Criteria

The inclusion criteria were: (1) case-control studies reporting slow and rapid acetylators of *NAT1* and *NAT2* polymorphisms in HNC patients and controls, (2) HNC patients were diagnosed clinically and pathologically, and (3) HNC patients had no other systemic diseases and controls were healthy or free of tumors. On the contrary, meta-analyses, review studies, articles with incomplete data, studies without a control group, animal studies, conference papers, book chapters, and comment papers were excluded.

2.4. Data Summary

The data of the articles involved in the meta-analysis were separately retrieved by two authors (M.S. and S.B.). Extracted data included names of the authors, publication year, study country, ethnicity, number of cases, tumor type, source of controls, genotyping method, quality score, age, and gender distribution.

2.5. Quality Evaluation

The quality scoring was completed by one author (M.S.) based on the Newcastle-Ottawa Scale (NOS) scale [30] that a study is judged on three broad perspectives: the selection (4 scores); the comparability (2 scores); and the outcome (3 scores) for non-randomized studies, respectively. The maximum possible score was nine and high-quality studies were those with a score of ≥ 7 .

2.6. Statistical Analysis

The effect sizes, odds ratios (OR) along with 95% confidence interval (CI), were calculated using the Review Manager 5.3 (RevMan 5.3; the Cochrane Collaboration, the Nordic Cochrane Centre, Copenhagen, Denmark) as well as subgroup analyses, quantifying the association between *NAT1* and *NAT2* polymorphisms and the HNC risk. A *p*-value (2-sided) < 0.05 was considered as a significant value. A random-effects model [31] was performed when I^2 statistic represented a significant heterogeneity ($P_{\text{heterogeneity}} < 0.1$ or $I^2 > 50\%$) and if the heterogeneity was insignificant, a fixed-effect model [32] was applied.

Subgroup analyses were performed based on the ethnicity of study participants, control source in the study, tumor type, sample size, and genotyping method used in a study. To adjust for the effect of sample sizes, gender, and age distribution of the subjects included in the studies, a meta-regression analysis was conducted.

Publication bias was assessed applying funnel plots, Egger's and/or Begg's tests with a *p*-value (2-sided) < 0.05 demonstrating the existence of publication bias. Sensitivity analyses ("one-study-removed" and "cumulative" analyses) were conducted to evaluate the stability of pooled ORs. The meta-regression, publication bias, and sensitivity analysis were analyzed using the Comprehensive Meta-Analysis version 2.0 (CMA 2.0) software (CMA 2.0; Biostat Inc., Englewood, NJ, USA).

To illustrate false-positive or negative conclusions from meta-analyses [33], trial Sequential Analysis (TSA) software (version 0.9.5.10 beta) (Copenhagen Trial Unit, Centre for Clinical Intervention Research, Rigshospitalet, Copenhagen, Denmark) was used to evaluate TSA for analyses [34]. A futility threshold can be checked by the TSA to determine the effectiveness or ineffectiveness before information size is reached. The required information size (RIS) and a two-sided boundary type were computed with an alpha risk of 5% and beta risk of 20%. There were enough studies where the Z-curve reached the RIS line or the boundary line or entered the futility area. Otherwise, the amount of information was not enough and more evidence was needed.

2.7. Primer Sequences

The primer sequences of *NAT1* and *NAT2* are shown in the studies of Katoh et al. [35] and Chen et al. [36], respectively.

3. Results

3.1. Study Selection

From the four electronic databases and manual searching, 265 records were identified. After excluding the duplicates and irrelevant records, 48 full-text articles met the eligibility criteria (Figure 1). Then, 20 full-texts were removed (five were meta-analyses, one was an umbrella review, three were reviews, one was a book, one was an animal study, five articles had no control groups, one article had insufficient data, and three articles did not report genotypes of slow and rapid). Finally, 28 articles were used in the meta-analysis.

3.2. Characteristics of Studies

Twenty-eight studies included in the analysis were published between 1998 and 2014 (Table 1). Fourteen articles [22,23,36–47] reported the results in Caucasians, nine [35,48–55] in Asians, and five [56–60] among participants of mixed ethnicity. The control source in eighteen articles [22,23,35,37,39,40,43–46,48,49,51–53,57,58,60] was hospitals and ten [36,38,41,42,47,50,54–56,59] recruited the controls from a general population. In total, the articles included 5154 HNC cases and 6194 controls. Age, gender distribution, sample size, tumor type, genotyping method, and the quality score are shown in Table 1.

Table 1. Characteristics of the articles included in the meta-analysis.

The First Author, Publication Year	Country	Ethnicity	Control Source	Number		Mean Year		Male Percentage		Type of Tumor	Genotyping Method	Quality Score
				Case	Control	Case	Control	Case	Control			
Gonzalez, 1998 [41]	Spain	Caucasian	PB	75	200	58.7	45	100	75	Oral, pharyngeal, laryngeal	PCR-RFLP	7
Katoh, 1998 [35]	Japan	Asian	HB	62	122	61.7	62.4	64.5	61.5	Oral	PCR-RFLP	7
Henning, 1999 [23]	Germany	Caucasian	HB	255	510	61.4	NA	90.6	NA	Laryngeal	PCR	7
Jourenkova-Mironova, 1999 [44]	France	Caucasian	HB	250	172	54.4	54.9	96	94.8	Oral, pharyngeal, laryngeal	PCR-RFLP	7
Morita, 1999 [54]	Japan	Asian	PB	145	164	59.0	49.8	86.9	62.2	Oral, pharyngeal, laryngeal	PCR	7
Olshan, 2000 [60]	USA	Mixed	HB	171	193	59.5	56.8	81.3	59.1	Oral, pharyngeal, laryngeal	PCR	7
Chen, 2001 [36]	USA	Caucasian	PB	341	552	NA	NA	70.4	71.6	Oral	PCR-RFLP	9
Fronhoffs, 2001 [39]	Germany	Caucasian	HB	291	300	59.8	47.1	80.1	58	Oral, pharyngeal, laryngeal	RT-PCR	6
Hahn, 2002 [42]	Germany	Caucasian	PB	94	92	61.5	45.1	65.9	51.1	Oral	PCR-RFLP	7
Lei, 2002 [51]	China	Asian	HB	62	56	60.2	58.2	NA	NA	Laryngeal	PCR-RFLP	7
Varzim, 2002 [47]	Portugal	Caucasian	PB	88	172	62.8	43.0	94.3	72.7	Laryngeal	PCR-RFLP	7

Cheng, 2003 [49]	Taiwan	Asian	HB	279	325	NA	NA	NA	NA	Pharyngeal	PCR-RFLP	6
Gajecka, 2005 [40]	Poland	Caucasian	HB	289	311	57.9	45.9	100	100	Laryngeal	PCR-RFLP	8
Rydzanicz, 2005 [45]	Poland	Caucasian	HB	266	143	61.6	53.1	95.1	100	Oral, pharyngeal, laryngeal	PCR-RFLP	8
Unal, 2005 [46]	Turkey	Caucasian	HB	45	104	53.5	50.0	93.3	65.4	Laryngeal	PCR-RFLP	7
Marques, 2006 [58]	Brazil	Mixed	HB	231	212	56.6	55.3	83.5	79.2	Oral	PCR-RFLP	8
Gara, 2007 [57]	Tunisia	Mixed	HB	64	160	50.7	53.6	65.6	45	Oral, pharyngeal, laryngeal	PCR-RFLP	7
Majumder, 2007 [53]	India	Asian	HB	297	342	NA	NA	NA	NA	Oral	PCR-RFLP	6
Boccia, 2008 [22]	Italy	Caucasian	HB	210	245	63.6	63.3	71.4	72.2	Oral, pharyngeal, laryngeal	PCR-RFLP	8
Buch, 2008 [56]	USA	Mixed	PB	182	399	58.7	58.7	87.4	75.7	Oral	PCR-RFLP	9
Harth, 2008 [43]	Germany	Caucasian	HB	312	300	59.7	47.2	80.4	58.7	Oral, pharyngeal, laryngeal	PCR-RFLP	6
Chatzimichalis, 2010 [37]	Greece	Caucasian	HB	88	102	66.5	62.5	87.5	74.5	Laryngeal	PCR-RFLP	8
Demokan, 2010 [38]	Turkey	Caucasian	PB	95	93	59.6	53.3	86.3	52.7	Oral, pharyngeal, laryngeal	PCR	8
Hou, 2011 [50]	China	Asian	PB	172	170	49.6	49.6	100	100	Oral, pharyngeal	PCR-RFLP and Taqman	9
Balaji, 2012 [48]	India	Asian	HB	157	132	53.1	55.1	54.8	34.8	Oral	Taqman	7
Majumder, 2012 [52]	India	Asian	HB	299	381	NA	NA	NA	NA	Oral	PCR	6
Tian, 2013 [55]	China	Asian	PB	233	102	60.0	60.0	NA	NA	Laryngeal	PCR	8
Marques, 2014 [59]	Brazil	Mixed	PB	101	141	NA	NA	NA	NA	Oral, pharyngeal, laryngeal	PCR-RFLP	7

Abbreviations: HB, hospital-based; PB, Population-based; PCR, Polymerase Chain Reaction; RT, Real Time; RFLP, Restriction Fragment Length Polymorphism; NA, Not Available. Taqman: The 5' Nuclease Assay.

Table 2 shows the prevalence of slow and rapid acetylators of *NAT1* and *NAT2* polymorphisms. Eight studies [23,35,38,39,44,47,52,60] included *NAT1* polymorphism with 1509 HNC cases and 1829 controls and twenty-five studies [22,23,35–38,40–51,53–59] included *NAT2* polymorphism with 4393 HNC cases and 5321 controls.

Table 2. Prevalence of the polymorphisms of *N-acetyltransferases 1* and *2* (*NAT1* and *NAT2*), (slow vs. rapid acetylators).

Author, Year	<i>NAT1</i>			
	Case		Control	
	Slow	Rapid	Slow	Rapid
Katoh, 1998 [35]	9	53	46	76
Henning, 1999 [23]	144	109	232	164
Jourenkova-Mironova, 1999 [44]	141	109	98	74
Olshan, 2000 [60]	83	88	108	85
Fronhoffs, 2001 [39]	195	96	206	94
Varzim, 2002 [47]	48	40	107	65
Demokan, 2010 [38]	53	42	42	51
Majumder, 2012 [52]	128	171	168	213
Author, Year	<i>NAT2</i>			
	Case		Control	
	Slow	Rapid	Slow	Rapid
Gonzalez, 1998 [41]	28	47	37	163
Katoh, 1998 [35]	7	55	7	115
Henning, 1999 [23]	138	117	286	224
Jourenkova-Mironova, 1999 [44]	142	108	91	81
Morita, 1999 [54]	18	127	17	147
Chen, 2001 [36]	198	143	302	250
Hahn, 2002 [42]	59	35	57	35
Lei, 2002 [51]	50	12	34	22
Varzim, 2002 [47]	47	41	76	96
Cheng, 2003 [49]	39	240	54	271
Gajecka, 2005 [40]	127	162	165	146
Rydzanicz, 2005 [45]	131	135	72	71
Unal, 2005 [46]	15	30	7	97
Marques, 2006 [58]	29	202	38	174
Gara, 2007 [57]	33	31	59	101
Majumder, 2007 [53]	190	107	205	137
Boccia, 2008 [22]	109	101	128	117
Buch, 2008 [56]	84	98	224	175
Harth, 2008 [43]	189	123	181	119
Chatzimichalis, 2010 [37]	39	49	65	37
Demokan, 2010 [38]	50	45	45	48
Hou, 2011 [50]	46	126	33	137
Balaji, 2012 [48]	100	57	67	65
Tian, 2013 [55]	189	44	56	46
Marques, 2014 [59]	48	53	51	90

3.3. Pooled Analyses

The pooled OR for the association between *NAT1* polymorphism and the risk of HNC from eight studies was 0.89 (95% CI: 0.77, 1.02; $p = 0.09$; $I^2 = 48\%$), (Figure 2). The pooled effect estimate was not significant demonstrating no association between *NAT1* polymorphism and the risk of HNC.

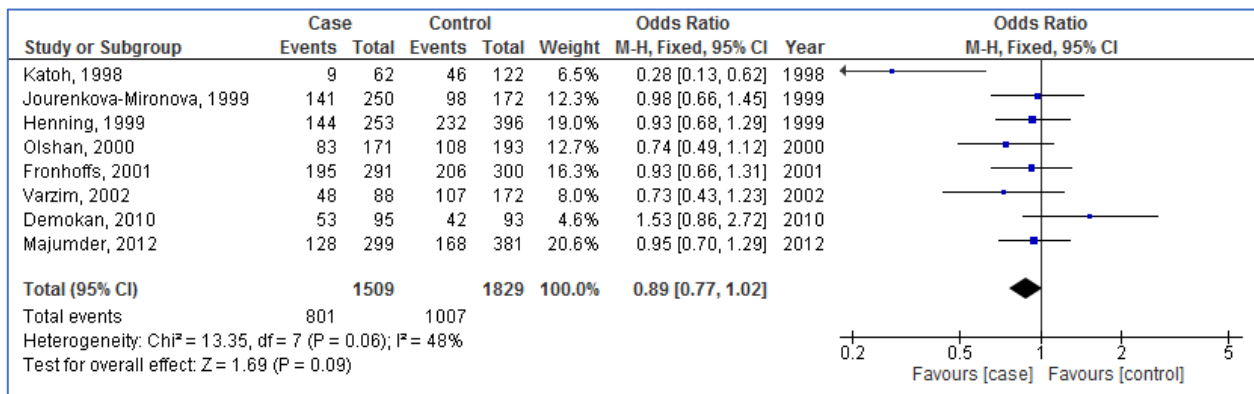


Figure 2. Forest plot for the association between *N-acetyltransferases 1 (NAT1)* polymorphism and the risk of head and neck cancer (slow vs. rapid acetylators). The diamond at the bottom of the forest plot illustrates the pooled result. The square in front of a individual study shows the result of the study and its horizontal line shows 95% confidence interval of the result.

Forest plot in Figure 3 illustrates that the pooled OR was 1.22 (95% CI: 1.02, 1.46; $p = 0.03$; $I^2 = 74%$) for the relationship between *NAT2* polymorphism and the HNC risk. This indicates that slow acetylators are related to high risk of HNC.

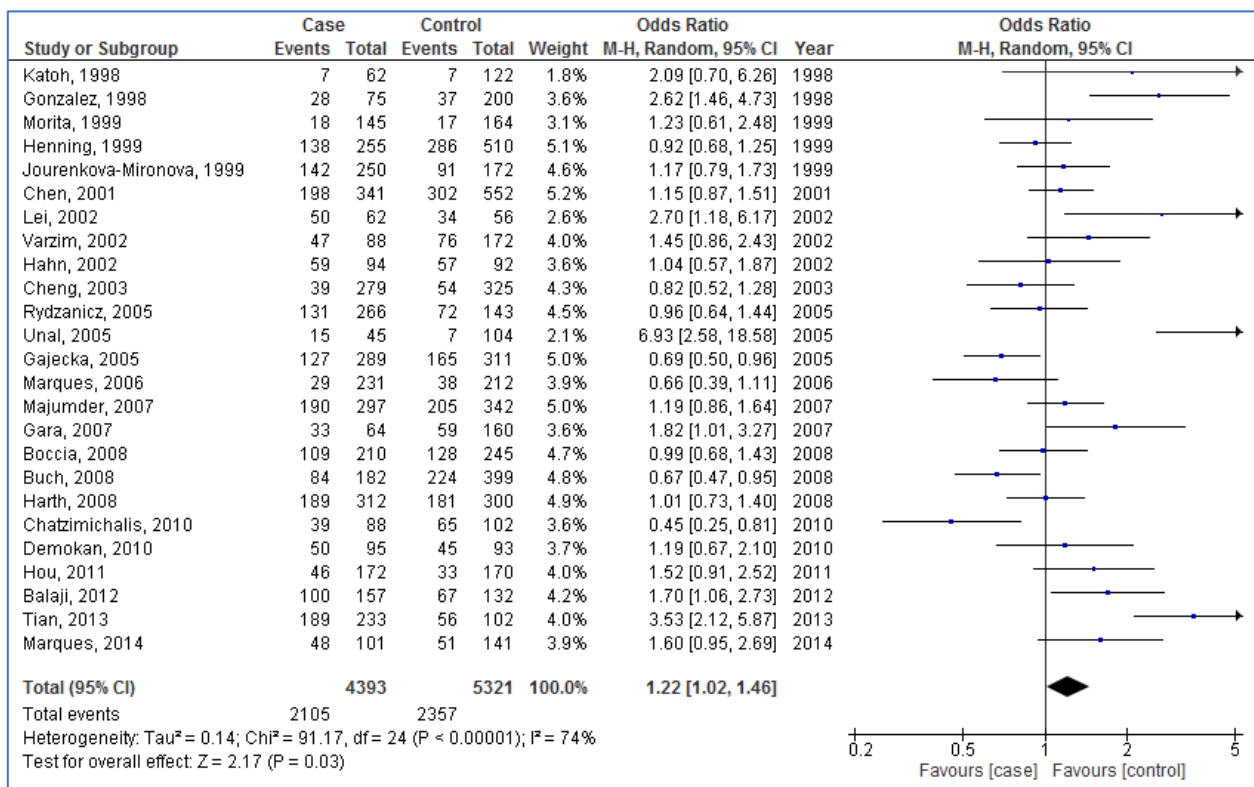


Figure 3. Forest plot demonstrating association between *N-acetyltransferases 2 (NAT2)* polymorphism and the risk of head and neck cancer (slow vs. rapid). The diamond at the bottom of the forest plot illustrates the pooled result. The square in front of a individual study shows the result of the study and its horizontal line shows 95% confidence interval of the result.

3.4. Subgroup Analyses

When there was one study for a subgroup, we could delete it [61]. Subgroup analyses were performed based on ethnicity, sample size, control source, genotyping method, and tumor type (Table 3). With regards to *NAT1* polymorphism, no subgroup differences were observed. For *NAT2* polymorphism, significant subgroup effects were observed for ethnicity and the control source. Slow acetylators among Asians and also the population-

based studies could be effective factors on the pooled result of the association between NAT2 polymorphism and the HNC risk.

Table 3. Subgroup analyses of association between *N-acetyltransferases 1 and 2* (NAT1 and NAT2) polymorphisms and the risk of head and neck cancer (slow vs. rapid acetylators).

Polymorphism	Variable (N)	OR	95% CI	p-Value	I ²	P _{heterogeneity}	
NAT1	Overall (8)	0.89	0.77, 1.02	0.09	48%	0.06	
	Ethnicity						
	Caucasian (5)	0.96	0.80, 1.15	0.64	0%	0.45	
	Asian (2)	0.55	0.17, 1.80	0.32	87%	0.005	
	Control source						
	Hospital-based (6)	0.87	0.74, 1.01	0.06	46%	0.10	
	Population-based (2)	1.05	0.51, 2.17	0.90	72%	0.06	
	Sample size						
	≥200 (6)	0.90	0.77, 1.04	0.15	0%	0.87	
	<200 (2)	0.67	0.13, 3.56	0.64	91%	0.0007	
	Genotyping method						
	PCR (4)	0.94	0.79, 1.14	0.54	26%	0.26	
	PCR-RFLP (3)	0.64	0.34, 1.18	0.15	74%	0.02	
	Tumor type						
	Oral (2)	0.55	0.17, 1.80	0.32	87%	0.005	
	Laryngeal (2)	0.87	0.67, 1.15	0.33	0%	0.43	
	Overall (25)	1.22	1.02, 1.46	0.03	74%	<0.00001	
NAT2	Ethnicity						
	Caucasian (13)	1.10	0.89, 1.37	0.38	71%	<0.0001	
	Asian (8)	1.60	1.13, 2.26	0.008	69%	0.002	
	Mixed (4)	1.04	0.61, 1.77	0.89	79%	0.003	
	Control source						
	Hospital-based (15)	1.10	0.88, 1.37	0.39	71%	<0.0001	
	Population-based (10)	1.41	1.04, 1.92	0.03	75%	<0.0001	
	Sample size						
	≥200 (20)	1.19	1.00, 1.42	0.05	70%	<0.00001	
	<200 (5)	1.49	0.68, 3.29	0.32	85%	<0.0001	
	Genotyping method						
	PCR (4)	1.47	0.77, 2.78	0.24	85%	0.0002	
	PCR-RFLP (19)	1.14	0.93, 1.39	0.21	72%	<0.00001	
	Tumor type						
	Oral (7)	1.05	0.80, 1.38	0.72	62%	0.01	
	Pharyngeal (2)	0.82	0.54, 1.24	0.35	0%	0.96	
	Laryngeal (8)	1.48	0.88, 2.51	0.14	88%	<0.00001	

Abbreviations: PCR, Polymerase Chain Reaction; RFLP, Restriction Fragment Length Polymorphism.

3.5. Meta-Regression

The meta-regression analyses assessing the effect of publication year, the sample size, and the mean age and gender distribution of cases and controls on the risk of HNC in NAT1 and NAT2 polymorphisms are shown in Table 4. Sample size, the mean age of cases, and the percentage of males in the controls were confounding factors for the pooled result of the association between NAT2 polymorphism and the HNC susceptibility. With an increase in sample size, age of the cases, and percentage of males in the controls, the OR decreased.

Table 4. Meta-regression analysis of association between *N-acetyltransferases 1 and 2* (NAT1 and NAT2) polymorphisms and the risk of head and neck cancer (slow vs. rapid acetylators).

Polymorphism	Variable		Point Estimate	Standard Error	Lower Limit	Upper Limit	Z-Value	p-Value	
NAT1	Publication year	Slope	0.01830	0.01361	-0.00837	0.04497	1.34462	0.17875	
		Intercept	-36.77098	27.26207	-90.20365	16.66169	-1.34880	0.17740	
	Sample size	Slope	0.00027	0.00045	-0.00060	0.00115	0.61240	0.54027	
		Intercept	-0.25993	0.24912	-0.74819	0.22833	-1.04340	0.29676	
	Mean age of cases	Slope	-0.01179	0.03248	-0.07546	0.05186	-0.36300	0.71660	
		Intercept	0.57037	1.93376	-3.21972	4.36047	0.29496	0.76803	
	Mean age of controls	Slope	-0.02263	0.03624	-0.09365	0.04839	-0.62459	0.53224	
		Intercept	1.17938	2.13386	-3.00290	5.36167	0.55270	0.58047	
	Male percentage of cases	Slope	-0.01131	0.01256	-0.03593	0.01331	-0.90074	0.36773	
		Intercept	0.86738	1.11137	-1.31087	3.04562	0.78046	0.43512	
	Male percentage of controls	Slope	-0.00268	0.00617	-0.01478	0.00942	-0.43474	0.066375	
		Intercept	0.03230	0.43459	-0.81948	0.88409	0.07433	0.94074	
	NAT2	Publication year	Slope	0.00944	0.01016	-0.01047	0.02934	0.092942	0.35267
			Intercept	-18.82284	20.36308	-58.73373	21.08806	-0.92436	0.35530
Sample size		Slope	-0.00080	0.00020	-0.00120	-0.00040	-3.91239	0.00009	
		Intercept	0.50882	0.11300	0.28733	0.73030	4.50265	0.00001	
Mean age of cases		Slope	-0.04050	0.01356	-0.06706	-0.01393	-2.098776	0.00281	
		Intercept	2.47888	0.80007	0.91077	4.04699	3.09832	0.00195	
Mean age of controls		Slope	-0.00438	0.00889	-0.02180	0.01305	-0.49203	0.62270	
		Intercept	0.34691	0.47403	-0.58217	1.27600	0.73184	0.46427	
Male percentage of cases		Slope	-0.0629	0.00393	-0.01399	0.00141	-1.60201	0.10915	
		Intercept	0.57366	0.33428	-0.08152	1.22884	1.71610	0.08614	
Male percentage of controls		Slope	-0.00785	0.00289	-0.01351	-0.00219	-2.71989	0.00653	
		Intercept	0.64373	0.22152	0.20956	1.07790	2.90598	0.00366	

3.6. Trial Sequential Analysis

TSA for both polymorphisms (NAT1 and NAT2) and the HNC risk is illustrated in Figure 4. The Z-curve (blue line) did not reach the RIS or the boundary lines or enter the futility area for either polymorphism and therefore, the amount of information was not large enough, suggesting the need for more studies.

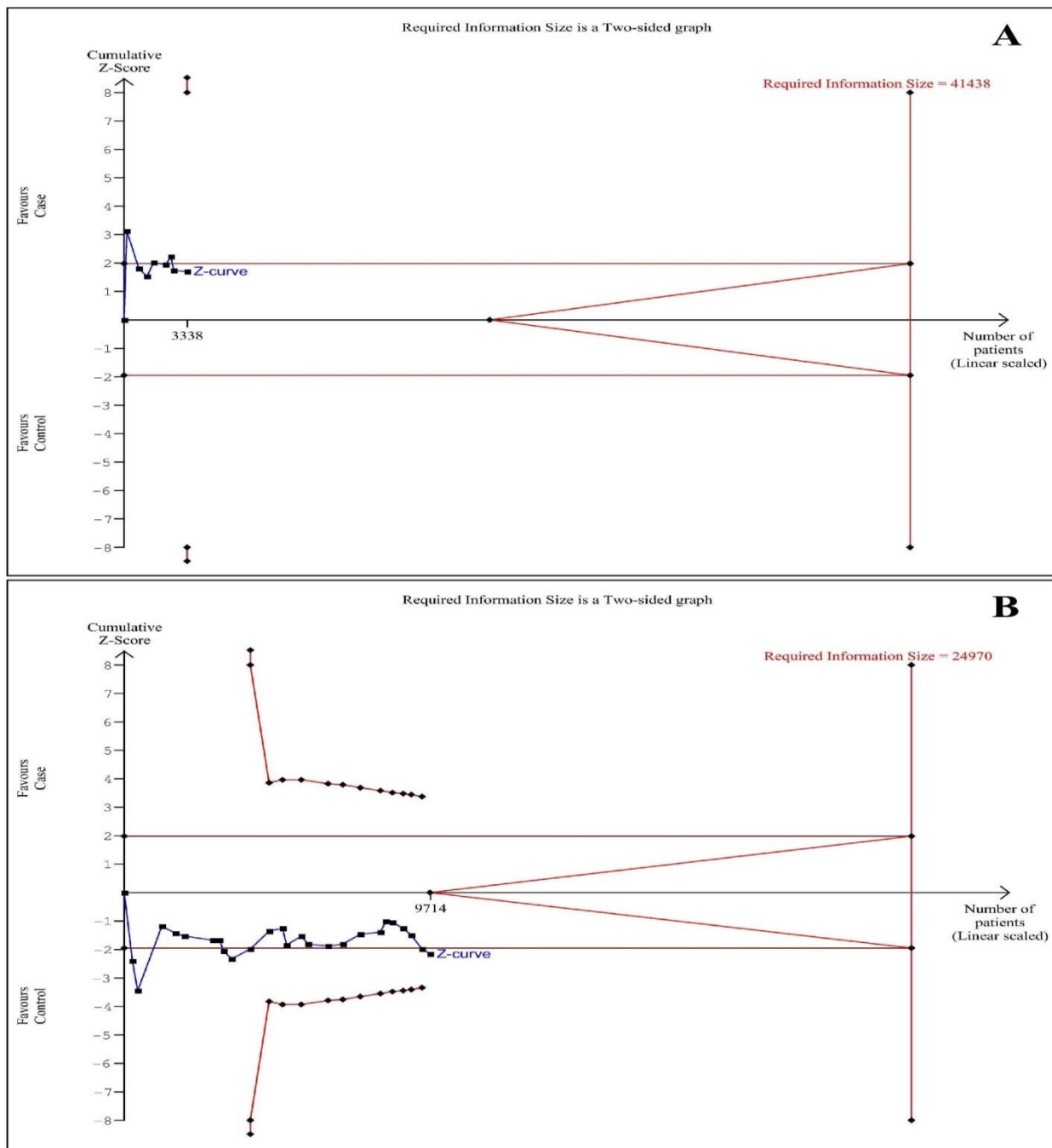


Figure 4. Trial sequential analysis of association between *N-acetyltransferases 1 and 2* (*NAT1* and *NAT2*) polymorphisms and the risk of head and neck cancer (slow vs. rapid acetylators) [$\alpha = 5\%$ and $1-\beta = 80\%$]. (A) *NAT1* [diversity or $D^2 = 52\%$] and (B) *NAT2* [$D^2 = 76\%$].

3.7. Sensitivity Analysis

Both “one-study-removed” and “cumulative analysis” illustrated the pooled data stability for *NAT1* and *NAT2* polymorphisms (data not presented). After removing one study [46] with outlier data, in concordance with previous analysis, the new pooled result did not report any relationship between *NAT2* polymorphism and the HNC susceptibility (OR = 1.17; 95% CI: 0.99, 1.39; $p = 0.07$; $I^2 = 70\%$). In addition, after removing the studies with a quality score of less than 7 for *NAT2* polymorphism [43,49,53], the new result remained similar (OR = 1.27; 95% CI: 1.03, 1.57; $p = 0.03$; $I^2 = 76\%$). Removal of studies with a quality score of less than 7 for *NAT1* polymorphism [39,52], did not change the pooled estimate (OR = 0.85; 95% CI: 0.75, 1.02; $p = 0.08$; $I^2 = 62\%$).

3.8. Publication Bias

The Egger's ($p = 0.240$) and Begg's ($p = 0.322$) tests did not reveal any publication bias for *NAT1* polymorphism, but both tests revealed the presence of publication bias for *NAT2* polymorphism (Egger's: $p = 0.012$ and Begg's: $p = 0.028$), (Figure 5).

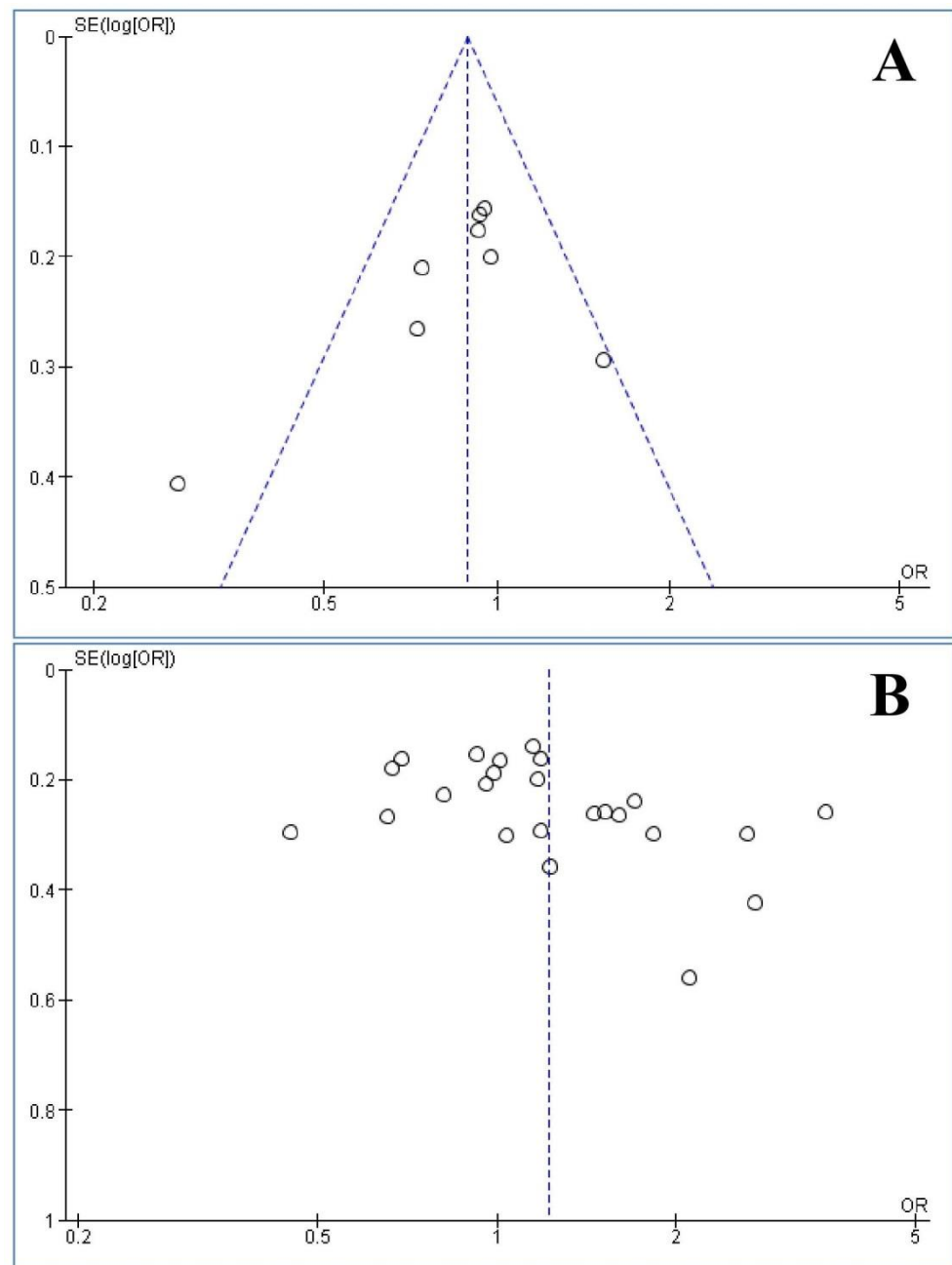


Figure 5. Funnel plot analyses of the association between *N-acetyltransferases 1 and 2* (*NAT1* and *NAT2*) polymorphisms and the risk of head and neck cancer (slow vs. rapid acetylators). (A) *NAT1* and (B) *NAT2*.

4. Discussion

This meta-analysis showed a significant relationship between *NAT2* polymorphisms and the HNC susceptibility with slow acetylators being at higher risk for HNC than rapid acetylators. For *NAT2* polymorphism, the ethnicity, the control source, and genotyping methods could modify the association of this polymorphism and the HNC risk. In

addition, TSA showed the amount of information for the association between the polymorphisms (*NAT1* and *NAT2*) and the HNC risk was not large enough.

The findings from studies exploring the association of *NAT1* polymorphism with other cancers and HNC are different. One meta-analysis [24] found *NAT1* polymorphism to be related to the risk of lung, colorectal, head and neck, bladder, and gastric carcinomas, but not with prostate, breast, and pancreatic carcinomas and non-Hodgkin's lymphoma. Varzim et al. [47] checked the association between *NAT1* polymorphism and the laryngeal cancer risk and found that the association depends on tumor location. Among the eight studies included in our meta-analyses [23,35,38,39,44,47,52,60] which evaluated the association between *NAT1* polymorphism and the HNC risk, just one study [35] reported a protective role of *NAT1* slow acetylators in the HNC patients while the rest of the studies did not find any association.

Comparing the individual studies included in the meta-analysis, differences were observed between the studies. For example, five studies [41,46,48,55] found an elevated risk of HNC for *NAT2* slow acetylators, one found a protective role of these acetylators in HNC patients, and three did not find any association between *NAT2* polymorphism and the HNC risk [23,45,49].

Effective factors on the association between NAT polymorphisms and the risk of HNC were not included in our analysis due to low numbers of studies, including smoking, gene combination, and the linkage disequilibrium. One study [41] found an elevated frequency of the *NAT2* slow acetylator genotypes among HNC patients who smoked less than those who smoked more frequently. Another study reported an association in cases with a smoking history ≤ 30 years in duration [35]. These contradictory results [35,41,46] suggest the need to evaluate the effect of NAT polymorphisms independent of the history of smoking. In addition, assessing the frequencies of gene-gene combination (*NAT2* with *GSTM1*, *XPB*, and *CYP1A1*) between cases with laryngeal cancer and the controls, the frequency of combinations was superior to cases than in controls where the numbers of combinations had an increased risk of laryngeal cancer and the numbers of other combinations had a protective role [40]. The linkage disequilibrium between the genes of *NAT1* and *NAT2* has been observed in HNC [23,38,62] and other cancers [63–65]. Research [66] showed the highest level of carcinogen-DNA adducts formation in cases with acetylation activity of *NAT1* rapid and *NAT2* slow. Therefore, future studies should consider the linkage between these polymorphisms.

The limitations of the present meta-analysis were: (1) low sample size in some studies. (2) In a number of the involved studies, the controls were not well matched to the cases. (3) Low numbers of studies entered to the analysis as shown by TSA. (4) Existence of publication bias and high heterogeneity between the analyses.

Conclusions

There was no association between *NAT1* polymorphism and susceptibility to HNC, whereas an association between *NAT2* polymorphism and the HNC risk was found. Slow acetylators of *NAT2* polymorphism were at greater risk for HNC than the rapid acetylators. Despite the stability of the results, the presence of high heterogeneity, publication bias, and confounding factors warrant the need for more studies to confirm the results of the present meta-analysis as well as TSA.

Author Contributions: Conceptualization: H.M. and M.S. (Masoud Sadeghi); Methodology: M.M.R., A.G., H.H., M.S. (Masoud Sadeghi), B.G., J.T., A.A.M., M.D., S.M., M.M., M.S. (Mojtaba Salehi), D.S.-B. and S.B.; Formal analysis and investigation: M.S. (Masoud Sadeghi); Writing—original draft preparation: M.M.R., A.G., H.H., M.S. (Masoud Sadeghi), B.G., J.T., A.A.M., M.D., S.M., M.M., M.S. (Mojtaba Salehi), D.S.-B. and S.B.; Writing—review and editing: M.M.R., A.G., H.H., M.S. (Masoud Sadeghi), B.G., J.T., A.A.M., M.D., S.M., M.M., M.S. (Mojtaba Salehi), D.S.-B. and S.B.; Funding acquisition: H.M. and S.B.; Resources: H.M.; Supervision: M.M.R., A.G., H.H., M.S. (Masoud Sadeghi), B.G., J.T., A.A.M., M.D., S.M., M.M., M.S. (Mojtaba Salehi), D.S.-B. and S.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: No new data were created or analyzed in this study. Data sharing is not applicable to this article.

Acknowledgments: We thank Undine Lang, medical director of the Psychiatric Clinics of the University of Basel (UPK; Basel, Switzerland) for financially support the open access publication.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Rezaei, F.; Mohammadi, H.; Heydari, M.; Sadeghi, M.; Mozaffari, H.R.; Khavid, A.; Godiny, M.; Brand, S.; M Dürsteler, K.; Brühl, A.B.; et al. Association between IL-8 (-251T/A) and IL-6 (-174G/C) Polymorphisms and Oral Cancer Susceptibility: A Systematic Review and Meta-Analysis. *Medicina* **2021**, *57*, 405.
2. Patterson, R.H.; Fischman, V.G.; Wasserman, I.; Siu, J.; Shrime, M.G.; Fagan, J.J.; Koch, W.; Alkire, B.C. Global burden of head and neck cancer: Economic consequences, health, and the role of surgery. *Otolaryngol.–Head Neck Surg.* **2020**, *162*, 296–303.
3. Vos, T.; Abajobir, A.A.; Abate, K.H.; Abbafati, C.; Abbas, K.M.; Abd-Allah, F.; Abdulkader, R.S.; Abdulle, A.M.; Abebo, T.A.; Abera, S.F. Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990–2016: A systematic analysis for the Global Burden of Disease Study 2016. *Lancet* **2017**, *390*, 1211–1259.
4. Foreman, K.J.; Marquez, N.; Dolgert, A.; Fukutaki, K.; Fullman, N.; McGaughey, M.; Pletcher, M.A.; Smith, A.E.; Tang, K.; Yuan, C.-W. Forecasting life expectancy, years of life lost, and all-cause and cause-specific mortality for 250 causes of death: Reference and alternative scenarios for 2016–40 for 195 countries and territories. *Lancet* **2018**, *392*, 2052–2090.
5. Nigro, C.L.; Denaro, N.; Merlotti, A.; Merlano, M. Head and neck cancer: Improving outcomes with a multidisciplinary approach. *Cancer Manag. Res.* **2017**, *9*, 363.
6. Weinberger, P.M.; Yu, Z.; Haffty, B.G.; Kowalski, D.; Harigopal, M.; Brandsma, J.; Sasaki, C.; Joe, J.; Camp, R.L.; Rimm, D.L. Molecular classification identifies a subset of human papillomavirus-associated oropharyngeal cancers with favorable prognosis. *J. Clin. Oncol.* **2006**, *24*, 736–747.
7. Johnson, D.E.; Burtneis, B.; Leemans, C.R.; Lui, V.W.Y.; Bauman, J.E.; Grandis, J.R. Head and neck squamous cell carcinoma. *Nat. Rev. Dis. Primers* **2020**, *6*, 1–22.
8. Mozaffari, H.R.; Rostamnia, M.; Sharifi, R.; Safaei, M.; Zavattaro, E.; Tadakamadla, S.K.; Imani, M.M.; Sadeghi, M.; Golshah, A.; Moradpoor, H. A PRISMA-compliant meta-analysis on association between X-ray repair cross complementing (XRCC1, XRCC2, and XRCC3) polymorphisms and oral cancer susceptibility. *Gene* **2021**, *781*, 145524.
9. Rezaei, F.; Doulatparast, D.; Sadeghi, M. Common polymorphisms of Interleukin-10 (-1082A/G, -592A/C, and -819C/T) in oral cancers: An updated meta-analysis. *J. Interferon Cytokine Res.* **2020**, *40*, 357–369.
10. Xia, S.; Wu, S.; Wang, M. The Association Between the XRCC1 Arg399Gln Polymorphism and the Risk of Head and Neck Cancer: An Updated Meta-Analysis Including 14586 Subjects. *Technol. Cancer Res. Treat.* **2021**, *20*, 15330338211033060, doi:10.1177/15330338211033060.
11. Wu, T.; Zhang, Z.T.; Li, L.; Liu, R.Y.; Bei, B.T. Correlation between hypoxia-inducible factor-1 α C1772T/G1790A polymorphisms and head and neck cancer risk: A meta-analysis. *World J. Surg. Oncol.* **2021**, *19*, 210, doi:10.1186/s12957-021-02324-0.
12. Vineis, P. Epidemiology of cancer from exposure to arylamines. *Environ. Health Perspect.* **1994**, *102*, 7–10.
13. Bartsch, H.; Malaveille, C.; Friesen, M.; Kadlubar, F.; Vineis, P. Black (air-cured) and blond (flue-cured) tobacco cancer risk IV: Molecular dosimetry studies implicate aromatic amines as bladder carcinogens. *Eur. J. Cancer* **1993**, *29*, 1199–1207.
14. Hein, D.W.; Doll, M.A.; Rustan, T.D.; Gray, K.; Feng, Y.; Ferguson, R.J.; Grant, D.M. Metabolic activation and deactivation of arylamine carcinogens by recombinant human NAT1 and polymorphic NAT2 acetyltransferases. *Carcinogenesis* **1993**, *14*, 1633–1638.
15. Hein, D.W.; Doll, M.A.; Fretland, A.J.; Leff, M.A.; Webb, S.J.; Xiao, G.H.; Devanaboyina, U.-S.; Nangju, N.A.; Feng, Y. Molecular genetics and epidemiology of the NAT1 and NAT2 acetylation polymorphisms. *Cancer Epidemiol. Prev. Biomark.* **2000**, *9*, 29–42.
16. Vatsis, K.P.; Weber, W.W. Structural heterogeneity of Caucasian N-acetyltransferase at the NAT1 gene locus. *Arch. Biochem. Biophys.* **1993**, *301*, 71–76.
17. Feki-Tounsi, M.; Khelifi, R.; Louati, I.; Fourati, M.; Mhiri, M.-N.; Hamza-Chaffai, A.; Rebai, A. Polymorphisms in XRCC1, ERCC2, and ERCC3 DNA repair genes, CYP1A1 xenobiotic metabolism gene, and tobacco are associated with bladder cancer susceptibility in Tunisian population. *Environ. Sci. Pollut. Res.* **2017**, *24*, 22476–22484.
18. Imam, H.; Imam, T.; Abbas, S.Z.; Ismail, M.; Muhammad, S.A. Association study of NAT2 gene polymorphism and risk of oral cancer in Southern Punjab, Pakistan. *J. Pak. Med Assoc.* **2021**, *71*, 1954–1958.
19. Sabbagh, N.; Delaporte, E.; Marez, D.; Lo-Guidice, J.-M.; Piette, F.; Broly, F. NAT2 genotyping and efficacy of sulfasalazine in patients with chronic discoid lupus erythematosus. *Pharmacogenetics* **1997**, *7*, 131–135.
20. Wang, L.; Minchin, R.F.; Butcher, N.J. Arylamine N-acetyltransferase 1 protects against reactive oxygen species during glucose starvation: Role in the regulation of p53 stability. *PLoS ONE* **2018**, *13*, e0193560.

21. Minchin, R.F. Acetylation of p-aminobenzoylglutamate, a folic acid catabolite, by recombinant human arylamine N-acetyltransferase and U937 cells. *Biochem. J.* **1995**, *307*, 1–3.
22. Boccia, S.; Cadoni, G.; Sayed-Tabatabaei, F.A.; Volante, M.; Arzani, D.; De Lauretis, A.; Cattel, C.; Almadori, G.; Van Duijn, C.M.; Paludetti, G. CYP1A1, CYP2E1, GSTM1, GSTT1, EPHX1 exons 3 and 4, and NAT2 polymorphisms, smoking, consumption of alcohol and fruit and vegetables and risk of head and neck cancer. *J. Cancer Res. Clin. Oncol.* **2008**, *134*, 93–100.
23. Henning, S.; Cascorbi, I.; Münchow, B.; Jahnke, V.; Roots, I. Association of arylamine N-acetyltransferases NAT1 and NAT2 genotypes to laryngeal cancer risk. *Pharmacogenetics* **1999**, *9*, 103–111.
24. Zhang, K.; Gao, L.; Wu, Y.; Chen, J.; Lin, C.; Liang, S.; Su, J.; Ye, J.; He, X. NAT1 polymorphisms and cancer risk: A systematic review and meta-analysis. *Int. J. Clin. Exp. Med.* **2015**, *8*, 9177.
25. Zheng, Y.; Li, Y.; Teng, Y.; Zhang, Z.; Cao, X. Association of NAT2 phenotype with risk of head and neck carcinoma: A meta-analysis. *Oncol. Lett.* **2012**, *3*, 429–434.
26. Zhuo, X.-L.; Ling, J.-J.; Zhou, Y.; Zhao, H.-Y.; Song, Y.-F.; Tan, Y.-H. NAT2 polymorphisms with oral carcinoma susceptibility: A meta-analysis. *Mol. Biol. Rep.* **2012**, *39*, 8813–8819.
27. Ying, X.-J.; Dong, P.; Shen, B.; Wang, J.; Wang, S.; Wang, G. Possible association of NAT2 polymorphism with laryngeal cancer risk: An evidence-based meta-analysis. *J. Cancer Res. Clin. Oncol.* **2011**, *137*, 1661–1667.
28. Zhang, L.; Xiang, Z.; Hao, R.; Li, R.; Zhu, Y. N-acetyltransferase 2 genetic variants confer the susceptibility to head and neck carcinoma: Evidence from 23 case–control studies. *Tumor Biol.* **2014**, *35*, 3585–3595.
29. Moher, D.; Liberati, A.; Tetzlaff, J.; Altman, D.G. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. *Int. J. Surg.* **2010**, *8*, 336–341.
30. Wells, G.A.; Shea, B.; O’Connell, D.; Peterson, J.; Welch, V.; Losos, M.; Tugwell, P. The Newcastle-Ottawa Scale (NOS) for Assessing the Quality of Nonrandomised Studies in Meta-Analyses. 2000. Available online: http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp (accessed on 12 January 2016).
31. DerSimonian, R.; Laird, N. Meta-analysis in clinical trials revisited. *Contemp. Clin. Trials* **2015**, *45*, 139–145.
32. Mantel, N.; Haenszel, W. Statistical aspects of the analysis of data from retrospective studies of disease. *J. Natl. Cancer Inst.* **1959**, *22*, 719–748.
33. Imberger, G.; Thorlund, K.; Gluud, C.; Wetterslev, J. False-positive findings in Cochrane meta-analyses with and without application of trial sequential analysis: An empirical review. *BMJ Open* **2016**, *6*, e011890.
34. Wetterslev, J.; Jakobsen, J.C.; Gluud, C. Trial sequential analysis in systematic reviews with meta-analysis. *BMC Med. Res. Methodol.* **2017**, *17*, 1–18.
35. Katoh, T.; Kaneko, S.; Boissy, R.; Watson, M.; Ikemura, K.; Bell, D.A. A pilot study testing the association between N-acetyltransferases 1 and 2 and risk of oral squamous cell carcinoma in Japanese people. *Carcinogenesis* **1998**, *19*, 1803–1807.
36. Chen, C.; Ricks, S.; Doody, D.R.; Fitzgibbons, E.D.; Porter, P.L.; Schwartz, S.M. N-Acetyltransferase 2 polymorphisms, cigarette smoking and alcohol consumption, and oral squamous cell cancer risk. *Carcinogenesis* **2001**, *22*, 1993–1999.
37. Chatzimichalis, M.; Xenellis, J.; Tzagaroulakis, A.; Sarof, P.; Banis, K.; Gazouli, M.; Bibas, A. GSTT1, GSTM1, GSTM3 and NAT2 polymorphisms in laryngeal squamous cell carcinoma in a Greek population. *J. Laryngol. Otol.* **2010**, *124*, 318–323.
38. Demokan, S.; Suoglu, Y.; Gözeler, M.; Demir, D.; Dalay, N. N-acetyltransferase 1 and 2 gene sequence variants and risk of head and neck cancer. *Mol. Biol. Rep.* **2010**, *37*, 3217–3226.
39. Fronhoffs, S.; Brüning, T.; Ortiz-Pallardo, E.; Bröde, P.; Koch, B.; Harth, V.; Sachinidis, A.; Bolt, H.M.; Herberhold, C.; Vetter, H. Real-time PCR analysis of the N-acetyltransferase NAT1 allele* 3,* 4,* 10,* 11,* 14 and* 17 polymorphism in squamous cell cancer of head and neck. *Carcinogenesis* **2001**, *22*, 1405–1412.
40. Gajecka, M.; Rydzanicz, M.; Jaskula-Sztul, R.; Kujawski, M.; Szyfter, W.; Szyfter, K. CYP1A1, CYP2D6, CYP2E1, NAT2, GSTM1 and GSTT1 polymorphisms or their combinations are associated with the increased risk of the laryngeal squamous cell carcinoma. *Mutat. Res. Fundam. Mol. Mech. Mutagenesis* **2005**, *574*, 112–123.
41. Gonzalez, M.; Alvarez, V.; Pello, M.; Menendez, M.; Suarez, C.; Coto, E. Genetic polymorphism of N-acetyltransferase-2, glutathione S-transferase-M1, and cytochromes P450IIE1 and P450IID6 in the susceptibility to head and neck cancer. *J. Clin. Pathol.* **1998**, *51*, 294–298.
42. Hahn, M.; Hagedorn, G.; Kuhlisch, E.; Schackert, H.K.; Eckelt, U. Genetic polymorphisms of drug-metabolizing enzymes and susceptibility to oral cavity cancer. *Oral Oncol.* **2002**, *38*, 486–490.
43. Harth, V.; Schäfer, M.; Abel, J.; Maintz, L.; Neuhaus, T.; Besuden, M.; Primke, R.; Wilkesmann, A.; Thier, R.; Vetter, H. Head and neck squamous-cell cancer and its association with polymorphic enzymes of xenobiotic metabolism and repair. *J. Toxicol. Environ. Health Part A* **2008**, *71*, 887–897.
44. Jourenkova-Mironova, N.; Wikman, H.; Bouchardy, C.; Mitrunen, K.; Dayer, P.; Benhamou, S.; Hirvonen, A. Role of arylamine N-acetyltransferase 1 and 2 (NAT1 and NAT2) genotypes in susceptibility to oral/pharyngeal and laryngeal cancers. *Pharm. Genom.* **1999**, *9*, 533–537.
45. Rydzanicz, M.; Wierzbicka, M.; Gajecka, M.; Szyfter, W.; Szyfter, K. The impact of genetic factors on the incidence of multiple primary tumors (MPT) of the head and neck. *Cancer Lett.* **2005**, *224*, 263–278.
46. Ünal, M.; Tamer, L.; Akbaş, Y.; Pata, Y.S.; Vayisoğlu, Y.; Değirmenci, U.; Çamdeviren, H. Genetic polymorphism of N-acetyltransferase 2 in the susceptibility to laryngeal squamous cell carcinoma. *Head Neck J. Sci. Spec. Head Neck* **2005**, *27*, 1056–1060.
47. Varzim, G.; Monteiro, E.; Silva, R.; Pinheiro, C.; Lopes, C. Polymorphisms of arylamine N-acetyltransferase (NAT1 and NAT2) and larynx cancer susceptibility. *ORL* **2002**, *64*, 206–212.

48. Balaji, L.; Krishna, B.S.; Bhaskar, L. An unlikely role for the NAT2 genotypes and haplotypes in the oral cancer of south Indians. *Arch. Oral Biol.* **2012**, *57*, 513–518.
49. Cheng, Y.-J.; Chien, Y.-C.; Hildesheim, A.; Hsu, M.-M.; Chen, I.-H.; Chuang, J.; Chang, J.; Ma, Y.D.; Luo, C.-T.; Hsu, W.-L. No association between genetic polymorphisms of CYP1A1, GSTM1, GSTT1, GSTP1, NAT2, and nasopharyngeal carcinoma in Taiwan. *Cancer Epidemiol. Prev. Biomark.* **2003**, *12*, 179–180.
50. Hou, Y.-Y.; Ou, H.-L.; Chu, S.-T.; Wu, P.-C.; Lu, P.-J.; Chi, C.-C.; Leung, K.-W.; Lee, C.-Y.; Wu, P.-H.; Hsiao, M. NAT2 slow acetylation haplotypes are associated with the increased risk of betel quid-related oral and pharyngeal squamous cell carcinoma. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endodontol.* **2011**, *112*, 484–492.
51. Lei, D.; Pan, X.; Guo, C.; Xu, F.; Zhang, L.; Liu, D.; Luan, X. Relationship between polymorphism of N-acetyltransferase 2 and genetic susceptibility to laryngeal carcinoma. *Zhonghua Zhong Liu Za Zhi [Chin. J. Oncol.]* **2002**, *24*, 154–156.
52. Majumder, M.; Ghosh, S.; Roy, B. Association between polymorphisms at N-acetyltransferase 1 (NAT1) & risk of oral leukoplakia & cancer. *Indian J. Med Res.* **2012**, *136*, 605.
53. Majumder, M.; Sikdar, N.; Ghosh, S.; Roy, B. Polymorphisms at XPD and XRCC1 DNA repair loci and increased risk of oral leukoplakia and cancer among NAT2 slow acetylators. *Int. J. Cancer* **2007**, *120*, 2148–2156.
54. Morita, S.; Yano, M.; Tsujinaka, T.; Akiyama, Y.; Taniguchi, M.; Kaneko, K.; Miki, H.; Fujii, T.; Yoshino, K.; Kusuoka, H. Genetic polymorphisms of drug-metabolizing enzymes and susceptibility to head-and-neck squamous-cell carcinoma. *Int. J. Cancer* **1999**, *80*, 685–688.
55. Tian, S.; Zhang, J.; Yuan, X.; Huang, M.; Guo, Z.; Chen, F.; Li, Q.; Guan, Z. The association between genetic polymorphisms of NAT1, NAT2 and susceptibility to laryngeal squamous carcinoma (LSCC) in Han population in Guangdong China. *J. Modern Oncol.* **2013**, *6*, 1213–1218.
56. Buch, S.C.; Nazar-Stewart, V.; Weissfeld, J.L.; Romkes, M. Case-control study of oral and oropharyngeal cancer in whites and genetic variation in eight metabolic enzymes. *Head Neck J. Sci. Spec. Head Neck* **2008**, *30*, 1139–1147.
57. Gara, S.; Abdennebi, M.; Chatti, S.; Touati, S.; Ladgham, A.; Guemira, F. Association of NAT2 gene substitution mutation T341C with increased risk for head and neck cancer in Tunisia. *Acta Oncol.* **2007**, *46*, 834–837.
58. Marques, C.F.; Koifman, S.; Koifman, R.J.; Boffetta, P.; Brennan, P.; Hatagima, A. Influence of CYP1A1, CYP2E1, GSTM3 and NAT2 genetic polymorphisms in oral cancer susceptibility: Results from a case-control study in Rio de Janeiro. *Oral Oncol.* **2006**, *42*, 632–637.
59. Marques, C.R.; Da Silva, T.M.; De Albuquerque, D.M.; Chaves, M.S.; Marques Filho, M.F.; Oliveira, J.S.; Di Pietro, G.; Sousa, S.M.B.; Simoes, A.L.; Rios-Santos, F. NAT2, XRCC1 and hOGG1 polymorphisms, cigarette smoking, alcohol consumption and risk of upper aerodigestive tract cancer. *Anticancer. Res.* **2014**, *34*, 3217–3224.
60. Olshan, A.F.; Weissler, M.C.; Watson, M.A.; Bell, D.A. GSTM1, GSTT1, GSTP1, CYP1A1, and NAT1 polymorphisms, tobacco use, and the risk of head and neck cancer. *Cancer Epidemiol. Prev. Biomark.* **2000**, *9*, 185–191.
61. Richardson, M.; Garner, P.; Donegan, S. Interpretation of subgroup analyses in systematic reviews: A tutorial. *Clin. Epidemiol. Glob. Health* **2019**, *7*, 192–198.
62. Hickman, D.; Risch, A.; Buckle, V.; Spurr, N.; Jeremiah, S.; McCarthy, A.; Sim, E. Chromosomal localization of human genes for arylamine N-acetyltransferase. *Biochem. J.* **1994**, *297*, 441–445.
63. Moslehi, R.; Chatterjee, N.; Church, T.R.; Chen, J.; Yeager, M.; Weissfeld, J.; Hein, D.W.; Hayes, R.B. Cigarette smoking, N-acetyltransferase genes and the risk of advanced colorectal adenoma. *Future Med.* **2006**, *7*, 819–829.
64. Cascorbi, I.; Roots, I.; Brockmüller, J. Association of NAT1 and NAT2 polymorphisms to urinary bladder cancer: Significantly reduced risk in subjects with NAT1* 10. *Cancer Res.* **2001**, *61*, 5051–5056.
65. Chen, J.; Stampfer, M.J.; Hough, H.L.; Garcia-Closas, M.; Willett, W.C.; Hennekens, C.H.; Kelsey, K.T.; Hunter, D.J. A prospective study of N-acetyltransferase genotype, red meat intake, and risk of colorectal cancer. *Cancer Res.* **1998**, *58*, 3307–3311.
66. Badawi, A.F.; Hirvonen, A.; Bell, D.A.; Lang, N.P.; Kadlubar, F.F. Role of aromatic amine acetyltransferases, NAT1 and NAT2, in carcinogen-DNA adduct formation in the human urinary bladder. *Cancer Res.* **1995**, *55*, 5230–5237.