RESEARCH ARTICLE

Effects of the NQO1 609C>T Polymorphism on Leukemia Susceptibility: Evidence from a Meta-analysis

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Abstract

A functional polymorphism in the NQO1 gene, featuring a 609C>T substitution, leading to proline to serine amino-acid and enzyme activity changes, has been implicated in cancer risk. However, individually published investigations showed inconclusive results, especially for leukemia. In this study, we therefore performed a metaanalysis of 21 publications with a total of 3,634 cases and 4,827 controls, mainly for leukemia. We summarized the data on the association between the NQO1 609C>T polymorphism and risk of leukemia and performed subgroup analyses by ethnicity and leukemia type. We found that the variant TT homozygous genotype o was associated with a modestly increased risk of leukemia (TT versus CT/CC: OR = 1.23, 95% CI = 1.00 - 1.51, heterogeneity = 0.76; $I^2 = 0\%$). Following further stratified analyses, increased risk was only observed in subgroups of Caucasians. This meta-analysis suggests that the NQO1 609T allele is a high-penetrance risk factor for leukemia in Caucasians. The effect on leukemia may be modified by ethnicity and leukemia type, and the small sample sizes of the subgroup analyses suggest that further larger studies are needed.

Keywords: NQO1 609C>T polymorphism - leukemia - meta-analysis

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Introduction

NQO1 (NAD(P)H dehydrogenase, quinone 1) is a member of the NAD(P)H dehydrogenase family and encodes a cytoplasmic 2-electron reductase. This FADbinding protein forms homodimers and reduces quinones to hydroquinones (Snyder et al., 1996; North et al., 2011). In organism, NQO1 functions as a gatekeeper of the 20S proteasomes (Asher et al., 2005), it binds to a subset of short-lived proteins (such as p53, p73 and ornithine decarboxilase) and protects them from 20S proteasomal degradation. The NQO1 609C>T polymorphism is characterized by a single proline-to-serine amino acid substitution, that decreases the half time of NQO1 from 18 h (wild-type) to only 1.2 h via ubiquitination and proteasome pathways. Moreover, other research demonstrated that cell lines and tissues genotyped as homozygous for the NQO1 609C>T polymorphism are deficient in NQO1 activity (Siegel et al., 2001).

NQO1 protein prevents one electron reduction of quinones that results in the production of radical species. Mutations in this gene have been associated with tardive dyskinesia (TD) (Pae et al., 2004; Pae, 2008), an increased risk of hematotoxicity after exposure to benzene (Iskander et al., 2005; Ross, 2005), increased risk of childhood asthma (David et al., 2003; Li et al., 2009), susceptibility to various forms of cancer (Sameer et al., 2010; Pandith et

al., 2011; Goode et al., 2013; Malik et al., 2013; Yang et al., 2013) and Alzheimer's disease (AD) (SantaCruz et al., 2004; Bian et al., 2008). Previous researches have revealed the association between NQO1 609C>T polymorphism and leukemia susceptibility. However, the results were conflicting, including an increased risk (Wiemels et al., 1999; Smith et al., 2001; Lanciotti et al., 2005; Yamaguti et al., 2009; Yamaguti et al., 2010; Yamaguti et al., 2010; Yamaguti et al., 2010; Yeoh et al., 2010), and no association (Seedhouse et al., 2002; Kracht et al., 2004; Wu et al., 2004; Zhang, 2005; Clavel et al., 2005; Eguchi-Ishimae et al., 2007; Bolufer et al., 2007; Boso et al., 2009; Chan et al., 2011; Lozic et al., 2011).

The aim of this article is to review and evaluate the association between NQO1609C>T polymorphism and leukemia risk, mainly focusing on different ethnicity types.

Materials and Methods

Identification and eligibility of relevant studies

To identify all published articles that examined the association between NQO1 609C>T polymorphism and leukemia risk, we conducted a search in the PubMed database (before 2012-12-15). We identified 90 articles

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Table 1. Characteristics of Studies on NQO1 609 C>T Polymorphism and Leukemia Risk

Year	Leukemia type	Age	First author	Ethnity	National		Cases	8			Contro	ls		$\mathrm{HW}\left(p\right)$
					-	C/C	C/T	T/T	Total	C/C	C/T	T/T	Total	-
2010	ALL	Children	Allen Eng-Juh Yeoh	Asian	Singapore		76	33	209	109	146	45	300	0.732382
2009	CLL		Asher Begleiter	Caucasian	Canada	219	93	11	312	196	96	7	299	0.229799
2011	Leukemia	Children	Bernarda Lozi	Caucasian	Croatia	14	10	0	24	30	8	0	38	0.468314
2002	Therapy-related AML		Claire Seedhouse	Caucasian	England	95	30	9	134	110	53	12	175	0.119939
2006	AML		Elad Malik	Mixed	Israel	96	55	5	196	274	137	7	418	0.368066
				Mixed		56	44	6	106	146	104	20	170	0.803388
2010	ALL		Gabriela G. Yamaguti	Caucasian	Brazil	51	47	1	99	73	26	0	99	0.132568
2009	AML		Gabriela G. Yamaguti	Mixed	Brazil	78	54	1	133	95	38	0	187	0.054594
2005	ALL	Children	Jacqueline Clavel	Caucasian	France	122	59	10	191	68	33	3	104	0.67273 10
	ANLL	Children				20	8	0	28	68	33	3	104	0.67273
						142	67	10	219	68	33	3	104	0.67273
011	ALL	Children	Jason Yong-Sheng Chan	Asian	Java	68	92	25	185	75	88	22	185	0.620394
999	Leukemia	Pediatric	Joseph L	Caucasian	England	68	61	6	136	67	32	1	100	0.1804167
005	ALL	Children	M Lanciotti	Caucasian	Italy	23	C/T+	FT/T 27	50	91	C/T+T/	T 56/	147	
007	AML		M. T. Voso	Caucasian	Italy	101	48	8	157	108	40	7	155	0.199683
002	ALL	Children	Maja Krajinovic	Asian	Singapore	100	76	33	209	109	146	45	300	0.732382
001	Acute leukemia	Adults	Martyn T. Smith	Caucasian	England	2850	C/T+T/T		490	562C	/T+T/T		836	_
							205				274			5
005	ALL		Minenori Eguchi-Ishimae	Asian	Japan	29	30	13	72	88	84	25	196	0.482312
	AML					10	19	2	31	88	84	25	197	0.482312
	Total					39	49	15	103					
007	CML		OlgaA. Gra	Caucasian	Russia	52	28	3	83	119	52	6	177	0.913237
2007	AML		Pascual Bolufer	Caucasian	Spain	163	94	16	273	268	160	19	447	0.422155 2
	ALL					65	41	14	120	268	160	19	447	0.422155
	Total					227	135	30	393	268	160	19	447	0.422155
004	ALL	Pediatric	Thorben Kracht	Caucasian	Germany	110	46	4	160	126	61	3	190	0.146514
010	ALL	Children	Vanessa da Silva Silveira	Mixed	Brazil	123	65	16	204	182	156	24	362	0.218281
2007	Therapy-related AML/MS		VM Guillem	Caucasian	Spain	49	30	2	81	41	34	5	80	0.55599
2004	Leukemia	Children	Wu YX	Asian	China	12	32	17	61	22	30	11	63	0.888452
2005	Acute leukemia	Adult	Zhang Juan	Asian	China	33	C/T+T	Г/Т 66	99	44	C/T+	T/T 55	5 99	

Relevant studies identified and screened (Pubmed: n=93)

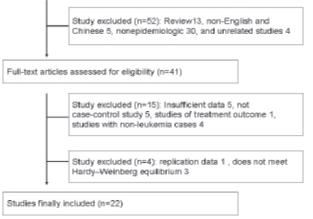


Figure 1. Flow Diagram of the Study Selection Process

with the search terms ("NAD(P)H Dehydrogenase (Quinone)" [MeSH] or "NQO1") and "leukemia" and limiting the search to studies in human populations. Articles with the following characteristics were excluded from the review: 1) non-English articles; 2) review articles; 3) non-epidemiological studies (e.g., studies on animals or cell culture); 4) treatment outcome studies (Figure 1); 5) studies with control that did not meet Hardy-Weinberg equilibrium (HWE). As of December 15, 2012, we had identified 22 published studies describing the association between NQO1 polymorphisms and leukemia included case-control analyses.

Data extraction and assessment of study quality Two authors (Fei-fei Han and Chang-long Guo)

extracted data and reached a consensus on all of the eligibility items, including author, journal and year of publication, location of study, selection and characteristics of cancer cases and controls, control source, age grades of patients, ethnicity, and leukemia types.

Meta-analysis

The risks (odds ratios, OR) of cancer associated with NQO1 609C>T polymorphism were estimated for each study independently. Also we estimated the risk for the NQO1 609C>T polymorphism and breast cancer, colorectal cancer separately.

Statistical analysis

The meta-analysis was performed in a fixed/random effect model. The OR and its 95% CI were estimated for each study. The chi-squared test-based Q-statistic was used to assess the between-study heterogeneity. Heterogeneity was significant for P < 0.10, and then the result of random effect model was selected. Otherwise, the result of fixed effect model was selected. Meanwhile, we measured the effect of heterogeneity by another measure, $I^2 = 100\% \times (Q-df)/Q$. The I²-statistic measures the degree of inconsistency in the studies by calculating what percentage of the total variation across studies is as a result of heterogeneity rather than by chance.

The effect of association was indicated as OR with the corresponding 95% confidence interval (CI). The combined OR was estimated using fixed effects (FE) models (Mantel-Haenszel) and random-effects (RE) models (DerSimonian and Laird) (Sarlauskas et al., 2004). We did the Q test to assess the heterogeneity between these studies, and it was considered statistically significant 31.3



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Ej	ffects of the NQO1 609C>T	Polymorphism on Leuke	mia Susceptibility: E	Evidence from a Meta-analysis
Table 2. Meta-Analysis	of the Risk of Leukemi	a for NOO1 609 C>T	Polymorphism	

Genotype	Populations		OR	$I^{2}(\%)$	$P_{\text{heterogeneity}}$	Р	Model
Ser/Ser + Pro/Ser	All populations		1.21 (1.02, 1.44)	69	< 0.00001	0.03	random
versus Pro/Pro		Asian	1.19 (0.87, 1.62)	72	0.006	0.41	random
		Caucasian	1.22 (1.00, 1.49)	59	0.003	0.05	random
	AML populations		0.96 (0.64, 1.44)	83	< 0.00001	0.85	random
		Caucasian	0.94 (0.76, 1.16)	30	0.23	0.57	fixed
	ALL populations		1.08 (0.81, 1.45)	69	0.0007	0.58	random
		Asian	0.93 (0.59, 1.46)	69	0.04	0.75	random
		Caucasian	1.36 (0.9, 2.06)	63	0.03	0.15	random
	Children		1.13 (0.83, 1.55)	71	0.0006	0.44	random
		Asian	1.01 (0.57, 1.79)	75	0.02	0.98	random
		Caucasian	1.41 (0.95, 2.09)	55	0.07	0.09	random
Ser/Ser versus	All populations		1.25 (1.02, 1.53)	0	0.94	0.03	fixed
Pro/Ser + Pro/Pro		Asian	1.19 (0.87, 1.62)	0	0.76	0.27	fixed
		Caucasian	1.44 (1.02, 2.01)	0	0.77	0.04	fixed
	AML populations		0.93 (0.64, 1.33)	4	0.4	0.68	fixed
		Caucasian	1.09 (0.69, 1.72)	0	0.55	0.72	fixed
	ALL populations		1.29 (0.99, 1.67)	0	0.47	0.06	fixed
		Asian	1.17 (0.84, 1.65)	0	0.73	0.36	fixed
		Caucasian	2.35 (1.27, 4.37)	0	0.64	0.007	fixed
	Children		1.28 (0.96, 1.7)	0	0.82	0.1	fixed
		Asian	1.20 (0.85, 1.69)	0	0.56	0.31	fixed
		Caucasian	2.03 (0.84, 4.93)	0	0.68	0.12	fixed

	Case		Contr			Odds Ratio	Odds Ratio
Study or Subgroup			Events			M-H, Random, 95% C	M-H, Random, 95% CI
Allen Eng-Juh Yeoh2010	109	209	191	300	5.6%	0.62 [0.43, 0.89]	-
Asher Begleiter2009	104	312	103	299	5.8%	0.95 [0.68, 1.33]	T
Bernarda Lozi2011	10	24	8	38	1.8%	2.68 [0.87, 8.25]	
Claire Seedhouse2002	39	134	65	175	4.7%	0.69 [0.43, 1.13]	-
Elad Malik2006-a	110	262	268	688	6.1%	1.13 [0.85, 1.51]	T
Gabriela G. Yamaguti2009	55	133	38	187	4.6%	2.76 [1.68, 4.54]	
Gabriela G. Yamaguti2010	48	99	26	99	3.9%	2.64 [1.46, 4.80]	
Jacqueline Clavel2005	77	219	36	104	4.6%	1.02 [0.63, 1.67]	-
Jason Yong-Sheng Chan2011	117	185	110	185	5.1%	1.17 [0.77, 1.78]	<u> </u>
Joseph L1999	67	136	33	100	4.3%	1.97 [1.15, 3.37]	
M Lanciotti 2005	27	50	56	147	3.6%	1.91 [1.00, 3.65]	
M. T. Voso2007	56	157	47	155	4.8%	1.27 [0.79, 2.05]	T
Martyn T. Smith2001	205	490	274	836	6.5%	1.48 [1.17, 1.86]	-
Minenori Eguchi-Ishimae20	64	103	109	196	4.7%	1.31 [0.80, 2.13]	
OlgaA. Gra2007	31	83	58	177	4.3%	1.22 [0.71, 2.11]	-
Pascual Bolufer2007	165	393	179	447	6.2%	1.08 [0.82, 1.43]	Ť
Slah Ouerhani 2012	98	106	91	100	2.1%	1.21 [0.45, 3.27]	
THORBEN KRACHT2004	50	160	64	190	4.9%	0.89 [0.57, 1.40]	
Vanessa da Silva 2010	81	204	180	362	5.7%	0.67 [0.47, 0.94]	-
VM Guillem2007	32	81	39	80	3.8%	0.69 [0.37, 1.28]	
Nu YX2004	49	61	41	63	2.8%	2.19 [0.97, 4.96]	
Zhang Juan2005	66	99	55	99	4.1%	1.60 [0.90, 2.85]	
Fotal (95% CI)		3700			100.0%		
Total (95% CI) Total events	1660	3700	2071	5027	100.0%	1.21 [1.02, 1.44]	*
		df = 21	(P < 0.00	001); l²	= 69%		0.01 0.1 1 10 Protective factor Risk factor
	P = 0.03)				= 69%	Odda Batia	Protective factor Risk factor
Heterogeneity: Tau ² = 0.11; Chi ² Test for overall effect: Z = 2.16 (P = 0.03) Cas	e	Con	trol		Odds Ratio	Protective factor Risk factor Odds Ratio
Test for overall effect: Z = 2.16 (Study or Subgroup	P = 0.03) Cas Events	e Total	Con Events	trol s Tota	I Weight	M-H, Fixed, 95% CI	Protective factor Risk factor
Test for overall effect: Z = 2.16 (Study or Subgroup Allen Eng-Juh Yeoh2010	P = 0.03) Cas <u>Events</u> 33	e Total 209	Con Event	trol <u>s Tota</u> 5 300	<u>I Weight</u>) 18.5%	M-H, Fixed, 95% Cl 1.06 [0.65, 1.73]	Protective factor Risk factor Odds Ratio
Test for overall effect: Z = 2.16 (<u>Study or Subgroup</u> Allen Eng-Juh Yeoh2010 Asher Begleiter2009	P = 0.03) Cas Events 33 11	e <u>Tota</u> 209 312	Con Events	trol <u>s Tota</u> 5 300 7 299	<u>I Weight</u> 18.5% 4.1%	M-H, Fixed, 95% CI 1.06 [0.65, 1.73] 1.52 [0.58, 3.99]	Protective factor Risk factor Odds Ratio
Test for overall effect: Z = 2.16 (<u>Study or Subgroup</u> Allen Eng-Juh Yeoh2010 Asher Begleiter2009 Bernarda Lozi2011	P = 0.03) Cas Events 33 11 0	e <u>Total</u> 209 312 24	Con Events	trol <u>s Tota</u> 5 300 7 299 0 38	I Weight 0 18.5% 9 4.1%	M-H, Fixed, 95% CI 1.06 [0.65, 1.73] 1.52 [0.58, 3.99] Not estimable	Protective factor Risk factor Odds Ratio
Test for overall effect: Z = 2.16 (<u>Study or Subgroup</u> Allen EngJuh Yeoh2010 Asher Begleiter2009 Bernarda Lozi2011 Claire Seedhouse2002	P = 0.03) Cas Events 33 11 0 9	e Total 209 312 24 134	Con Event: 45	trol 5 Tota 5 300 7 299 0 38 2 175	<u>I Weight</u> 0 18.5% 9 4.1% 5 5.8%	M-H, Fixed, 95% CI 1.06 [0.65, 1.73] 1.52 [0.58, 3.99] Not estimable 0.98 [0.40, 2.39]	Protective factor Risk factor Odds Ratio
Test for overall effect: Z = 2.16 (<u>Study or Subgroup</u> Alen Eng-Juh Yeoh2010 Sher Begleite/2009 Bernarda Lo22011 Laire Seedhouse2002 Ead Malik2006-a	P = 0.03) Cas Events 33 11 0 9 11	e Total 209 312 24 134 262	Con Event: 45	trol <u>5 Tota</u> 5 300 7 299 0 38 2 175 7 688	I Weight 0 18.5% 9 4.1% 8 5.8% 8 5.8%	M-H, Fixed, 95% CI 1.06 [0.65, 1.73] 1.52 [0.58, 3.99] Not estimable 0.98 [0.40, 2.39] 1.07 [0.52, 2.20]	Protective factor Risk factor Odds Ratio
Test for overall effect: Z = 2.16 (<u>Study or Subgroup</u> Allen Eng-Juh Yech2010 Asher Begleiter2009 Bernarda Lozi2011 Jairo Seedhouse2002 Elad Malik2006-a Sabriela G. Yamaguti2009	P = 0.03) Cas Events 33 11 0 9 11	e Total 209 312 24 134 262 133	Con Event: 45 12 27	trol <u>5</u> 300 7 299 0 38 2 175 7 688 0 187	<u>I Weight</u> 0 18.5% 9 4.1% 3 5.8% 3 8.5% 7 0.2%	M-H, Fixed, 95% CI 1.06 [0.65, 1.73] 1.52 [0.58, 3.99] Not estimable 0.98 [0.40, 2.39] 1.07 [0.52, 2.20] 4.25 [0.17, 105.02]	Protective factor Risk factor Odds Ratio
Test for overall effect: Z = 2.16 (<u>Study or Subgroup</u> Nen Eng-Juh Yeoh2010 Sher Bogleite/2009 Bernarda Lo220110 Laire Seedhouse2002 Laid Maik2006-ja 2006-ja Sabriela G. Yamaguit2010	P = 0.03) Cas Events 33 11 0 9 11 1 1	e 209 312 24 134 262 133 99	Con Events 45 12 12 27 ()	trol <u>5 Tota</u> 5 300 7 299 0 38 2 175 7 688 0 187 0 99	Weight 18.5% 4.1% 5 5 8 7 0.2% 9	M-H, Fixed, 95% CI 1.06 [0.65, 1.73] 1.52 [0.58, 3.99] Not estimable 0.98 [0.40, 2.39] 1.07 [0.52, 2.20] 4.25 [0.17, 105.02] 3.03 [0.12, 75.30]	Protective factor Risk factor Odds Ratio
Test for overall effect: Z = 2.16 (<u>Study or Subgroup</u> Alien Eng-Juh Yeoh2010 Asher Begleier2009 Bernards Lozi2011 Zahrida Lozi2012 Zahrida G. Yamaguti2009 Sabriela G. Yamaguti2010 Jacqueline Clavel2005	P = 0.03) Cass Events 33 11 0 9 11 1 1 1 1 1	e 209 312 24 134 262 133 99 219	Con Events 45 12 21 (((trol <u>5 Tota</u> 5 300 7 299 0 38 2 175 7 688 0 187 0 99 3 104	Weight 18.5% 4.1% 5 5 8 7 0.2% 9 3 2	M-H, Fixed, 95% CI 1.06 [0.65, 1.73] 1.52 [0.58, 3.99] Not estimable 0.98 [0.40, 2.39] 1.07 [0.52, 2.20] 4.25 [0.17, 105.02] 3.03 [0.12, 75.30] 1.61 [0.43, 5.98]	Protective factor Risk factor Odds Ratio
Fest for overail effect: Z = 2.16 (<u>Btudy or Subgroup</u> Men Eng-Juh Yeoh2010 Sahre Bogleiez009 Bernarda Lo22021 Den Maik2000 Sahriala G. Yamagut2000 Sahriala G. Yamagut2000 Jacruelan Clavel2005 Jacovalene Clavel2005 Jason Yong-Shong Chan2011	P = 0.03) Cas Events 111 0 9 111 1 1 10 25	e 209 312 24 134 262 133 99 219 185	Con Events 12 12 27 12 27 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	trol <u>5 Tota</u> 5 300 7 299 0 38 2 175 7 688 0 187 0 99 3 104 2 185	Weight 18.5% 4.1% 5 5 8 5 7 0.2% 4.2.3% 5 11.3%	M-H, Fixed, 95% Cl 1.06 [0.65, 1.73] 1.52 [0.58, 3.99] Not estimable 0.98 [0.40, 2.39] 1.07 [0.52, 2.20] 4.25 [0.17, 105.02] 3.03 [0.12, 75.30] 1.61 [0.43, 5.98] 1.16 [0.63, 2.14]	Protective factor Risk factor Odds Ratio
Test for overall effect: Z = 2.16 (Study or Subgroup Mien Eng-Juh Yeoh2010 Asher Begeleier2009 Benards Lozi2011 Barnids Lozi2011 Barbiela G. Yamagut2009 Babriela G. Yamagut2010 Jacquelien Clavel2005 Jason Jong-Shong Chan2011 Joseph L1999	P = 0.03) Cas Events 33 11 0 9 11 1 1 1 10 255 6	e 209 312 24 134 262 133 99 219 185 136	Con Events 12 12 27 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	trol <u>5 Tota</u> 5 300 7 299 0 38 2 175 2 175 0 185 0 99 3 104 2 185 1 100	Weight 18.5% 4.1% 5 5.8% 8.5% 0.2% 0.3% 11.3% 0.7%	M-H, Fixed, 95% Cl 1.06 [0.65, 1.73] 1.52 [0.58, 3.99] Not estimable 0.98 [0.40, 2.39] 1.07 [0.52, 2.20] 4.25 [0.17, 105.02] 3.03 [0.12, 75.30] 1.61 [0.43, 5.98] 1.16 [0.63, 3.54]	Protective factor Risk factor Odds Ratio
Test for overall effect: Z = 2.16 (<u>Bitudy or Subgroup</u> <u>Ulen Engular School</u> <u>Bitudy or Subgroup</u> <u>Bitudy or Subgroup</u> <u>Bitudy or Subgroup</u> <u>Bitudy or Subgroup</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitudy</u> <u>Bitud</u>	P = 0.03) Cas Events 33 11 0 9 11 1 1 1 10 25 6 8	e Total 209 312 24 134 262 133 99 219 185 136 157	Con Events 45 12 27 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	trol <u>s Tota</u> 5 300 7 299 0 38 2 175 2 175 0 185 0 99 3 104 2 185 1 100 7 155	Weight 18.5% 4.1% 5 5 6 7 0.3% 2.3% 5 1.1.3% 0.7% 4.0%	<u>M-H, Fixed, 95% C1</u> 1.06 [0.65, 1.73] 1.52 [0.58, 3.99] Not estimable 0.98 [0.40, 2.30] 1.07 [0.52, 2.20] 4.25 [0.17, 105,02] 3.03 [0.12, 75,30] 1.61 [0.43, 5.98] 1.16 [0.53, 2.14] 4.57 [0.54, 38.57] 1.14 [0.40, 3.21]	Protective factor Risk factor Odds Ratio
Test for overail effect: Z = 2.16 (<u>Bluck or Subprove</u> Allen Eng-Juh Yeo:2010 Alare Eng-Juh Yeo:2010 Calero Sendhouse2002 Esabriela C, Yamagui2009 Sabriela C, Yamagui2009 Sabriela C, Yamagui2010 Jacquillen Clavel/2008 Jacquillen Clavel/2008 M. T, Yoso2007 Mirrenori Bguchi-Hshimae20	P = 0.03) Cas Events 33 11 0 9 11 1 1 10 25 6 8 8 15	e Total 209 312 24 134 262 133 99 219 185 136 157 103	Con Events 45 12 0 0 0 0 0 0 0 0 0 0 0 0 0	trol <u>Tota</u> <u>5</u> 300 7 295 3 104 2 175 7 688 0 187 0 95 3 104 2 185 1 100 7 155 5 196	I Weight 18.5% 18.5% 5 5.8% 5 5.8% 6 0.3% 9 0.3% 9 0.3% 9 0.3% 9 0.3% 9 0.3% 9 0.3% 5 11.3% 0 0.7% 5 4.0% 5 8.7%	<u>M-H, Fixed, 95% Cl</u> 1.06 [0.65, 1.73] 1.52 [0.58, 3.99] Not estimable 0.98 [0.40, 2.39] 1.07 [0.52, 2.20] 4.25 [0.17, 105.02] 3.03 [0.12, 75.30] 1.61 [0.63, 2.14] 4.57 [0.54, 38.67] 1.14 [0.63, 2.14] 4.57 [0.54, 2.32]	Protective factor Risk factor Odds Ratio
Test for overail effect: Z = 2.16 (Btudy or Subaroup Mon Eng-Juh Yooh2010 Maher Begleiter2009 Bernarda Loz2010 Janie Skendhouse2001 Zahriela G, Yamagui2009 Zahriela G, Yamagui2009 Zahriela G, Yamagui2009 Zahriela G, Yamagui2009 Minedon G Buuchi-Bhimae20 Minedon G Buuchi-Bhimae20	P = 0.03) Cas Events 33 11 0 9 11 1 1 1 1 10 25 6 8 8 5 3	e Total 209 312 24 134 262 133 99 219 185 156 157 103 83	Con Events 45 12 12 27 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	trol <u>Tota</u> <u>5</u> 300 7 299 0 38 2 175 7 688 0 185 0 185 100 7 155 5 196 5 196 5 197	Weight 18.5% 4.1% 5 5 6 7 0.2% 0.3% 2.3% 11.3% 0.7% 4.0% 5 8.7%	M-H. Fixed, 95% CI 1.06 [0.65, 1.73] 1.52 [0.58, 3.99] Not estimable 0.98 [0.40, 2.39] 1.07 [0.52, 2.20] 1.03 [0.12, 75.30] 1.61 [0.43, 5.98] 1.16 [0.63, 2.14] 1.16 [0.43, 5.98] 1.16 [0.63, 2.14] 1.17 [0.56, 2.32] 1.17 [0.56, 2.52]	Protective factor Risk factor Odds Ratio
Test for overail effect: Z = 2.16 (Black or Subprose Alem Ergs Juhn Yosh2010 Alem Ergs Juhn Yosh2010 Alem Ergs Juhn Yosh2010 Bernarda Loz2021 Claire Seenhouse2020 Eabriela C, Yamagut2010 Jacqueline Clavel2005 Jacon Yong S-Borg Chan2011 M. T. Vos2007 Mirrenori Eguch-Hshimac20 DigaA, Cira2007 Pascual Boldref2007	P = 0.03) Cass Events 33 111 0 9 11 1 1 1 1 1 1 1 25 6 8 8 15 3 30	e 209 312 24 134 262 133 99 219 185 136 157 106 157 333 393	Con Events 48 12 12 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	trol 5 Tota 5 300 7 299 0 38 2 179 2 179 3 104 2 185 1 100 2 185 5 196 3 177 9 447 9 447	Weight 18.5% 4.1% 5 5 8 9 0.3% 2.3% 11.3% 0.7% 4.0% 8.5% 7 2.3% 11.3% 0.3% 2.3% 4.0% 5 7 9.8%	<u>M-H, Fixed, 95% C1</u> 1.06 [0.65, 1.73] 1.52 [0.58, 3.99] Not estimable 0.98 [0.40, 2.39] 1.07 [0.52, 2.20] 4.25 [0.17, 105.02] 3.03 [0.12, 75.30] 1.61 [0.63, 2.14] 4.57 [0.54, 38.57] 1.14 [0.40, 3.211] 1.17 [0.58, 2.32] 1.07 [0.26, 4.38] 1.88 [1.03, 3.38]	Protective factor Risk factor Odds Ratio
Test for overnil effect: Z = 2.16 (Study or Subaroup Men EngJuh Yeoh2010 Asher Begleher2009 Bernarda Los2011 Clare Seenhouxe2002 Gabriela G, Yamagui2010 Gabriela G, Yamagui2010 Gabriela G, Yamagui2010 M, T. Vanos2007 M, Sanos2007 M, Sanos2007 M, Sanos2007 M, Sanos2007 M, Sanos2007 M, Sanos2007 Sanos2007 M, Sanos2007 Sanos20	P = 0.03) Cass Events 3 33 111 0 9 111 1 10 25 6 8 8 15 3 30 8	e Total 209 312 24 134 262 133 99 219 185 136 157 103 83 393 106	Con Events 45 12 12 27 12 27 12 27 12 27 12 12 27 12 12 12 12 12 12 12 12 12 12 12 12 12	trol <u>Tota</u> 5 300 7 299 0 38 2 175 7 688 0 185 9 98 104 2 185 1 100 7 155 5 196 3 177 9 445 9 445 9 100	I Weight 18.5% 4.1% 3 5.5.8% 3 8.5% 7 0.2% 4 2.3% 5 11.3% 5 4.0% 5 4.0% 6 8.7% 7 2.2% 9 5.1%	M-H. Fixed, 95% CI 1.06 [0.65, 1.73] 1.52 [0.58, 3.99] Not estimable 0.98 [0.40, 2.39] 1.07 [0.52, 2.20] 1.03 [0.12, 75.30] 1.61 [0.43, 5.98] 1.16 [0.63, 2.14] 1.67 [0.43, 5.98] 1.16 [0.63, 2.14] 1.67 [0.43, 5.98] 1.68 [0.43, 5.98] 1.68 [0.43, 5.98] 1.68 [0.43, 5.98] 1.68 [0.43, 5.98] 1.68 [0.43, 5.98] 0.68 [0.43, 5.98] 1.68 [0.43, 5.98] 0.68 [0.43, 5.98] 0.68 [0.43, 5.98] 0.68 [0.43, 5.98] 0.68 [0.43, 5.98] 0.68 [0.43, 5.98] 0.68 [0.43, 5.98] 0.68 [0.43, 3.86] 0.68 [0.43, 3.36] 0.88 [0.43, 3.36] 0.88 [0.43, 2.23] 0.88 [0.41, 2.23]	Protective factor Risk factor Odds Ratio
Test for overall effect: Z = 2.16 (Blady or Subprose Min Eng. Jun Yook2010 Min Eng. Jun Yook2010 Min Eng. Jun Yook2011 Dares Bendhouxe2001 Laires Bendhouxe2002 Bud Malk2006 Jachela C. Yamagut2010 Jacqueline Clavel2005 Jachela C. Yamagut2010 Jacqueline Clavel2005 Jacov Hondro Eurol Char2011 Minerol Eguch-Inimae20 JapaA Eng2007 Salcoul Boldref2007 Siln Ouerhani 2012 HOREBK IRACHT2004	P = 0.03) Cas Events 333 111 0 9 111 1 1 1 1 1 1 1 0 25 6 8 8 5 3 3 0 8 8 4 4	e Total 209 312 24 133 99 219 135 136 157 103 83 393 106 160	Con Events 14 12 22 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	trol <u>5 Tota</u> 5 300 7 299 0 38 2 175 7 688 0 187 0 99 3 104 2 185 1 100 7 155 5 196 5	Weight 18.5% 4.1% 5 5 6 7 9 7 9.8% 0 7 9.8% 0 7 9.8% 0 1.6%	$\begin{array}{c} \underline{M-H}, \underline{Fixed}, \underline{95\%}, \underline{C1}\\ 1, 06 (0, 65, 1.73)\\ 1, 52 (0, 58, 3.99)\\ Not estimable\\ 0, 80 (1, 0, 2, 30)\\ 1, 07 (0, 52, 2.20)\\ 4.25 (0, 17, 105, 02)\\ 3.03 (0, 12, 75, 30)\\ 1, 61 (0, 43, 5.98)\\ 1, 16 (0, 63, 2, 14)\\ 1, 57 (0, 54, 3.6, 57)\\ 1, 14 (0, 40, 3, 21)\\ 1, 17 (0, 58, 2.32)\\ 1, 07 (0, 26, 4, 38)\\ 0, 83 (0, 31, 2, 23)\\ 1, 60 (0, 55, 7.25)\\ \end{array}$	Protective factor Risk factor Odds Ratio
Test for overail effect: Z = 2.16 (<u>Study of Subaroup</u> Allen EngJuh Yeob2010 Aher Begleiter2009 Bernarda Loz2011 Claire Sendhouse2002 Elab Mall2006 Sabriela C, Yamagut2009 Jacon Yong-Sheng Chan2011 Joseph L1999 M. T. Voss2007 Mercol Eguch-Hahmae20 DigaA. Craz007 DigaA. Craz007 DigaA. Craz007 DigaA. Craz007 DigaA. Craz007 DigaA. Craz007 THOREBK NRRACH72004 Woressd ad Silva 2010	P = 0.03) Cass Events 33 11 0 9 11 1 1 10 25 6 6 8 15 30 30 8 4 16 15 15 15 15 15 15 15 15 15 15	e Total 209 312 24 133 99 219 185 1366 1577 103 83 393 106 160 204	Con Events 1 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	trol <u>5</u> Tota <u>5</u> 300 <u>7</u> 299 <u>9</u> 382 <u>7</u> 688 <u>9</u> 185 <u>9</u> 485 <u>9</u> 445 <u>9</u> 100 <u>8</u> 100 <u>9</u> 445 <u>9</u> 100 <u>8</u> 1000 <u>8</u> 10000 1000 1000 10000 10000 10000 10000 10000	Weight 18.5% 4.1% 5 5 6 7 0.2% 0.3% 5 11.3% 0.7% 5 4.0% 8.7% 2.2% 9.8% 5.1% 2.51% 2.9.5%	M-H, Fixed, 95% CI 1.06 (0.65, 1.73) 1.52 (0.58, 3.99) Not estimable 0.88 (0.40, 2.39) 1.07 (0.52, 2.20) 4.25 (0.17, 105 0.2) 3.03 (0.12, 75.30) 1.61 (0.43, 5.98) 1.16 (0.63, 2.14) 4.57 (0.54, 38, 57) 1.14 (0.40, 3.21) 1.17 (0.56, 4.38) 1.86 (1.03, 3.36) 0.83 (0.31, 2.23) 1.60 (0.35, 7.25) 1.20 (0.62, 2.31)	Protective factor Risk factor Odds Ratio
Test for overail effect: Z = 2.16 (Btudy or Subaroup Mon Eng-Juh Yooh2010 Maher Begleiter2009 Bernarda Lua2011 2014 (State 2006) 2014 (Stat	P = 0.03) Cas Events 333 111 0 9 111 1 1 1 1 1 1 1 0 25 6 8 8 5 3 3 0 8 8 4 4	e Total 209 312 24 133 99 219 185 1366 1577 103 83 393 106 160 204	Con Events 45 12 27 12 27 12 27 12 27 12 27 12 12 27 12 12 27 12 12 27 12 12 12 12 12 12 12 12 12 12 12 12 12	trol <u> Tota</u> 30(7 29% 29% 217% 68% 100 16% 100 15% 3 100 218% 100 15% 3 100 3 1	I Weight 18.5% 4.1% 5 5.8% 6 5.8% 7 0.2% 9 0.3% 5 11.3% 6 8.7% 7 2.2% 7 2.8% 0 1.6% 2 9.8% 0 2.95%	$\begin{array}{c} \underline{M-H}, \underline{Fixed}, \underline{95\%}, \underline{C1}\\ 1, 06 (0, 65, 1.73)\\ 1, 52 (0, 58, 3.99)\\ Not estimable\\ 0, 80 (1, 0, 2, 30)\\ 1, 07 (0, 52, 2.20)\\ 4.25 (0, 17, 105, 02)\\ 3.03 (0, 12, 75, 30)\\ 1, 61 (0, 43, 5.98)\\ 1, 16 (0, 63, 2, 14)\\ 1, 57 (0, 54, 3.6, 57)\\ 1, 14 (0, 40, 3, 21)\\ 1, 17 (0, 58, 2.32)\\ 1, 07 (0, 26, 4, 38)\\ 0, 83 (0, 31, 2, 23)\\ 1, 60 (0, 55, 7.25)\\ \end{array}$	Protective factor Risk factor Odds Ratio
Test for overail effect: Z = 2.16 (Buildr or Subaroup Men Eng-Juh Yeoh2010 Maher Begleiter2009 Bernarda Loz2012 Jaine Seehouse2002 Jaine Seehouse2002 Sabriela G. Yamagut2009 Sabriela G. Yamagut2009 Sabriela G. Yamagut2009 Sabriela G. Yamagut2009 Japan Gra2007 Japan Gra2007 Japan Gra2007 Japan Gra2007 Japan Gra2012 Japan Gra2012 Jap	P = 0.03) Cas Events 33 11 0 9 11 1 1 1 1 1 1 25 6 8 3 30 8 4 16 2 2 16 16 16 16 16 16 16 16 16 16	e <u>Total</u> 209 3122 244 262 133 99 219 185 136 157 103 83 393 106 160 204 81 83 83 83 83 83 83 83 83 83 83	Con Events 45 12 27 12 27 12 27 12 27 12 27 12 12 27 12 12 27 12 12 27 12 12 12 12 12 12 12 12 12 12 12 12 12	trol Tota Tota Tota Tota Tota Tota Tota Tot	I Weight 18.5% 4.1% 5 5.8% 6 5.8% 7 0.2% 9 0.3% 4 2.3% 5 11.3% 6 8.7% 7 2.2% 9 9.8% 0 5.1% 1 1.6% 2 9.5%	$\begin{array}{c} \underline{M} \textbf{H}, \underline{Freed}, \underline{g} \\ \underline{S} \\ 1, 06 \\ (0.66, 1.73) \\ 1.52 \\ (0.53, 2.60) \\ Not estimable \\ 0.98 \\ (0.40, 2.39) \\ 1.07 \\ (0.52, 2.20) \\ 4.25 \\ (0.47, 150, 22) \\ 1.07 \\ (0.52, 2.20) \\ 1.07 \\ (0.52, 2.20) \\ 1.07 \\ (0.52, 2.20) \\ 1.07 \\ (0.52, 2.20) \\ 1.07 \\ (0.52, 2.20) \\ 1.07 \\ (0.53, 2.14) \\ 1.17 \\ (0.54, 2.15) \\ 1.16 \\ (0.53, 2.14) \\ 1.16 \\ (0.53, 2.14) \\ 1.16 \\ (0.53, 2.14) \\ 1.16 \\ (0.53, 2.14) \\ 1.16 \\ (0.53, 2.14) \\ 1.16 \\ (0.53, 2.14) \\ 1.16 \\ (0.53, 2.14) \\ 1.16 \\ (0.53, 2.15) \\ 1.16 \\ (0.53, 2.15) \\ 1.16 \\ (0.53, 2.15) \\ 1.00 \\ (0.53, 2.25) \\ 1.20 \\ (0.62, 2.31) \\ 1.20 \\ (0.62, $	Protective factor Risk factor Odds Ratio
	P = 0.03) Cas Events 33 11 0 9 11 1 1 1 1 1 1 25 6 8 3 30 8 4 16 2 2 16 16 16 16 16 16 16 16 16 16	e <u>Total</u> 209 312 24 134 262 213 99 99 219 185 136 157 103 83 393 106 160 204 81 61 3061	Con Events 45 12 27 12 27 12 27 12 27 12 27 12 12 27 12 12 27 12 12 27 12 12 12 12 12 12 12 12 12 12 12 12 12	trol <u> Tota</u> Tota So Tota So	I Weight 18.5% 4.1% 3 5.5.8% 8 5.5.8% 8 8.5% 7 0.2% 9 0.3% 8 2.3% 11.3% 0.7% 9 0.8% 9 0.7% 9 0.8% 9 0.7% 9 0.8% 9 1.6% 9 2.9% 9 2.9% 9 4.6%	$\begin{array}{c} \underline{M} \underline{H}, \underline{Freed}, \underline{556}, \underline{C1}\\ 1.06 (065, 1.73)\\ 1.52 (053, 2.90)\\ Not estimable\\ 0.98 (040, 2.39)\\ 1.07 (052, 2.20)\\ 4.25 (017, 1050,2)\\ 3.03 (012, 73.30)\\ 1.61 (1043, 5.98)\\ 1.16 (1043, 5.98)\\ 1.16 (1043, 5.98)\\ 1.16 (1043, 5.98)\\ 1.16 (1043, 5.98)\\ 1.16 (1043, 5.98)\\ 1.16 (1043, 5.98)\\ 1.16 (1043, 5.98)\\ 1.17 (1058, 2.32)\\ 1.07 (1026, 3.38)\\ 1.08 (103, 3.36)\\ 0.83 (035, 7.25)\\ 1.20 (062, 3.31)\\ 0.38 (007, 2.02) \end{array}$	Protective factor Risk factor Odds Ratio
Test for overail effect: Z = 2.16 (Bluck or Subprose Main Ergs Juh Yeos2010 Asher Begaleter2009 Bernards Loz2020 Estel Mailt2006 Estel Mailt2007 Bin Oberhami 2012 Sin Oberhami 2012 Wit Spikorof Estel Mailt2007 Bin Oberhami 2012 Wit Spikorof Wit Spikorof Wit Spikorof	P = 0.03) Cas Events 33 111 0 9 9 111 1 1 1 1 1 1 1 1 1 1 0 255 6 8 8 3 300 8 8 4 4 16 2 2 17 7 210	e <u>Total</u> 209 312 244 1334 2622 133 99 99 219 1855 1366 167 103 833 393 393 1066 1600 204 81 61 30661	Con Events 48 12 27 0 0 12 27 0 0 12 27 0 0 12 27 0 0 12 12 12 12 12 12 12 12 12 12 12 12 12	trol <u> Tota</u> Tota So Tota So	I Weight 18.5% 4.1% 3 5.5.8% 8 5.5.8% 8 8.5% 7 0.2% 9 0.3% 8 2.3% 11.3% 0.7% 9 0.8% 9 0.7% 9 0.8% 9 0.7% 9 0.8% 9 1.6% 9 2.9% 9 2.9% 9 4.6%	$\begin{array}{c} \underline{M} \textbf{H}, \underline{Freed}, \underline{g} \\ \underline{S} \\ 1, 06 \\ (0.66, 1.73) \\ 1.52 \\ (0.53, 2.60) \\ Not estimable \\ 0.98 \\ (0.40, 2.39) \\ 1.07 \\ (0.52, 2.20) \\ 4.25 \\ (0.47, 150, 22) \\ 1.07 \\ (0.52, 2.20) \\ 1.07 \\ (0.52, 2.20) \\ 1.07 \\ (0.52, 2.20) \\ 1.07 \\ (0.52, 2.20) \\ 1.07 \\ (0.52, 2.20) \\ 1.07 \\ (0.53, 2.14) \\ 1.17 \\ (0.54, 2.15) \\ 1.16 \\ (0.53, 2.14) \\ 1.16 \\ (0.53, 2.14) \\ 1.16 \\ (0.53, 2.14) \\ 1.16 \\ (0.53, 2.14) \\ 1.16 \\ (0.53, 2.14) \\ 1.16 \\ (0.53, 2.14) \\ 1.16 \\ (0.53, 2.14) \\ 1.16 \\ (0.53, 2.15) \\ 1.16 \\ (0.53, 2.15) \\ 1.05 \\ (0.53, 2.15) \\ 1.00 \\ (0.53, 2.25) \\ 1.00 \\ (0.62, 2.31) \\ 1.00 \\ 1.$	Protective factor Risk factor Odds Ratio

Figure 2. Forest Plot of Leukemia Risk Associated with NQO1 609 C>T Polymorphism Analysis

with P < 0.10 (Yuan et al., 2010). The heterogeneity was quantified by I² metric (I² = 100% × (Q-df)/Q), which is independent of the number of studies in the meta-analysis (I² < 25% no heterogeneity; I² = 25–50% moderate heterogeneity; I² > 50% extreme heterogeneity) and Pvalue (P > 0.1 no heterogeneity). Publication bias was investigated by funnel plot and Egger's linear regression test (Egger et al., 1997). The significant of asymmetry was determined by t test and P < 0.05 was considered as a significant publication bias. Hardy-Weinberg equilibrium (HWE) was tested by the Chi-square test. Meta-analysis was performed using Review Manager 5.0 software. Sensitivity analysis was performed by sequential remove (statistics of study remove) of individual studies (Review Manager 5.0 software).

Results

Eligible studies for meta-analysis

This study is focusing on NQO1 609C>T polymorphism and leukemia risk. After a careful evaluation of the published literature, only 22 studies met our inclusion criteria for this meta-analysis (Table 1). The retrieved papers were then read in entirety to assess their appropriateness for the inclusion in this study. The basic information, including leukemia type, ethnicity, the number of cases and controls of each study, are listed in Table I. In all studies, the controls were free of leukemia. In the total 22 studies, 8 articles provided the data of AML (acute myeloid leukemia) patients and 10 articles provided the data of ALL (acute lymphoblastic leukemia) patients. All of the researches were then conducted in different ethnicity, mainly Asian and Caucasian: 13 studies provided Caucasian and 5 studies provided Asian data.

Leukemia susceptibility analysis

22 studies (3700 cases and 5027controls) examining the association between NQO1 609C>T polymorphism and leukemia were included. Significant heterogeneity was observed in dominant genetic model (Ser/Ser + Pro/Ser versus Pro/Pro) and the original data were combined by means of the random effect model. In this model there showed no association of NQO1 609C>T polymorphism with leukemia (OR=1.21, 95%CI = 1.02-1.44, Heterogeneity<0.00001; I² =69%),), and there is an association of NQO1 609C>T polymorphism with leukemia in recessive genetic model (Ser/Ser versus Pro/Ser + Pro/Pro: OR = 1.25, 95%CI = 1.02-1.53, Heterogeneity=0.94; I² = 0%). The forest plot (Figure 3A.) showed that the distribution of the ORs from individual

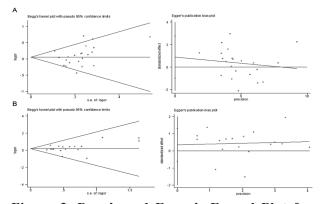


Figure 3. Begg's and Egger's Funnel Plot for Publication bias Test in the Recessive Genetic Model Analysis

studies in relation to their respective standard deviation was symmetric in funnel plot. Similarly, the Egger's test provided no evidence of publication bias in reviewed studies for dominant genetic model (t = 1.22, P = 0.235) and for recessive genetic model (t = 1.01, P = 0.328) (Figure 3B).

Ethnicity analysis

In different ethnicity populations we found the results are different. Both results of dominant genetic model and recessive genetic model showed the associations of NQO1 609C>T polymorphism with leukemia (Ser/Ser + Pro/Ser versus Pro/Pro OR = 1.22, 95% CI = 1.22-1.45, P = 0.05; Ser/Ser versus Pro/Ser + Pro/Pro OR = 1.25, 95% CI = 1.02-1.53, P = 0.04) in Caucasian population. The Egger's test provided no evidence of publication bias in reviewed studies (t = 0.36, P = 0.772 for recessive genetic model and t = 0.22, P = 0.832 for dominant genetic model). In Asian population there are no significant results (Ser/Ser + Pro/Ser versus Pro/Pro OR = 1.19, 95% CI = 0.78-1.82, P = 0.41; Ser/Ser versus Pro/Ser + Pro/Pro OR = 1.19, 95% CI = 0.87-1.62, P = 0.27).

Leukemia type analysis

Most of studies involved in this research provided the data of AML and ALL. 7 studies (1111 cases and 1829 controls) examining the association between NQO1 609C>T polymorphism and AML were included. We found no association of NQO1 609C>T polymorphism with AML (Ser/Ser + Pro/Ser versus Pro/Pro OR = 0.96, 95% CI = 0.64-1.44, P = 0.85; Ser/Ser versus Pro/Ser + Pro/Pro OR = 0.93, 95% CI = 0.64-1.33, P = 0.68). 10 studies (1248 cases and 1489 controls) examining the association between NQO1 609C>T polymorphism and ALL population were included. And we found an association of NQO1 609C>T polymorphism with ALL in recessive genetic model but not in dominant genetic model (Ser/Ser + Pro/Ser versus Pro/Pro OR = 1.18,95%) CI = 0.77-1.81, P = 0.45; Ser/Ser versus Pro/Ser + Pro/Pro OR = 1.33,95% CI = 1.02-1.75, P = 0.04). The forest plot showed that the distribution of the ORs from individual studies in relation to their respective standard deviation was symmetric in funnel plot. Similarly, we performed an analysis for ethnicity in AML and ALL populations. The results were shown in Table 2.

Age phase analysis

Because of some studies applied the data of children or pediatric, we performed an analysis of NQO1 609C>T polymorphism and children leukemia. Nine studies (1248 cases and 1489 controls) examining the association between NQO1 609C>T polymorphism and children leukemia were included. We found there is no association between NQO1 609C>T polymorphism and children leukemia (Ser/Ser + Pro/Ser versus Pro/Pro OR = 1.13, 95% CI = 0.83-1.55, P = 0.44; T/T versus C/T+C/C OR = 1.28, 95% CI = 0.96-1.7, P = 0.1). The forest plot (Figure 3C) showed that the distribution of the ORs from individual studies in relation to their respective standard deviation was symmetric in funnel plot.

Discussion

The NQO1, which is generally involved in Xenobiotic-Metabolizing, has been studied extensively on its relationship with different types of cancer, such as breast cancer, colorectal cancer, leukemia and so on. Previous conclusions of numerous studies on association between NQO1 609C>T polymorphism and leukemia remain conflicting and contradictory, this was largely attributed to the small samples or the relatively low statistical power of published studies. Meta-analysis is a powerful method for resolving inconsistent findings with a relatively large number of subjects. So, this meta-analysis was applied to provide a quantitative approach for combining the different results. To the author's knowledge, this is the most comprehensive meta-analysis investigating the genetic susceptibility of NQO1 gene C609T polymorphism to leukemia.

In the present meta-analysis with 3700 cases and 5027controls, the variant TT homozygous genotype and the combined CT/TT genotype of the NQO1 609 C>T polymorphism was found to be associated with a increased risk of leukemia, especially in Caucasian populations. These findings suggested that the NQO1 609C>T polymorphism may modify the risk of leukemia mainly in Caucasian populations but not in Asian populations. Publication bias was not observed in this study. In the subgroup analysis of age phase we found that NQO1 609C>T polymorphism was not associated with children leukemia.

Several limitations of this meta-analysis should be pointed out. First, although the Begg's test and Egger's test did not show any publication bias, selection bias could have occurred, because only studies published in English and Chinese were included in our meta-analysis. Second, this analysis was based on unadjusted published estimates, and hence, it was unable to adjust them by possible confounders such as sex, smoking status and living environment risk factors. Furthermore, due to a limited number of published studies available to be included, it was unable to perform further subgroup analyses for AML in Asian populations.

In summary, this meta-analysis provided robust evidence of the association between NQO1 609 C>T polymorphism and leukemia risk on Caucasian population, supporting the results of published paper that NQO1 609 C>T polymorphism is a strong susceptibility marker of leukemia, especially in Caucasian population. Moreover, sophisticated gene-gene interaction should be considered in future analysis, which would lead a better, comprehensive understanding of the association between NQO1 609 C>T polymorphism and leukemia risk.

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