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Research Article

Role of Polymorphisms in MicroRNA-196a2, MicroRNA-499 and MicroRNA-146a for Prostate and Gastric Cancer Risk: An Updated Meta-analysis

Maheen Zahra[1] | Saman | Rauf Ramay[1] | Haseeb Nisar[1]* | Mubbasher Munir[2] | Anum Javaid[3] | Muhammad Khurram[1] | Samiah Shahid[4] | Wardah Nisar[5] | Komal Javed[1]

[1]Department of Life-Sciences, University of Management and Technology, Lahore, Pakistan
[2]Department of Economics and Statistics, University of Management and Technology, Lahore, Pakistan
[3]School of Biological Sciences, University of the Punjab, Lahore, Pakistan
[4]Institute of Molecular Biology and Biotechnology, The University of Lahore, Lahore, Pakistan
[5]Department of Public Health, University of Health Sciences, Lahore, Pakistan

***Correspondence**
Haseeb Nisar
Email:
haseeb.nisar@umt.edu.pk

Keywords:
Meta-analysis, Prostate Cancer, Gastric Cancer, Polymorphism

ABSTRACT

Numerous studies have reported the polymorphisms in miR-196a2, miR-499 and miR-146a were associated with different types of cancers. However, the results have been inconsistent and varied. Therefore, we conducted a meta-analysis with the addition of the latest articles to explain the effect of these polymorphisms on Prostate (PCa) and Gastric cancer (GC). A total of 27 articles were recruited after a thorough literature analysis by two independent authors under the PRISMA guidelines in which 7 studies were related to PCa and 20 studies were of GC. We used STATA for performing the meta-analysis. The results from our analysis showed that miR-196a2 rs11614913 polymorphism is associated with PCa in allelic model in Asian population (CvsT: OR=1.207, 95%CI: 1.023-1.425, P=0.026), heterozygous model in Asian population (CvsT: OR=1.264, 95%CI: 1.008-1.585, P=0.042) while miR-499 rs3746444 is associated with PCa in allelic model overall population (AvsC: OR=1.201, 95%CI: 1.039 -1.388, P=0.013) and in Asian subjects (AvsC OR=1.23, 95%CI: 1.030-1.469, P=0.022). The miR-499 rs3746444 is also associated with GC in all four genetic models. Our results concluded that miR-196a2 rs11614913 and miR-499 rs3746444 may be involved in the development of PCa in Asian subjects while miR-499 rs3746444 may be related to GC prognosis.

1. INTRODUCTION

MicroRNAs (miRNAs) are small 17-25 non-coding nucleotides that play a significant role in gene expression regulation and controlling various physiological developments including many cellular processes, proliferation, differentiation, and apoptosis. They undergo their function by repressing translational mechanisms or degrading mRNA by binding to the 3´-untranslated region (3´-UTR) of target mRNAs [1][2][3]. The role of single nucleotide polymorphisms (SNPs), which are the most common type of mutations found in the human genome, within these miRNAs have been instrumental in altering these biological mechanisms controlled by these small nucleotides resulting in progression of various types of cancers [4][5][6].

The function of miRNAs that regulate genes essential for prostate cell growth and survival can be impacted by certain polymorphisms. For instance, variations in miRNAs that target genes such as the androgen receptor (AR) or prostate-specific antigen (PSA) might affect androgen signaling pathways, which in turn

can accelerate the development of prostate cancer. Apoptosis avoidance, unchecked cell proliferation, and the advancement of cancer are all possible outcomes of disruptions in the regulation of miRNA. Similar to this, polymorphisms in GC can impact miRNAs that control genes related to inflammation, gastric mucosal integrity, and the body's reaction to Helicobacter pylori infection, a key risk factor for GC. Epithelial-mesenchymal transition (EMT), metastasis, and chronic inflammation can all be influenced by polymorphisms in miRNAs, including miR-146a, miR-196a2, and miR-499. These polymorphisms might result in altered expression or function. The development and progression of gastric cancer can be impacted by these alterations in oncogenes and tumor suppressors (e.g., KRAS, PIK3CA, TP53).

Recently studies have reported the most frequent SNPs in these miRNAs to be associated with Prostate (PCa) and Gastric Cancer (GC). Some of these notable SNPs include rs2910164 C>G present in miR-146a, rs11614913 C>T located in miR-196a2, and rs37346444 A>G present in miR499 have been extensively studied in cancer risk and its susceptibility [7][8][9][10][11]. Although role of these SNPs have been studied in relation with possible association with cancer, nevertheless the results have been variable. For instance, Zhou et al. found a significant role of rs37346444 A>G in progression of cervical squamous cell carcinoma while Tian et al. and Srivastava et al. have failed to find a possible role of this SNP in lung and gall-bladder cancer respectively [12][13]. Similarly, some researchers have revealed the role of minor allele C in rs2910164 in reducing the PCa cancer (PCa) risk in the Chinese Han population [14] while others have reported no significant evidence of this variant in PCa development in other populations [15]. The reason behind this substantial inconsistency in the SNPs data may be due to different ethnic backgrounds, varied sample sizes, and the corrected physiological status [16, 17].

One of the strategies for resolving these inconsistencies in these reported studies is to undergo a more precise statistical analysis. Meta-analysis provides a more logical methodology for removing these heterogeneities by combining, summarizing, and critically analyzing the previous research work conducted across different sample sizes and ethnic communities. We conducted an up-to-date meta-analysis on all available case-control studies by combining data to achieve a bigger sample and increasing the statistical power to assess the relationship between miRNAs and cancer risk.

2. MATERIALS AND METHODOLOGY

2.1 Literature Search for Identification of Eligible Studies and Data Extraction

The meta-analysis was performed according to the guidelines of Preferred Reporting Items for Meta-analyses (PRISMA) [18]. Two authors (Zahra M. and Ramay S.R) performed an extensive literature review using PubMed, Springer-Link, and Google Scholar as databases up to October 21st, 2022 with the following keywords: “miR-146a”, “miR-196a2”, “miR499”, “Prostate cancer”, “Gastric cancer” and “polymorphism”. We assessed relevant articles by evaluating their titles and abstracts for inclusion in our meta-analysis. Studies including review articles, comments, meta-analysis, and data from family-based linkage analysis and/or overlapping data were eliminated. The studies suitable for our analysis met with certain inclusion criteria: (a) original case-control studies with odds ratios (ORs), 95% confidence intervals (CIs), and mentioned *p* values, (b) articles published in English, (c) accessibility of genotypes and allele frequencies from each relevant article.

Similar articles present in different databases were removed to avoid repetition. Each article that met our inclusion criteria was scrutinized independently by two reviewers. The information extracted from each study were included: the author's name, year of publication, ethnicity, genotyping method, the sample size in both case and control categories, genotype, and allelic frequencies.

2.2 Statistical Analysis

The strength of the association between rs2910164, rs11614913, and rs37346444 and PCa and GC was analyzed by the ORs and their corresponding 95%CI. We used four genetic models (allelic, wild-type homozygote, recessive allele homozygote, and heterozygous) to obtain the pooled ORs. Additionally, stratified subgroup analysis was performed by ethnicity (Asian or Caucasian). Chi-squared based Q statistic test (To evaluate whether a difference between two categorical variables is the result of chance or a relationship between them, the Chi-Square test is a statistical procedure that can be used to determine the difference between observed and expected data) was used to identify differences between studies [19]. Using the I^2 statistic test (It indicates the percentage of variation in the observed effect that results from variation in the genuine effects as opposed to sampling error), the impact of heterogeneity was calculated and classified as high (I^2 > 50%), moderate (25% I^2 50%), and low (I^2 25%). The I^2 statistic test was performed to assess the impact of heterogeneity that was classified as high (I^2 > 50%), moderate (25% I^2 50%), and low (I^2 25%) [20]. Results with p-value< 0.10 and an I^2> 50%, indicates high level of heterogeneity, thereby applying random effect model. Alternatively, when there was no heterogeneity, a fixed effect model was used. To ascertain whether publication bias existed, funnel plots and Egger's linear regression tests were performed [21]. A two-sided *p-value* <0.05 was considered as statistically significant. All the analyses were performed by using STATA software version 14.1 (USA).

3. RESULTS

3.1 Study Characteristics

The main characteristics of selected studies investigating the association of miR-196a2 rs11614913, miR-146a rs2910164, and miR-499 rs3746444 with PCa and GC are shown in Table 1 and Table 3. There were 4611 articles relevant to searching words found in PubMed and Google Scholar. 1812 were excluded because they were meta-analysis and comments. After performing an in-depth analysis of selecting relevant studies using the inclusion and exclusion criteria, a total of 54 articles were included in our study. In total, 27 articles were enrolled in the current study after removing duplicates and studies that do not include SNPs related to our analysis. A flow chart of studies selected using PRISMA guidelines is indicated in Figure 1.

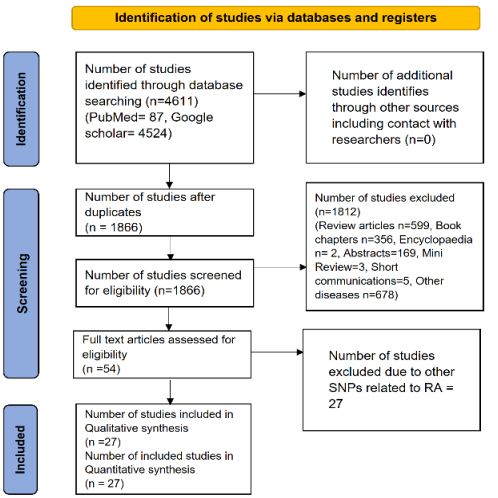

Figure 1. PRISMA Chart showing the approach adopted during the literature search among the associated studies

Table 1. Characteristics of all studies related to PCa Cancer in the meta-analysis

SNP rs11614913 C>T															
Author	Year	Ethnicity	Genotyping Method	Subjects Enrolled		Genotype of Cases				Genotype of Controls						Association P value
				Cases	Control	CC	CT	TT	C	T	CC	CT	TT	C	T	
George et al	2011	Asia	PCR-RFLP	159	230	55	101	3	211	107	106	114	10	326	134	0.18
Damodaran et al	2020	Asia	PCR-RFLP	100	100	32	51	17	115	85	47	36	17	130	70	1
Nikolić et al	2015	Europe	HRMA	355	312	150	161	40	461	241	121	147	41	389	229	N/A
Hashemi et al	2016	Asia	T-ARMS-PCR	169	182	64	88	17	216	122	77	93	12	247	117	0.300
Nouri et al	2019	Asia	PCR-RFLP	150	150	48	73	29	169	131	48	80	22	176	124	0.563
SNP rs2910164 C>G																
Author	Year	Ethnicity	Genotyping Method	Subjects Enrolled		Genotype of Cases				Genotype of Controls						Association P value
				Cases	Control	CC	CG	GG	C	G	CC	CG	GG	C	G	
George et al	2011	Asia	PCR-RFLP	159	230	116	107	7	339	121	76	79	4	231	87	0.074
Nikolić et al	2014	Europe	Taqman	286	199	184	90	12	458	114	129	63	7	321	77	0.89
Hashemi et al	2016	Asia	T-ARMS-PCR	169	182	25	131	13	181	157	24	147	11	195	169	0.974
Xu et al	2010	Asia	PCR-RFLP	251	280	68	135	48	271	231	54	150	76	258	302	0.01
Damodaran et al	2020	Asia	PCR-RFLP	100	100	52	38	10	142	58	51	39	10	141	59	1
SNP rs3746444 A>C																
Author	Year	Ethnicity	Genotyping Method	Subjects Enrolled		Genotype of Cases				Genotype of Controls						Association P value
				Cases	Control	AA	AC	CC	A	C	AA	AC	CC	A	C	
George et al.	2011	Asia	PCR-RFLP	159	230	48	98	13	194	124	104	92	34	300	160	0.23
Nikolić et al.	2015	Europe	PCR-RFLP	355	312	190	147	18	527	183	180	110	17	470	144	0.35
Hashemi et al.	2016	Asia	PCR-RFLP	169	182	62	82	25	206	132	85	64	33	234	130	0.39
Nouri et al.	2019	Asia	PCR-RFLP	150	150	29	72	49	130	170	35	83	32	153	147	0.06

Abbreviations: PCR-RFLP (Polymerase chain reaction-restriction fragment length polymorphism), T-ARMS-PCR (tetra-primer amplification refractory mutation system polymerase chain reaction, HRMA (High resolution melt analysis)

Table 2. Total and stratified group analysis according to ethnicity for the three miRNAs polymorphisms and PCa susceptibility

Genetic Model rs11614913 C>T	Comparison Group & Subgroup		Test of Association			Test of Heterogeneity			Publication Bias P value
			OR	95% CI	P value	Model	I^2	P value	
Dominant	5	Total	0.822	0.617-1.097	0.183	R	55.09	0.063	0.161
	4	Asian	0.735	0.583-0.928	0.01	F	20.04	0.289	0.752
	1	European	1.159	0.849-1.583	0.351	F	0	N/A	0
Recessive	5	Total	1.033	0.769 -1.388	0.83	F	14.43	0.322	0.793
	4	Asian	1.188	0.810-1.742	0.379	F	12.7	0.332	0.162
	1	European	0.841	0.528-1.339	0.465	F	0	N/A	0
Heterozygous	5	Total	1.183	0.868- 1.614	0.287	R	63.24	0.028	0.362
	4	Asian	1.264	1.008-1.585	0.042	F	64.51	0.037	0.73
	1	European	N/A						
Allele C	5	Total	0.922	0.807-1.054	0.234	F	25.34	0.252	0.264
	4	Asian	N/A						
	1	European	1.126	0.899-1.411	0.302	F	0	N/A	0
Allele T	5	Total	1.084	0.949-1.239	0.234	F	25.34	0.252	0.376
	4	Asian	1.207	1.023-1.425	0.026	F	0	0.866	0.323
	1	European	0.888	0.709-1.113	0.302	F	0	N/A	0
Genetic Model rs2910164 C>G	Comparison Group & Subgroup		Test of Association			Test of Heterogeneity			Publication Bias P value
			OR	95% CI	P value	Model	I^2	P value	
Dominant	5	Total	1.157	0.948-1.412	0.153	F	0	0.565	0.915
	4	Asian	1.234	0.975-1.560	0.08	F	0	0.589	0.509
	1	European	0.979	0.670 -1.429	0.912	F	0	0	0
Recessive	5	Total	0.824	0.603-1.125	0.223	F	0	0.428	0.123
	4	Asian	0.787	0.566-1.095	0.155	F	0	0.367	0.091
	1	European	1.201	0.464-3.107	0.705	F	0	0	0
Heterozygous	5	Total	0.944	0.781-1.140	0.547	F	0	0.963	0.315

	4	Asian	0.93	0.749-1.153	0.506	F	0	0.914	0.45
	1	European	0.991	0.672-1.463	0.965	F	0	0	0
Allele C	5	Total	1.114	0.971-1.277	0.124	F	9.6	0.352	0.175
	4	Asian	1.15	0.988-1.338	0.071	F	13.84	0.323	0.274
	1	European	0.964	0.698-1.330	0.822	F	0	0	0
Allele G	5	Total	0.898	0.783-1.030	0.124	F	9.6	0.352	0.175
	4	Asian	0.87	0.747-1.012	0.071	F	13.84	0.323	0.274
	1	European	1.038	0.752-1.432	0.822	F	0	0	0
Genetic Model rs3746444 A>C	Comparison Group & Subgroup		Test of Association			Test of Heterogeneity			Publication Bias P value
			OR	95% CI	P value	Model	I^2	P value	
Dominant	4	Total	0.701	0.574-0.857	0.001	F	0	0.406	0.627
	3	Asian	0.629	0.483-0.820	0.001	F	0	0.499	0.439
	1	European	0.812	0.597-1.106	0.187	F	0	0	0
Recessive	4	Total	0.922	0.540-1.574	0.767	R	67.89	0.025	0.231
	3	Asian	0.917	0.443-1.897	0.815	R	78.4	0.01	0.257
	1	European	0.911	0.461-1.801	0.789	F	0	0	0
Heterozygous	4	Total	1.414	0.901-2.220	0.132	R	80.47	0.002	0.993
	3	Asian	1.469	0.749-2.881	0.263	R	86.2	0.001	0.054
	1	European	1.266	0.924-1.734	0.142	F	0	0	0
Allele A	4	Total	0.833	0.720-0.962	0.013	F	0	0.853	0.304
	3	Asian	0.813	0.681-0.971	0.022	F	0	0.748	0.416
	1	European	0.882	0.686-1.134	0.329	F	0	0	0
Allele C	4	Total	1.201	1.039 -1.388	0.013	F	0	0.853	0.304
	3	Asian	1.23	1.030-1.469	0.022	F	0	0.748	0.416
	1	European	1.133	0.882-1.457	0.329	F	0	0	0

Abbreviations: F (Fixed effect model), R (Random effect model)

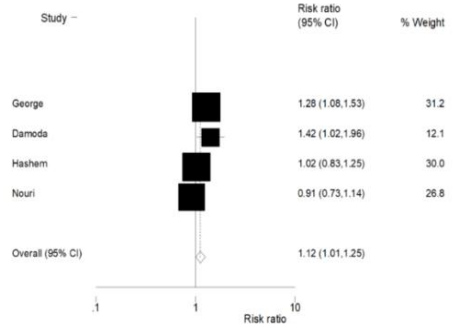

Fig2A) Meta-analysis of the relationship between miR-196a2 rs11614913 C>T polymorphism and prostate cancer risk for different region (Heterozygous Asian Model)

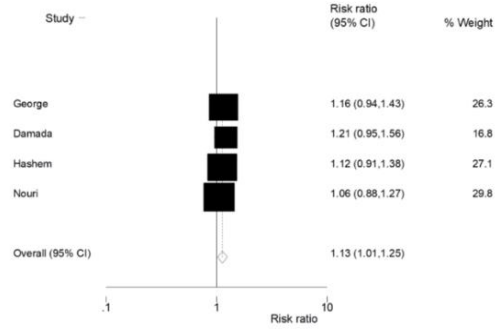

Fig2B) Meta-analysis of the relationship between miR-196a2 rs11614913 C>T polymorphism and prostate cancer risk for different region (T allele Asian Model)

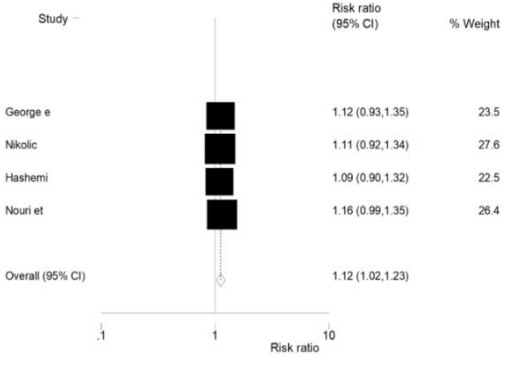

Fig2C) Meta-analysis of the relationship between miR-499 rs3746444 A>C polymorphism and prostate cancer risk for different region (Allele C Overall Model)

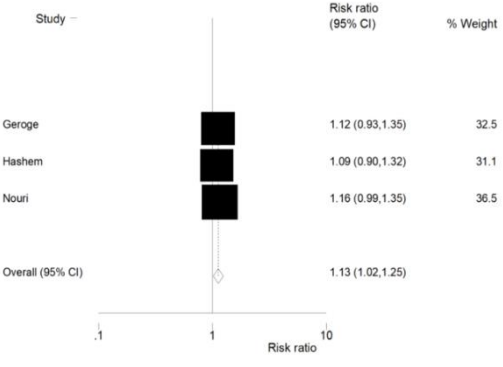

Fig2D) Meta-analysis of the relationship between miR-499 rs3746444 A>C polymorphism and prostate cancer risk for different region (Allele C Asian Model)

Figure 2. Meta-analysis of the relationship between miR-196a2 rs11614913 C>T polymorphism and prostate cancer risk for different regions. A) (Heterozygous Asian Model); B) T allele Asian Model; C) Allele C Overall Model; D) Allele C Asian Model

Table 3. Characteristics of all studies related to GC in the meta-analysis

SNP rs11614913 C>T															
Author	Year	Ethnicity	Genotyping Method	Subjects Enrolled		Genotype of Cases					Genotype of Controls					Association P value
				Cases	Control	CC	TC	TT	C	T	CC	TC	TT	C	T	
Okubo	2010	Asian	PCR-RFLP	552	697	105	281	166	491	613	124	350	223	598	796	NA
Peng	2010	Asian	PCR-RFLP	213	213	76	94	43	246	180	56	107	50	219	207	0.936
Dikeakos	2013	Caucasian	PCR-RFLP	163	480	102	46	15	250	76	79	229	172	387	573	< 0.0001
Kupcinskas	2014	Caucasian	Taqman	363	351	144	184	35	472	254	159	145	46	463	237	0.567
Ahn	2013	Asian	PCR-RFLP	461	447	100	242	119	442	480	87	232	128	406	488	0.419
Pu	2014	Asian	PCR-RFLP	220	530	39	95	25	173	145	101	324	86	526	496	0.197
Wang	2013	Asian	TaqMan	753	900	152	371	226	675	823	220	448	232	888	912	0.898
Gu	2016	Asian	PCR-RFLP	186	186	39	96	51	174	198	57	98	31	212	160	0.02
Jiang	2016	Asian	MassARRAY	898	992	166	423	300	755	1023	198	487	290	865	1067	0.171
Xin	2022	Asian	TaqMan	386	341	89	183	114	361	411	87	158	96	332	350	0.46
SNP rs2910164 C>G																
Author	Year	Ethnicity	Genotyping Method	Subjects Enrolled		Genotype of Cases					Genotype of Controls					Association P value
				Cases	Control	CC	CG	GG	C	G	CC	CG	GG	C	G	
Zeng	2010	Asian	PCR-RFLP	304	304	89	153	62	331	277	119	132	53	370	238	0.009
Okubu	2010	Asian	PCR-RFLP	552	697	236	243	73	715	389	254	322	121	830	564	NA
Hishida	2010	Asian	PCR-CTPP	583	1742	230	271	82	731	435	633	825	229	2091	1283	N/A
Zhou F	2012	Asian	Taqman MGB	1686	1895	286	822	578	1394	1978	393	951	551	1737	2053	0.001
Ahn	2013	Asian	PCR-RFLP	461	447	159	231	71	549	373	164	221	62	549	345	0.549
Pu	2014	Asian	PCR-RFLP	220	530	65	96	36	226	168	143	274	96	560	466	0.18
Zhou Y	2012	Asian	Locus-specific PCR	299	416	107	146	46	360	238	127	215	74	469	363	NA
Jiang	2016	Asian	MassARRAY	898	992	303	441	154	1047	749	325	457	207	1107	871	0.107
Gu	2016	Asian	PCR-RFLP	186	186	29	91	66	149	223	25	90	70	140	230	0.81
Dikeakos	2013	Caucasian	PCR-RFLP	163	480	105	45	13	255	71	307	149	24	763	197	0.17
Kupcinskas	2014	Caucasian	Taqman	363	351	16	94	252	126	598	16	108	223	140	554	0.119

Xin	2022	Asian	TaqMan	386	341	129	188	69	446	326	114	155	60	383	275	0.87
SNP rs3746444 A>C																
Author	Year	Ethnicity	Genotyping Method	Subjects Enrolled		Genotype of Cases					Genotype of Controls					Association P value
				Cases	Control	AA	AC	CC	A	C	AA	AC	CC	A	C	
Rogoveanu	2017	Caucasians	Taqman	142	288	80	58	4	218	66	173	107	8	453	123	0.53
Poltronieri-Oliveira	2017	Caucasians	PCR-RFLP	151	249	97	49	5	243	59	143	100	6	386	112	270
Cai	2015	Asian	Mass ARRAY	363	969	261	89	13	611	115	765	179	25	1709	229	0.002
Wu	2013	Asian	PCR-RFLP	201	213	149	47	4	345	55	166	42	3	374	48	NA
Pu	2014	Asian	PCR-RFLP	220	530	141	50	5	332	60	366	121	17	853	155	0.576
Ahn	2013	Asian	PCR-RFLP	461	447	323	123	15	769	153	299	134	14	732	162	0.514
Okubu	2010	Asian	PCR-RFLP	552	697	364	151	37	879	225	466	198	33	1130	264	NA
Tang	2019	Asian	SNP scan	1063	1677	695	311	34	1701	379	1214	419	41	2847	501	NA
Rong	2021	Asian	SNP scan	490	1476	340	130	17	810	164	1058	380	34	2496	448	0.149
Xin	2022	Asian	TaqMan	386	341	272	106	8	650	122	254	79	8	587	96	0.32

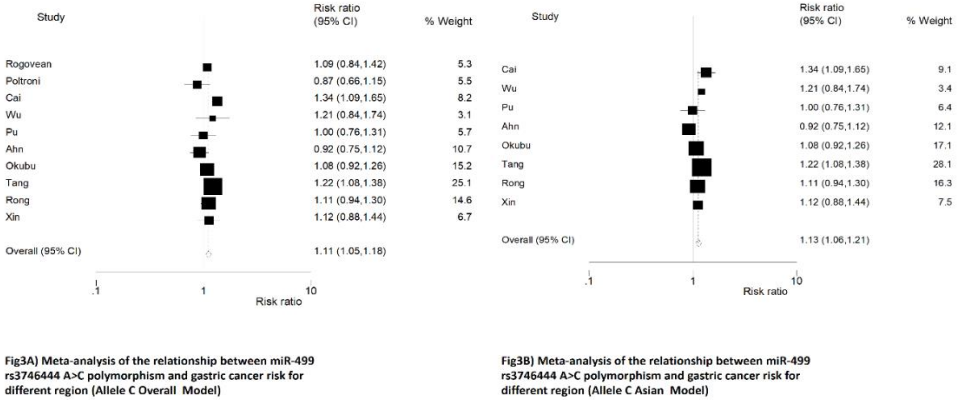

Figure 3. Meta-analysis of the relationship between miR-499 rs3746444 A>C polymorphism and gastric cancer risk for different regions. A) Allele C Overall Model; B) Allele C Asian Model

Table 4. Total and stratified group analysis according to ethnicity for the three miRNAs polymorphisms and GC susceptibility

Genetic Model rs11614913 C>T	Comparison Group & Subgroup		Test of Association			Test of Heterogeneity			Publication Bias P value
			OR	95% CI	P value	Model	I2	P value	
Dominant	10	Total	1.196	0.816-1.753	0.359	R	92.96	0	0.228
	8	Asian	0.984	0.827-1.172	0.861	R	57.35	0.022	0.41
	2	European	2.579	0.252-26.429	0.425	R	98.85	0	0
Recessive	10	Total	0.899	0.699-1.157	0.408	R	83.12	0	0.128
	8	Asian	1.087	0.983-1.202	0.105	F	47.29	0.066	0.751
	2	European	0.361	0.096-1.366	0.133	R	92.44	0	0
Hetrozygous	10	Total	0.934	0.802-1.088	0.38	R	66.63	0.001	0.387
	8	Asian	1.018	0.874-1.185	0.82	R	61.73	0.011	0.238
	2	European	0.797	0.242-2.622	0.708	R	95.84	0	0
Allele C	10	Total	1.131	0.888-1.440	0.318	R	93.49	0	0.171
	8	Asian	0.96	0.647-1.425	0.839	R	96.39	0	0.91
	2	European	2.146	0.433-10.637	0.35	R	98.72	0	0
Allele T	10	Total	0.884	0.694-1.126	0.318	R	93.49	0	0.171
	8	Asian	1.029	0.917-1.154	0.63	R	66.05	0.004	0.575
	2	European	0.466	0.094-2.309	0.35	R	98.72	0	0
Genetic Model rs2910164 C>G	Comparison Group & Subgroup		Test of Association			Test of Heterogeneity			Publication Bias P value
			OR	95% CI	P value	Model	I2	P value	
Dominant	12	Total	0.991	0.869-1.131	0.897	R	60.64	0.003	0.738
	10	Asian	1.009	0.876-1.161	0.902	R	64.9	0.002	0.574
	2	European	1.006	0.724-1.398	0.971	F	0	0.875	0
Recessive	12	Total	1.056	0.919-1.213	0.444	R	54.73	0.012	0.63
	10	Asian	0.94	0.805-1.099	0.438	R	60.65	0.006	0.044
	2	European	1.33	0.999-1.770	0.051	F	0	0.512	0
Hetrozygous	12	Total	0.969	0.905-1.037	0.365	F	0.31	0.44	0.845
	10	Asian	0.949	0.884-1.019	0.148	F	31.84	0.154	0.342

	2	European	1.271	0.579-2.793	0.55	R	88.58	0.003	0
Allele C	12	Total	0.995	0.906-1.094	0.921	R	67.95	0	0.465
	10	Asian	1.013	0.913-1.124	0.806	R	72.55	0	0.263
	2	European	0.827	0.676-1.012	0.066	F	0	0.928	0
Allele G	12	Total	1.005	0.914-1.104	0.921	R	67.95	0	0.465
	10	Asian	0.987	0.890-1.095	0.806	R	72.55	0	0.263
	2	European	1.146	0.937-1.401	0.186	F	0	0.608	0
Genetic Model rs3746444 A>C	Comparison Group & Subgroup		Test of Association			Test of Heterogeneity			Publication Bias P value
			OR	95% CI	P value	Model	I2	P value	
Dominant	10	Total	0.873	0.800-0.952	0.002	F	38.85	0.099	0.286
	8	Asian	0.856	0.781-0.937	0.001	F	33.74	0.159	0.572
	2	European	1.066	0.693-1.640	0.771	R	54.31	0.139	0
Recessive	10	Total	1.288	1.028-1.614	0.028	F	0	0.972	0.105
	8	Asian	1.296	1.026-1.638	0.030	F	0	0.917	0.115
	2	European	1.188	0.505-2.797	0.693	F	0	0.72	0
Heterozygous	10	Total	1.111	1.015-1.215	0.022	F	44.03	0.065	0.411
	8	Asian	1.134	1.032-1.246	0.009	F	37.15	0.133	0.752
	2	European	0.917	0.568-1.482	0.724	R	62.07	0.104	0
Allele A	10	Total	0.878	0.815- 0.947	0.001	F	27.76	0.189	0.228
	8	Asian	0.864	0.798-0.935	0.000	F	25.26	0.227	0.494
	2	European	1.029	0.806- 1.315	0.817	F	23.95	0.251	0
Allele C	10	Total	1.139	1.056-1.228	0.001	F	27.76	0.189	0.228
	8	Asian	1.158	1.069-1.253	0.000	F	25.26	0.227	0.494
	2	European	0.971	0.760-1.241	0.817	F	23.95	0.251	0

Abbreviations: F (Fixed effect model), R (Random effect model)

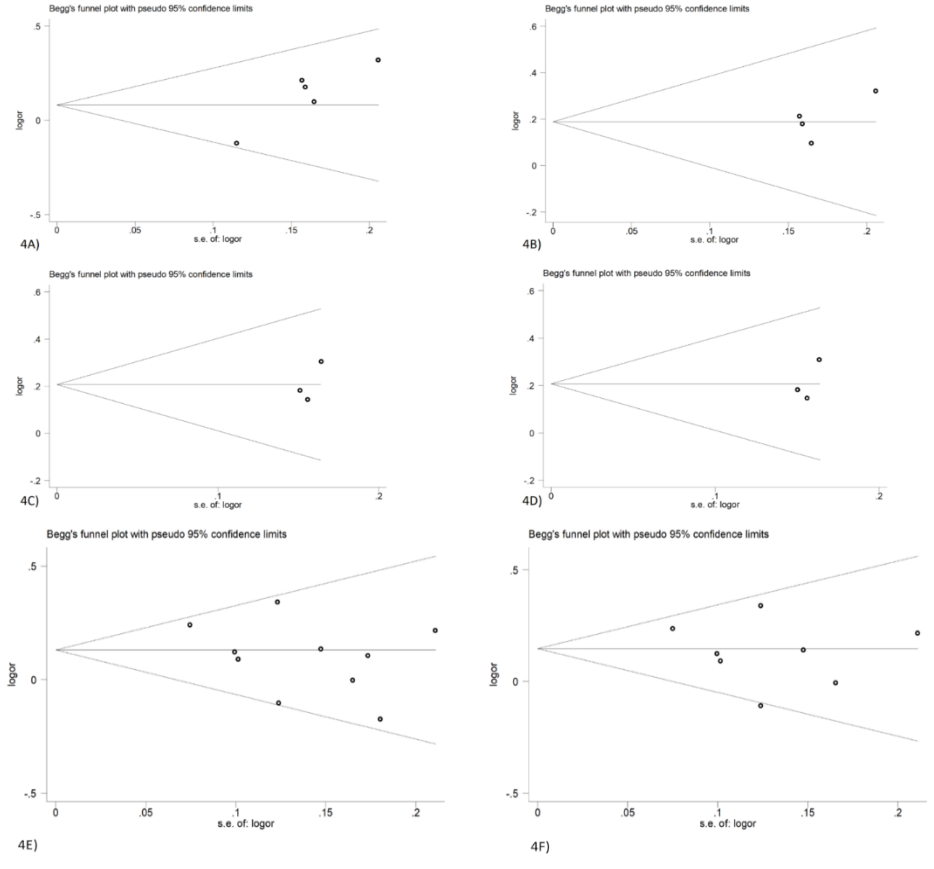

Figure 4. Begg's funnel plot for publication bias of miR-196a2 rs11614913 polymorphism and prostate cancer risk. A) T allelic overall model; 4B) T allelic Asian model; 4C) C allelic overall model; 4D) C allelic Asian model; 4E) C allelic overall model; 4F) C allelic Asian model. **Each point represents a separate study for the indicated association. Logor represents the natural logarithm of OR. The horizontal line means the magnitude of the effect. A funnel plot with pseudo 95% confidence limits was used*

3.2 Association between miR-196A2, miR-146a and miR-499 Polymorphism and PCa

For PCa cancer, a total of seven studies were included with samples ranging from 100-355. There were 5 studies from Asian ethnicity and 2 studies from European ethnicity. Controls in each study were age and gender-related to cases. Results from the study showed that miR-196a2 rs11614913 C>T polymorphism might be associated with PCa susceptibility in two genetic models. The estimated allele model in the Asian population (CvsT: OR=1.207, 95%CI: 1.023-1.425, P=0.026) and heterozygous model in the Asian population (CvsT: OR=1.264, 95%CI: 1.008-1.585, P=0.042) were significantly associated with PCa. The miR-146a SNP rs2910164 C>G didn't show any significant association with any genetic models while the miR499 rs3746444 A>C showed significant association in the allele model in the overall population (AvsC: OR=1.201, 95%CI: 1.039 -1.388, P=0.013) and also in Asian subjects (AvsC OR=1.23, 95%CI: 1.030-1.469, P=0.022) (Table 2 and Figure 2). *Association between miR-196A2, miR-146a and miR-499 polymorphism and GC.*

For GC, total 20 studies were included with sample ranging from 142-1895. There were 16 studies from Asian ethnicity and 4 studies from European ethnicity (Table 4). Controls in each study were age and gender-related to cases. Results from the study showed miR-499 rs3746444 A>C polymorphism might be associated with increased GC susceptibility in all four genetic models i.e. allele model P=0.228, dominant model P=0.002, heterozygote model P=0.02, recessive model P=0.028. In the subgroup analysis for ethnicity, a significant association between miR-499 rs3746444 A>C and risk of GC was found in Asian subjects in all genetic models i.e. allele model P=0.000, dominant model P=0.001, heterozygote model P=0.030, recessive model P=0.009. The miR-196a2 rs11614913 C>T and miR-146a SNP rs2910164 C>G didn't show any significant association with any genetic models in the overall study and subgroup analysis according to ethnicity (Table 4 and Figure 3) *Evaluation of Publication Bias*

In order to access the publication bias of literature, we used Begg's funnel plot (Figure 4) and Eggers test. The shape of the funnel plots did not reveal any evidence of obvious asymmetry for all genetic models in the overall meta-analysis. Egger's test was used to provide statistical evidence of funnel plot asymmetry. Egger's test only detected evidence of publication bias in the subgroup analysis of European subjects in prostate and gastric cancer for all genetic models ($P<0.05$). However, Egger's test was not applied in some comparisons due to the small number of studies.

4. DISCUSSION

The role of SNPs in cancer susceptibility is the most studied type of human genetic variation due to advancements in screening technologies. Efforts to understand the role of SNPs present in precursor and mature miRNA and their effects on the progression of various diseases have been an area of interest. Researchers have shown that SNPs in miRNA precursor and mature miRNA may influence the process of gene coding and splicing, affecting different types of cancer susceptibility. Through modifying the primary transcript of miRNA processing, maturation, and miRNA-miRNA interaction, these alterations also affect cancer susceptibility and play a significant role in the development of primary and metastatic cancers [22][23]24]. Thus it is important to find the association between miRNA SNPs and cancer to further explicate the mechanisms of carcinogenesis. Even though numerous studies have been published on the association of miR-196a2 rs11614913, miR-146a rs2910164, and

miR499 rs3746444 polymorphism and cancer risk, the results have been heterogynous that might be due to small sample size and differences in cancer type and ethnicities. Therefore, to achieve a uniform conclusion and a more precise assessment of the relationship, we performed this meta-analysis. The current study is an updated meta-analysis of the association between miR-196a2, miR-146a and miR-499 polymorphisms and the risk of PCa and GC. New case control studies from recent years were included in the current investigation, and some new conclusions have been suggested.

The results from our study have demonstrated that the minor allele in miR-499 rs3746444 T→C might contribute to PCa in overall study while minor allele variant in miR-196a2 rs11614913 C→T might be involved in increasing the risk for PCa in Asian population. The results from GC have shown that miR-499 rs3746444 T→C were significantly associated in all genetic models being studied in overall and Asian subjects when stratified by ethnicity. The miR-196a2 gene produces two unique mature miRNAs (miR-196a-3P and miR-196a-5P), which are processed from the same precursor miRNA [25], its varied expression patterns may have an impact on the miR-196a's possible targets. The processing or target selection of miRNAs [26, 27], which is known to be a major factor in oncogenesis and helps to control the translation or breakdown of messenger RNA, may be affected by SNPs in miRNA genes (mRNA) [28]. Previous meta-analysis being published to determine the correlation between rs3746444 and the risk of GC didn't find any significant association that might be due to limited studies [17, 29-33]. More than 150 genetic association studies have been conducted to examine the relationship between the human miR-196a2 rs11614913 variant and susceptibility to various cancers; however, these research have produced conflicting results. To elucidate a more definite role and examine the role of rs11614913 of miR-196a2 that is linked to the likelihood of developing cancer, several meta-analyses have been carried out in the past [34][35]. It should be noted that these meta-analyses also lacked certain promising and recent studies that need to be taken into account to determine the exact relationship between this variation and cancer susceptibility. In order to produce a concrete result, we thus carried out this meta-analysis, incorporating the greatest number of association studies carried out in various cohorts or races.

Despite the fact that this study combined all previous research and examined the relationship between rs11614913, rs2910164 and rs3746444 with PCa and GC development, some limitations also need to be addressed. Firstly, although we rigorously checked the papers and carefully retrieved the data, the variations in the subject selection could not be completely removed. Secondly we only include Asian and Caucasian ethnicities in our sub group analysis and the influence of differences in racial descent should not be avoided. Thirdly, studies from languages other than English were excluded, thus including potential language biasness. Fourthly, it could be beneficial to confirm our findings with a different cohort. However, a cohort analysis was not carried out because there were insufficient data. Finally, despite the fact that we find the connection between rs11614913, rs3746444, and the establishment of PCa and GC, the purpose and mechanism of this polymorphism remained a mystery.

In conclusion, polymorphisms in the miR-196a2 rs11614913 and miR-499 rs3746444 may have a role in the onset of PCa and GC. It may be helpful as a candidate marker for the diagnosis of various diseases and may potentially increase the risk of developing cancer in Asian population. Additionally, larger-scale research and investigations

focusing on specific cancer types or ethnicities should be conducted to confirm the findings.

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REFERENCES

1. Cai Y, Yu X, Hu S, Yu J. A brief review on the mechanisms of miRNA regulation. Genomics Proteomics Bioinformatics. 2009;7(4):147-154. https://doi.org/10.1016/S1672-0229(08)60044-3
2. Fabian MR, Sonenberg N. The mechanics of miRNA-mediated gene silencing: a look under the hood of miRISC. Nat Struct Mol Biol. 2012;19(6):586-593. https://doi.org/10.1038/nsmb.2296
3. Kloosterman WP, Plasterk RH. The diverse functions of microRNAs in animal development and disease. Dev Cell. 2006;11(4):441-450. https://doi.org/10.1016/j.devcel.2006.09.009
4. Landi D, Gemignani F, Pardini B, et al. Polymorphisms affecting micro-RNA regulation and associated with the risk of dietary-related cancers: a review from the literature and new evidence for a functional role of rs17281995 (CD86) and rs1051690 (INSR), previously associated with colorectal cancer. Mutat Res. 2011;717(1-2):109-115. https://doi.org/10.1016/j.mrfmmm.2010.10.002
5. Liu X, Chen Z, Yu J, Xia J, Zhou X. MicroRNA-499-5p promotes cellular invasion and tumor metastasis in colorectal cancer by targeting FOXO4 and PDCD4. Carcinogenesis. 2011;32(12):1798-1805. https://doi.org/10.1093/carcin/bgr213
6. Hu Z, Chen X, Zhao Y, et al. Serum microRNA signatures identified in a genome-wide serum microRNA expression profiling predict survival of non-small-cell lung cancer. J Clin Oncol. 2010;28(10):1721-1726. http://jco.ascopubs.org/cgi/doi/10.1200/JCO.2009.24.9342
7. Nouri R, Ghorbian S. Association of single nucleotide polymorphism in hsa-miR-499 and hsa-miR-196a2 with the risk of prostate cancer. Int Urol Nephrol. 2019;51(5):811-816. https://doi.org/10.1007/s11255-019-02099-0
8. Damodaran M, Paul SFD, Venkatesan V. Genetic polymorphisms in miR-146a, miR-196a2 and miR-125a genes and its association in prostate cancer. Pathol Oncol Res. 2020;26(1):193-200. https://doi.org/10.1007/s12253-018-0412-x
9. Peng S, Kuang Z, Sheng C, Zhang Y, Xu H, Cheng Q. Association of microRNA-196a-2 gene polymorphism with gastric cancer risk in a Chinese population. Dig Dis Sci. 2010;55(8):2288-2293. https://doi.org/10.1007/s10620-009-1007-x
10. Nikolić Z, Lazić Z, Jović N, et al. Assessment of association between genetic variants in microRNA genes hsa-miR-499, hsa-miR-196a2 and hsa-miR-27a and prostate cancer risk in Serbian population. Exp Mol Pathol. 2015;99(1):145-150.

https://doi.org/10.1016/j.yexmp.2015.06.009

11. Wang F, Sun G, Zou Y, et al. Lack of association of miR-146a rs2910164 polymorphism with gastrointestinal cancers: evidence from 10206 subjects. PLoS One. 2012;7(6). https://doi.org/10.1371/journal.pone.0039623

12. Tian T, Shu Y, Chen J, et al. A functional genetic variant in microRNA-196a2 is associated with increased susceptibility of lung cancer in Chinese. Cancer Epidemiol Biomarkers Prev. 2009;18(4):1183-1187. https://doi.org/10.1158/1055-9965.EPI-08-0814

13. Srivastava K, Srivastava A, Mittal B. Common genetic variants in pre-microRNAs and risk of gallbladder cancer in North Indian population. J Hum Genet. 2010;55(8):495-499. https://doi.org/10.1038/jhg.2010.54

14. Xu B, Feng NH, Li J, et al. A functional polymorphism in Pre-miR-146a gene is associated with prostate cancer risk and mature miR-146a expression in vivo. Prostate. 2010;70(5):467-472. https://doi.org/10.1002/pros.21080

15. George GP, Gangwar R, Mandal RK, Sankhwar SN, Mittal RD. Genetic variation in microRNA genes and prostate cancer risk in North Indian population. Mol Biol Rep. 2011;38(3):1609-1615. https://doi.org/10.1007/s11033-010-0270-4

16. Dar SA, Haque S, Mandal RK, et al. Interleukin-6-174G > C (rs1800795) polymorphism distribution and its association with rheumatoid arthritis: A case-control study and meta-analysis. Autoimmunity. 2017;50(3):158-169. https://doi.org/10.1080/08916934.2016.1261833

17. Xu Z, Shen J, Liao Z, et al. The association between miR-499 polymorphism and cancer susceptibility: a meta-analysis. Onco Targets Ther. 2015;8:2179-2186. https://doi.org/10.2147/OTT.S88224

18. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting Items for systematic reviews and meta-analyses: The PRISMA Statement. Ann Intern Med. 2009;151(4):264-269. doi:10.7326/0003-4819-151-4-200908180-00135

19. Wu R, Li B. A multiplicative-epistatic model for analyzing interspecific differences in outcrossing species. Biometrics. 1999;55(2):355-365. https://doi.org/10.1111/j.0006-341X.1999.00355.x

20. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med. 2002;21(11):1539-1558 https://doi.org/10.1002/sim.1186

21. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ. 1997;315(7109):629-634. https://doi.org/10.1136/bmj.315.7109.629

22. Hu Z, Liang J, Wang Z, et al. Common genetic variants in pre-microRNAs were associated with increased risk of breast cancer in Chinese women. Hum Mutat. 2009;30(1):79-84. https://doi.org/10.1002/humu.20837

23. Sluijter JP, van Mil A, van Vliet P, et al. MicroRNA-1 and -499 regulate differentiation and proliferation in human-derived cardiomyocyte progenitor cells. Arterioscler Thromb Vasc Biol. 2010;30(4):859-868. https://doi.org/10.1161/ATVBAHA.109.197434

24. Chen C, Zhang Y, Zhang L, Weakley SM, Yao Q. MicroRNA-196: critical roles and

clinical applications in development and cancer. J Cell Mol Med. 2011;15(1):14-23. https://doi.org/10.1111/j.1582-4934.2010.01219.x

25. Wei J, Gao W, Zhu CJ, et al. MiR-196a2 rs11614913 T > C polymorphism and risk of esophageal cancer in a Chinese population. Hum Immunol. 2013;74(9):1199-1205. https://doi.org/10.1016/j.humimm.2013.06.012

26. Parlayan C, Ikeda S, Sato S, et al. Association analysis of single nucleotide polymorphisms in miR-146a and miR-196a2 on the prevalence of cancer in elderly Japanese: a case-control study. Asian Pac J Cancer Prev. 2014;15(5):2101-2107. https://doi.org/10.7314/APJCP.2014.15.5.2101

27. Ryan BM, Robles AI, Harris CC. Genetic variation in microRNA networks: the implications for cancer research. Nat Rev Cancer. 2010;10(6):389-402. https://doi.org/10.1038/nrc2867

28. Yan W, Gao X, Zhang S. Association of miR-196a2 rs11614913 and miR-499 rs3746444 polymorphisms with cancer risk: a meta-analysis. Oncotarget. 2017;8(69):114344-114359. https://doi.org/10.7314/APJCP.2014.15.5.2101

29. Yang X, Li X, Zhou B. A Meta-Analysis of miR-499 rs3746444 Polymorphism for Cancer Risk of Different Systems: Evidence From 65 Case-Control Studies. Front Physiol. 2018;9:737. https://doi.org/10.3389/fphys.2018.00737

30. Jiang SG, Yang ZH, Yang L, et al. Lack of association between Hsa-Mir-499 rs3746444 polymorphism and cancer risk: meta-analysis findings. Asian Pac J Cancer Prev. 2015;16(1):339-344. https://doi.org/10.7314/APJCP.2015.16.1.339

31. Chen C, Yang S, Chaugai S, Wang Y, Wang DW. Meta-analysis of Hsa-mir-499 polymorphism (rs3746444) for cancer risk: evidence from 31 case-control studies. BMC Med Genet. 2014;15:126. https://doi.org/10.1186/s12881-014-0126-1

32. Xu Q, Liu JW, Yuan Y. Comprehensive assessment of the association between miRNA polymorphisms and gastric cancer risk. Mutat Res Rev Mutat Res. 2015;763:148-160. https://doi.org/10.1016/j.mrrev.2014.09.004

33. Umar M, Upadhyay R, Haque S, et al. Evaluation of common genetic variants in pre-microRNA in susceptibility and prognosis of esophageal cancer. Mol Carcinog. 2013;52 Suppl 1. https://doi.org/10.1002/mc.21931

34. Ma XP, Zhang T, Peng B, Yu L, Jiang de K. Association between microRNA polymorphisms and cancer risk Based on the findings of 66 case-control studies. PLoS One. 2013;8(11). https://doi.org/10.1371/journal.pone.0079584

35. Chu H, Wang M, Shi D, et al. Hsa-miR-196a2 Rs11614913 Polymorphism contributes to cancer susceptibility: evidence from 15 Case-control studies. PLoS One. 2011;6(3). https://doi.org/10.1371/journal.pone.0018108