REVIEW

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Meta-Analysis of Polymorphic Variants Conferring Genetic Risk to Cervical Cancer in Indian Women Supports *CYP1A1* as an Important Associated Locus

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Abstract

Objective: Association of multiple polymorphic variants with cervical cancer has been elucidated by several candidate gene based as well as genome-wide association studies. However, contradictory outcomes of those studies have failed to estimate the true effect of the polymorphic variants on cervical cancer. **Methods:** Literature mining of the PubMed database was done to gather all the publications related to genetic association with cervical cancer in India. Out of 98 PubMed hits only 29 genetic association studies were selected for meta-analysis based on specific inclusion criteria. A fixed-effect meta-analysis was performed to evaluate the overall association of the genetic polymorphisms with cervical cancer. Cochran's Q test was performed to assess between study heterogeneity. Publication bias was also estimated by funnel plots and Egger's regression test. Further, sub-group analysis was conducted by fixed-effect meta-regression to assess the impact of polymorphisms on cervical cancer in the presence of Human Papilloma Virus (HPV). **Result:** Following a fixed-effect model, meta-analysis was conducted that revealed 2 polymorphic variants viz. 'deletion polymorphism (Del2) (OR=1.79, 95% CI= 1.08-2.95, P=0.023) in GSTM1' and 'rs1048943 (OR = 2.34, 95% CI=1.37-3.99, P=0.0018) in *CYP1A1*' to be associated with cervical cancer. However, multiple testing correction showed only rs1048943 of *CYP1A1* to be significantly associated (P-value=0.029) with cervical cancer with significant publication bias (P-value=0.0113) as estimated by Egger's regression test. The polymorphic variants 'rs1801131', 'rs1801133', 'rs2430561', 'rs1799782', 'rs25486' and 'rs25487' showed significant ($p<0.05$) evidence of heterogeneity between studies by Cochran's Q test and also by heterogeneity index (I^2) calculation. **Conclusion:** Therefore, our study revealed significant association of rs1048943 in *CYP1A1*, but a nominal association of deletion polymorphism (Del2) in GSTM1 with cervical cancer, which provides a comprehensive insight on the true effect of the polymorphisms, reported in various case-control studies, on the risk of the development of cervical cancer in Indian women.

Keywords: Cervical cancer- meta-analysis- polymorphism- HPV- logistic regression

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Introduction

Cervical cancer is the fourth most prevalent cancer in women worldwide with nearly 85% of reported incidences in the lesser socioeconomically developed regions of the world (Ferlay et al., 2015). Highest incidence and mortality rates of cervical cancer are reported from sub-Saharan Africa, Central and South America, South-eastern Asia, Central and Eastern Europe (Ferlay et al., 2015). A statistically significant decline in the incidence of cervical cancer in India is evident from long-term hospital based patient registries (Sreedevi et al., 2015; Yeole et al., 2004). However, India still shares 25% of global incidences with highest age standardized occurrence in South Asia. In India, nearly122,844 women are diagnosed with cervical cancer and 67,477 die from the disease every year (Sreedevi et al., 2015) ranking second in cancer related deaths in women (International Collaboration of Epidemiological Studies of Cervical, 2006). Aizawl district of Mizoram, a north-eastern state of India, accounts for the highest reported incidences of cervical cancer followed by Barshi and Bengaluru (Sreedevi et al., 2015).

Cancer of the cervix is generally classified into two broad histotypes based on their site of origin, viz. squamous cell carcinoma from ectocervix and adenocarcinoma from endocervix (Sreedevi et al., 2015). Several epidemiological and clinical reports states the development of cervical cancer to be multifactorial where human papillomavirus (HPV) infection is considered to be a major player in the etiology of cervical cancer

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(Bruni et al., 2017; Vaccarella et al., 2006) with evidences of also being a risk factor for other anogenital cancers, such as, anal cancer, vulval cancer, vaginal cancer, penile cancer and head and neck cancers (Bruni et al., 2017). Nearly 70% of all reported cervical cancer cases worldwide are caused mainly by HPV serotypes 16 and 18 (Bruni et al., 2017; Khaliq et al., 2012). Apart from HPV infection, other factors like smoking (Vaccarella et al., 2006; Ylitalo et al., 1999), immunosuppression (International Collaboration of Epidemiological Studies of Cervical, 2006; Vaccarella et al., 2006) high parity (Hildesheim et al., 2001; International Collaboration of Epidemiological Studies of Cervical, 2006), chronic use of oral contraceptives (Smith et al., 2003; Ylitalo et al., 1999), age of first pregnancy (International Collaboration of Epidemiological Studies of Cervical Cancer., 2006) also act as modifiers conferring risk to the development of cervical cancer. Interestingly, HPV negative incidences of cervical cancer (Barreto et al., 2013; de Sanjose et al., 2010; Guan et al., 2012; Igidbashian et al., 2014; Lai et al., 2007; Li et al., 2011) have been reported to be associated with adenocarcinoma of the uterine endocervix with poor disease free survival (DFS) (Rodriguez-Carunchio et al., 2015). HPV infection of the cervical epithelium are transient in nature that elicits immune responses to counter the infection (Hakama and Day, 1986). Interestingly, 80-90% of HPV infections are neutralized by the immune system within few years, but persistent infections of the cervical epithelium due to compromised immunity are associated with intraepithelial lesions that turn into invasive cervical carcinoma (Moscicki et al., 2012; Shvetsov et al., 2009). This differential nature of immune action is well attributed to the variations in the genes involved in generation and modification of immune responses to viral antigens.

Candidate gene based association studies have identified the impact of genetic polymorphisms in the genes belonging to xenobiotic metabolism (Abbas et al., 2014; Jain et al., 2017; Joseph et al., 2006; Satinder et al., 2017; Sobti et al., 2006), DNA repair (Konathala et al., 2017; Nagpal et al., 2002; Singhal et al., 2013), cell cycle (Katiyar et al., 2003; Pillai et al., 2002; Saranath et al., 2002; Satinder et al., 2008; Singhal et al., 2013; Thakur et al., 2009) and immune responses (Gangwar et al., 2009; Kordi Tamandani et al., 2008; Shekari et al., 2012; Singh et al., 2008; Singh et al., 2009; Singhal et al., 2015; Sobti et al., 2008), in modifying the risk of cervical cancer. Several genome-wide association studies (Chen et al., 2013; Chen et al., 2016; Leo et al., 2017; Lin et al., 2016; Miura et al., 2016) revealed polymorphisms in human leukocyte antigen (HLA)/MHC genes involved in immune response pathways, pertaining to cervical carcinogenesis. The HLA genes are very complex and are difficult to assess the influence of the SNPs on cervical cancer risk based on standard SNP gene effect model (Wang et al., 2015). However, most of these reports were based on Caucasian, Chinese and Japanese populations (Chen et al., 2013; Chen and Gyllensten , 2015; Chen et al., 2016; Leo et al., 2017; Lin et al., 2016; Miura et al., 2016), and most of the identified risk alleles have not been adequately evaluated in Indian population. Till date, the overall effects of the genes and the genetic variations on cervical cancer risk in Indian population have not been determined due to contradictory findings. In one study conducted on 147 patients and 165 controls in south Indian population at Thiruvananthapuram, the GSTT1 null allele shows no significant association with cervical cancer (Joseph et al., 2006), but in another study conducted on 150 patients and 150 controls in north Indian population of Chandigarh, the same null allele of GSTT1shows significant association with cervical cancer (Satinder et al., 2017).The conflicting results in two different reports on the same variant could be due to small sample sizes, racial and/or ethnic differences and/or clinical and genetic heterogeneity between populations.

Therefore, the combined effect of the variant(s) on the Indian population to get an accurate estimate of association between a genetic variant and cervical cancer risk needs to be assessed by meta-analysis (Lee, 2015), which is a powerful statistical tool to evaluate the true effect of polymorphisms on the disease status by pooling the individual study data. The study aims to assess the overall influence of genetic variants on cervical cancer risk in the context of Indian populations.

Materials and Methods

Data extraction

Data mining was done from the PubMed database (www.ncbi.nlm.nih.gov/pubmed) for the appropriate studies using the following search strings: (polymorphisms/ single nucleotide polymorphisms /SNP/SNPs/SNVs/SNV/ Mutation) AND (cervical cancer /cervical carcinoma) AND (India). Selection of the studies for inclusion in the meta-analysis was done in accordance with the following criteria: (a) the studies should be based samples from Indian population, (b) the studies should have case-control genotype data for the polymorphic variant reported, (c) the studies should be full research articles, (d) the studies should have reports on covariate risk factors, such as HPV status and/or smoking status and/or use of oral contraceptives and/or age of menarche, menopause and first pregnancy and/or parity, (e) all the candidate genes based association studies were considered that were published till November 2017, and (f) the polymorphisms with reports in at least two different studies were considered. The following data were extracted from the selected reports: (1) first author, (2) year of publication, (3) mean age with standard deviation, (4) sex, (5) smoking status, (6) HPV status, (7) age of menarche and menopause, (8) age of marriage and first pregnancy, (9) parity, (10) use of oral contraceptives, (11) genetic polymorphisms and (12) genotype specific case-control data. On the basis of the aforementioned criteria 29 papers involving 16 polymorphisms from 11 genes were selected for further meta-analysis (Figure 1).

Selection of genetic model

Apart from Del1, Del2, rs1695, rs1800871 and rs1801133, an additive (allelic) model was followed for all the 11 polymorphisms that assume the effect of the heterozygous genotype as intermediate between the

two homozygotes. Del1, Del2, rs1695, rs1800871 and rs1801133 were analyzed in a recessive model.

Meta-analysis

Meta-analysis was performed in R, 3.4.2 (R Core Team, 2017) package 'metafor' (Viechtbauer, 2010) considering fixed-effect model (Borenstein et al., 2010), on genetic association reports pertaining to Indian population (Sengupta et al., 2017) ignoring ethnicity and geographical distribution stratification due to lack of sufficient data.

Evaluation of heterogeneity between selected studies

Cochran's Q test (P<0.10) (Huedo-Medina et al., 2006) was done to evaluate the heterogeneity of inter-study variations. Further, heterogeneity index (I^2) was calculated that measures the degree of inconsistency across studies using the formula: $I^2 = (Q-(n-1)\ n)/QX100\ \%$, where 'n' is the number of studies. I2 value is expressed as percentage with grade cut-offs as 25%, 50% or 75%, which signifies the presence of low-, mid- or high-grade heterogeneity, respectively (Bedi et al., 2011; Jiang et al., 2012; Jin et al., 2009; Vyas et al., 2013). Hence, heterogeneity between studies for all SNPs were evaluated using H^2 metrics at 10% level of significance and I^2 metric, (Sengupta et al. 2017).

Evaluation of publication bias among the selected studies

Qualitative estimation of publication bias was done by visual inspection of funnel plots (Sterne and Egger, 2001). Further, quantitative estimation of publication bias was done by Egger's regression test that evaluate the asymmetry of the funnel plots (P<0.05) using a weighted regression model with multiplicative dispersion, for only those polymorphic variants that are reported in 3 or more studies. Symmetry of the funnel plots shows absence of publication bias, whereas asymmetry depicts the presence of publication bias.

Evaluation of genetic association of reported polymorphisms with cervical cancer

Individual study level odds ratios and 95% confidence intervals (95% CI) for both additive (allelic) and recessive model along with their corresponding standard errors were first determined to evaluate the statistical association (p<0.05) between the reported polymorphisms and cervical cancer risk. Further a logistic regression of cancer status on variant genotype coded as 0 (homozygous wild type), 1 (heterozygous) and 2 (homozygous variant) was done. For, the four polymorphisms (Del1, Del2, rs1695, rs1800871 and rs1801133); 0 (homozygous wild type + heterozygous), 1 (homozygous variant) coding was used.

Results

Study characteristics

Extensive mining of PubMed (www.ncbi.nlm.nih.gov/pubmed) database generated 98 articles for the aforementioned search strings. Further textmining of the 98 articles identified 29 articles (Table 1) that actually fit all the inclusion criteria of the study proposed. The reported variables of the studies included in this meta-analysis are summarized in (Table A1). However, case-control genotype data of all the 16 polymorphisms were extracted from 29 selected articles (Table A2). The case-control data for covariates, particularly tobacco smoking, mean age at sampling, HPV status, age at menarche, age at menopause, age at first pregnancy, parity and use of oral contraceptives, were recorded from 29 selected articles for meta-analysis (Table A3).

Meta-analysis of reported polymorphisms to determine the overall association with cervical cancer through

Meta-analysis was performed in R package 'metafor' using Fixed-effect model for 16 polymorphisms from 11 genes reported on Indian population. For 12 polymorphisms, study-specific crude Odds Ratio (OR) estimates and 95% Confidence Interval (CI) were determined using additive (allelic) model and for the remaining 4 polymorphisms recessive model was used. Due to few available study reports for the 16 polymorphisms in the context of Indian population, stratification based on the geographical distribution was not performed for the meta-analysis due to lack to consistent data. The polymorphic variants 'rs1801131', 'rs1801133', 'rs2430561', 'rs1799782', 'rs25486' and 'rs25487' were found to exhibit significant heterogeneity (P <0.10) between studies as revealed by Cochran's Q test. The variants 'rs1799782' and 'rs25486' of XRCC1 gene showed high-grade heterogeneity according to I^2 metric (I2>75%) while the variants 'rs1801131', 'rs1801133' and 'rs25487' showed mid-grade heterogeneity (I^2>50%). The remaining 10 polymorphic variants showed low-grade or no heterogeneity (Table 2). After meta-analysis, 2 polymorphisms, viz. del2 (OR=1.79, 95% CI= 1.08-2.95, P=0.023) and rs1048943 (G versus A: crude OR = 2.34,

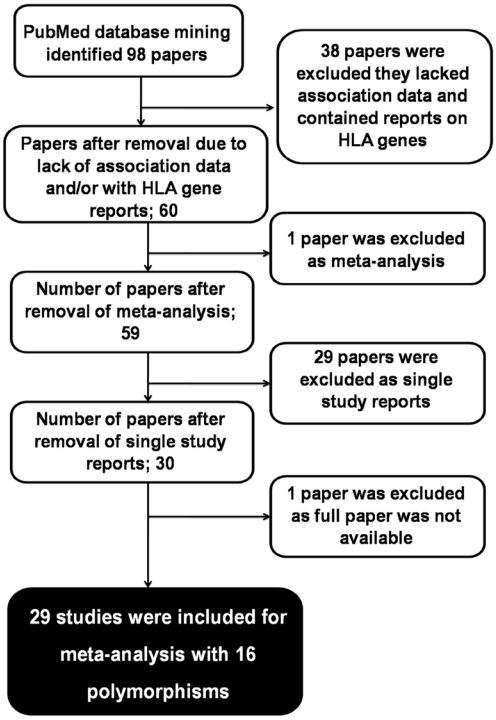

Figure 1. A Workflow for the Selection of Studies from PubMed Repository

Table 1. The List of Candidate Gene-Based Association Studies Included in the Meta-Analysis along with the Study Numbers, First Author, Year of Publication, Covariate Counts and Reference Urls for PubMed Search

First Author Year	Study Number	Number of Cases	Number of Controls	Smokers in Cases	Smokers in Controls	Non-Smokers in Cases	Non-Smokers in Controls	Mean Age of Cases	Mean Age of Controls	Number of HPV positives in Cases	Number of HPV positives in Controls	Number of HPV negative in Cases	Number of HPV negative in Controls	Geographical Location of the Study	Reference URLs
Jain V et al.2017	Study 20	100	100	0	0	0	0	50.9±8.2	44.05±10.4	0	0	0	0	Raipur	https://www.ncbi.nlm.nih.gov/pubmed/28433806
Satinder K et al.2017	Study 25	150	150	0	0	0	0	48.5±9.4	46.1±11.2	0	0	0	0	Chandigarh	https://www.ncbi.nlm.nih.gov/pubmed/28361858
Bajpai D et al.2016	Study 28	65	68	13	5	52	63	43.9±0.0	43.2±10.3	61	12	4	56	New Delhi	https://www.ncbi.nlm.nih.gov/pubmed/25812040
Konathala G et al.2016	Study 29	125	150	0	0	0	0	0	0	0	0	0	0	Visakhapatnam	https://www.ncbi.nlm.nih.gov/pubmed/27942558
Sharma A et al. 2015	Study 3	135	457	22	39	113	418	42.1±11.7	41.1±8.9	89	91	46	366	New Delhi	https://www.ncbi.nlm.nih.gov/pubmed/26434855
Abbas M et al. 2014	Study 1	200	208	13	9	74	119	48.54±9.529	48.09±8.347	0	0	0	0	Lucknow	https://www.ncbi.nlm.nih.gov/pubmed/24657182
Singhal P et al.2014	Study 5	256	250	0	0	0	0	48.6±11	46.07±6.6	229	12	27	238	New Delhi	https://www.ncbi.nlm.nih.gov/pubmed/25412954
Singhal P et al.2013	Study 12	182	182	0	0	0	0	49±0.0	49±9.64	166	8	16	174	New Delhi	https://www.ncbi.nlm.nih.gov/pubmed/23210739
Shekari M et al.2012	Study 7	200	200	90	62	110	138	48.55±9.43	48.81±9.64	0	0	0	0	Chandigarh	https://www.ncbi.nlm.nih.gov/pubmed/22157213
Prasad et al.2011	Study 16	62	241	0	0	0	0	0	0	0	0	0	0	Andhra Pradesh	https://www.ncbi.nlm.nih.gov/pubmed/21934341
Kohar I et al. 2010	Study 18	203	231	0	0	0	0	49.4±12.4	48.2±10.2	173	2	30	229	New Delhi	https://www.ncbi.nlm.nih.gov/pubmed/19793004
Singh H et al.2009	Study 6	150	162	0	0	0	0	47.2±8.8	48.3±8.3	0	0	0	0	Lucknow	https://www.ncbi.nlm.nih.gov/pubmed/19823053
Gangwar R et al.2009	Study 8	200	230	62	32	138	198	44.8±9.3	46.7±9.9	0	0	0	0	Lucknow	https://www.ncbi.nlm.nih.gov/pubmed/19681846
Thakur N et al.2009	Study 26	200	200	0	0	0	0	49±11.3	49±11.3	0	0	0	0	New Delhi	https://www.ncbi.nlm.nih.gov/pubmed/19489683
Kordi TMK et al.2008	Study 9	200	200	90	62	110	138	48.55±9.43	48.81±9.9	0	0	0	0	Chandigarh	https://www.ncbi.nlm.nih.gov/pubmed/18806746
Singh H et al.2008	Study 10	150	162	0	0	0	0	47.2±8.8	48.3±9.64	0	0	0	0	Lucknow	https://www.ncbi.nlm.nih.gov/pubmed/18333945

Table 1. Continued

First Author Year	Study Number	Number of Cases	Number of Controls	Smokers in Cases	Smokers in Controls	Non-Smokers in Cases	Non-Smokers in Controls	Mean Age of Cases	Mean Age of Controls	Number of HPV positives in Cases	Number of HPV positives in Controls	Number of HPV negative in Cases	Number of HPV negative in Controls	Geographical Location of the Study	Reference URLs
Sobti RC et al.2008	Study 11	150	200	65	57	83	74	48.55±9.43	48.81±8.3	0	0	0	0	Chandigarh	https://www.ncbi.nlm.nih.gov/pubmed/18154955
Nandan NK et al.2008	Study 17	142	77	0	0	0	0	39±5	39±5	0	0	0	0	New Delhi	https://www.ncbi.nlm.nih.gov/pubmed/19356065
Shekari M et al.2008	Study 19	200	200	2	6	110	138	48.55±9.43	48.81±9.64	0	0	0	0	Chandigarh	https://www.ncbi.nlm.nih.gov/pubmed/18351371
Satinder K et al.2008	Study 27	150	150	67	62	83	88	48.5±9.4	46.1±11.2	0	0	0	0	Chandigarh	https://www.ncbi.nlm.nih.gov/pubmed/18548202
Joseph T et al.2006	Study 2	222	90	0	0	0	0	46±10.3	47±9.2	111	32	36	280	Thiruvananthapuram	https://www.ncbi.nlm.nih.gov/pubmed/16360200
Sobti RC et al.2006	Study 4	103	103	45	13	58	90	48.6±9.9	48±11.3	0	0	0	0	Chandigarh	https://www.ncbi.nlm.nih.gov/pubmed/16631467
Bhattacharya P et al.2005	Study 15	120	205	0	0	0	0	0	0	82	84	38	121	Kolkata	https://www.ncbi.nlm.nih.gov/pubmed/16054204
Mitra S et al. 2004	Study 21	61	94	0	0	0	0	46.89±10.29	47.44±12.53	52	17	9	77	Kolkata	https://www.ncbi.nlm.nih.gov/pubmed/15623478
Katiyar S et al.2003	Study 23	163	74	0	0	0	0	0	0	128	0	35	74	New Delhi	https://www.ncbi.nlm.nih.gov/pubmed/14577584
Pillai MR et al.2002	Study 13	311	110	0	0	0	0	0	0	201	14	110	96	Thiruvananthapuram	https://www.ncbi.nlm.nih.gov/pubmed/12458344
Bhattacharya P et al.2002	Study 14	55	201	0	0	0	0	0	0	46	84	9	117	Kolkata	https://www.ncbi.nlm.nih.gov/pubmed/12406566
Nagpal JK et al. 2002	Study 22	111	29	0	0	0	0	0	0	77	11	34	18	Cuttack	https://www.ncbi.nlm.nih.gov/pubmed/12534455
Saranath D et al. 2002	Study 24	337	164	0	0	0	0	0	0	258	37	79	127	Mumbai	https://www.ncbi.nlm.nih.gov/pubmed/12144822

Table 2. Combined Statistical Association of the Respective Variants with Cervical Cancer; with Crude Odds Ratio (OR), 95% CI and Heterogeneity Indices, I2, H2. P-values are adjusted by Benjamini-Höchberg FDR Correction.

Variants	Number of Studies	Odds Ratio (OR)	95% CI	P-value	I^2	H^2	Het.stat	Het.P.value	P_{adj}
del 1	4	0.8347	0.468-1.487	0.5399	30.2584	1.4339	3.0935	0.3774	0.7853
del 2	4	1.7881	1.081-2.955	**0.0234**	16.6965	1.2004	1.8402	0.6062	0.1873
rs1695	2	1.2124	0.411-3.574	0.7270	0.0271	1.0003	0.0234	0.8784	0.8331
rs1800871	2	0.6886	0.360-1.316	0.2592	0.0151	1.0002	0.0175	0.8949	0.5428
rs1801133	4	1.0987	0.645-1.870	0.7290	**67.7116**	3.0971	9.6305	**0.0220**	0.8331
rs1801131	2	0.5838	0.287-1.183	0.1357	**69.8727**	3.3192	3.6057	**0.0576**	0.4844
rs4646903	4	1.3821	0.899-2.123	0.1400	41.2724	1.7028	3.8478	0.2784	0.4844
rs1048943	**3**	**2.3384**	**1.371-3.987**	**0.0018**	**11.2109**	**1.1263**	**1.0149**	**0.6020**	**0.0290**
rs1800872	2	1.3058	0.773-2.205	0.3185	40.0435	1.6679	1.5370	0.2151	0.5662
rs2430561	2	1.0410	0.608-1.780	0.8835	**67.7555**	3.1013	3.3542	**0.0670**	0.8835
Rs16944	2	1.4198	0.760-2.651	0.2714	15.9649	1.1900	0.7187	0.3966	0.5428
rs1042522	8	0.8134	0.600-1.101	0.1816	23.3077	1.3039	5.4461	0.6057	0.4844
rs603965	2	1.2190	0.708-2.096	0.4740	19.9871	1.2498	0.8427	0.3586	0.7584
rs1799782	2	0.9418	0.488-1.814	0.8578	**92.3336**	13.0439	13.7906	**0.0002**	0.8835
rs25486	2	1.5943	0.817-3.110	0.1713	**85.5546**	6.9226	7.5016	**0.0062**	0.4844
rs25487	2	1.1416	0.614-2.121	0.6752	**66.7560**	3.0081	3.2455	**0.0716**	0.8331

P value<0.05*; Cochran's Q test P-value<0.10

Table 3. Egger's Regression Test for Funnel Plot Asymmetry

Gene name	Variant I.D	t	df	P-value*
GSTT1	del 1	0.6324	2	0.5918
GSTM1	del 2	0.1516	2	0.8934
MTHFR	rs1801133	-0.5631	2	0.6301
XRCC1	rs25487	1.6167	4	0.1812
TP53	rs1042522	-0.4649	6	0.6584
CYP1A1	rs4646903	-0.6117	2	0.603
CYP1A1	**rs1048943**	**56.5441**	**1**	**0.0113**

*P<0.05

95% CI=1.37-3.99, P=0.0018) were found to be associated (p< 0.05) with cervical cancer. Further, multiple-testing adjustment by Benjamini-Höchberg FDR correction (p<0.05) for 16 variants revealed only rs1048943 of CYP1A1 to be associated (P=0.029) with cervical cancer based on additive (allelic) model in Indian population (Table 2). The association of rs1048943 of CYP1A1 and Del2 of GSTM1 with cervical cancer are shown in forest plots (Figure 2-a, Figure 3-a). Publication bias was qualitatively assessed by visual inspection of funnel plots that revealed evidence of publication bias for rs1048943 of CYP1A1gene but not for Del2 of GSTM1 (Figure 2-b, Figure 3-b). Further, Egger's regression test for the quantitative assessment of funnel plot asymmetry (critical p<0.05) also showed evidence of significant publication bias (P=0.0113) for rs1048943 of CYP1A1gene (Table 3). However, the individual and overall study effects for each of the remaining fourteen variants (OR estimates, 95% CI) are also depicted in forest plots (Figures 1-14; Supplement and Supporting Data).

Evaluation of covariate stratified genetic association of reported polymorphic variants with cervical cancer

The effect of the polymorphic variants on the development of cervical cancer influenced by the covariate risk factors, such as HPV status, was estimated. We collected HPV stratified summary data from the literature for only rs1042522 of TP53 due availability of sufficient data (Table A4). Study specific summary data viz. β-coefficient (log OR) and standard errors (SE) was obtained 7 studies for rs1042522 respectively. For some articles, stratified β-coefficient and SE were calculated using logistic regression from the covariate stratified genotype counts provided in the tables. The remaining 15 polymorphic variants were not stratified based on any of the covariate risk factors due to lack of consistent covariate stratified summary data in the selected studies.

Sub-group meta-analysis of rs1042522 of TP53 was done following the fixed-effect model (Borenstein et al., 2010). Using the covariate specific summary data separately within the "HPV positive" and "HPV negative" sub-groups. The sub-group meta-analysis stratified by

Table 4. Sub-Group Meta- Analysis of rs1042522 of TP53 with Cervical Cancer, Stratified by HPV Status, i.e. HPV Positive and HPV Negative. OR, *Odds Ratio (OR); 95% CI and Heterogeneity indices, I^2, H^2*

Sub-Groups	Number of Studies	Odds Ratio (OR)	95% CI	P-value	I^2	H^2	Het-stat	P-value (Het.)
For rs1042522 of TP53								
HPV positive	7	1.0743	0.7154-1.6132	0.7290	25.1340	1.3357	4.1915	0.6508
HPV negative	7	1.0177	0.6641-1.5594	0.9350	27.9676	1.3883	4.8911	0.5578

*P-value< 0.05

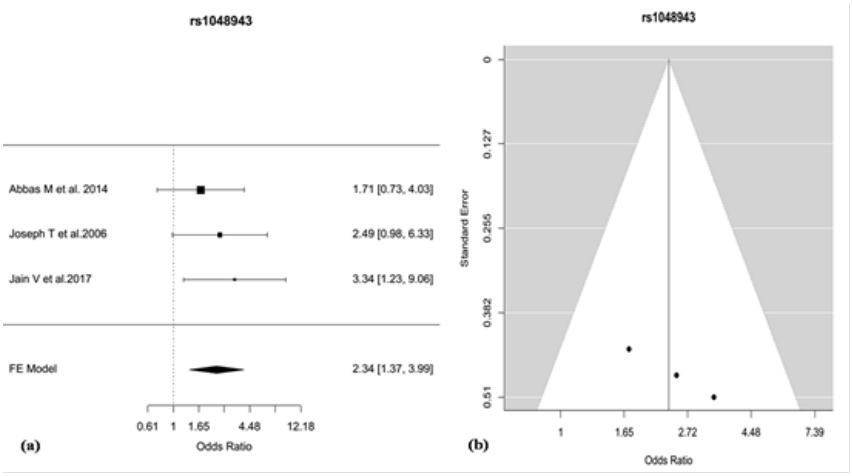

Figure 2. (a) Forest Plot for the Odds Ratios (ORs) of the SNP rs1048943 of *CYP1A1* for the Association with Cervical Cancer, (b) Funnel Plot, Showing Evidence of Publication Bias between the Studies Reporting the SNP rs1048943.

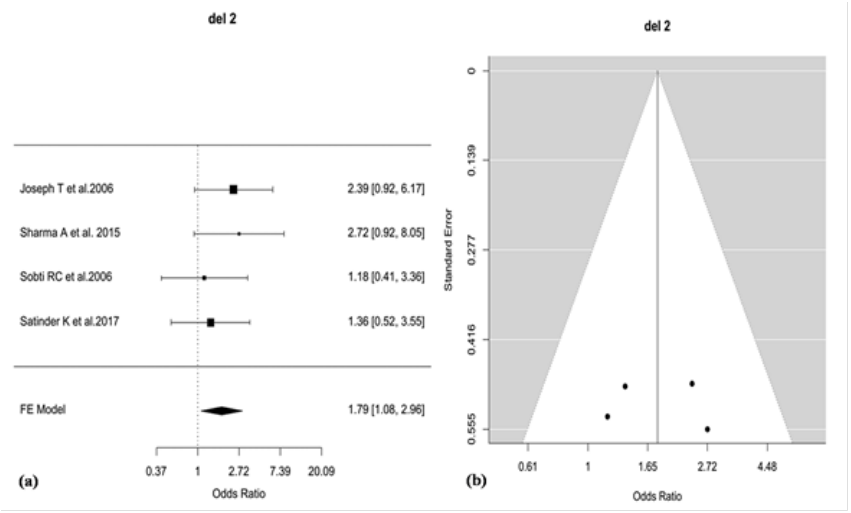

Figure 3. (a) Forest Plot for the Odds Ratios (ORs) of the Deletion Polymorphism (del2) of *GSTM1* for the Association with Cervical Cancer, (b) Funnel Plot, Showing no Evidence of Publication bias between the Studies Reporting del2.

HPV status revealed lack of significant associations with cervical cancer (Table 4) as depicted in forest plots (Figure 4).

Test for effect modification of rs1042522 of TP53 by HPV status using a fixed effect meta-regression model was done with all the sub-group specific summaries and specifying covariate status as a moderator variable in R package 'metafor' (Viechtbauer 2010). The difference in effect size among HPV positive individuals and HPV negative individuals as captured by the coefficient of the moderator term revealed that rs1042522 has no significant effect modification on cervical cancer risk

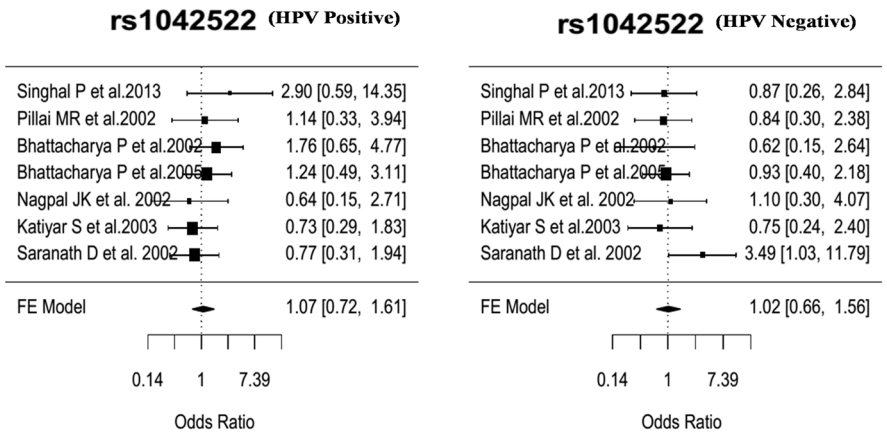

Figure 4. Forest Plot for the Odds Ratios (ORs) of the rs1042522 of TP53 for the Association with Cervical Cancer Stratified by HPV Status in Sub-Group Analysis between HPV Positive and HPV Negative.

by HPV status (Moderator Effect size (θ)= 0.05415944, θlow= -0.5352891, θhigh= 0.6435892; P= 0.85).

Discussion

Cervical cancer is a complex disease and is essentially an outcome of virus induced carcinogenesis. Human Papilloma virus (HPV) is the primal cause of cervical carcinogenesis but other environmental and social factors, like tobacco smoke, age of marriage and first pregnancy, use of oral contraceptive, number of sexual partners, modify the risk of the cancer (Fonseca-Moutinho, 2011; Hildesheim et al., 2001; Igidbashian et al., 2014; Vaccarella et al., 2006; Ylitalo et al., 1999). HPV infection acts as the primary etiological factor for the development of cervical cancer but all HPV infections do not result in cervical cancer. The immune system is well equipped to counter the viral attack and in most of the infected individuals the viral load is cleared out or become dormant by the immune responses. Persistent HPV infection along with other risk modifiers synergistically act to cause cellular transformations that leads to cervical cancer (Bosch et al., 2002; Hakama and Day, 1986; Moscicki et al., 2012). Recent candidate gene based investigations show genetic association of several polymorphisms with cervical cancer (Abbas et al., 2014; Bajpai et al., 2016; Bhattacharya et al., 2002; Bhattacharya and Sengupta, 2005; Gangwar et al., 2009; Jain et al., 2017; Joseph et al., 2006; Katiyar et al., 2003; Kohaar et al., 2010; Konathala et al., 2017; Kordi Tamandani et al., 2008; Mitra et al., 2005; Nagpal et al., 2002; Pillai et al., 2002; Prasad and Wilkhoo 2011; Saranath et al., 2002; Satinder et al., 2008; Satinder et al., 2017; Sharma et al., 2015; Shekari et al., 2008; Shekari et al., 2012; Singh et al., 2008; Singh et al., 2009; Singhal et al., 2013; Singhal et al., 2015; Sobti et al., 2006; Sobti et al., 2008; Thakur et al., 2009). A genome-wide association study (GWAS) revealed three haplotypes, HLA-DRB1*15/HLA-DQB1*0602/HLA-DQA1*0102, HLA-B*0702/HLA-C*0702, and HLA-DRB1*0401/HLA-DQA1*0301, to be associated with increased risk of both HPV16 and HPV18-driven development of both squamous cell carcinoma and adenocarcinoma of the cervix in populations of European descent (Leo et al., 2017). Another GWAS reported four new SNPs, viz. rs6812281, located at 4q34.3 ($P < 5.0 \times 10^{-8}$) rs4590782, located at 10q26.2 ($P = 1.59 \times 10^{-5}$), rs1742101 located at 14q32.11 ($P = 7.11 \times 10^{-6}$), and rs1364121 located at 16q23.3 ($P = 3.15 \times 10^{-6}$), that exhibits strong evidence of associations with response to neoadjuvant chemotherapy in cervical cancer in Chinese women (Li et al., 2017). More GWAS reports on Swedish population supported the association of previously identified loci at 6p21.3 (rs9271898, $P = 1.2 \times 10^{-24}$; rs2516448, 1.1×10^{-15}; and rs3130196, 2.3×10^{-9}, respectively) with cervical cancer. The study also confirmed associations of cervical cancer with reported classical HLA alleles including HLA-B*07:02, -B*15:01, -DRB1*13:01, -DRB1*15:01, -DQA1*01:03, -DQB1*06:03 and -DQB1*06:02. Further, an independent signal at rs73730372 at 6p21.3 ($P = 3.0 \times 10^{-19}$) was found to be an expression quantitative trait locus (eQTL) of both HLA-DQA1 and HLA-DQB1 conferring protection to cervical cancer (CIN3) (Chen et al., 2016).

This study is the first comprehensive meta-analysis from India on the available candidate gene based genetic association studies that gives us the most risk polymorphic variant responsible for the development of cervical cancer based on the available reports. Our meta-analysis revealed rs1048943 (exon 7, A>G) of CYP1A1 gene to be significantly associated with cervical cancer (Figure 2a) after Benjamini-Höchberg FDR correction (p=0.029). The absence of inter-study heterogeneity for the variant rs1048943 of CYP1A1 suggests that the association of the polymorphic variant with cervical cancer holds true. Moreover, the studies for the variant rs1048943 of CYP1A1 exhibits significant publication bias as confirmed by Egger's regression test (Table 3) and visual inspection of funnel plots (Figure 2b), which indicates a probable overestimation under the influence of publication bias. For rs1048943 of CYP1A1, sufficient covariate-stratified summary data is absent to perform sub-group analysis that led to the failure of the determination of the true effect of the variant under the influence of covariate risk factors. Therefore, more studies are needed to gain a comprehensive insight on the overall and covariate-stratified effect of rs1048943 of CYP1A1 on cervical cancer biology.

Meta-analysis of rs1042522 of TP53 stratified by HPV status also revealed lack of significant association with cervical cancer in any of the sub-groups. However, No significant statistical interaction (effect modification) was found for rs1042522 of TP53 with HPV infection through our meta-regression analysis, which implies that the polymorphism has no modifier effect on cervical cancer by the status of HPV infection in Indian women. However, we surmise that there may be interaction in the biological mechanisms conferring susceptibility towards the development of cervical cancer. Due to wide variation in covariate-adjusted or covariate-stratified summary data in the various studies, moderate degree of heterogeneity could have led to loss of power in detecting effect modification. Due to inconsistency in reporting subgroup level summaries or covariate adjusted summaries across studies, we restricted our sub-group analysis with only rs1042522 of TP53. Further, we were unable to perform sub-group analysis based on covariates, such as age, sex, cancer subtypes, age of marriage, age at first pregnancy, use of oral contraceptives due to lack of sufficient reports on Indian population.

The SNP rs1048943 of CYP1A1 was found to be significantly associated with cervical cancer mostly in Caucasian and East Asian population (Wang et al., 2015) and lung cancer in Indian population (Sengupta et al., 2017), which shows colinearity of our finding on Indian population. The CYP1A1 (Cytochrome P4501A1; 15q22-24) gene encodes an essential phase I xenobiotic metabolism enzyme (512 aa length) found mainly in the endoplasmic reticulum and cytosol. The enzyme generates highly reactive electrophilic compounds from pro-carcinogenic xenobiotics, like polycyclic aromatic

hydrocarbons (PAHs) and enhances the formation of genotoxic DNA adducts that results in mutagenesis and cellular transformation. CYP1A1 is also involved in the metabolism different drugs and endogenous steroid hormone molecules like oestrogen. The SNP rs1048943A>G in the exon 7 of CYP1A1 gene is a point mutation that results in the substitution of isoleucine by valine (Ile>Val) in the crucial heme-binding domain, which increases enzyme activity and confers risk of cervical carcinogenesis. Cervical carcinogenesis occurs due the synergistic effect of HPV infection and oestrogen metabolism (den Boon et al., 2015). In the development of cervical carcinogenesis the expression of oestrogen receptor alpha (ER-α) ablates in tumor cells but retained in tumor-associated stromal fibroblasts that alters several cross signaling pathways between stroma and tumor (den Boon et al., 2015). Cytochrome P4501A1 enzyme is involved in oxidation of oestrogen to catechols and oestrogen quinones (Spink et al., 1992; Zhang et al., 2007) that forms mutagenic stable and depurinating DNA adducts (Zhang et al., 2007; Zhu and Conney, 1998) facilitating HPV integration in the host genome (Joseph et al., 2006). Moreover, a polymorphism in CYP1A1 has been found to be associated with extended time for clearance of high risk HPV infection from cervical epithelia (Sudenga et al., 2014).

Our meta-analysis also revealed a marginal association of GSTM1 null genotypes with cervical cancer. Glutathione-S-transferases (GSTs) are a family of multifunctional enzymes that catalyzes the conjugation of glutathione to electrophilic substrates resulting in their enhanced renal clearance reducing carcinogenic load from the cell. The null genotype of GSTM1 has been reported to be associated with enhanced risk to cervical cancer (Hasan et al., 2015; Nunobiki et al., 2015) that justified our finding. Cervical cancer is an outcome of the combined effect of HPV infection with environmental and hormonal influence. Interestingly, our meta-analysis pooled out 11 genes that includes genes belonging to xenobiotic metabolism, DNA repair, cell cycle and immune response regulation. Probably these genes play as 'modifiers' that enhances the chance and severity of HPV infection in the cervical epithelia. Tobacco smoking has been reported as one of the major modifiers in cervical cancer risk (Fonseca-Moutinho, 2011; Ylitalo et al., 1999).

With the inclusion of more studies on genetic association with cervical cancer on different populations and sub-populations with contradictory outcomes; meta-analysis would become a very important tool to estimate the true effect of those variants on the cancer status considering the covariate stratifications of the studied population. Therefore, identification of the genetic variants for which there is evidence of influence on cervical cancer risk through meta-analysis, would provide new insights into the candidate biological pathways involved in the development of cervical cancer. This would further benefit in the assessment of population specific risk for accurate decision making, which could be of potential value in targeting primary prevention and population specific cervical cancer screening modalities and therapeutic interventions.

Our meta-analysis showed rs1048943A>G, in exon 7 of CYP1A1 to be associated with cervical cancer even after Benjamini-Höchberg FDR correction (P=0.029). However, the remaining variants failed to show significant association with cervical cancer after multiple-testing adjustment, which might be due to small sample size, ethnic differences or probably due to lack of sufficient data. Therefore, more studies on Indian population are needed along with covariate-adjusted summary data from individual studies, to gain more statistical power and accuracy for such a study.

Authors Contribution Statement

D. Sengupta and M. Sengupta conceptualized and designed the study. U. Guha, S. Mitra, S. Ghosh and D. Sengupta searched literature and extracted data. S. Bhattacharjee, M. Sengupta and D. Sengupta analyzed the data. D. Sengupta drafted the manuscript with important intellectual inputs from S. Bhattacharjee and M. Sengupta. S. Bhattacharjee and M. Sengupta critically revised the manuscript, figures, tables and supplementary data and supervised the entire work. All the authors gave approval for submission of the current version of the manuscript for publication and have full access to the study data.

Competing Financial Interests

The author(s) declare no competing financial interests.

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