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Family history of cancer and the risk of squamous cell carcinoma of oesophagus: a case–control study in Kashmir, India

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Background: Only a few studies have examined the association between family history of cancer (FHC) and the risk of oesophageal squamous cell carcinoma (ESCC) in high incidence areas of ESCC. We conducted a case–control study to evaluate the relationship between FHC and ESCC risk in Kashmir, India, with analysis of detailed epidemiological data and information on multiple gene polymorphisms.

Methods: We collected detailed information on FHC and a number of socio-demographic and lifestyle factors, and also obtained blood samples for genetic analysis from 703 histopathologically confirmed ESCC cases and 1664 individually matched controls. Conditional logistic regression models were used to calculate odds ratios (ORs) and 95% confidence intervals (95% CIs).

Results: Participants who had FHC showed a strong association with ESCC risk, and the risk was stronger when first-degree relatives (FDRs) had FHC (OR = 6.8; 95% CI = 4.6–9.9). Having a sibling with a cancer showed the strongest association (OR = 10.8; 95% CI = 6.0–19.3), but having a child with a cancer was not associated with ESCC risk. A history of any cancer in the spouse was also associated with ESCC risk (OR = 4.1; 95% CI = 1.6–10.2). Those with two or more relatives with FHC were at a higher risk of ESCC. After restricting FHC to familial ESCC only, the above associations were strengthened, except when spouses were affected with ESCC (OR = 2.5; 95% CI = 0.7–8.9). When we examined the associations between several single-nucleotide polymorphisms and ESCC in those with and without FHC, the associations of variant genotypes in cytochrome P450 (*CYP) 2C19* and *CYP2D6* and the wild genotype of *CYP2E1* with ESCC were much stronger in those with FHC. The FHC had an additive interaction with several risk factors of ESCC in this population.

Conclusion: Our results showed that FHC was strongly associated with ESCC risk in Kashmir. It seems both genetic factors and shared environment are involved in this association.

Currently, oesophageal cancer is the eighth most common malignancy and its poor prognosis makes it the sixth most common cause of deaths from cancer globally (Jemal *et al*, 2011; Levine and Rubesin, 2005). The predominant histological type of oesophageal cancer is the oesophageal squamous cell carcinoma (ESCC), for which 90% of cases are from Asian regions (Gholipour *et al*, 2008; Jemal *et al*, 2011) like northeastern Iran (Islami *et al*, 2009) and northern China (Tran *et al*, 2005). The other high-risk regions of the world include Transkei region in South Africa (Pink *et al*, 2011), Calvados region in France (Desoubeaux *et al*, 1999), northeast Italy and Uruguay (Franceschi *et al*, 1995; De Stefani *et al*, 2003). The ESCC incidence not only differs between high-

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and low-risk areas (where difference might go up to 200-fold (Lukanich, 2003), but it also shows a huge variation among its high-risk regions. The geographical variation in ESCC incidence is attributed to the presence or absence of different environmental or lifestyle risk factors of ESCC in different populations.

Under similar exposures, the ESCC risk is not same across different populations, ethnic groups or even in men and women (Louwman *et al*, 2010; Thrift *et al*, 2012; Zhong *et al*, 2013). This inter-individual difference in ESCC risk under similar exposures is partially attributed to genetic markers harboured by an individual that modulate the effect of environmental and other exposures (Wang *et al*, 2004; Guengerich, 2008), which are most likely inherited into descendants. The frequent occurrence of cancers in a family suggests a heritable genetic predisposition for ESCC. In other words, family history of cancer (FHC) can be a proxy of genetic vulnerability among the family members (especially in the first-degree relatives, FDRs), who usually have common genetic background. The outcome of prospective twin cohort (Lichtenstein *et al*, 2000), segregation and migration studies (Zhang *et al*, 2000) as well as findings like onset of ESCC at a younger age (Jia *et al*, 2014) and the familial clustering of ESCC (Chang-Claude *et al*, 1997) strongly support the inheritance of genetic susceptibility of ESCC. In addition, FHC can also reflect sharing of same environmental exposures by members of a family (Kato *et al*, 1990).

Unlike low-risk regions of the ESCC (Dhillon *et al*, 2001), the FHC has been positively associated with the ESCC risk in high incidence areas (Gao *et al*, 2009; Turati *et al*, 2013). Till date, only one study has reported some genetic loci/markers, linked with hereditary of ESCC risk (Ko *et al*, 2014). Identification of more loci/genetic markers will help to understand the genetic component of ESCC risk associated with FHC further. Xenobiotic metabolising enzymes (XMEs) are involved in several defense mechanisms by handling the toxic environmental exposures (Xing *et al*, 2003; Yang *et al*, 2005a, b). The various polymorphisms in XME genes result in their activity differences, hence individuals who harbour different variants of such genes can unlikely have a similar risk of a cancer. Hence, XME markers can be important in studying the genetic bases of FHC. The analysis of the ESCC risk due to XME markers in combination with FHC is not explored yet. However, a study (Wu *et al*, 2011b) has analysed the modifying effect of FHC on relationship of oesophageal cancer with its various environmental risk factors.

In Kashmir, ESCC is the most common cancer (Rasool *et al*, 2012) both in males and females with age-standardised incidence rates of 42.6 in men and 27.5 in women per 10^5 person-years (Khuroo *et al*, 1992). A number of risk factors have been associated with the ESCC susceptibility in Kashmir, including tobacco use (Dar *et al*, 2012), low socioeconomic status (Dar *et al*, 2013b), daily and close animal contact (Dar *et al*, 2014b), poor oral hygiene (Dar *et al*, 2013a), salt tea intake (Dar *et al*, 2014a) and consumption of N-nitrosamine containing food items (Siddiqi *et al*, 1988) as well as certain genetic markers (Bhat *et al*, 2014; Makhdoomi *et al*, 2015). Neither relative risks of such factors nor their prevalence in Kashmir is of a reasonable magnitude to explain the high incidence of ESCC in Kashmir. Nevertheless, these finding, suggest the exposure of the Kashmiri population to a range of toxic substances, which are handled by the XMEs. In addition, Kashmir is a non-migrant pure ethnic Muslim-dominated population (Ayub *et al*, 2011) and consanguineous marriages are common (Fareed and Afzal, 2014), hence the frequent admixture of genetic markers is unlikely to happen. Therefore, studying the possible familial factors (genetic as well as environmental) might give deeper insight into the ESCC aetiology. Hence, we carried out a case–control study to investigate in detail the relationship of FHC with ESCC risk and modifying effects of FHC on various risk factors of ESCC.

MATERIALS AND METHODS

Case–control selection and data collection. Details of the subject selection and recruitment are provided elsewhere (Dar *et al*, 2012). Briefly, all ESCC cases were recruited at the Regional Cancer Centre and Department of Radiation Oncology of Sher-i-Kashmir Institute of Medical Sciences (SKIMS), Srinagar, from September 2008 to January 2012. All cancer patients in Kashmir are referred to SKIMS, the only available tertiary care hospital in Kashmir. Cases with histopathologically confirmed ESCC, who were above the age of 18 years, had not received any treatment and did not have any personal history of cancer, were invited to participate in this study. For each case subject, we attempted to recruit at least one hospital-based control, individually matched to the case for sex, age (± 5 years) and place of residence. The controls were recruited from in-patient wards of SKIMS, the Government Medical College Hospital, Srinagar, and district hospitals to ensure the controls are residence matched to their respective cases. The refusal rate for cases and controls was only 4% (30 refusals, out of 733 invited) and 2% (33 refusals out of 1697 invited), respectively. The majority of those who refused were too ill to participate in the study. The maximum interval between recruitment of cases and their controls was 6 months. We included a large number of diseases that allowed us to catch a larger section of the population and increased the representation of the population. However, only those patients were enrolled as controls when the disease for which they had been admitted, did not have a strong association with tobacco or alcohol consumption (the two main factors associated with ESCC risk) and did not affect the dietary habits of the patients (like diabetes). The major reasons for hospitalisation of the enrolled controls are provided elsewhere (Dar *et al*, 2014b, 2015). This study was reviewed and approved by the Institutional Ethics Committee of SKIMS.

After obtaining informed consent from all the participants, questionnaire developed for the population was administered in face-to-face interviews in the local language. Cases and controls were interviewed during their hospital stay and information on various socio-demographic characteristics, lifestyle factors and dietary information was obtained, the details of which are provided elsewhere (Dar *et al*, 2012).

Detailed information on FHC was obtained from all the participants. If participants had FHC, further information on type of relation was asked to know whether their FDRs (including father, mother, siblings and offspring), second-degree relatives (including cousins, uncles, aunts, stepsiblings) or spouses had been diagnosed with any type of cancer. Further information on the type(s) and site(s) of cancer as well as age at which malignancy was diagnosed in relatives was also obtained. The information on FHC was checked by cross interviewing the relative of the participants who attended or visited them in the hospital, wherever possible.

In addition, blood sample was collected from each subject for DNA extraction. DNA was analysed for genotyping in XME genes, cytochrome p450 (*CYP*), glutathione -S-transferases (*GSTs*), alcohol dehydrogenases 2 *(ADH2)* and aldehyde dehydrogenases 2 *(ALDH2)*. DNA extraction and genotypes for *CYP* and *GSTs* and their association with ESCC in Kashmir is provided elsewhere (Bhat *et al*, 2014; Makhdoomi *et al*, 2015), however, details of genotype analysis for *ALDH* and *ADH* genes are provided in the Supplementary Text 1.

Statistical analysis. Conditional logistic regression models were used to calculate unadjusted and adjusted odds ratios (ORs) and 95% confidence intervals (95% CIs) as well as to assess interactions. Confounders against which adjustment was made include age, ethnicity, religion, animal contact, oral hygiene, *hookah* smoking, *nass* chewing, alcohol consumption, daily fresh fruit and vegetable

intake and salt tea drinking. The confounders are known risk factor of ESCC particularly in the study population (Dar *et al*, 2012, 2013a, b, 2014b, 2015). Age was included in the multivariate models, because the matching for age was not perfect (± 5 years). Adjustments were done for cumulative wealth scores, place of residence and education level as indicators of socioeconomic status. To assess the socioeconomic status, we built a composite score for wealth, based on appliances ownership and other variables by using multiple correspondence analysis (Islami *et al*, 2009). Information on multiple correspondence analysis-based wealth score calculation is provided elsewhere (Dar *et al*, 2013b). Fruit and vegetable intake data (g per day) were transformed to logarithmic values following the addition of 0.1 to original values.

Parents, siblings and children were included as FDRs, whereas relatives like cousins, uncle, aunt, stepsiblings were categorised as second-degree relatives or other relatives and participants with FHC in spouse were put in a separate category. Cancers (including head and neck, breast, liver, pancreas, urogenital, skin and leukaemias and lymphomas), which were modest in number were grouped as 'other cancers'. The participants who had FHC or history of cancer in FDRs (FHC-FDRs) were grouped together, and those with family history of ESCC (FH-ESCC) were put in a separate group for further analysis. FHC was considered positive when at least one FDRs had been diagnosed with any type of cancer. In case of FH-ESCC, FHC was regarded positive when at least one FDR or two other relatives in a family had ESCC.

The participants were also grouped based on the frequency and type of genotype allele they carried. With respect to *CYP* genes, individuals had either homozygous wild or variant genotype (variant genotype has at least one or both mutant alleles). The wild genotypes of *CYP2A6a, CYP2A6b, CYP2A6c, CYP2A13, CYP2C19, CYP2D6* and *CYP2E1* are **1A/*1A*, **1/*1*, **1A/*1A*, **C/*C*, **1/*1, G/G* and *c1/c1,* whereas the variant genotypes are **1A/*6 + *6/*6, *1/*4 + *4/*4, *1A/*4C + *4C/*4C, *C/*T + *T/*T, *1/*2 + *2/*2, G/A + *A/*A* and *c1/c2 + c2/c2,* respectively. In case of *GSTs,* the wild and null genotype conditions were represented as $GSTM^+$, $GSTT1^+$ and $GSTTM^-$, $GSTT1^-$, respectively. However, in case of *ALDH2* and *ADH2,* the wild genotypes in both the genes were represented as *2*1/2*1*, whereas mutant homozygous had *2*2/2*2 + 2*2/2*2* combination. All statistical analysis was done using STATA software, version 12 (STATA Corp., College Station, TX, USA). Two-sided *P* values <0.05 were considered as statistically significant.

RESULTS

The general characteristics of the participants are presented in Table 1. Table 2 shows the distribution of FHC among participant relatives and its association with ESCC risk. A strong increase in ESCC risk was observed in subjects who had FHC (OR = 5.8; 95% CI = 4.1–8.3). The risk showed a slight increase when analysis was limited to participants who had FHC-FDRs (OR = 6.8; 95% CI = 4.6–9.9). On analysing the sex wise data, both sexes showed similar risk as seen in above subgroups as well as among themselves. On stratifying the subjects on the basis of the relation type, the strongest risk of ESCC was found when siblings had FHC (OR = 10.8; 95% CI = 6.0–19.3). Association between FHC and ESCC risk was also noticed in participants who had FHC among their spouses (OR = 4.1; 95% CI = 1.6–10.2). The association was not significant when the analysis was done in participants whose children were affected. Increased risk of ESCC was found when several relatives were affected with cancer. The enhanced risk of ESCC was also observed across various groups based on age at diagnosis of cancer in family members.

Strong ESCC risk was observed (OR = 11.8; 95% CI = 6.8–20.3) when analysis was limited to subjects who had FH-ESCC (Table 3). The risk turned out stronger, when participants were grouped as FHC-FDRs together (OR = 16.1; 95% CI = 8.5–30.0), or separately as parents (OR = 11.4; 95% CI = 5.0–24.9) or siblings (OR = 28.1; 95% CI = 9.1–85.8). Similarly, very high risk of ESCC was found when participants with FH-ESCC were grouped sex wise. The association was not significant when participant's spouse had FH-ESCC (OR = 2.5; 95% CI = 0.7–8.6). The association of ESCC with FH-ESCC was higher when more than one relatives were affected with ESCC. When FHC was further stratified on the basis of organ affected, a substantial increase in ESCC risk was observed only in case of same site FHC (Supplementary Table 1).

The ESCC risk could be estimated only in a few genotypes when the analysis was limited to subjects who had FHC (Table 4). The ESCC risk was very strong in subjects who carried variant genotypes of *CYP2C19 (*1/2 + *2/*2)* (OR = 15.5; 95% CI = 7.4–32.3) and *CYP2D6 (*G/A + *A/*A)* (OR = 9.7; 95% CI = 3.6–25.9) or wild genotype of *CYP2E1 (c1/c1)* (OR = 9.7; 95% CI = 2.5–37.5).

The analysis of the association of major risk factors with ESCC in the presence of FHC is presented in Supplementary Table 2. When FHC was reported, the association of ESCC risk with the known risk factors was appreciably increased. On assessing the interaction between the various environmental exposures and different genotypes of participants with FHC, significant interaction persisted only in adobe house dwellers ($P_{interaction} < 0.001$; Supplementary Table 3).

DISCUSSION

Our analysis showed substantial elevation of ESCC risk with positive FHC and risk was appreciably increased when blood relations had cancer or subjects had same organ cancer history in their families. A strong risk was also found when more than one relative had positive FHC. No risk was found when children of participants suffered from cancer and in case of spouse affected group, the association of ESCC risk did not persist when the analysis was limited to FH-ESCC. ESCC risk did not change when subjects with FHC were classified sex wise. A modifying effect of FHC was observed on ESCC risk in the presence of some important exogenous risk determinants and vulnerable genotypes.

The positive association between FHC and ESCC risk in the current study is consistent with previous studies (Garavello *et al*, 2005; Akbari *et al*, 2006; Gao *et al*, 2009; Turati *et al*, 2013) from high ESCC risk regions. As compared with these studies from high-risk ESCC, (Garavello *et al*, 2005; Tran *et al*, 2005; Akbari *et al*, 2006; Gao *et al*, 2009; Wu *et al*, 2011b), our study showed overall stronger association between ESCC risk and FHC. However, a few epidemiological studies from low-risk regions have reported insignificant associations of FHC-FDRs and oesophageal cancer risk (Lagergren *et al*, 2000; Dhillon *et al*, 2001). This inconsistency in different areas might be due to variation in the frequency of oesophageal susceptibility alleles or differences in major attributable exogenous factors and or a combination of both (Akbari *et al*, 2006). FHC can be a stand-in of shared environmental factors or genetic susceptibility and in the following discussion we have tried to explain the possible contribution of both familial factors in determining the ESCC risk in the context of our results.

The association of FHC with risk of ESCC in participants with affected relatives even at younger age cannot explain the association of FHC and cancer occurrence exclusively based on shared exposure, rather it suggests a role of genetic predisposition as well. Findings from high-risk areas (Hiyama *et al*, 2007; Wu *et al*, 2011b) support the role of genetics in ESCC development. In addition, the consistent molecular profiles within some chromosomal regions from high-risk regions could also support the positive associations of some genes with ESCC risk (Ko

Table 1. Characteristics of ESCC patients and matched controls from Kashmir, 2008–2012[a]

Characteristics	Cases (%)	Controls (%)	*P*-value
Age, mean (s.d.), years	61.6 (± 11.1)	59.8 (± 11.1)	0.480
Sex			0.78
Men Women	393 (55.9) 310 (44.1)	920 (55.3) 744 (44.7)	
Fresh fruit and vegetable, median g per day (IQR) intake	1.3 (0.8–2.0)	6.1 (2.1–72.1)	<0.001
Place of residence			<0.001
Urban Rural	29 (4.1) 674 (95.9)	146 (8.8) 1518 (91.2)	
House type			<0.001
Concrete Adobe	271 (38.6) 432 (61.4)	1251 (75.2) 413 (24.8)	
Cooking fuel			<0.001
Other Biomass	16 (2.3) 685 (97.7)	298 (18.0) 1358 (82.0)	
Education			<0.001
No schooling Primary (<5th) Middle (5–8th) High school (9th–12th) Graduate and higher	626 (89.0) 33 (4.7) 24 (3.4) 16 (2.3) 04 (0.6)	1074 (64.5) 203 (12.2) 123 (7.4) 149 (8.9) 115 (7.0)	
Wealth score			<0.001
Cat 1[b] Cat 2 Cat 3	397 (56.5) 112 (15.9) 194 (29.6)	337 (20.3) 328 (19.7) 999 (60.0)	
Animal contact			<0.001
No or occasional contact Daily contact Daily and close contact	164 (23.3) 175 (24.9) 364 (51.8)	774 (46.5) 616 (37.0) 274 (16.5)	
Oral hygiene			<0.001
Do not brush Ones/week Twice or thrice/week Daily	161 (22.9) 366 (52.1) 94 (13.4) 81 (11.6)	101 (6.2) 785 (47.9) 345 (21.1) 405 (24.8)	
Smoking			<0.001
Never Ever	271 (38.6) 432 (61.4)	835 (50.2) 829 (49.8)	
Nass chewing			<0.001
Never Ever	502 (71.5) 201 (28.5)	1471 (88.4) 193 (11.6)	
Alcohol consumption			<0.001
Never Ever	695 (98.9) 08 (1.1)	1664 (100.00) 0 (0.00)	
Tea type			0.041
Other Salt tea	09 (1.3) 693 (98.7)	51 (3.1) 1612 (96.9)	

Abbreviations: ESCC = oesophageal squamous cell carcinoma; IQR = inter-quartile range.
[a]Although cases and controls were individually matched, the percentages of cases and controls are not necessarily equal in each sex category, because some cases have one matched control and others have more controls. Numbers may not add up to the total numbers due to missing data in some variables. *P* values calculated using χ^2 tests for categorical variables (χ^2 for trend in variables with more than two categories) and Wilcoxon Rank Sum tests for continuous variables.
[b]Cat1, cat2 and cat3 represent the wealth scores in increasing order.

et al, 2014). Certain cancer vulnerability increasing alleles can modulate the risk of ESCC, which is attributed to a range of biological phenomenon including elevation of the toxicity of certain compounds. For example, in the current study, subjects harbouring vulnerable genotypes of *CYP2C19*, *CYP2D6* and *CYP2E1* and also having FHC had an increased risk of ESCC. Increased ESCC risk among subjects harbouring a variant genotypes of *CYP2C19* and *CYP2D6* may be possible due to their reduced metabolic activity towards carcinogens, which are usually formed due to activation of toxic compounds present in tobacco smoke (Yadav *et al*, 2010). The splice mutation at 1934 *G* to *A* in case of *CYP2D6* results in the loss of enzyme activity towards its substrates as compared with its wild-type allele (Gaikovitch *et al*, 2003) or formation of a truncated protein by a single base pair mutation at *681G* to *A* in case of *CYP2C19* results lower enzyme expression and reduced metabolism of preformed or activated carcinogens (Shi *et al*, 2012). The reduced metabolic activity owing to such gene variants results in increased production of toxic metabolites that could lead to the formation of different biochemical end products including DNA adducts in substrate-specific tissues, hence tissue-specific toxicity. However, *CYP2E1* has been involved in the activation of nitrosamines-specific pro-carcinogens (Yang *et al*, 1990), which results in the increased production of activated carcinogens and hence a possible reason of an increased ESCC risk of wild genotypes (LeMarchand *et al*, 1999; Tan *et al*, 2001).

On ESCC, there are four original genome-wide association studies (GWAS; Abnet *et al*, 2010; Wang *et al*, 2010; Wu *et al*, 2011a; Wu *et al*, 2012) and their two combinational analysis (Wang *et al*, 2014; Wu *et al*, 2014) available till date. The five most common loci reported at least in two GWAS are *PLCE1 (rs2274223)*, *ALDH2 (rs671)*, *ADH1B (rs1229984)*, *CY1A1 (rs1048943)* and *CASP-8 (rs3834129)*. Out of which three loci *ALDH2*, *ADH1B* and *CY1A1* fall in XME genes, which we have analysed in this study or elsewhere (Bhat *et al*, 2014). The other two common GWAS identified loci *CASP-8 (rs3834129)* and *PLCE1 (rs2274223)* have either showed no association (with *CASP8*; Malik *et al*, 2011) or weak association (*PLCE1*; Malik *et al*, 2014) with ESCC risk in Kashmir.

In addition, various interesting findings in our study further substantiate the role of genetic predisposition in the familial aggregation of ESCC. First, those who were not exposed to known risk factors in Kashmir including tobacco smoking and *nass* chewing still showed association of FHC with ESCC risk (Supplementary Table 2). Second, multiple affected relatives showed a dose-dependent relationship with ESCC and higher risk of ESCC was found when FDRs were affected. Third, the elevated risk of ESCC at a younger age as in previous studies (Garavello *et al*, 2005; Wen *et al*, 2006). Fourth, the risk of ESCC was not significantly enhanced in subjects with family history of other environment and lifestyle-induced cancer (for example, lung cancer, which is strongly related to smoking; Supplementary Table 1), supports the high risk due to certain cancer risk-increasing alleles in gene pool of families. Fifth, increased ESCC risk in siblings and parents in the current study, like earlier findings (Shao *et al*, 1997), could be attributed to high penetrant dominant effects of genetic inheritance (Hemminki *et al*, 2001). Sixth, disappearance of association of various analysed genotypes with ESCC when FHC was absent. Seventh, strongest risk when there was FH-ESCC, although this equally can support shared ESCC risk-increasing exposures in a family. All such results indicate that FHC can be a manifestation of genetic predisposition.

However, the close relationship between FHC and cancer occurrence can also be due to same-shared exposures within a family. In our study, the high risk of ESCC when spouses were affected supports the potential effect of shared risk habits, as supported by migrant studies that show the cancer patterns of immigrants are largely set after the first 2 decades of their life as immigrants (Hemminki and Li, 2002; Hemminki *et al*, 2002). Besides environmental factors, viral infections like human papilloma virus, could be one of the possible aetiological factors for cervical, penile carcinomas and ESCC, however, conclusive

Table 2. The distribution of family history of cancer and its association with ESCC in Kashmir

Variable	Cases (%; *N* = 703)	Controls (%; *N* = 1664)	UAOR (95% CI)	AOR (95% CI)[a]
FHC				
No[b]	462 (65.7)	1544 (92.8)	Referent	Referent
Yes[c]	241 (34.3)	120 (7.2)	6.4 (4.9–8.2)	5.8 (4.1–8.3)
FDRs	187 (26.6)	89 (5.3)	6.6 (4.8–8.8)	6.8 (4.6–9.9)
Other	54 (7.7)	31 (1.7)	5.6 (3.4–9.0)	4.0 (2.1–7.6)
Gender				
Male	130 (18.5)	63 (3.8)	6.22 (4.4–8.8)	5.5 (3.4–8.9)
Female	111 (15.8)	57 (3.4)	6.51 (4.4–9.5)	6.2 (3.7–10.3)
Affected relatives[d]				
Parents	91 (12.9)	58 (3.5)	4.9 (3.4–7.0)	4.1 (2.4–6.8)
Father	54 (8.0)	29 (1.8)	6.0 (3.7–9.9)	5.4 (2.7–11.0)
Mother	36 (5.3)	23 (1.4)	4.4 (2.6–7.6)	3.1 (1.5–6.7)
Sibling	98 (13.9)	30 (1.8)	10.5 (6.6–16.6)	10.8 (6.0–19.3)
Brother	66 (9.8)	18 (1.1)	11.0 (6.1–19.9)	10.9 (5.3–22.6)
Sister	31 (4.6)	11 (0.7)	9.8 (4.6–21.2)	10.1 (3.6–28.1)
Children	04 (0.6)	07 (0.4)	1.8 (0.5–6.5)	3.8 (0.5–30.2)
Spouse	24 (3.7)	16 (1.0)	5.1 (2.6–10.1)	4.1 (1.6–10.2)
Other relatives[e]	25 (3.4)	15 (0.9)	4.8 (2.5–9.5)	2.6 (1.0–6.8)
Affected relatives				
1	187 (26.6)	112 (6.7)	5.2 (3.9–6.8)	4.76 (3.3–6.9)
≥2	54 (7.7)	13 (0.8)	13.4 (7.0–25.5)	11.86 (4.9–28.3)
P for trend			<0.001	<0.001
Age at diagnosis of relative (in years)				
1–40	11 (1.6)	13 (0.8)	3.2 (1.3–7.8)	4.1 (1.3–13.4)
41–50	42 (6.0)	28 (1.7)	4.7 (2.8–7.9)	4.1 (1.9–8.9)
>50	188 (26.7)	79 (4.8)	7.3 (5.4–9.8)	6.5 (6.3–9.8)
P for trend			<0.001	<0.001

Abbreviations: AOR = adjusted odds ratio; CI = confidence interval; FDR = first-degree relative; FHC = family history of cancer; UAOR = unadjusted odds ratio.
[a]Adjusted for age, place of residence, education, ethnicity, tobacco smoking, *nass* consumption, fruit and vegetables, animal contact, oral hygiene, wealth score, ever alcohol..
[b]Subject without family histories of any cancer.
[c]Family history of any cancer in first or other degree relatives.
[d]Relatives were analysed separately to find out their individual effects.
[e]Second-degree relatives like stepbrothers, stepsisters, cousins, uncle, aunt, niece, nephew.

Table 3. Association of FHC with ESCC exclusively in participants who had positive oesophageal cancer family history

Variable	Cases (%; *N* = 703)	Controls (%; *N* = 1664)	UAOR (95% CI)	AOR (95% CI)[a]
No	462 (73.6)	1544 (97.8)	Referent	Referent
Yes[b]	172 (27.1)	35 (2.2)	15.3 (9.9–23.5)	11.8 (6.8–20.3)
FDRs	140 (22.1)	23 (1.5)	18.6 (11.1–30.9)	16.1 (8.5–30.5)
Other	32 (5.0)	12 (0.8)	8.6 (4.0–18.4)	4.2 (1.6–11.2)
Gender				
Male	96 (15.1)	16 (1.0)	16.3(8.9–29.8)	13.4 (6.2–29.4)
Female	76 (12.0)	19 (1.1)	14.3 (7.7–26.4)	10.4 (4.9–21.9)
Affected relatives				
Parents	73 (11.5)	19 (1.2)	12.7 (7.0–22.7)	11.4 (5.0–24.9)
Siblings	67 (10.6)	04 (0.3)	40.0 (14.3–112.0)	28.1 (9.1–85.8)
Spouse	11(1.7)	08 (0.5)	5.1 (1.7–15.0)	2.5 (0.7–8.6)
Other	21 (3.3)	04 (0.3)	12.6 (4.2–37.8)	8.6 (1.7–38.0)
Number of affected relatives				
1	74 (13.2)	85 (5.2)	2.8 (1.9–3.9)	3.5 (2.2–5.6)
2 or more	23 (4.1)	08 (0.5)	9.9 (3.9–24.6)	4.6 (1.4–15.0)
P for trend			<0.001	< 0.001
Age at diagnosis of relative (in years)				
1–40	04 (0.6)	02 (0.1)	14.3 (2.4–88.4)	10.5 (1.2–86.6)
41–50	29 (4.3)	02 (0.1)	28.0 (6.6–118.8)	17.4 (3.2–94.9)
>50	139 (21.9)	31 (1.9)	14.3 (8.9–22.4)	11.3 (6.3–20.0)
P for trend			<0.001	<0.001

Abbreviations: AOR = adjusted odds ratio; CI = confidence interval; ESCC = oesophageal squamous cell carcinoma; FDR = first-degree relative; FHC = family history of cancer; UAOR = unadjusted odds ratio.
[a]Adjusted for age, place of residence, education, ethnicity, tobacco smoking, *nass* consumption, fruit and vegetables, animal contact, oral hygiene, wealth score, ever alcohol.
[b]Positive family history of oesophageal cancer.

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Table 4. Association of gene polymorphisms with ESCC in subjects with and without FHC

	FHC$^-$				FHC$^+$			
Genotypes#	**Cases *N* (%)**	**Controls *N* (%)**	**UAOR (95% CI)**	**AOR[a] (95% CI)**	**Cases *N* (%)**	**Controls *N* (%)**	**UAOR (95% CI)**	**AOR[a] (95% CI)**
CYP2A6a								
[b]*1A/*6, *6/*6	55 (17.2)	111 (24.4)	Referent	Referent	32 (18.5)	12 (31.6)	Referent	Referent
[c]*1A/*1A	264 (82.8)	343 (75.6)	1.5 (1.0–2.3)	1.2 (0.5–2.7)	141 (81.5)	26 (68.4)	2.0 (0.2–22.1)	—
CYP2A6b								
[d]*1/*4,*4/*4	69 (21.6)	120 (26.4)	Referent	Referent	38 (21.9)	07 (18.4)	Referent	Referent
[e]*1/*1	250 (50.8)	334 (67.9)	1.3 (0.9–1.9)	1.0 (0.6–1.6)	135 (78.9)	31 (8.6)	—	—
CYP2A6c								
[f]*1A/*4C,*4C/*4C	68 (13.8)	162 (32.9)	Referent	Referent	37 (21.4)	10 (26.3)	Referent	Referent
[g]*1A/*1A	251 (78.7)	292 (64.3)	1.9 (1.3–2.8)	1.9 (0.8–4.1)	136 (78.6)	28 (73.7)	4.0 (0.4–35.8)	—
CYP2A13								
[h]*C/*T,*T/*T	65 (20.4)	134 (29.5)	Referent	Referent	42 (24.3)	11 (28.9)	Referent	Referent
[i]*C/*C	254 (79.6)	320 (70.5)	1.4 (0.9–2.0)	1.2 (0.7–2.1)	131 (75.7)	27 (71.1)	4.0 (0.4–35.8)	—
CYP2C19								
[j]*1/*1	104 (32.6)	232 (51.1)	Referent	Referent	56 (32.4)	24 (63.2)	Referent	Referent
[k]*1/*2,*2/*2	215 (67.4)	222 (48.9)	2.2 (1.6–3.2)	4.1 (1.8–9.2)	117 (23.8)	14 (36.8)	14.9 (8.0–27.6)	15.5 (7.4–32.3)
CYP2D6								
[l]G/G	251 (78.7)	386 (85.0)	Referent	Referent	118 (68.2)	31 (81.6)	Referent	Referent
[m]G/A,*A/*A	68 (21.3)	68 (15.0)	1.7 (1.1–2.6)	2.00 (1.2–3.4)	55 (11.2)	07 (1.4)	9.7 (4.3–21.8)	9.7 (3.6–25.9)
CYP2E1								
[n]c1/c2, c2/c2	78 (24.4)	84 (18.5)	Referent	Referent	41 (23.7)	06 (15.8)	Referent	Referent
[o]c1/c1	241 (75.6)	370 (81.5)	1.3 (0.9–1.9)	1.5 (0.7–3.5)	132 (26.8)	32 (6.5)	3.0 (0.3–28.8)	9.7 (2.5–37. 5)-
GSTM1								
[p]GSTM1$^+$	203 (65.3)	300 (65.5)	Referent	Referent	125 (69.1)	28 (82.3)	Referent	Referent
[q]GSTM1$^-$	108 (34.7)	158 (34.5)	1.1 (0.7–1.5)	1.0 (0.5–1.9)	56 (30.9)	06 (17.7)	2.0 (0.2–22.1)	—
GSTT1								
[r]GSTT1$^+$	198 (63.7)	342 (74.7)	Referent	Referent	108 (59.7)	25 (73.5)	Referent	Referent
[s]GSTT1$^-$	113 (36.3)	116 (25.3)	1.5 (1.0–2.1)	—	73 (40.3)	09 (26.5)	3.0 (0.3–28.8)	—
ALDH2								
[t]2*1/2*1	211 (97.2)	327 (98.2)	Referent	Referent	120 (97.6)	21 (100.0	Referent	Referent
[u]2*2/2*2, 2*2/2*2	03 (2.4)	00 (0.0)	0.0 (0.0–0.0)	—	06 (2.8)	06 (1.8)	2.00 (0.5–8.0)	—
ADH2								
[v]2*1/2*1	79 (36.4)	124 (37.1)	Referent	Referent	51 (41.5)	06 (28.6)	Referent	Referent
[w]2*2/2*2, 2*2/2*2	138 (63.6)	210 (62.9)	1.2 (0.7–1.8)	2.5 (0.8–7.8)	72 (58.5)	15 (71.4)	1.00 (0.1–7.1)	—

Abbreviations: AOR = adjusted odds ratio; CI = confidence interval; ESCC = oesophageal squamous cell carcinoma; FHC = family history of cancer; UAOR = unadjusted odds ratio. [#]Numbers may not add up to the total number of participants due to some missing information in certain selected pairs of variables/genotypes. [a]AOR for age, ethnicity, gender, place of residence, religion, education level, wealth score, animal contact frequency, oral hygiene, fruits and vegetables, tobacco smoking, *nass* consumption, alcohol drinking and salted tea. [b,d,f] and [h] represent protective variant genotypes of *CYP2A6a, b, c* and *CYP2A13* genes. [c,e,g] and [i] represent vulnerable wild genotypes of *CYP2A6a, b, c* and *CYP2A13* genes. [j,l,n,p,r,t] and [v] represent the protective genotypes of *CYP2C19, 2D6, 2E1, GSTM1, T1, ALDH2* and *ADH2* genes, respectively. [k,m,o,q,s,u,] and [w] represent the vulnerable genotypes of *CYP2C19, 2D6, 2E1, GSTM1, T1, ALDH2* and *ADH2* genes, respectively (it is pertinent to mention that variant group represent combination of both homozygous mutant and heterozygous condition and the protective and vulnerable genotype have been assigned to a particular genotype, based on their role in the development of ESCC risk in our other studies and available literature).

evidence on HPV association with ESCC is lacking (Kamangar *et al*, 2006). Like other studies (Tran *et al*, 2005; Akbari *et al*, 2006; Gao *et al*, 2009; Wu *et al*, 2011b), we found the higher risk when siblings than parents were affected by a cancer or when more than one relative were affected, however, we could not find this association in children-affected participants. This indicates that the siblings most likely share the same environmental exposures as children do with their parents.

Additive or multiplicative association of FHC with known risk factors of ESCC in Kashmir including *hookah* smoking, daily and close animal contact, alkaline salted tea intake, low socioeconomic status, living in adobe houses and use of biomass fuels shows shared environmental factors besides genetic predisposition can also elevate ESCC risk in a family.

Statistically insignificant interaction among the various risk factors and genetic markers with FHC indicates both the genetic factors and environmental risk factors act independently. However, the interaction with the adobe house shows that certain exposures in dwellers of such houses and the genetic alleles harboured by them work in a biologically synergistic way to influence the risk of ESCC. Participants living in poorly ventilated adobe houses possibly get high exposure to cooking fumes as well as household smoke from tobacco or biomass cooking fuel use. Use of animal dung and cooking fumes from adobe houses have been closely associated with the development of ESCC from other studies also (Wornat *et al*, 2001; Deziel *et al*, 2013).

Family history data were based on self-reported information from cases and controls rather than medically verified cancers in family members, which may be a source of bias (Glanz *et al*, 1999), and is a limitation of our study, irrespective of the fact that we had cross interviewed the attendants of the participants wherever possible about the history, site and type of malignancy among the relatives in a family. There are several strengths of this study. This is the first large hospital-based strictly matched case–control study from high-risk area of ESCC-Kashmir, addressing the modifying effect of FHC on ESCC risk in detail and exploring the interaction between FHC, lifestyle risk factors and certain genetic markers and adjustments of the results for multiple potential confounding

factors. There is representation of participants from all the regions of the Kashmir. Also, there are least chances of subjective extrapolation and recall bias because of limited number of interviewers who got information in local language (Dar *et al*, 2012; Bhat *et al*, 2014). Finally, the similar hospital setting of the interview for both cases and controls should have improved the quality of collected information.

CONCLUSION

The study shows FHC, as a proxy of genetic, and shared environmental risk factors could be responsible for the high risk of ESCC in Kashmir.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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