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Contribution of *TMC6* and *8* (*EVER1* and*2*) variants to cervical cancer susceptibility

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Abstract

Cervical cancer (CxCa) is caused by persistent human papillomavirus (HPV) infection; genetic predisposition is also suspected to play a role. The present study is a targeted candidate gene follow-up based on: i) strong clinical evidence demonstrating that mutations in the *TMC6* and *TMC8* (*EVER1* and *EVER2*) genes associate with the HPV-associated disease Epidermodysplasia Verruciformis (EV), and ii) recent epidemiological data suggesting a genetic susceptibility conferred by polymorphisms in such genes for skin and cervical cancer. Clarifying the association of the *TMC6/8* genes with risk of CxCa will help in understanding why some HPV-infected women develop persistent infection, cervical lesions and eventually cancer while others do not. Twenty-two single nucleotide polymorphisms (SNP) harbouring the TMC6/8 genes were genotyped in 2,989 cases with cervical intraepithelial neoplasia grade III (CINIII) or invasive cervical cancer (ICC) and 2,281 controls from the Swedish population. Association was evaluated in logistic regression models. Two SNPs displayed association with cervical disease: rs2290907 (OR_{GGvsAA} = 0.6, 95% CI: 0.3 - 0.9, p = 0.02) and rs16970849 (OR_{AGvsGG} = 0.8, 95% CI: 0.66 - 0.98, p = 0.03). The present data supports the involvement of the TMC6/8 region in CxCa susceptibility but further analyses are needed to replicate our findings, fully characterize the region and understand the function of the genetic variants involved.

Keywords

Cervical cancer; EVER1; EVER2; polymorphism; TMC6; TMC8

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Introduction

Cervical cancer (CxCa) is the second most common cancer in the world among women and caused by persistent infection by oncogenic mucosatropic Human papillomaviruses (HPV)[1] of the alpha genus. Although HPV infection is a necessary cause of CxCa it is not sufficient and the majority of infected women never develop cancer. It has been shown that genetic susceptibility factors are also important[2] and the association of genes in immunological pathways with susceptibility to CxCa has been demonstrated in several studies[3-6].

Mutations in the TMC6 and 8 genes, also known as the *EVER1* and *EVER2* genes, are implicated in the development of the rare autosomal recessive disease Epidermodysplasia Verruciformis (EV)[7-9]. EV is characterised by extreme susceptibility to infection by cutaneous HPV of the beta genus[10]. A polymorphism in the *TMC8* gene has also been associated with increased risk of squamous cell carcinoma of the skin and the presence of HPV antibodies in control subjects[11]. The *TMC6/8* genes may be involved in controlling HPV gene expression and replication in epidermal keratinocytes or they may directly affect innate and adaptive immune responses which control the clearance of HPV-infected keratinocytes in EV cases[12].

The link between the TMC6/8 genes and vulnerability to HPV makes these genes interesting candidate genes in CxCa. The genes are located adjacently on chromosome 17q25. A recent report by Wang *et al* indicated an association between genetic variation in the TMC6/8 region and persistent HPV infection as well as risk of CxCa[13]. The findings warrant replication in larger studies and in different populations. There is also a need for further investigations of genetic variation in the *TMC6/8* genes to understand the possible mechanisms of susceptibility to persistent HPV infection and development of CxCa.

This study aims to evaluate the association of single nucleotide polymorphisms (SNP) in the genomic region harbouring the *TMC6* and *TMC8* genes with susceptibility to CxCa. A previous observation[13] indicated that the *TMC6/8* genes may influence risk of CxCa and the current study provides comprehensive data on the association of polymorphisms in the *TMC6/8* region with CxCa in the Swedish population.

Material and methods

Study population

The current study comprises two Swedish cohorts, here denoted as cohort 1 and 2, and the total sample set consisted of 2,989 cases and 2,281 controls. Of the cases, 2,767 (93 %) were diagnosed with cervical intraepithelial neoplasia grade III (CINIII) and 222 (7 %) with invasive cervical carcinoma (ICC).

Cohort 1 was based on a nested study within the Northern Sweden Health and Disease Study Cohort and has been previously described[6]. It is derived from the Swedish province of Västerbotten which comprises the Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA) Project and the Västerbotten Intervention Program. The MONICA Project contains material from population-based screenings for cardiovascular disease that were carried out in 1986, 1990, 1994, 1999, and 2004. There were approximately 14,000 sampling occasions for approximately 9,000 persons, 50% of whom are included in the Västerbotten Intervention Program. Established in 1985, the Västerbotten Intervention Program is a long-term health promotion project that by 2004 included approximately 74,000 persons, of whom approximately 70,000 had donated blood. The samples are collected annually from all residents aged 40, 50, and 60 years in Västerbotten County and are stored as plasma, erythrocytes and buffy coats at –80°C at the university hospital of the

University of Umeå, Umeå, Sweden. Cervical cancer cases were traced through the Swedish Cancer Registry. For the current investigation 1,002 cases (867 CINIII and 135 ICC) and 1,734 controls were included. All controls were selected from female subjects alive and free of CxCa at the time of diagnosis of the case in each respective subcohort, and matching the index case on age at diagnosis (±5 years). Mean ages of the cases and controls were 48.0 years (range, 20.1–69.7) and 49.6 years (range, 26.3–70.0), respectively. Genomic DNA was extracted from buffy coats using standard procedures and the study was approved by the Institutional Review Board of the Umeå University.

Cohort 2 has also been described previously[14] and included 1,987 cases from families with at least two affected women (1,900 CIN III, 87 ICC) from all of Sweden. The current study includes 942 individuals with one first degree relative (mother, sister or daughter) and 39 individuals with two first degree relatives also participating in the study. The remaining 1006 individuals were the sole participants of their family. The families were originally identified by cross-linking the Swedish Cancer Registry and the National Family Registry. The registry identified 5,400 individuals from families with at least two affected. A total of 4,145 individuals were invited and blood was available from 2,135 (51%). [2, 14]. All cases were from the Swedish population. To avoid any potential overlapping of individuals between cohorts all participants in cohort 2 coming from the region of Västerbotten (Umeå pathology department) were excluded. Cohort 2 also included 547 unrelated controls (265 male and 282 female). These were blood donors at Uppsala University Hospital (n=257) and healthy non-obese adolescents from a Swedish obesity study (n=289)[15]. The controls were of Swedish descent (both parents of Swedish origin). DNA was extracted from peripheral blood using standard methods. The study was approved by the regional ethical review board in Uppsala and all cases provided written informed consent.

Genotyping

The study was designed to capture variations across the 58 kb genomic region on chr. 17q25 harbouring the *TMC6* and *TMC8* genes (17:73600594 – 73659033). The region was defined as 20 kb upstream from *TMC6* gene and 8.4 kb downstream of the *TMC8* gene. Tag SNPs were selected using data from the HapMap CEU population (Utah residents with Northern and Western European ancestry from the CEPH collection) in The International HapMap Project (Rel24/phase II Nov08, on NCBI B36 assembly) and the Tagger algorithm in Haploview[16-17]. Aiming for 100% coverage of the region resulted in 25 selected SNPs, which tag a total of 68 SNPs (Figure 1, Supplementary material). A multiplex genotyping assay was developed according to previously described methods[6]. Briefly, the assay was based on newly designed multiplex PCR and hybridization using allele-specific probes coupled to fluorescently labelled polystyrene beads in a Luminex system. Genotypes were assigned by plotting the ratio of both hybridization signals (expressed as median fluorescence intensity values, MFI) of the allele-specific probe pairs for each tested sample using the software Spotfire 2.1 (TIBCO Software Inc, California, USA). The accuracy of the assay was validated using a HapMap reference panel of 91 samples.

In total, 22 SNP were successfully included in the assay (Table 1) resulting in 88.5 % coverage of the genetic variation in the target region. In order to ensure robustness of genotyping 192 samples were typed twice. Additional quality control samples such as negative PCR controls (no template) and CEPH reference samples were randomly distributed among the study samples. Genotyping of samples from both cohorts was performed simultaneously in one single laboratory.

Statistical analysis

Basic statistical analysis was performed using SAS 9.1.3 (SAS Institute, Cary, NC, US) and the analysis tool PLINK (http://pngu.mgh.harvard.edu/purcell/plink/)[18]. Initial quality control, included calculating the genotyping success rate for the SNPs and individuals and assessing the Hardy-Weinberg equilibrium in controls with p = 0.05 as threshold. Testing for heterogeneity among the two cohorts was performed using the Breslow-Day test, as described previously[19].

Risk estimates were computed as genotype odds ratios (OR) with 95% confidence intervals (95% CI) in unconditional logistic regression models. In order to account for differences between cohorts, multivariate analysis was performed including cohort as a categorical covariate in the model. In addition, analysis was performed restricted to cases diagnosed with ICC (n = 222) versus female controls. Haplotype blocks were identified as per the definition of GABRIEL et al. (2002)[20], using Haploview software[17]. Then haplotype analyses were performed in SAS/GENETICS using the PROC HAPLOTYPE procedure, where all haplotype blocks were tested adjusting by cohort. This procedure utilizes the Expectation Maximization (EM) algorithm to predict the maximum likelihood estimates of the haplotype frequencies assuming Hardy-Weinberg equilibrium.

The statistical power to find genetic susceptibility factors was increased by inclusion of cases from a family cohort, as these samples are more likely to share such factors than randomly selected sporadic cases. However, the use of related subjects may lead to inflated associations simply because of family structure. The software tool PedGenie provided in Genie 2.7.2[21-22] can be used to accommodate a study design which combines data from related and unrelated individuals. PedGenie calculates empirical p-values and confidence intervals corrected for family structure based on N simulations. The input to PedGenie is genotype data and pedigree information describing the family structure. Unrelated individuals are entered as being the only member of a family. PedGenie calculates allele frequencies based on genotype data and assigns genotypes to all founders accordingly. For each simulation a genotype distribution is simulated based on founder genotypes and then combined with the true phenotype to calculate the simulated statistic. Summarizing all simulated statistics that are larger than the observed statistic and dividing by N gives p-values and confidence intervals corrected for family structure. In the current study PedGenie was used to investigate the influence of family structure in a separate analysis of the Uppsala cohort. Empirical 95% CI for genotype-based OR were calculated by 10,000 gene-drop simulations in PedGenie and compared with the 95% CI from standard unadjusted analysis. The empirical and the unadjusted p-values were comparable; therefore, no correction for relatedness was implemented in the pooled analysis presented below (data not shown).

The aim of this study was to analyze a candidate region previously implicated in CxCa susceptibility[13], therefore no correction for multiple testing was applied and $p < 0.05$ was considered statistically significant.

Results

The robustness of genotyping was evaluated by repeated typing of 192 samples yielding >99 % consistent genotypes. Analysis of the genotyping success rate resulted in removal of 124 (2.4 %) samples due to < 90% call rate. In the remaining samples all SNPs were successfully genotyped and the total genotyping rate was 99.5%. None of the SNPs displayed deviations from Hardy-Weinberg equilibrium in controls of the combined and the separated cohorts.

Large p-values for the Breslow-Day test (between 0.07 and 0.97, data not shown) indicates no significant cohort differences for all the tested SNPs, except for the SNP rs3813026

(p=0.01). In addition, in order to avoid possible heterogeneity among the two Swedish cohorts, CxCa risk estimates for all markers were calculated by logistic regression modelling with adjustment for cohort (Table 2). Two markers remained significantly associated with CxCa after controlling for cohort differences: rs2290907 (OR_{GGvsAA} = 0.6, 95% CI: 0.3 - 0.9, p = 0.02), and rs16970849 (OR_{AGvsGG} = 0.8, 95% CI: 0.66 - 0.98, p = 0.03).

Figure 1 shows the location of all genotyped SNPs on chromosome 17. The two associated SNPs are located in the intronic regions of the *TNRC6C* (SNP rs2290907) and *TMC8 genes* (SNP rs16970849).

When the analysis was restricted to cases diagnosed with ICC (n = 222) the OR for the two SNPs associated with overall disease were similar, although statistical significance was not reached due to lack of power: rs2290907 (OR_{GGvsAA} = 0.2, 95% CI: 0.03 - 1.5, p = 0.1) and rs16970849 (OR_{AGvsGG} = 0.8, 95% CI: 0.5 - 1.3, p = 0.3). Risk estimates for CXCa and ICC did not differ when analysis was done excluding males from the controls group of cohort 2 (data not shown). Haplotype analysis did not show any additional association (data not shown).

Discussion

The aim of the present study was to evaluate genetic variation in the genomic region harbouring the *TMC6* and *TMC8 genes* with susceptibility to CxCa. Our approach was a targeted candidate gene follow-up based on: first, strong clinical evidence that demonstrate a genetic predisposition to EV acquisition in patients carrying mutations in these genes and second, epidemiological data coming from skin and cervical cancer studies suggesting the involvement of polymorphisms in the mentioned genes. Clarifying the potential association of the *TMC6/8* genes with risk of CxCa will help in understanding why some HPV-infected women develop persistent infection, cervical lesions and eventually cancer, while others do not.

Some evidence for an association between *TMC6/8* genes and CxCa was observed in the Swedish population. The *TMC6/8* region on chromosome 17 displayed limited linkage disequilibrium indicated by the fact that 25 SNPs were needed in order to completely capture the variation provided by 68 SNPs. When results were adjusted by cohort, two markers located in the *TNRC6C* (rs2290907) and *TMC8* (rs16970849) genes remained associated with disease. The effects of the associated SNPs were modest but consistent in analyses restricted to invasive cancers and controls.

The present work is the largest study to date to investigate the role of EVER polymorphisms in cervical cancer susceptibility. It comprises a total sample size of 2,989 cases and 2,281 controls from a single population. We estimate to have covered around 92% of the genomic variation in the *TMC6/TMC8* region. An additional benefit is the inclusion of cases from a family study enriched for genetic susceptibility factors. The overall genotyping performance was good for all tested markers, call rates were >99.7% for the two CxCa-associated SNPs in the present study.

The present investigation was instigated by the report of Wang *et al.* [12] who studied common genetic variants in 305 candidate genes and reported an association in the *TMC6/8* region. In total, they investigated 26 SNPs in this region in 416 cases and 425 controls from a population-based cohort in Guanacaste, Costa Rica. They found that EVER region was statistically significantly associated with progression to CINIII/cancer and inside the region one particular SNPs, the rs9893818 displayed the strongest association ($OR_{ACvs.CC}$= 2.76 95% CI: 1.59-4.77, p_{trend} = 0.0003). This SNP was not included in the current study as it is monomorphic in HapMap-CEU population. The SNP rs9893818 is located downstream the

TMC8 gene and is approximately 8 kb from our associated SNP rs16970849, which is located in the *TMC8* gene. Examination of this region in HapMap-CEU and YRI populations revealed no marker in high or even moderate LD with rs9893818. Furthermore we have observed among the CEU and YRI populations the formation of 5 haplotypes blocks along the TMC6/8 region which are overlapped among both populations. Interestingly the marker associated in the Costa Rican population lie outside of these extended block haplotypes (Supplementary material, figure 1). Overall, 13 SNPs from the previous paper were typed in the current study. As expected, the allele frequencies for these SNPs vary between the studies. The populations are ethnically distinct and only four variants displayed less than 5% allele frequency difference among controls.

HPV16 as well as other high risk HPV are recognized as causal factors for the development of CxCa. Recent studies suggest a direct link between TMC gene products and the HPV E5 protein. The TMC6 and TMC8 proteins form a complex with the Zn2+ transporter 1 (ZnT-1) in the ER membrane. HPV16 E5 binds to the TMC/ZnT complex and prevents complex-mediated inhibition of the Zn-sensoring transcription factor MTF-1 and c-jun activity[23]. Following a model proposed by Lazarczyk and coworkers[12] the TMC proteins constitute a cellular barrier for HPV replication. The interaction of the E5 protein of mucosal alpha-papillomaviruses like HPV16 with the TMC/ZnT complex in keratinocytes would result in increased intracellular free Zn2+ levels and increased activity of transcription factors such as AP-1 which would then lead to increased viral replication. Genetic polymorphisms in the *TMC6/8* genes that result in amino acid changes might affect TMC protein function or changes in regulatory regions might affect expression levels, RNA stability or splice variants. Either change could affect HPV replication, and thereby the viral load.

In a recent population-based case-control study of head-and-neck cancer in the greater Boston-Massachusetts area, homozygotes for the T allele of *TMC8* SNP rs7208422, encoding an amino acid change from Asn to Ile at position 306, was found to be associated with seropositivity to HPV 16L1 (OR_{TT}: 0.49, 95% CI 0.27 – 0.86) and also to be protective against head-and-neck cancer (OR_{AT}: 0.70, 95% CI 0.52 – 0.93, OR_{TT}: 0.62, 95% CI 0.44 – 0.86) (Heather H. Nelson, Caihua Liang, Anita Patel, Tim Waterboer, Michael Pawlita, Michael McClean, Karl T. Kelsey, unpublished observation). In contrast with this report, but in agreement with Wang et al., we did not find any statistically significant association of this SNP with CxCa after adjusting by cohort. The discordant findings may be explained by differences on the rs7208422 allele frequencies among different studies and ethnic groups. If the effects of this polymorphism vary with HPV type then analysis stratified by HPV type might reveal associations with cervical cancer susceptibility. However, one of the limitations of the present study is the lack of HPV data among cases to allow for analysis stratified by HPV type or phylogenetic groups. Although HPV information exists for about 50 % of the cases, the number of cases positive for HPV16 (the most common HPV type detected in cervical cancer) was not sufficient to allow for a proper evaluation of effects by HPV status of cases.

The *EVER* SNPs found associated with CxCa in our study and their tagged variants all are located in intronic regions or 3'-untranslated sequences and their putative functional consequences are currently unknown. The variant rs1048591 located in the 3' UTR of *TNRC6C* and the variant rs16970849 located in an intron *of TMC8* tag several other SNPs (Supplementary material, figure 1) located also in intronic regions of the TMC genes. Although, there is evidence that TNRC6C gene plays a role in RNA-mediated gene silencing by micro-RNAs (miRNAs) [24-26], there is no observation for a possible participation of this gene in cancer development.

Several steps have been taken in order to account for differences among the two pooled Swedish cohorts in the present study: i) We performed all analysis by using a Monte Carlo significance testing (meta genetic) which did not show any differences on the risk estimates for all associated and non-associated SNPs. ii) We tested for heterogeneity among the cohorts by using the Breslow-Day test. This analysis did not result in any significant heterogeneity for the included SNPs, except for the SNP rs3813026 (p=0.01), which showed no association with disease. iii) We calculated all risk estimates adjusting for Cohort differences. Nonetheless, we acknowledge the need for caution in interpreting our findings, and the need for additional replication efforts before the association between EVER genes and cervical cancer is considered established.

In conclusion, our observations support the involvement of the *TMC6/8* region in susceptibility to CxCa. Caution in the statistical analysis for genetic studies is needed even when studying very homogeneous populations such as ours. More work such as deep-sequencing of the region is required in order to establish the full spectrum of variation and identify the functional variants. Future studies would benefit from including HPV type in the analysis. It would also be of interest to investigate the contribution of associated variants in other HPV-related cancers, and to consider the association of TMC6/8 variation with HPV antibody responses. The latter would be not only relevant for clarifying the observed associations of TMC6/8 genes with HPV seropositivity against alpha and beta-types, but also for understanding the mechanisms of these genes in HPV infection.

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References

1. Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, Snijders PJ, Peto J, Meijer CJ, Munoz N. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol. 1999; 189:12–9. [PubMed: 10451482]
2. Magnusson PK, Sparen P, Gyllensten UB. Genetic link to cervical tumours. Nature. 1999; 400:29–30. [PubMed: 10403244]
3. Ivansson EL, Gustavsson IM, Magnusson JJ, Steiner LL, Magnusson PK, Erlich HA, Gyllensten UB. Variants of chemokine receptor 2 and interleukin 4 receptor, but not interleukin 10 or Fas ligand, increase risk of cervical cancer. Int J Cancer. 2007; 121:2451–7. [PubMed: 17688234]
4. Ivansson EL, Magnusson JJ, Magnusson PK, Erlich HA, Gyllensten UB. MHC loci affecting cervical cancer risk: distinguishing the effects of HLA-DQB1 and non-HLA genes TNF, LTA, TAP1 and TAP2. Genes Immun. 2008
5. Ivansson EL, Juko-Pecirep I, Gyllensten UB. Interaction of immunological genes on chromosome 2q33 and IFNG in susceptibility to cervical cancer. Gynecol Oncol. 2009
6. Castro FA, Haimila K, Sareneva I, Schmitt M, Lorenzo J, Kunkel N, Kumar R, Forsti A, Kjellberg L, Hallmans G, Lehtinen M, Hemminki K, et al. Association of HLA-DRB1, interleukin-6 and cyclin D1 polymorphisms with cervical cancer in the Swedish population--a candidate gene approach. Int J Cancer. 2009; 125:1851–8. [PubMed: 19585495]
7. Ramoz N, Rueda LA, Bouadjar B, Favre M, Orth G. A susceptibility locus for epidermodysplasia verruciformis, an abnormal predisposition to infection with the oncogenic human papillomavirus type 5, maps to chromosome 17qter in a region containing a psoriasis locus. J Invest Dermatol. 1999; 112:259–63. [PubMed: 10084299]
8. Ramoz N, Rueda LA, Bouadjar B, Montoya LS, Orth G, Favre M. Mutations in two adjacent novel genes are associated with epidermodysplasia verruciformis. Nat Genet. 2002; 32:579–81. [PubMed: 12426567]

9. Ramoz N, Taieb A, Rueda LA, Montoya LS, Bouadjar B, Favre M, Orth G. Evidence for a nonallelic heterogeneity of epidermodysplasia verruciformis with two susceptibility loci mapped to chromosome regions 2p21-p24 and 17q25. J Invest Dermatol. 2000; 114:1148–53. [PubMed: 10844558]
10. Orth G. Host defenses against human papillomaviruses: lessons from epidermodysplasia verruciformis. Curr Top Microbiol Immunol. 2008; 321:59–83. [PubMed: 18727487]
11. Patel AS, Karagas MR, Pawlita M, Waterboer T, Nelson HH. Cutaneous human papillomavirus infection, the EVER2 gene and incidence of squamous cell carcinoma: a case-control study. Int J Cancer. 2008; 122:2377–9. [PubMed: 18224692]
12. Lazarczyk M, Cassonnet P, Pons C, Jacob Y, Favre M. The EVER proteins as a natural barrier against papillomaviruses: a new insight into the pathogenesis of human papillomavirus infections. Microbiol Mol Biol Rev. 2009; 73:348–70. [PubMed: 19487731]
13. Wang SS, Gonzalez P, Yu K, Porras C, Li Q, Safaeian M, Rodriguez AC, Sherman ME, Bratti C, Schiffman M, Wacholder S, Burk RD, et al. Common Genetic Variants and Risk for HPV Persistence and Progression to Cervical Cancer. PLoS One. 5:e8667. [PubMed: 20084279]
14. Engelmark M, Beskow A, Magnusson J, Erlich H, Gyllensten U. Affected sib-pair analysis of the contribution of HLA class I and class II loci to development of cervical cancer. Hum Mol Genet. 2004; 13:1951–8. [PubMed: 15238505]
15. Jacobsson JA, Danielsson P, Svensson V, Klovins J, Gyllensten U, Marcus C, Schioth HB, Fredriksson R. Major gender difference in association of FTO gene variant among severely obese children with obesity and obesity related phenotypes. Biochem Biophys Res Commun. 2008; 368:476–82. [PubMed: 18249188]
16. de Bakker PI, Yelensky R, Pe'er I, Gabriel SB, Daly MJ, Altshuler D. Efficiency and power in genetic association studies. Nat Genet. 2005; 37:1217–23. [PubMed: 16244653]
17. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics. 2005; 21:263–5. [PubMed: 15297300]
18. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet. 2007; 81:559–75. [PubMed: 17701901]
19. Breslow NE, Day NE. Statistical methods in cancer research. Volume I - The analysis of case-control studies. IARC Sci Publ. 1980:5–338. [PubMed: 7216345]
20. Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, Higgins J, DeFelice M, Lochner A, Faggart M, Liu-Cordero SN, Rotimi C, et al. The structure of haplotype blocks in the human genome. Science. 2002; 296:2225–9. [PubMed: 12029063]
21. Curtin K, Wong J, Allen-Brady K, Camp NJ. PedGenie: meta genetic association testing in mixed family and case-control designs. BMC Bioinformatics. 2007; 8:448. [PubMed: 18005446]
22. Allen-Brady K, Wong J, Camp NJ. PedGenie: an analysis approach for genetic association testing in extended pedigrees and genealogies of arbitrary size. BMC Bioinformatics. 2006; 7:209. [PubMed: 16620382]
23. Lazarczyk M, Pons C, Mendoza JA, Cassonnet P, Jacob Y, Favre M. Regulation of cellular zinc balance as a potential mechanism of EVER-mediated protection against pathogenesis by cutaneous oncogenic human papillomaviruses. J Exp Med. 2008; 205:35–42. [PubMed: 18158319]
24. Jakymiw A, Lian S, Eystathioy T, Li S, Satoh M, Hamel JC, Fritzler MJ, Chan EK. Disruption of GW bodies impairs mammalian RNA interference. Nat Cell Biol. 2005; 7:1267–74. [PubMed: 16284622]
25. Meister G, Landthaler M, Peters L, Chen PY, Urlaub H, Luhrmann R, Tuschl T. Identification of novel argonaute-associated proteins. Curr Biol. 2005; 15:2149–55. [PubMed: 16289642]
26. Till S, Ladurner AG. RNA Pol IV plays catch with Argonaute 4. Cell. 2007; 131:643–5. [PubMed: 18022355]

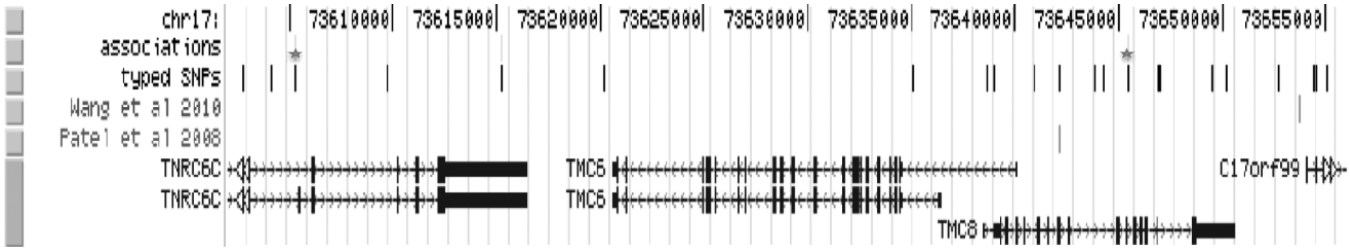

Figure 1. The chromosomal region on chromosome 17 harbouring the TMC6 and TMC8 genes

The image displays the position of the two CxCa associated SNP in the present study (rs2290907, and rs16970849) which are marked with stars. Variants previously reported to be associated with squamous cell carcinoma of the skin[11] and CxCa[13] are denoted by author and study year. Created with the UCSC Genome Browser looking at the Human genome assembly March 2006 (NCBI36/hg18).

Table 1

SNP genotyped in the current study

SNP	Gene	Chr. Position[a]	Location[a]
rs16970811	TNRC6C	73602775	Intronic
rs2311001	TNRC6C	73604148	Intronic
rs2290907	TNRC6C	73605272	Intronic
rs16970829	TNRC6C	73609776	Intronic
rs1048591	TNRC6C	73615279	UTR-3
rs9807014	TMC6	73620232	nearGene-3
rs3813026	TMC6	73635123	Intronic
rs11658760	TMC6	73638671	Intronic
rs383603	TMC6	73639026	intron UTR-5
rs450474	TMC6	73640937	nearGene-5
rs7208422	TMC8	73642170	306 Asn-Ile
rs412611	TMC8	73643854	Intronic
rs8068430	TMC8	73644287	Intronic
rs16970849	TMC8	73645503	Intronic
rs17773842	TMC8	73646997	Intronic
rs17773854	TMC8	73647007	Intronic
rs11654773	TMC8	73649528	UTR-3
rs4789015	TMC8	73650172	UTR-3
rs9915090	TMC8	73652728	Upstream
rs748708	UNQ464/PRO809[b]	73654390	Intronic
rs7217374	UNQ464/PRO809[b]	73654604	Intronic
rs11656744	UNQ464/PRO809[b]	73655029	Intronic

[a] Chromosome position and location based on information from UCSC Genome Browser assembly March 2006 (NCBI B36 assembly)

[b] Also described as uncharacterized protein C17orf99.

Table 2

Polymorphisms associated with CxCa (CINIII + ICC) in combined and adjusted analysis

SNP	Genotype	Cases	Controls	Combined analysis				Adjusted by cohort			
				OR	95% CI		P_{trend} [a]	OR	95% CI		P_{trend} [a]
rs16970811	CC	8	7	0.9	0.3	2.4	0.7	1.0	0.3	3	0.5
	AC	273	216	1.0	0.8	1.2		0.9	0.8	1.1	
	AA	2624	2001	1.0	--	--		1.0	--	--	
rs2311001	GG	288	222	1.0	0.8	1.2	0.5	0.9	0.7	1.1	0.3
	CG	1302	961	1.1	0.95	1.2		1.0	0.9	1.1	
	CC	1324	1043	1.0	--	--		1.0	--	--	
rs2290907	GG	38	46	0.6	0.4	0.97	0.3	**0.6**	**0.3**	**0.9**	0.1
	AG	670	508	1.0	0.9	1.1		1.0	0.8	1.1	
	AA	2204	1667	1.0	--	--		1.0	--	--	
rs16970829	GG	8	15	0.4	0.2	0.9	0.04	0.4	0.2	1.1	0.1
	AG	361	304	0.9	0.8	1		0.9	0.7	1.1	
	AA	2547	1908	1.0	--	--		1.0	--	--	
rs1048591	GG	8	15	0.4	0.2	0.9	0.03	0.4	0.2	1.1	0.05
	CG	354	300	0.9	0.7	1		0.9	0.7	1	
	CC	2540	1899	1.0	--	--		1.0	--	--	
rs9807014	TT	93	54	1.4	0.97	1.9	0.03	1.3	0.9	1.9	0.4
	CT	801	577	1.1	0.97	1.2		1.0	0.9	1.2	
	CC	2019	1598	1.0	--	--		1.0	--	--	
rs3813026	GG	14	15	0.7	0.3	1.5	0.2	0.9	0.4	2	0.5
	AG	383	315	0.9	0.8	1.1		1.1	0.9	1.3	
	AA	2515	1893	1.0	--	--		1.0	--	--	
rs11658760	TT	623	535	0.9	0.8	1	0.1	1.0	0.8	1.2	0.8
	GT	1461	1069	1.0	0.9	1.2		1.0	0.9	1.2	
	GG	809	613	1.0	--	--		1.0	--	--	
rs383603	GG	160	100	1.3	0.98	1.7	0.04	1.3	0.9	1.7	0.7
	CG	1070	785	1.1	0.97	1.2		0.9	0.8	1.1	
	CC	1686	1342	1.0	--	--		1.0	--	--	

SNP	Genotype	Cases	Controls	Combined analysis				Adjusted by cohort			
				OR	95% CI		P_{trend} [a]	OR	95% CI		P_{trend} [a]
rs450474	CC	30	22	1.0	0.6	1.8	0.3	1.2	0.7	2.3	0.5
	CT	474	397	0.9	0.8	1.1		0.9	0.8	1.1	
	TT	2336	1774	1.0	--	--		1.0	--	--	
rs7208422	TT	680	573	0.8	0.7	0.9	0.01	1.0	0.8	1.2	0.8
	AT	1472	1134	0.9	0.8	1		0.9	0.8	1.1	
	AA	764	521	1.0	--	--		1.0	--	--	
rs412611	TT	10	3	2.6	0.7	9.3	0.2	3.5	0.9	13.7	0.97
	CT	265	188	1.1	0.9	1.3		0.9	0.7	1.1	
	CC	2636	2034	1.0	--	--		1.0	--	--	
rs8068430	CC	111	115	0.7	0.5	0.9	0.03	0.9	0.6	1.2	0.98
	CT	919	720	0.9	0.8	1.1		1.1	0.9	1.2	
	TT	1882	1390	1.0	--	--		1.0	--	--	
rs16970849	AA	6	9	0.5	0.2	1.4	0.003	0.6	0.2	1.8	**0.02**
	AG	279	266	0.8	0.7	0.9		**0.8**	**0.7**	**0.98**	
	GG	2629	1951	1.0	--	--		1.0	--	--	
rs17773842	TT	615	509	0.9	0.7	0.995	0.04	1.0	0.8	1.2	0.9
	GT	1451	1121	0.9	0.8	1		1.0	0.8	1.1	
	GG	851	599	1.0	--	--		1.0	--	--	
rs17773854	AA	243	191	1.0	0.8	1.2	0.6	1.0	0.8	1.3	0.6
	AG	1190	926	1.0	0.9	1.1		0.9	0.8	1	
	GG	1464	1104	1.0	--	--		1.0	--	--	
rs11654773	GG	2	2	0.8	0.1	5.4	0.5	0.7	0.1	6.1	0.7
	GT	170	140	0.9	0.7	1.2		1.0	0.7	1.2	
	TT	2736	2075	1.0	--	--		1.0	--	--	
rs4789015	AA	611	402	1.3	1.1	1.6	0.0004	1.1	1	1.4	0.1
	AG	1459	1089	1.2	1.03	1.3		1.1	0.9	1.3	
	GG	842	733	1.0	--	--		1.0	--	--	
rs9915090	TT	460	291	1.3	1.1	1.6	0.001	1.2	1	1.4	0.1
	CT	1433	1066	1.1	1.01	1.3		1.1	0.9	1.2	
	CC	978	830	1.0	--	--		1.0	--	--	

SNP	Genotype	Cases	Controls	Combined analysis				Adjusted by cohort			
				OR	95% CI		P_{trend} [a]	OR	95% CI		P_{trend} [a]
rs748708	AA	41	40	0.8	0.5	1.2	0.1	0.9	0.5	1.4	0.7
	AG	635	519	0.9	0.8	1.1		1.0	0.9	1.1	
	GG	2177	1650	1.0	--	--		1.0	--	--	
rs7217374	AA	571	472	0.9	0.7	1	0.05	0.9	0.8	1.1	0.3
	AG	1424	1106	0.9	0.8	1		0.9	0.8	1.1	
	GG	905	639	1.0	--	--		1.0	--	--	
rs11656744	AA	71	72	0.7	0.5	1	0.03	0.7	0.5	1.1	0.3
	AG	738	598	0.9	0.8	1		1.0	0.9	1.1	
	GG	2107	1557	1.0	--	--		1.0	--	--	

OR, odds ratios from the unconditional logistic regression model; 95% CI, 95% confidence intervals

[a] P-value for the test of trend (P_{trend}) for the number of copies of the variant allele (0,1,2); SNPs rs16970829 and rs1048591 were in high LD (r2>0.8).