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Merkel cell carcinoma and Merkel cell polyomavirus: a systematic review and meta-analysis

SHORT RUNNING TITLE: Correlation between Merkel cell carcinoma and Merkel cell polyomavirus

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What is already known about this topic?
Several studies have assessed the correlation between merkel cell carcinoma and merkel cell polyomavirus with inconsistent results.

What does study add?

A systematic review and a meta-analysis including all studies up to August 2014.

BACKGROUND: several observational studies have assessed the correlation between merkel cell carcinoma and merkel cell polyomavirus with variable results.

OBJETIVE: to determine whether there is a correlation between merkel cell carcinoma and merkel cell polyomavirus.

METHODS: Studies assessing the relationship between merkel cell carcinoma and merkel cell polyomavirus up to August 2014 were pooled from MEDLINE, EMBASE, PubMed, Cochrane Database of Systemic Reviews and Google Scholar. From each study, first author´s last name, publication year, origin country, type of study design, characteristics of participants, possible variables incorporated into the multivariable analyses, and the relative risk (RR) for merkel cell carcinoma associated with merkel cell polyomavirus joint with the corresponding 95% confidence interval (95% CI) were collected. Methodological assessment of the study was evaluated using the Newcastle-Ottawa scale (NOS). Crude RR was calculated from the data provided in each article. Meta-analyses for the global RR and for the proportion of positives in both case and control samples were performed. In addition, in order to explore the sources of heterogeneity among the studies, meta-regression and sensitivity analyses are also provided.

RESULTS: A total of 22 studies were identified for the analysis. The pooled RR from random effects analysis was determined to be 6.32 (95% CI, 4.02-9.93). Global proportions of positive samples were 0.79 (95% CI, 0.72-0.84) and 0.12 (95% CI, 0.08-0.19) in the case and control groups, respectively.

CONCLUSIONS: The findings support the association between merkel cell carcinoma and Merkel cell polyomavirus. However, some find a non-negligible percentage of positive in controls. Some caution must be taken in the interpretation of these results because we found heterogeneity between studies.

INTRODUCTION

Merkel cell carcinoma (MCC) is a rare and aggressive malignant skin cancer which appears in the elderly (1). Whether MCC are derived from Merkel cells, epidermal stem cells or pluripotent dermal stem cells is yet to be determining (2). MCC resembles a neuroendocrine tumor with expression of synaptophysin and chromogranin A and characteristically paranuclear dot-like expression pattern of cytokeratin 20, which distinguishes it from other neuroendocrine tumors (1).

Polyomaviruses are small double-stranded DNA viruses that are suspected as etiologic agents of human cancer. Feng and colleagues (3) reported the identification of 5th human polyoma virus that was designed by Merkel cell polyomavirus (MCV) based on its detection in MCC. This finding supports the hypothesis that MCV is a major contributor to the pathogenesis of MCC. The understanding of the biology of MCC disease, has allowed the development of targeted/immune therapies in MCC. Currently new clinical trials are investigating this approach as antibody drug conjugates, immunotherapies like cytotoxic T-Lymphocyte antigen 4 (CTLA-4, CD152), programmed Death 1 (PD-1) (4, 5) and the development of vaccines. A therapeutic MCV DNA vaccine is a compelling option for the MCC treatment (6).

The aim of this work was to examine the relationship between MCC and MCV. With this goal, a systematic review and a meta-analysis of all published data related to this topic has been performed.

METHODS:

Data Sources and Searches: Published studies that assess the association between MCC and MCP were searched in MEDLINE; EMBASE and PubMed databases covering the period from January 2008 up to August 2014. In addition, we searched Cochrane Database of Systematic Reviews and Google Scholar. Literature search was carried out in first stage using the Medical Subject Headings (Mesh) terms “merkel cell carcinoma” and “merkel cell polyomavirus” in PubMed (“Merkel cell carcinoma” [Mesh] AND “Merkel cell polyomavirus” [Mesh] AND (“2008/01/01” [PDAT]: “2014/08/01” [PDAT]) meanwhile in EMBASSE we searched for “merkel cell carcinoma” and “merkel cell polyomavirus” using “explosion” terms or “major focus” (`merkel cell

carcinoma´/mj AND `merkel cell polyomavirus´ AND [2008-2014]/py) or (`merkel cell carcinoma´/exp/mj AND `merkel cell polyomavirus´/exp/mj AND [embase]/lim AND [2008-2014]/py).

In a second stage, the total hits obtained from the initial search were screened by reading the "title" and "abstract". Studies not satisfying the inclusion criteria were excluded at this stage. The studies selected for inclusion in the second stage were further screened for suitability during stage three by reading the selected manuscripts. The reference lists of retrieved papers were also checked for the identification of additional studies. This process was conducted by two independent reviewers (CG and PC).

The study inclusion selection criteria are listed below:

1. Prevalence of MCV in cutaneous MCC is the studied variable.
2. The presence of MCV is confirmed by polymerase chain reaction.
3. To have original data.
4. To have a control group.
5. To provide risk ratio (RR) estimates with confidence intervals or enough data to calculate them.

Data Extraction and Quality Assessment: For each study, we extracted the following information: the first author´s last name, publication year, country of origin, study design, and RR of MCV associated with MCC along with the corresponding 95% confidence interval (CI) (Table 1). From the data provide in each article, crude RR was also calculated. To measure study quality we use the Newcastle-Ottawa scale (NOS) (7) (Table 2). All evaluations were performed by the same author (JSJ). The variables are categorized into three dimension including, selection, comparability, and exposure for case-control studies. The selection contains four items, the comparability contains one item, and the exposure contains two items. A star system is used to allow a semi-quantitative assessment of study quality. A study can be awarded a maximum of one star for each numbered item within the selection comparability and exposure categories. The NOS ranges from zero up to seven stars. We consider high quality studies those that achieve more than six stars, medium quality study between four and five stars.

Data Synthesis and analysis: for each study, we constructed separate 2x2 tables to calculate the RR and 95% confidence intervals (CI) to assess the association between MCV and MCC. Q test was performed to evaluate the between-study heterogeneity of the study. The degree of heterogeneity among studies was assessed using chi-square and I^2 test. An I^2 greater than >50% is conventionally considered substantial heterogeneity. In this case, the DerSimonian and Laird random effect models were considered in order to compute the global RR. To explore sources of heterogeneity among the studies and determine how they would influence the estimates we perform meta-regression and sensitivity analyses.

The presence of publication bias was investigated graphically by constructing a funnel plot. In addition, the association between variance was analyzed by the L´Abbe plot.

Summary relative risks (RR) and positive proportions for both cases and controls were computed from the usual random and fixed effect models. The Mantel-Haenszel method was used in order to compute the variability intra-study while the DerSimonian-Laird estimator was used for approximating the value of τ^2 (tau-square, variability among studies). A continuity correction of 0.5 was employed in those studies with zero cell frequencies. Main results were summarized by forest plots which included global and particular 95% confidence intervals. In addition, an analysis including the standard procedure for investigating: heterogeneity, publication bias, influence analysis, and meta-regressions, was performed only for RR. The package Meta and Metafor for the statistical environment R, all of them freely downloaded from the CRAN (www.r-project.org) were used for all computes.

All the protocol was designed according to the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) reporting guidelines, and an elaborated checklist was followed. Besides, although the performed systematic review has not been registered and therefore it has not a registered number; it satisfies most of the PRISMA statements (8).

RESULTS

Finally, only 22 papers fulfilled the inclusion criteria (1, 3, 9-28). Due to clearly set inclusion/exclusion criteria the two teams performing independent screening selected the same papers. A flowchart of the identified studies in this review is presented in 4.1.

Due to our restrictive selection criteria all papers herein included obtained a minimum of five stars in the NOS scale. Three of them obtained the maximum NOS score (seven stars). Eighteen papers obtained six stars; within this group all studies lack a star in the control groups. Only one study obtained five stars, in addition to the absence of a star in the control group item it lack the star in the comparability item. The results derived from the three studies with seven stars were really variable but not different to the rest of included studies (see figures 2, 6 and 7). The pooled RR from random effects analysis for the association between MCC and MCV was determined to be 6.32 (95%CI, 4.02-9.93) (Figure 2).

The indicators suggest that the heterogeneity is high: τ^2=0.63; H = 2.19 [1.80; 2.67]; I^2 = 79.2% [69.2%; 86%]. The Q of Chocarme has a p-value less than 0.001. Figure 3 (left) shows the plot of L'Abble heterogeneity.

Evidence for publication bias was found (Egger´s test p<0.001). The series included generally show very few controls, most of them being negative. Only series with enough controls find a relatively high percentage of positivity in this, as indicated by visual inspection of the funnel plot (Loyo et al, Martel-Jantin et al) (Figure 3, right). This issue is clearer pointed out in Figure 7.

The sensitivity analysis shows a variation that ranges from 5.81 to 7.13. This procedure performs k different meta-analysis (k stands for the number of included studies) excluding, each time, one of the papers. This process allows determining the robustness of the obtained results and detecting the possible influence points (papers) (Figure 4).

The meta-regression studies the influence of the factor year, continent, and influence outcomes. Particularly, no trend was found by publication year (p-value=0.276). Relative risks ranged between 2.44 in 2008 and 20.11 in 2011. In regard to the continent, there are not significant differences noted between them (p-value=0.521). Figure 5 depicts the bubbles plot for both publication year and continent; size of the points is according with the sample size.

Finally, meta-analysis on the proportions of positives found in the cases and controls were performed. In both cases the heterogeneity is not negligible; in the cases group: τ^2=0.398; H = 1.63 [1.3; 2.05]; I^2 = 62.5% [40.6%; 76.3%]; and in the controls group: τ^2= 0.8011; H = 2.32 [1.92; 2.81]; I^2 = 81.5% [72.8%; 87.3%]. Random effects models

estimated a positive proportion of 0.79 [0.72; 0.84] in cases and a non-negligible 0.12 [0.08; 0.19] in controls. Figures 6 and 7 show the respective forest plot.

DISCUSSION

A systematic review and a meta-analysis is a systematic approach to identifying, appraising, synthesizing, and, when appropriate, combining the results of relevant studies to draw conclusions about a body of research. In addition, meta-analyses allow knowing the state of the art, strength and weakness of the considered topic (29). Due to the possible increase in the between studies variability, the application of formal meta-analytic methods to observational studies has been controversial. Stroup et al. recommended a checklist to be followed in meta-analyses of observational studies and conclude that; in spite of the obvious limitations, meta-analyses of observational studies are valuable tool for helping to understand and quantify sources of variability in result across studies and thus the number of published meta-analyses concerning observational studies in health sciences has significantly increased in the last decades (30, 31).

The MCC is one of the most lethal cutaneous malignancies, with a five-year overall survival of approximately 50%. To date, MCC remains an orphan disease. The study of its association with the MCV may change the therapeutic approach to treat MCC. At this moment new therapies are being tested (4). PCR is not the only technique suitable for detection of MCV, this virus can also be detected by immunohistochemistry with highly sensitive and specific monoclonal antibodies (CM2B4) directed against the large T antigen (22, 25, 28).

In the last years, many articles have been published on the relationship between MCC and MCV. Less attention has been paid to the presence of polyomavirus in others cutaneous tumors or controls. In order to understand the true role of MCV in the MCC this information seems essential.

We have not found previous meta-analyses focused on the correlation between MCV and MCC. In the meta-analysis herein presented, we include 22 studies that met inclusion criteria. The studies included have a high quality due to restrictive inclusion criteria used. Although the prevalence in cases was generally high (overall mean of 79%), the prevalence in controls was not negligible (12%), especially, in the studies that

included more controls. The studies that have reported the highest prevalence in controls are the works of Loyo et al (42%) and Mantel-Jantin et al (39%). We found statistically significant association among MCC and MCV in 18 of the 22 studies. As usual in this type of analyses, provided results were not adjusted by possible confounders (any of the original papers provided adjusted RRs). This is a clear limitation which suggests that results must be taken carefully and not causal conclusion can be derived.

There are several limitations to our meta-analysis and to the studies that this review is based upon. There are various sources of heterogeneity that exist in the studies (L´Abbe´s test). This evidence of heterogeneity must be valued on the combined results. A major difficulty in integrating the findings from various studies stems from the diverse nature of the studies being combined. The studies may differ, for example, in terms of patient characteristics or methods employed for diagnosis. To account for such inter-study differences, the random effects model proposed by DerSimonian and Laird was used (32). A number of technical aspects (quality of the DNA, sensibilities of the PCR methods) may have contributed to the wide range of observed detection proportions. For instance, in the same work the detection rate was always higher in DNA extracted from fresh or frozen tumor samples that in DNA from FFPE tissues (13,19). This is, most likely, linked to the degradation of the DNA in fixed tissue which decreases PCR sensitivity. However, we must notice that these technical limitations affect in the same way cases and controls and the RR of these works are not the greatest, Kassem et al and Chun et al found on FFPE tissues the highest RR. On the other hand to overcome the different PCR sensitivity resulting from the use of primers, several primers were used in most studies (Table 1). In most studies performed on relatively large series, around 10 to 20% of the samples from MCC tumors are found negative for MCV detection using several specific primers (1,3,9,13,19,21). It should be noted that the interpretation of the meta-analysis when heterogeneity or variability among studies are present is controversial. We could confirm the publication bias in our meta-analysis by performing a funnel plot and Egger and Begg´s tests. Funnel plots are a visual tool for investigating publication bias (the association of publication probability with the statistical significance of study results). If studies showing no statistically significant effects remain unpublished, then such publication bias will lead to an asymmetrical appearance of the funnel plot. Editorial criterion usually prioritize works with large

effects size finding and then, studies with small sample sizes are only available (published) when the size effects is large which produces a big publication bias. Also, the control samples often have various flaws; few, not comparable samples (blood donors). We want to point out the need for studies that include more cases, and especially larger number of well-selected controls. Hence, it is apparent that despite the differences of the included studies, a correlation between MCC and MCV could be possible.

TABLES LEGEND

Table 1. F.A: First author. Study: CCR: case-controls retrospective. Material FFPE: formalin-fixed paraffin-embedded material; Fr: frozen material. N.R: not reported. Crude RR: crude relative risk. 95% CI: 95% confidence interval.

Table 2. Scale NOS each of the selected items. 1: Suitable case definition. 2: representativeness of cases. 3: Selection of controls. 4: Definition of controls. 5: Comparability. 6: Knowledge of exposure. 7: There is some method to discern enter cases and controls.

FIGURES LEGEND

Figure 1: Flowchart of selection process. Course of systematic literature review on MCC and MCV.

Figure 2: Principal forest plot (Risk Ratio). Studies are ordered by publication´s year. The point estimate (center of each blue square) and the statistical size (proportional area of square) are represented. Horizontal lines indicate 95% confidence intervals. The pooled odds ratio (diamond) was calculated by means of a random effects model. RR, relative risk; CI, confidence interval.

Figure 3. Publication bias funnel plots for the primary outcome, at left. Graphic of heterogeneity of L´Abbé, at right.

Figure 4. Influence analysis. Relative risks are represented by omitting the aforementioned study.

Figure 5. Bubble plot for the meta-regression with year and continent factor. The size of the bubbles is proportional to the weight of the groups.

Figure 6. Forest plot for the proportion of cases.

Figure 7. Forest plot for the proportion of controls.

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F.A, Year, Ref	Country	Study / material	Cases	Controls	Crude RR (95%CI)	PCR primers
Feng (2008) (3)	U.S	CCR/Fr	9	84	7.26 (3.57-14.75)	LT1, LT3, VP1.
Kassem (2008) (1)	Germany	CCR/FFPE	39	45	70.27 (4.44-1112.12)	LT1, LT3, VP1, M1/2.
Becker (2009) (9)	Germany	CCR/FFPE	53	24	6.79 (2.34-19.70)	LT1, LT3
Garneski (2009)(10)	U.S/Australia	CCR/N.R	21	30	5.71 (1.35-24.26)	LT1.
Hembold (2009)(11)	Germany	CCR/FFPE	98	44	10.10 (3.96-25.76)	MCPyV.
Shito (2009) (12)	Finland	CCR/FFPE	114	22	35.96 (2.32-558.25)	LTA.
Touze (2009) (13)	France	CCR/FFPE/Fr	32	9	12.57 (0.84-188.73)	LT1, VP1.
Varga (2009) (14)	Hungary	CCR/FFPE	7	29	43.27 (2.68-698.74)	LT1, LT3, VP1.
Wieland (2009) (15)	Germany	CCR/N.R	34	95	4.66 (3.02-7.18)	LT1, LT3
Wetzels (2009) (16)	Netherlands	CCR/FFPE/Fr	5	18	16.82 (0.94-300.59)	LT1, LT3, VP1.
Andres (2010) (17)	Germany	CCR/FFPE	33	33	3.50 (1.62-7.55)	LTA (MCV 138), STA (MCV 191).
Bathia (2010) (18)	U.S	CCR/FFPE	23	52	38.43 (5.43-271.81)	MCPyV (EU375804)
Foulogne (2010) (19)	France	CCR/FFPE/Fr	11	24	3.27 (1.55-6.91)	LT1, LT3, VP1.
Loyo (2010) (20)	US	CCR/FFPE/Fr	7	286	2.06 (1.48-2.87)	LT3, VP1.
Mangana (2010)(21)	Switzerland	CCR/FFPE	30	19	26.21 (1.68-408.91)	LT1, LT3, VP1.
Kuwamoto(2011) (22)	Japan	CCR/FFPE	22	3	6.38 (0.49-83.28)	LT3, MCVPS1, MCVKW3.
Jung (2011) (23)	Korea	CCR/FFPE	11	24	2.18 (1.21-3.92)	LT1, LT3, VP1 LT1-1, LT1-1a, LT3a.
Martel-Jantin(2012) (24)	France	CCR/FFPE	36	31	2.44 (1.56-3.83)	LT3, MerkT.
Rodig (2012) (25)	U.S	CCR/FFPE	51	6	13.00 (0.91-186.42)	LT2, Set 6, 7, 9 LT3.
Chun (2013) (26)	Korea	CCR/FFPE	7	32	56.33 (3.55-894.24)	LTA (MCV 138), STA (MCV 191)
Fukumoto (2013) (27)	Japan	CCR/FFPE/Fr	30	183	4.47 (3.02-6.62)	STA, LT1,LT3, VP1, VP2, VP3.
Hattori (2013) (28)	Japan	CCR/FFPE/Fr	26	21	38.13 (2.46-592.19)	LT1, LT3, VP1.

Table 1. F.A: First author. Study: CCR: case-controls retrospective. Material FFPE: formalin-fixed paraffin-embedded material; Fr: frozen material. N.R: not reported. Crude RR: crude relative risk. 95% CI: 95% confidence interval.

	1. CD	2. RC	3. CS	4. CD	5. CP	6. KE	7. DCS	Total
Feng (2008) (3)	*	*		*	*	*	*	**6**
Kassem (2008) (1)	*	*	*	*	*	*	*	**7**
Becker (2009) (9)	*	*		*	*	*	*	**6**
Garneski (2009) (10)	*	*		*	*	*	*	**6**
Hembold (2009) (11)	*	*	*	*	*	*	*	**7**
Shito (2009) (12)	*	*		*	*	*	*	**6**
Touze (2009) (13)	*	*		*	*	*	*	**6**
Varga (2009) (14)	*	*		*	*	*	*	**6**
Wieland (2009) (15)	*	*		*	*	*	*	**6**
Wetzels (2009) (16)	*	*		*	*	*	*	**6**
Andres (2010) (17)	*	*		*	*	*	*	**6**
Bathia (2010) (18)	*	*		*	*	*	*	**6**
Foulogne (2010) (19)	*	*		*	*	*	*	**6**
Loyo (2010) (20)	*	*		*	*	*	*	**6**
Mangana (2010) (21)	*	*		*	*	*	*	**6**
Kuwamoto (2011) (22)	*	*		*		*	*	**5**
Jung (2011) (23)	*	*		*	*	*	*	**6**
Martel-Jantin (2012) (24)	*	*	*	*	*	*	*	**7**
Rodig et al (2012) (25)	*	*		*	*	*	*	**6**
Chun (2013) (26)	*	*		*	*	*	*	**6**
Fukumoto (2013) (27)	*	*		*	*	*	*	**6**
Hattori (2013) (28)	*	*		*	*	*	*	**6**

Table 2. Scale NOS each of the selected items. 1: Suitable case definition. 2: representativeness of cases. 3: Selection of controls. 4: Definition of controls. 5: Comparability. 6: Knowledge of exposure. 7: There is some method to discern enter cases and controls.

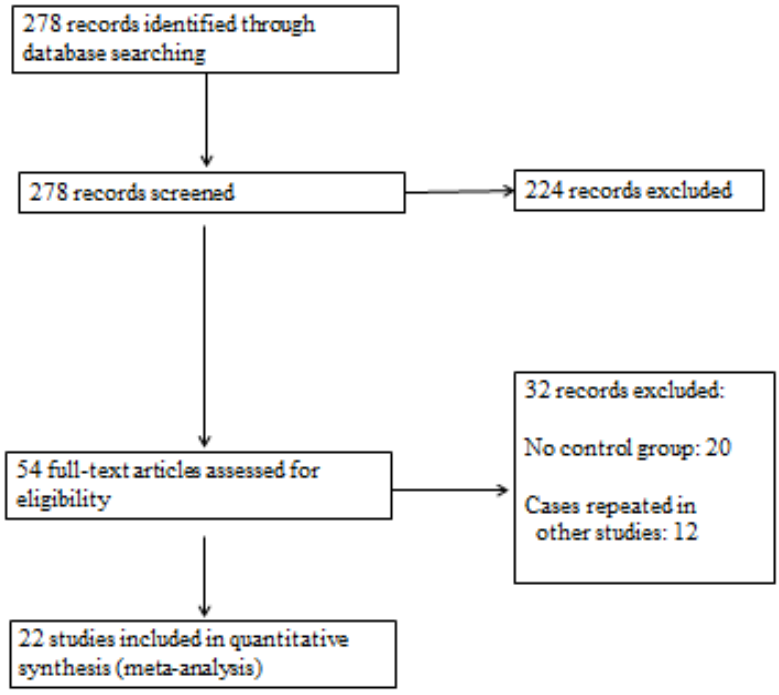

Figure 1. Flowchart of selection process. Course of systematic literature review on Merkel cell carcinoma and Merkel polyomavirus.

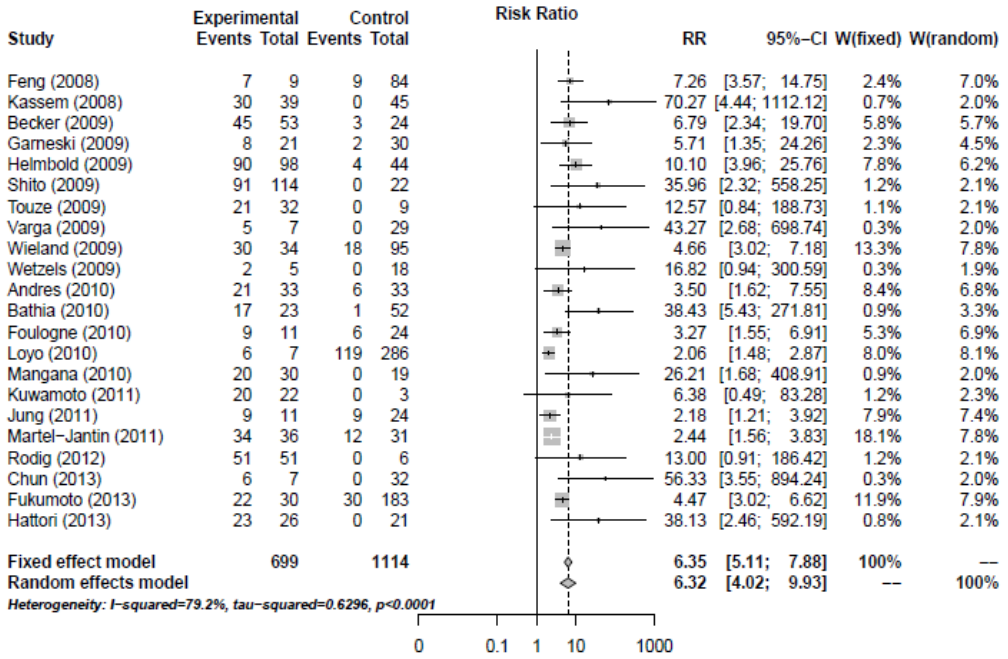

Figure 2. Principal forest plot (Risk Ratio). Studies are ordered by publication´s year. The point estimate (center of each blue square) and the statistical size (proportional area of square) are represented. Horizontal lines indicate 95% confidence intervals. The pooled odds ratio (diamond) was calculated by means of a random effects model. RR, relative risk; CI, confidence interval.

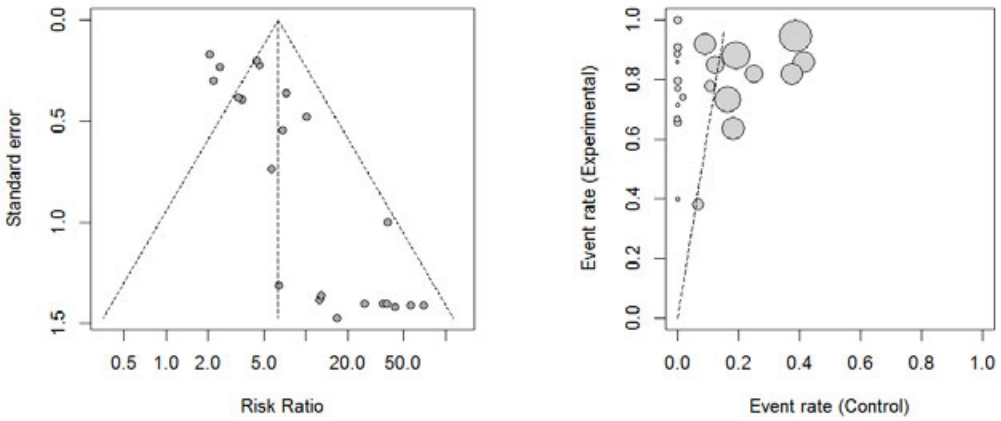

Figure 3. Publication bias funnel plots for the primary outcome, at left. Graphic of heterogeneity of L´Abbé, at right.

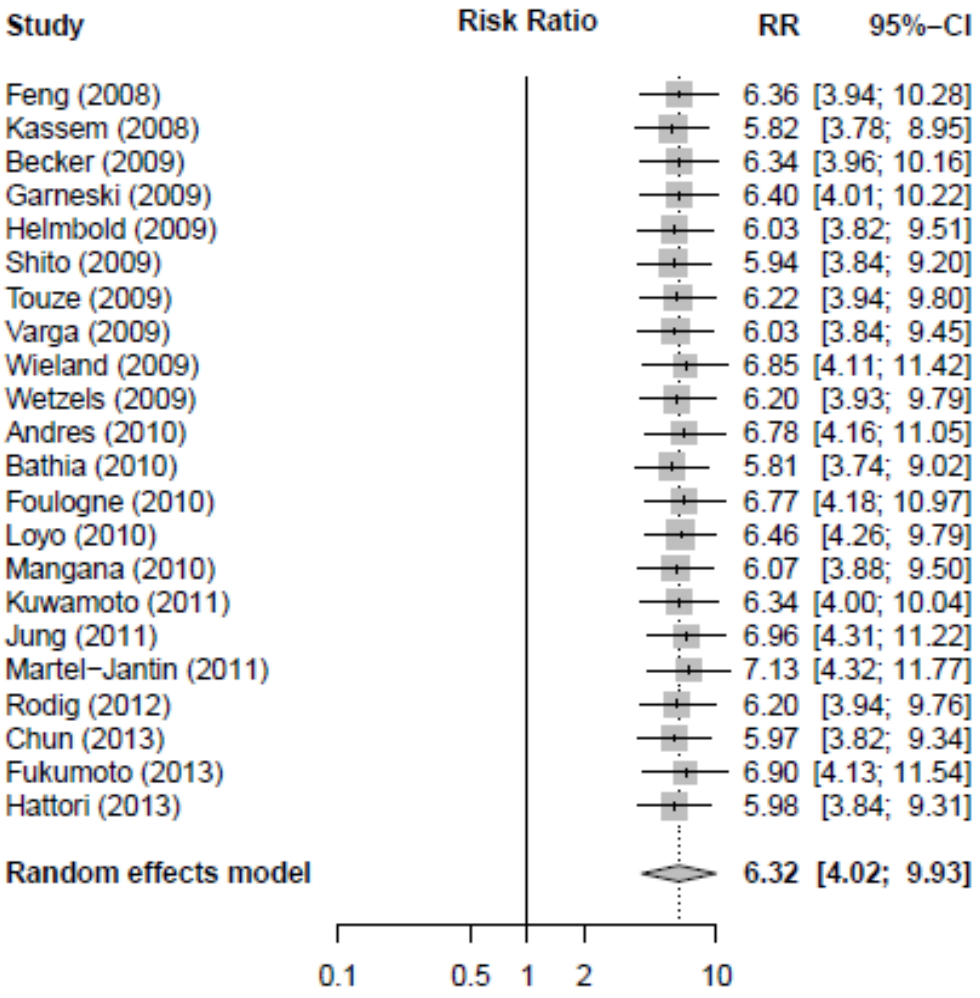

Figure 4. Inluence analysis. Relative risks are represented by omitting the aforementioned study.

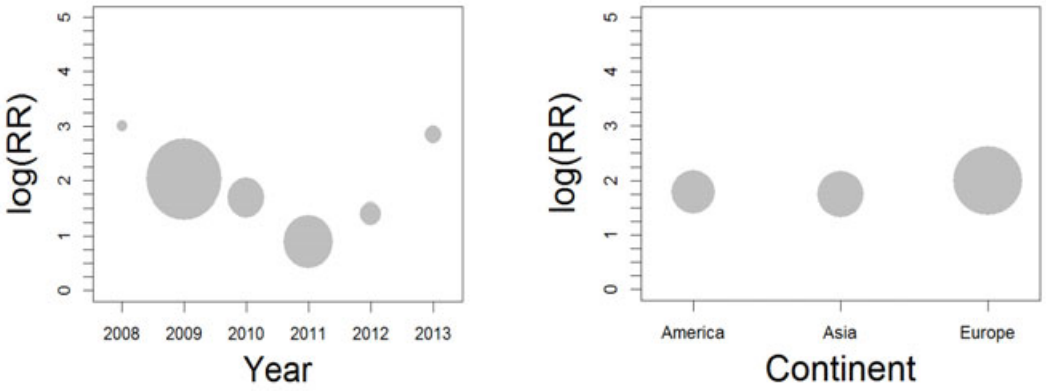

Figure 5. Bubble plot for the meta-regression with year and continent factor. The size of the bubbles is proportional to the weight of the groups.

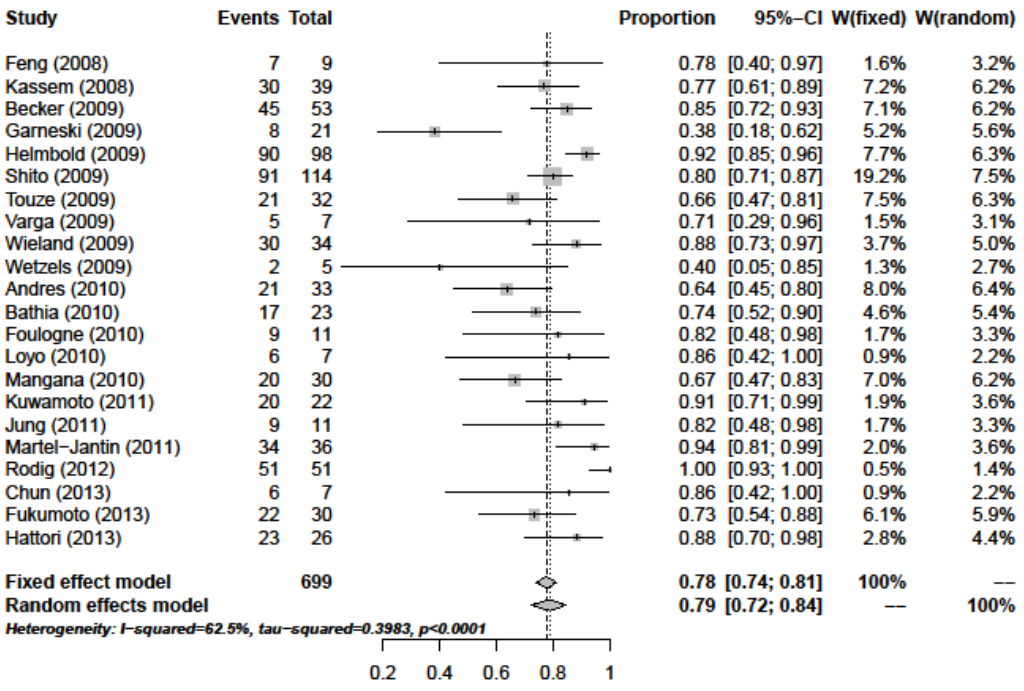

Figure 6. Forest plot for the proportion of cases

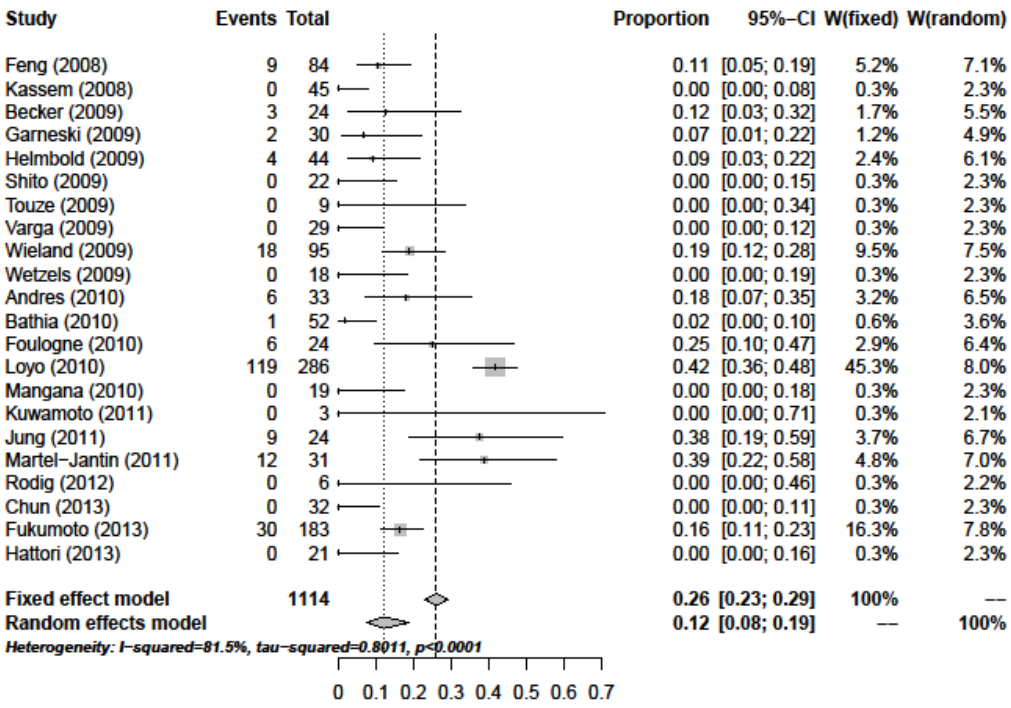

Figure 7. Forest plot for the proportion of controls