Prevalence of \textit{EGFR} Tyrosine Kinase Domain Mutations in Head and Neck Squamous Cell Carcinoma: Cohort Study and Systematic Review

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Abstract. Background: Mutations in the epidermal growth factor receptor (\textit{EGFR}) tyrosine kinase domain (TKD) are associated with response and resistance to targeted therapy. The \textit{EGFR} mutation status in patients with advanced oral and oropharyngeal squamous cell carcinoma (OOSCC) was evaluated. A systematic literature review was undertaken to summarize current evidence and estimate the overall prevalence of \textit{EGFR} TKD mutations in patients with head and neck squamous cell carcinoma (HNSCC). Materials and Methods: Genomic DNA was extracted from formalin-fixed, paraffin-embedded tumor samples of 113 patients with OOSCC. Pyrosequencing was performed to investigate mutations in \textit{EGFR} exons 18 to 21. Medline databases were searched for relevant studies. Studies reporting mutations in the \textit{EGFR} TKD in HNSCC were eligible for inclusion in the systematic review. Results: No mutations in the \textit{EGFR} TKD were observed in 113 samples of OOSCC. A total of 53 eligible studies were included in the systematic review. In total, from the review, 117 patients harboring a total of 159 \textit{EGFR} TKD mutations were reported among 4122 patients with HNSCC. The overall prevalence of \textit{EGFR} TKD mutations in HNSCC was 2.8%. Conclusion: Large-scale studies are warranted to provide further evidence regarding the mutation status of \textit{EGFR} in patients with HNSCC.

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summarize current evidence regarding the *EGFR* mutation status in HNSCC. The present study aimed to estimate the overall prevalence of *EGFR* TKD mutations in patients with HNSCC and whether differences in the prevalence of *EGFR* mutations exist between patients with HNSCC across different countries and geographic regions.

Materials and Methods

*Population of cohort study.* This study included 113 patients diagnosed with primary locally advanced OOSCC who underwent neoadjuvant chemoradiation followed by tumor resection at the Department of Radiotherapy and Department of Cranio-Maxillofacial and Oral Surgery at the Medical University of Vienna between 2000 and 2009. All of them had: biopsy-proven OOSCC, available pretreatment biopsy tumor tissues, clinical TNM stage III or IV disease, no distant metastasis, no previous history of head and neck cancer, performance status and laboratory parameters permitting chemoradiotherapy and surgery, and had undergone complete resection (R0) of the primary tumor. The multimodal treatment comprised neoadjuvant chemotherapy with mitomycin C (15-20 mg/m², i.v. bolus injection on day 1) and 5-fluorouracil (750 mg/m²/day, continuous infusion on days 1-5), concurrent with radiation therapy delivered over 5 weeks up to a total dose of 50 Gy (25 fractions of 2 Gy per day) followed by post-treatment radical surgery. Surgery was performed 4-8 weeks after the end of radiotherapy. Patients were followed-up regularly for further 5 years.

Patient data were obtained from the Vienna General Hospital Patient Information System (AKIM). Clinical and pathological TNM staging was based on the seventh edition of the classification Union for International Cancer Control (UICC) (18). The surgical specimens were histopathologically evaluated by means of an institutional standard protocol. Pathological complete response (pCR) was defined by the absence of residual cancer within both the primary tumor site and regional lymph nodes. A study-specific patient number was given to patients to protect their identity. The Ethics Committee of the Medical University of Vienna approved this research (approval number: 774/2008).

**HPV testing.** E6/E7-specific quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) was used to determine the HPV16/18 status. Results were corroborated by p16 (cyclin-dependent kinase inhibitor 2A) immunohistochemical staining.

*EGFR mutation testing.* EGFR mutations were studied in DNA extracted from formalin-fixed, paraffin-embedded tissue blocks routinely archived at the Department of Pathology at the Medical University of Vienna. For each patient, one section of an appropriate tumor block was stained with hematoxylin and eosin to confirm the presence of viable carcinoma cells. DNA was extracted from tumor samples confirmed to have >50% cancer cells. From each block, 5-μm-thick sections were cut for DNA extraction performed using the QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer’s protocol. *EGFR* mutation analysis was carried out with Therascreen® Pyro Kit (Qiagen, Hilden, Germany). The *EGFR* kit enables detection and quantitation of the most common mutations in codon 719 (exon 18), exon 19 deletions, codon 768 and 790 (exon 20) and codon 858-861 (exon 21) of the *EGFR* gene.

Table I. Patient clinical and histopathological characteristics.

| Characteristic       | Total No. (%) | Pathological response, n (%) |
|----------------------|--------------|------------------------------|
|                      |              | pCR  | Non-pCR |
| Patients             | 113 (100)    | 46 (41) | 67 (59) |
| Age (years)          |              |      |        |
| ≤60                  | 72 (64)      | 29 (40) | 43 (60) |
| >60                  | 41 (36)      | 17 (42) | 24 (58) |
| Gender               |              |      |        |
| Male                 | 83 (73)      | 28 (34) | 55 (66) |
| Female               | 30 (27)      | 18 (60) | 12 (40) |
| Smoking              |              |      |        |
| Current              | 94 (83)      | 37 (39) | 57 (61) |
| Former or never      | 19 (17)      | 9 (47)  | 10 (53) |
| Alcohol use          |              |      |        |
| Current              | 83 (73)      | 36 (43) | 47 (57) |
| Former or never      | 30 (27)      | 10 (33) | 20 (67) |
| Tumor site           |              |      |        |
| Oral cavity          | 97 (86)      | 40 (41) | 57 (59) |
| Oropharynx           | 16 (14)      | 6 (37)  | 10 (63) |
| Clinical TNM stage   |              |      |        |
| Stage III            | 6 (5)        | 4 (67)  | 2 (33)  |
| Stage IV             | 107 (95)     | 42 (39) | 65 (61) |
| HPV status           |              |      |        |
| HPV−                 | 104 (92)     | 44 (42) | 60 (58) |
| HPV+                 | 9 (8)        | 2 (22)  | 7 (78)  |

pCR, Pathological complete response; HPV, human papillomavirus.

Figure 1. Kaplan–Meier estimates of the probability of overall survival in 113 patients with oral and oropharyngeal cancer according to pathological response to neoadjuvant treatment.
After the extraction of genomic DNA, EGFR was amplified by PCR with HotStarTaq DNA Polymerase (Qiagen) after the DNA concentration of each sample was adjusted to 2 ng/μl. The PCR conditions were 95°C for 15 min for the initial activation of HotStarTaq DNA Polymerase, followed by a 3-step-cycling: denaturation at 95°C for 20 s, annealing at 53°C for 30 s and extension at 72°C for 20 s for 42 cycles. Finally, incubation at 72°C for 5 min was accomplished for the final extension. After amplification, the immobilization of PCR products on Streptavidin Sepharose High-Performance (GE Healthcare, Uppsala, Sweden) beads was performed using a volume of 10 μl PCR product which was added to a 24-well PCR plate containing 70 μl master mix [2 μl Streptavidin Sepharose High-Performance beads, 40 μl of PyroMark Binding Buffer (Qiagen) and 28 μl water]. The next step was the preparation of single-stranded DNA with a PyroMark Q24 Vacuum Workstation (Qiagen) and the annealing of the sequencing primer (included in the Therascreen® Pyro Kit; Qiagen) to the template. Pyrosequencing of the samples was then carried out on a PyroMark Q24 MDx system (Qiagen). The results were analyzed with PyroMark Q24 software (version 2.0.6; Qiagen). Pyrosequencing results in the initial round of sequencing were confirmed by subsequent runs of independent PCRs and pyrosequencing, as well as by Sanger sequencing.

Systematic literature review: Data sources, search strategy, selection of studies, and data extraction. This study followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (19). Medline databases (hosts: PubMed and OVID) from inception up to October 20, 2016 were searched for relevant studies using the key words “head and neck cancer”, “EGFR”, and “mutation”. No search restriction was applied. The complete search strategy can be found in Appendix A. In addition, manual searches were conducted on the web and by reviewing the reference lists of the retrieved articles.

Studies reporting the mutation status of the EGFR TKD in tumor tissues of patients with HNSCC were eligible for inclusion in the systematic review. For quantitative synthesis, only studies reporting the prevalence of EGFR TKD mutations in HNSCC were considered. Letters and unpublished research were not included in the present review. Case reports were considered as qualitative evidence. Two reviewers (CP and RP) independently carried out study selection and data extraction. Any disagreements between
reviewers were resolved by consensus involving a third reviewer (JE). The reviewers independently screened all records that were identified by the search strategy. Duplicate publications were excluded both electronically and manually. The selected records were pooled, retrieved as full-text publications, and assessed for eligibility. The two reviewers independently extracted data from each eligible study using a predefined data-abstraction sheet. The following data were collected: name of the first author, year of publication, study location, characteristics of study cohorts (sample size, tumor stage, tumor site), source of tumor profiled, exon location and type of EGFR mutations, detection methods, prognostic effect of EGFR mutations, and the prevalence of EGFR mutations. The PRISMA flow diagram was used to describe the study selection processes.

Statistical analysis. For the cohort study, patient characteristics were summarized using descriptive statistics. Categorical variables are described with frequencies and percentages. Patient demographic, clinical, and tumor characteristics were tested for association with pathological response using the chi-square test for categorical variables. Overall survival was defined as the time from surgery to death from any cause or to date of last follow-up. The Kaplan–Meier method was used for overall survival assessment and the log-rank test to compare differences in survival between groups. A two-sided p-value of less than 0.05 was considered statistically significant.

The systematic review was quantitatively analyzed to pool the overall prevalence of EGFR mutations in HNSCC. Subgroup analysis was performed to assess the prevalence of EGFR mutations according to geographic region. Prevalence of EGFR mutations was defined as the proportion of patients with EGFR-mutated tumors among patients who underwent the mutation testing and was assessed as percentage with the 95% confidence interval (CI) (20). Subgroups of geographic regions (Europe, North America, Southeast Asia, and South Asia) were generated if two or more studies on a specific geographic region were present. Study location was defined based on the country where the patients were recruited in the study. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS®, version 21.0; IBM Corp., Armonk, NY, USA).

Results

Description of patient cohort and survival analysis. The clinical and histopathological characteristics of 113 study patients are presented in Table I. The median age of patients was 58 years (range=24-79 years) and most of the patients were male (73%) and current smokers (83%). The primary tumor was predominantly located in the oral cavity (86%). Pathological complete response to neoadjuvant chemoradiotherapy was observed in 46 patients (41%). Nine patients (8%) were HPV-positive. Among HPV-positive patients, two (22%) achieved a complete pathological response. Of 16 oropharyngeal tumors, two samples (13%) were positive for HPV, compared to seven HPV-positive samples (7%) out of 97 oral cavity tumors. At 2 years, the overall survival rate of the cohort was 66% and at 5 years 46%. The median follow-up time was 4.6 years by which time 56 (50%) patients had died. The overall survival of the 113 patients with OOSCC was assessed according to the pathological response status using the Kaplan–Meier method (Figure 1). The median overall survival was significantly higher in patients with tumors showing pathological complete response compared with those having non-complete tumor regression (7.9 versus 2.8 years respectively, log-rank p=0.001).

Mutation status of EGFR. The kinase domain of EGFR (exons 18-21) was analyzed in tumor samples from 113 treatment-naïve patients with primary locally advanced OOSCC. Using pyrosequencing technology, no EGFR mutations were detected in any of the 113 tumor tissues (Figure 2).

Characteristics of studies included in the systematic review. Overall, 53 eligible studies were included in the present systematic review. The literature search of databases and reference lists yielded 1203 records. After removing duplicates, 886 records remained to be screened. After screening of titles and abstracts, 824 studies that reported on unrelated topics were excluded. The full-text papers of the remaining 62 studies were reviewed in depth. A total of nine studies did not provide sufficient information regarding the EGFR TKD mutation status and were discarded, thus 53 studies were included in the qualitative review and the quantitative synthesis. The PRISMA flow diagram was used to illustrate the study selection process (Figure 3).
Table II shows the main characteristics of the studies included in the systematic review. All studies were published between 2005 and 2013 in peer-reviewed journals. Most of the studies were conducted in the USA (n=19), followed by the Republic of Korea (n=5), and India (n=4). The source for tumor DNA mutation profiling was fresh frozen tumor samples (n=26), formalin-fixed paraffin-embedded tumor tissue (n=23), or both (n=4). Three studies investigated the prognostic impact of EGFR mutation status on HNSCC survival using the Kaplan–Meier method/Cox proportional hazards regression model (21-23).

Overall prevalence of EGFR TKD mutations in HNSCC and distribution of mutations by exon location and type. In total, 117 patients harboring a total of 159 EGFR TKD mutations were reported among 4122 patients with HNSCC. The overall pooled prevalence of EGFR TKD mutations in HNSCC was 2.8% (95% CI=2.4-3.4%). The distribution of mutations in the EGFR TKD categorized by exon location and mutation type is shown in Figure 4. Among EGFR-mutated HNSCCs, the majority of TKD mutations were located in exon 19 (41.5%, 95% CI=33.8-49.6%), followed by exon 20 (32.1%, 95% CI=25.0-40.0%), exon 21 (17.0%, 95% CI=11.7-23.9%), and exon 18 (9.4%, 95% CI=5.6-15.3%). Of all EGFR mutations, missense mutations in exons 18-21 occurred in 7.5% (95% CI=4.1-13.1%) and 2.5% (95% CI=0.8-6.7%) of all EGFR mutations, respectively.

Prevalence of EGFR TKD mutations in HNSCC by study location. The EGFR mutation prevalence classified by study location (countries and geographic regions) is shown in Table III. The prevalence of EGFR-mutated HNSCCs was estimated in four geographic regions: the highest prevalence was found in Southeast Asia (4.9%, 95% CI=3.7-6.4%), followed by North America (2.7%, 95% CI=2.1-3.6%; all 19 studies were conducted in the USA), and Europe (1.3%, 95% CI=0.7-2.6%), whereas in South Asia (all four studies were conducted in India), no EGFR TKD mutations were detected. Additionally, one study from South America stated the finding of two EGFR mutations in 45 tumor samples (prevalence 4.4%, 95% CI=0.8-16.4%) and one study from Australia reported one EGFR mutation in 60 tumor samples (prevalence 1.7%, 95% CI=0.1-10.1%). When considering EGFR TKD mutations in individual countries, the Republic of Korea had the highest prevalence with 15.1% (95% CI=11.2-20.1%), followed by the Czech Republic (6.9%, 95% CI=1.2-24.2%), and Greece (3.3%, 95% CI=0.8-9.9%).

Discussion

In this study, pyrosequencing technology was used to identify the EGFR mutation status in pretreatment tumor samples of patients with locally advanced OOSCC. No EGFR TKD mutations were observed among 113 cases of
| First author (Reference) | Year | Study location | Site/source of tumor profiled | Stage | No. of patients | Patients with *EGFR* mutation, n (%) | Exon location of *EGFR* mutation | Detection method | Prognostic role impact of *EGFR* mutations |
|--------------------------|------|----------------|-------------------------------|-------|----------------|-------------------------------------|--------------------------------|-----------------|------------------------------------------|
| Current study            | 2016 | Austria        | OC/OP – FFPE                 | III/IV| 113            | 0 (0)                               |                                | PCR, pyrosequencing, Sanger  | NA                                        |
| Ock et al. (28)          | 2016 | Republic of Korea | H&N – FFPE                  | I-IV  | 71             | 19 (26.7)                          | 18-21                           | Targeted NGS, Sanger              | NA                                        |
| Feldmann et al. (34)     | 2016 | USA            | H&N – FFPE                   | NM+M  | 360            | 3 (0.8)                             | 19, 20                           | Targeted NGS                  | NA                                        |
| Chau et al. (35)         | 2016 | USA            | H&N – FFPE                   | I-IV  | 213            | 0 (0)                               |                                | Targeted NGS                  | NA                                        |
| Wu et al. (36)           | 2016 | USA            | H&N – FFPE                   | I-IV  | 214            | 42 (20)                            | 18-20                           | MALDI-TOF MS                  | NA                                        |
| Huang et al. (37)        | 2016 | Republic of Korea | H&N – fresh frozen           | IV    | 18             | 0 (0)                               |                                | Whole exome sequencing           | NA                                        |
| TCGA (25)                | 2015 | USA            | H&N – fresh frozen           | I-IV  | 279            | 1 (0.4)                             | 18                              | Whole exome sequencing           | NA                                        |
| Vetter et al. (38)       | 2015 | Singapore      | Tongue – fresh frozen        | I-IV  | 78             | 0 (0)                               |                                | Whole exome/ targeted NGS      | NA                                        |
| Kim et al. (39)          | 2015 | Republic of Korea | H&N – FFPE/ Fresh frozen     | III/IV| 33             | 2 (6)                               | 19-21                           | Targeted NGS                  | NA                                        |
| Seiwert et al. (26)      | 2015 | USA            | H&N – fresh frozen           | III/IV| 120            | 0 (0)                               |                                | Targeted NGS                  | NA                                        |
| Pickering et al. (40)    | 2014 | USA            | Tongue – fresh frozen        | I-IV  | 42             | 0 (0)                               |                                | Whole exome sequencing           | NA                                        |
| Wang et al. (41)         | 2014 | China          | Larynx – FFPE                | I-IV  | 132            | 3 (2.3)                             | 20, 21                           | Multiplex PCR                  | NA                                        |
| Tan et al. (42)          | 2014 | Singapore      | Tongue – fresh frozen        | I-IV  | 66             | 0 (0)                               |                                | PCR, MALDI-TOF MS              | NA                                        |
| McBride et al. (43)      | 2014 | USA            | H&N – FFPE                   | I-IV  | 64             | 1 (2)                               | 20                              | PCR, sequencing                | NA                                        |
| Mehta et al. (44)        | 2014 | India          | OC – fresh frozen            | NA    | 40             | 0 (0)                               |                                | PCR, sequencing                | NA                                        |
| Bocek et al. (45)        | 2014 | Belgium        | OP/Larynx – FFPE             | III/IV| 52             | 0 (0)                               |                                | HRMA                          | NA                                        |
| Nagalakshmi et al. (46)  | 2014 | India          | H&N – fresh frozen           | I-IV  | 129            | 0 (0)                               |                                | PCR, SSCP, sequencing           | NA                                        |
| Gaykalova et al. (47)    | 2014 | USA            | H&N – fresh frozen           | I-IV  | 37             | 0 (0)                               |                                | Targeted NGS                  | NA                                        |
| Argris et al. (48)       | 2013 | USA            | H&N – FFPE                   | IV    | 69             | 1 (1.4)                             | 20                              | PCR, pyrosequencing             | NA                                        |
| Mati et al. (49)         | 2013 | India          | H&N – fresh frozen           | I-IV  | 148            | 0 (0)                               |                                | PCR, SSCP, sequencing           | NA                                        |
| Lechner et al. (50)      | 2013 | UK             | OP – FFPE                    | I-IV  | 40             | 0 (0)                               |                                | Targeted NGS                  | NA                                        |
| ICGC (51)                | 2013 | India          | OC – fresh frozen            | II-IV | 50             | 0 (0)                               |                                | Whole exome sequencing          | NA                                        |
| Pickering et al. (52)    | 2013 | USA            | OC – fresh frozen            | II-IV | 35             | 0 (0)                               |                                | Whole exome sequencing          | NA                                        |
| Fanjul-Fernandez et al.  | 2013 | Spain          | Larynx – fresh frozen        | IV    | 4              | 0 (0)                               |                                | Whole exome sequencing          | NA                                        |
| Bontognali et al. (54)   | 2013 | Switzerland    | H&N – fresh frozen           | II-IV | 6              | 0 (0)                               |                                | PCR, sequencing                | NA                                        |
| Bahassi et al. (55)      | 2013 | USA            | Larynx – FFPE                | IV    | 1              | 1 (100)                             | 18                              | PCR, sequencing                | NA                                        |
| Smilek et al. (56)       | 2012 | Czech Republic | H&N – FFPE                   | III/IV| 29             | 2 (6.9)                             | 19                              | Real-Time PCR                  | Negative impact on TR                  |
| Tan et al. (57)          | 2012 | Singapore      | H&N – FFPE                   | III-IV| 15             | 2 (13.3)                            | 18, 19                          | PCR, sequencing                | NA                                        |
| Friedland et al. (58)    | 2012 | Australia      | H&N – FFPE                   | NA    | 60             | 1 (1.6)                             |                                | PCR, SSCP, sequencing           | NA                                        |
| Szabo et al. (59)        | 2011 | Hungary        | H&N – FFPE                   | I-IV  | 71             | 0 (0)                               |                                | Real-time PCR, HRMA             | NA                                        |
| Hsie et al. (60)         | 2011 | Taiwan         | OC – FFPE                    | I-IV  | 56             | 2 (3.57)                            | 21                              | PCR, sequencing                | NA                                        |
| Morris et al. (61)       | 2011 | USA            | H&N – fresh frozen           | I-IV  | 31             | 0 (0)                               |                                | PCR, sequencing                | NA                                        |
| Agrawal et al. (62)      | 2011 | USA            | H&N – fresh frozen           | II-IV | 32             | 0 (0)                               |                                | Whole exome sequencing          | NA                                        |
| Stransky et al. (27)     | 2011 | USA            | H&N – fresh frozen           | P/R   | 74             | 0 (0)                               |                                | Whole exome sequencing          | NA                                        |
| Murray et al. (22)       | 2010 | Greece         | H&N – FFPE                   | IV    | 92             | 3 (3.3)                             | 19, 21                          | PCR, sequencing                | NS impact on OS                      |
| Szymanska et al. (63)    | 2010 | South America  | UADT – fresh frozen          | I-IV  | 45             | 2 (4.4)                             | 19, 21                          | PCR, sequencing                | NA                                        |

Table II. Continued
The identification of activating mutations in the \textit{EGFR} TKD in a subset of NSCLC and their association with substantial sensitivity to gefitinib, erlotinib or afatinib represents an important milestone in the therapy of this malignancy (14, 17, 24). Driven by the paradigm in NSCLC, several studies in HNSCC attempted to define the mutational spectrum of the \textit{EGFR} TKD. To date, genomic data from whole exome sequencing and targeted next-generation sequencing studies have provided a comprehensive landscape of genomic alterations in HNSCC (25-27). In a recent study, Ock \textit{et al.} using targeted next-generation sequencing identified \textit{EGFR} TKD mutations in 19 out of 71 (26.7%) HNSCCs (28). The Cancer Genome Atlas data from whole exome sequencing of HNSCCs demonstrated, however, that only one out of 279 (0.4%) tumor samples from HNSCCs harbored a missense mutation in the \textit{EGFR} TKD (25). Given this background, the present systematic review aimed to summarize current evidence regarding the \textit{EGFR} mutation status in HNSCC. Based on the quantitative data analysis, this study demonstrated that the overall prevalence of \textit{EGFR} TKD mutations in HNSCC is 2.8%. This study revealed that the \textit{EGFR} mutation prevalence in patients with HNSCC varies modestly across geographic regions, with the highest prevalence shown (4.9%) in Southeast Asia and the lowest in South Asia (0%). The \textit{EGFR} mutation prevalence within the population of Southeast Asia varies by country, from approximately 1% in Taiwan to 15% in the Republic of

\begin{table}[h]
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\begin{tabular}{|l|l|l|l|l|l|l|l|l|}
\hline
First author (Reference) & Year & Study location & Site/source of tumor profiled & Stage & No. of patients & Patients with \textit{EGFR} mutation, n (%) & Exon location of \textit{EGFR} mutation & Detection method & Prognostic role impact of \textit{EGFR} mutations \\
\hline
Van Damme \textit{et al.} (64) & 2010 & Belgium & Tonsil – FFPE & NA & 24 & 0 (0) & – & PCR, sequencing & NA \\
Keller \textit{et al.} (65) & 2010 & USA & OP/Larynx – fresh frozen & I-IV & 60 & 2 (3.3) & 19 & PCR, sequencing & NA \\
Hama \textit{et al.} (21) & 2009 & Japan & H&N – fresh frozen & I-IV & 82 & 5 (6.1) & 18, 20, 21 & PCR, sequencing & Positive impact on DFS \\
Huang \textit{et al.} (66) & 2009 & Taiwan & OC – fresh frozen & I-IV & 172 & 0 (0) & – & PCR, sequencing & NA \\
Carlson \textit{et al.} (67) & 2009 & USA & H&N – fresh frozen & NA & 20 & 0 (0) & – & PCR, sequencing & NA \\
Jin \textit{et al.} (68) & 2009 & China & H&N – fresh frozen & I-IV & 96 & 0 (0) & – & PCR, sequencing & NA \\
Schwentner \textit{et al.} (69) & 2008 & Austria & H&N – FFPE/ fresh frozen & NA & 127 & 3 (2.4) & 19, 20 & PCR, sequencing & NA \\
Sheikh Ali \textit{et al.} (70) & 2008 & Japan & H&N – fresh frozen & I-IV & 91 & 0 (0) & – & PCR, sequencing & NA \\
Chiang \textit{et al.} (71) & 2008 & Taiwan & OC – FFPE & I-IV & 20 & 0 (0) & – & PCR, sequencing & NA \\
Na \textit{et al.} (23) & 2007 & Republic of Korea & Tongue/tonsil – FFPE & I-IV & 108 & 17 (15.7) & 19-21 & PCR, sequencing & Positive impact on OS \\
Temam \textit{et al.} (72) & 2007 & USA/France & H&N – FFPE/ fresh frozen & I-IV & 134 & 0 (0) & – & PCR, sequencing & NA \\
Lemos-Gonzalez \textit{et al.} (73) & 2007 & Spain & H&N – fresh frozen & NA & 31 & 0 (0) & – & PCR, SSCP, sequencing & NA \\
Peronne \textit{et al.} (74) & 2006 & Italy & OP – FFPE & I-IV & 40 & 1 (2.5) & 19 & PCR, sequencing & NA \\
Chung \textit{et al.} (75) & 2006 & USA & H&N – fresh frozen & I-IV & 52 & 0 (0) & – & PCR, sequencing & NA \\
Willmore-Payne \textit{et al.} (76) & 2006 & USA & H&N – FFPE & NA & 24 & 2 (8.3) & 19, 20 & PCR, HRMA, sequencing & NA \\
Cohen \textit{et al.} (77) & 2005 & USA & H&N – FFPE/ fresh frozen & NA & 82 & 0 (0) & – & PCR, sequencing & NA \\
Lee \textit{et al.} (78) & 2005 & Republic of Korea & H&N – FFPE & NA & 41 & 3 (7.3) & 19 & PCR, SSCP, sequencing & NA \\
\hline
\end{tabular}
\caption{Continued}
\end{table}

\begin{itemize}
\item NA, Not available; NS, non-significant; \textit{EGFR}, epidermal growth factor receptor; TKD, tyrosine kinase domain; OC, oral cavity; OP, oropharynx; H&N, head and neck; UADT, upper aerodigestive tract (included oral cavity, pharynx, larynx and oesophagus); FFPE, formalin-fixed paraffin-embedded; OS, overall survival; DFS, disease-free survival; TR, treatment response; TCGA, The Cancer Genome Atlas; ICGC, International Cancer Genome Consortium; MALDI-TOF MS, matrix-assisted laser desorption ionization time-of-flight mass spectrometry; MFI, median fluorescence intensity; HRMA, high resolution melting analysis; SSCP, single-strand conformational polymorphism; NGS, next-generation sequencing.
\end{itemize}
Korea. In addition, this study showed that the EGFR mutation status in HNSCC has been insufficiently assessed worldwide as evident from the limited number of studies conducted in Australia (n=1) and South America (n=1), and the lack of data from several large geographic regions, particularly Africa, Central America, the Middle East, and Central Asia. Therefore, it is apparent that large-scale and multicenter studies are necessary to provide more definitive answers regarding the prevalence of EGFR mutations across geographic regions and countries and to assess their potential clinical value in patients with HNSCC.

In this systematic review, the overall EGFR mutation status in HNSCC according to exon location and mutation type was explored. The data showed that the most prevalent EGFR kinase domain mutations, accounting for 73% of all EGFR mutations in HNSCC, are missense mutations in exons 18-21. The L858R substitution, well-known in NSCLC, which comprises about 40% of all EGFR mutations in NSCLC and is associated with sensitivity to EGFR TKIs, was found in only 2.5% of all EGFR-mutated HNSCCs (29). The missense mutation T790M in exon 20, which is associated with acquired resistance to EGFR TKIs in about half of all patients with NSCLC, was found in 7.5% of all EGFR mutations in HNSCC (17). In-frame deletions in exon 19, which account for about 45% of all EGFR mutations in NSCLC and are linked to responsiveness to EGFR TKIs, were observed in 22% of all EGFR-mutated HNSCCs (24). Insertion mutations in exon 20, which occur in about 3% of all EGFR mutations in NSCLC and are frequently associated with resistance to EGFR TKIs, were observed in 5% of all EGFR mutations in HNSCC (30). Taken together, it is clear that substantial differences exist between HNSCC and NSCLC regarding the distribution of mutations within exons 18-21 of the EGFR TKD. Unlike NSCLC, EGFR mutations in HNSCC do not involve specific hotspots but are rather scattered throughout exons 18 to 21. Thus, mutation screening in HNSCC should not be limited to the NSCLC hotspot regions in exons 19 and 21 of EGFR. Moreover, given that the overall prevalence of EGFR TKD mutations in HNSCC is 2.8%, it is challenging to identify specific EGFR mutations related to response or resistance to anti-EGFR therapy or other targeted therapies (31).

The present cohort study has some weaknesses, including its retrospective nature and the relatively small sample size. Additionally, next-generation sequencing methods to compare and validate the results of the EGFR mutation testing by pyrosequencing were not used. However, recent studies have shown that pyrosequencing has the ability to detect EGFR mutations at a low ratio of mutant to wild-type alleles and thus provides high analytical sensitivity for identifying EGFR mutations (32, 33). The systematic review is limited in several ways. Firstly, high heterogeneity has to be assumed across the study populations given the differences in study location, tumor site, stage and interventions. Secondly, various mutation testing methods with different sensitivities in detecting EGFR mutations were used across studies. Thirdly, a number of studies limited their EGFR mutation testing to hotspot regions in exons 19 and 21, thus the true prevalence of EGFR mutations might in fact be under-reported. Taken together, the results of the quantitative synthesis should be interpreted cautiously.

In the emerging era of personalized medicine, the identification of clinically useful prognostic and, most importantly, predictive biomarkers to guide treatment decision in patients with cancer is urgently needed. In this study, no mutations were detected using pyrosequencing when analyzing the EGFR TKD mutation status in a cohort of 113 patients with advanced OOSCC. In addition, the systematic review demonstrated that EGFR TKD mutations are rare in HNSCC, with an overall prevalence of 2.8% and modest variation in the prevalence across countries and

| Geographic region/country | No. of studies* | Patients with EGFR mutation/total patients | EGFR mutation prevalence, % (95% CI) |
|---------------------------|----------------|------------------------------------------|-----------------------------------|
| Overall                   | 53             | 117/4122                                  | 2.8 (2.4-3.4)                     |
| Europe                    | 13             | 9/670                                     | 1.3 (0.7-2.6)                     |
| Austria                   | 2              | 3/240                                     | 1.3 (0.3-3.9)                     |
| Spain                     | 2              | 0/35                                      | 0                                 |
| Belgium                   | 2              | 0/76                                      | 0                                 |
| UK                        | 1              | 0/40                                      | 0                                 |
| Switzerland               | 1              | 0/6                                       | 0                                 |
| Czech Republic            | 1              | 2/29                                      | 6.9 (1.2-24.2)                    |
| Hungary                   | 1              | 0/71                                      | 0                                 |
| Greece                    | 1              | 3/92                                      | 3.3 (0.8-9.9)                     |
| France                    | 1              | 0/41                                      | 0                                 |
| Italy                     | 1              | 1/40                                      | 2.5 (0.1-14.7)                    |
| North America             | 19             | 52/1901                                   | 2.7 (2.1-3.6)                     |
| USA                       | 19             | 52/1901                                   | 2.7 (2.1-3.6)                     |
| Southeast Asia            | 15             | 53/1079                                   | 4.9 (3.7-6.4)                     |
| Republic of Korea         | 5              | 41/271                                    | 15.1 (11.2-20.1)                  |
| Taiwan                    | 3              | 2/248                                     | 0.8 (0.1-3.2)                     |
| Singapore                 | 3              | 2/159                                     | 1.3 (0.2-4.9)                     |
| Japan                     | 2              | 5/173                                     | 2.9 (1.1-7.0)                     |
| China                     | 2              | 3/228                                     | 1.3 (0.3-4.1)                     |
| South Asia                | 4              | 0/367                                     | 0                                 |
| India                     | 4              | 0/367                                     | 0                                 |
| South America             | 1              | 2/45                                      | 4.4 (0.8-16.4)                    |
| Australia                 | 1              | 1/60                                      | 1.7 (0.1-10.1)                    |

*The study of Temam et al. (72) included patients from two distinct study locations: 41 patients from France and 93 from the United States, who were categorized into European and North American populations, respectively. The study of Bahassi et al. (55) was not considered for prevalence estimation (case report).
geographic regions. Large-scale studies are warranted to provide further up-to-date evidence regarding the mutation status of EGFR in patients with HNSCC and to investigate whether the EGFR mutation profile of individual tumors is associated with sensitivity or resistance to targeted therapy.

Conflicts of Interest

None declared.

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None.

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