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Diagnostic accuracy of high-resolution melting curve analysis for discrimination of oncology-associated *EGFR* mutations: a systematic review and meta-analysis

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Abstract

Objective: To investigate the diagnostic value of high-resolution melting (HRM) analysis for oncology-associated epidermal growth factor receptor (*EGFR)* gene mutations.

Methods: We systematically searched Embase, PubMed, and Web of Science for HRM and *EGFR* mutation detection studies published through September 2024. True and false positives and negatives were extracted to evaluate the diagnostic accuracy of HRM to detect *EGFR* mutations. The study was registered at INPLASY (INPLASY202490062).

Results: Twenty-six articles were obtained from 416 references. The overall diagnostic sensitivity and specificity were high at 0.95 [95% confidence interval (CI), 0.94–0.96] and 0.99 (95% CI, 0.99–0.99), respectively. Other indicators, including the positive likelihood ratio, negative likelihood ratio, and diagnostic odds ratio, were 144.91 (95% CI: 69.07–304.04), 0.08 (95% CI: 0.04–0.13), and 2405.21 (95% CI: 1231.87–4696.13), respectively. The Q value of the summary receiver operating characteristic curve was 0.979, and the area under the curve was 0.997.

Conclusion: As a pre-screening method, the high specificity, sensitivity, low cost, rapid turnaround, and simplicity of HRM make it a good alternative for clinical practice, but positive results

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must still be obtained for diagnostic confirmation. This study provides a transparent overview of relevant studies in design and conduct.

Keywords
High-resolution melting curve, epidermal growth factor receptor mutation, diagnostic accuracy, oncology-associated disease, systematically evaluate, literature review

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Introduction

The activation of signaling pathways by epidermal growth factor receptor (*EGFR*) plays an important role in the development of tumor-associated diseases. The *EGFR* gene, which has tyrosine kinase activity, is a member of the human epidermal growth factor receptor (HER) family composed of *HER1* (*erbB1*, *EGFR*), *HER2* (*erbB2*, *NEU*), *HER3* (*erbB3*), and *HER4* (*erbB4*). Over-expression of *EGFR* is critical for lung, breast, and gastric cancer and squamous cell carcinoma of the head and neck.[1–3] Activation of *EGFR* launches a series of cellular signaling pathways that promote cancer proliferation, invasion, and metastasis and protects carcinoma cells from apoptosis via an anti-apoptosis pathway.[4,5] Tyrosine kinase inhibitors (TKIs), such as gefitinib and erlotinib, can inhibit this pathway and consequently offer efficacy for patients with an *EGFR* mutation.[6–8] Therefore, *EGFR* gene mutational status is the most sensitive target for TKI therapy selection.

EGFR mutations are located on exons 18, 19, 20, and 21 of *EGFR*, and most include an in-frame deletion of codons 746 to 750 in exon 19 and a missense mutation at codon 858 in exon 21. An activating mutation in *EGFR* can be found in high incidence in non-smokers, women, those with adenocarcinoma, and individuals of Asian ethnic background.[9,10] Currently, several genotypic methods to screen gene mutations and expand the knowledge of drug-gene relationships have been developed, such as DNA sequencing,[11] single-strand conformation polymorphism analysis,[12] denaturing high-performance liquid chromatography,[13,14] allele-specific polymerase chain reaction (PCR),[15] array analysis,[16] pyrosequencing,[17] and high-resolution melting (HRM) curve analysis.[18,19] Some of these methodologies require sample separation on a gel or matrix; others require expensive fluorescently labeled probes or special instruments. However, HRM analysis is performed in a closed-tube system that protects the amplified DNA from cross-contamination, which is the main advantage of HRM analysis, and it has been proven as a rapid, cost-effective method that uses few or no probes.[20] As an alternative molecular testing platform for genotyping of polymorphisms, HRM analysis has been applied to various diseases, such as oncological, infectious, and inherited diseases.[21–23]

HRM curve analysis is a relatively mature method based on the melting profiles of double-stranded PCR products that is widely used in diagnostic laboratories for identification in disease-associated genotyping, sequence matching, methylation studies, single nucleotide polymorphism

analysis, and mutation scanning.[24] It reveals a different melting curve based on DNA duplex melting temperature changes.[25] As the temperature rises, intercalating dye is released, and the fluorescence intensity decreases; then, mutations are distinguished by changes in melt curve shapes compared with a reference profile.[26] Compared with DNA sequencing, HRM analysis requires minimal investment, thus the technology is broadly available, but it also has high sensitivity, rapid turnaround, low cost, and nondestructive and closed tube operations.[27] In selecting a molecular testing platform for genotyping polymorphisms, an important consideration is the rapid delivery of genetic information to meet the need for increasing clinical treatment.

Since its first application for genotyping in 2003, HRM analysis has been extensively used to detect mutations such as *KRAS, BRAF, EGFR,* and *TP53*.[7,18,19,28–30] A recent study suggested that HRM analysis is a promising method to detect *EGFR* mutations.[31] However, its diagnostic accuracy for *EGFR* identification has not been systematically evaluated. It is essential to investigate the *EGFR* mutation signature in tumor-associated diseases, which leads to more suitable decision making for treatment by physicians. Therefore, we performed a meta-analysis to evaluate the accuracy of HRM analysis for *EGFR* mutation identification.

Materials and methods

We performed this meta-analysis according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[32] Our research was registered at INPLASY, registration number INPLASY202490062 (DOI: 10.37766/inplasy2024.9.0062).

Literature search strategy

Excerpta Medica Database (Embase), Medline (using PubMed as the search engine), and the Web of Science were searched to identify relevant publications in English until 12 September 2024, using the search strategy 'epidermal growth factor receptor' or 'EGFR' or 'EGF-R' or 'EGF-receptor' or 'EGF receptor' or 'receptor, epidermal growth factor' or 'transforming growth factor alpha receptor' or 'ERBB-1 proto-oncogene protein' or 'receptor, transforming-growth factor alpha' or 'receptor, transforming growth factor alpha' or 'C-ERBB-1 protein' or 'receptors, epidermal growth factor' or 'receptor, EGF' or 'urogastrone receptor' or 'TGF-alpha receptor' or 'epidermal growth factor receptor kinase' or 'epidermal growth factor receptor protein–tyrosine kinase' or 'epidermal growth factor receptor protein tyrosine kinase' **AND** 'HRMA' or 'HRM' or 'HRMCA' or 'HRMC' or 'high resolution melting analysis' or 'high resolution melting' or 'high resolution melting curve analysis' or 'high resolution melting curve'. We also carried out manual searches for additional eligible studies.

Inclusion and exclusion criteria

Studies were included if HRM was used to study *EGFR* mutations in humans, DNA sequencing (including direct DNA sequencing and pyrosequencing) was used as a reference standard, and true and false positives and negatives (TP, TN, FP, FN) could be calculated from the provided information. The exclusion criteria included studies using only HRM, studies for which the reference standard was not direct DNA sequencing or pyrosequencing, studies for which only positive HRM samples were sequenced, studies examining methylation or epigenetic mechanisms rather than genetic mutations, or studies that were

duplicated, a review, a conference abstract, a letter, or a comment.

Data extraction and quality assessment

We extracted the following data: author's name, publication year, country of origin, specimen sources, mutation prevalence, instruments, disease types, sample number, amplicon length, dye types, and disease-associated mutations. Outcome parameters such as TN, FN, TP, and FP values were calculated based on PCR amplicons, not based on tissue or blood samples. Two authors performed data collection, and disagreements were resolved by discussion or consensus with a third author. We assessed the quality of each study based on the Quality Assessment for Studies of Diagnostic Accuracy (QUADAS-2),[33] which includes four primary domains to evaluate bias and applicability of included studies by assessing patient selection methods, an index test, reference standards, and patient flow through studies.[34]

Statistical analysis

We determined the accuracy of each study with standard methods using Meta-Disc (version 1.4, http://www.hrc.es/investigacion/metadisc_en.htm) and STATA 12.1 software (Stata Corp., College Station, TX, USA). The sensitivity, specificity, positive and negative likelihood ratios (PLRs, NLRs), and diagnostic odds ratio (DOR) from studies and corresponding 95% confidence intervals (CIs) were computed using fixed or random effects models depending on the presence of significant heterogeneity. Degrees of heterogeneity were evaluated with a chi-square test of heterogeneity (Cochran's Q statistical test) and an inconsistency index (I-square). Alternatively, to quantify the effect of heterogeneity, significant heterogeneity was defined as a Q test with $p < 0.10$ or $I^2 > 50\%$. The threshold effect was performed using summary receiver operating characteristic (SROC) curves for each study to ascertain the presence of a "shoulder-arm" pattern, which would suggest a threshold effect.[35] The Spearman correlation coefficient between the logit of sensitivity and logit of 1−specificity for each study was also calculated to assess any threshold effect. A positive correlation ($p < 0.05$) would suggest a threshold effect. Publication bias was determined using funnel plot analysis with STATA 12.1 software.

Meta-regression analysis and subgroup analysis

Meta-regression analysis was performed to explore heterogeneity sources using Meta-Disc (version 1.4) software. A multivariable regression model was applied, and a backward stepwise algorithm with covariates including disease type, specimen source, instruments, and dye type was used. Variables were retained in the regression model if $p < 0.05$. Subgroup analysis was performed if reasons for heterogeneity could be found.

Results

Literature search outcome

The results of the literature search and the stage-wise exclusion process are illustrated in Figure 1. All 416 references were found by searching multiple sources and databases. One hundred ninety-seven records were excluded because of duplicates. After reviewing the titles and abstracts, 182 records were eliminated, and 37 articles were deemed potentially relevant for the next detailed screening. Eleven records were excluded for reasons shown in Figure 1. Finally, 26 articles were retrieved in this meta-analysis and divided into 34

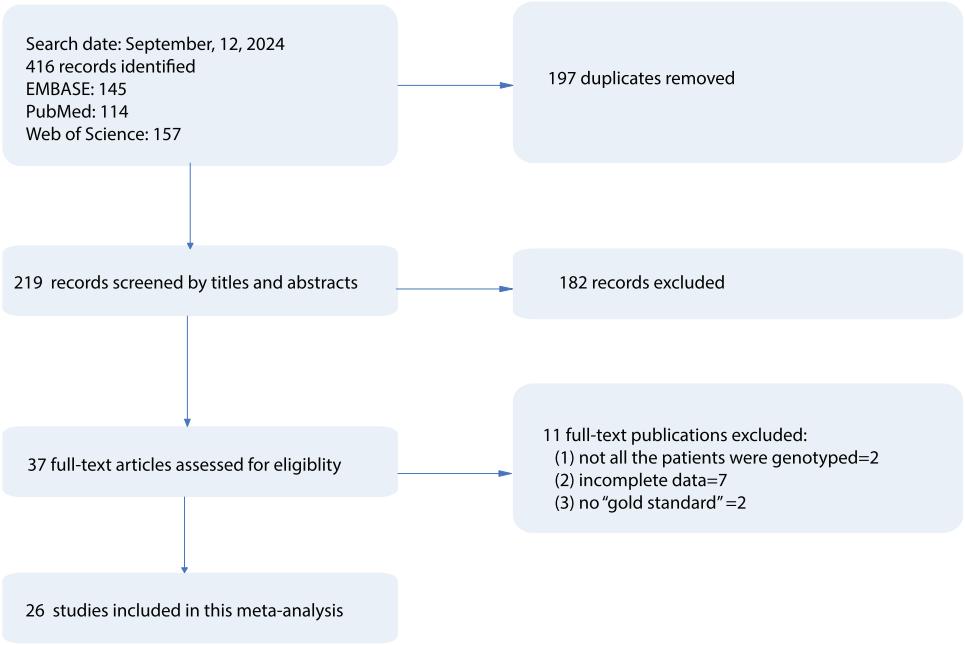

Figure 1. Flow chart of the literature search and study selection.

subsets for statistical analysis according to specimen sources.

Characteristics of the studies

We found 26 eligible studies,[11,18,20,29,36–57] that reported evaluations of diagnostic accuracy of HRM analysis for human disease-associated mutations, as detailed in Table 1. All samples screened by HRM were followed up with direct sequencing. The flow and timing domain was labeled as "unclear risk". The patient selection, index test, and reference standard domains were labeled as "low risk" both for risk of bias and applicability concerns.

Diagnostic accuracy

The diagnostic sensitivity and specificity were 0.95 [95% CI: 0.94–0.96] and 0.99 (95% CI: 0.99–0.99), respectively (Figure 2(a) and 2(b)). As shown in Figure 2(c) and 2(d), a high PLR of 144.91 (95% CI: 69.07–304.04) and a low NLR of 0.08 (95% CI: 0.04–0.13) indicated that HRM analysis had an excellent ability to identify the presence of *EGFR* mutations. Additionally, the DOR supported that HRM analysis was effective for *EGFR* mutation screening (Figure 3(a)). Chi-square and I2 tests for heterogeneity confirmed significant heterogeneity for the specificity and sensitivity of the pooled results. The SROC curve is shown in Figure 3(b). The SROC curve from our data showed a Q value of 0.979, while the area under the curve (AUC) was 0.997, further indicating a high overall accuracy of HRM analysis.

Threshold effect and publication bias

Although the Spearman correlation coefficient between the log (sensitivity) and the log (1−specificity) was 0.092, $p = 0.605$, the typical "shoulder-arm" pattern in the SROC curve suggested a threshold effect.

Table 1. Characteristics of the 26 studies included in this meta-analysis.

No.	Author	Year	Country	Diease	Number of amplicons	Prevalence	Specimen source	Instrument	Dye	Sequence analyzed	Amplicon size	TP*	FP	FN	TN
1	Willmore-Payne, C.[18]	2006	America	Lung adenocarcinoma	156	5%	FFPE	LightCycler	LCG1	Exons 18–21	186–248	6	0	1	149
2	Willmore-Payne, C.[36]	2006	America	Squamous cell carcinoma	120	1.7%	FFPE	LightCycler	LCG1	Exons 18–21	186–259	2	0	0	118
3	Nomoto, K.[37]	2006	Japan	NSCLC	74	28%	Cytologic slides	LightCycler	LCG1	Exon-21 L858R mutation/ -19 DEL	51–83	19	0	2	53
4	Takano, T.[38]	2007	Japan	NSCLC	132	28%	Methanol-fixed	LightCycler	LCG1	Exon-21 L858R mutation/ -19 DEL	51–83	36	0	1	95
	Takano, T.[38]	2007	Japan	NSCLC	56	28%	Cytologic slides	LightCycler	LCG1	Exon-21 L858R mutation/ -19 DEL	51–83	14	0	2	40
	Takano, T.[38]	2007	Japan	NSCLC	126	29%	FFPE	LightCycler	LCG1	Exon-21 L858R mutation/ -19 DEL	51–83	34	0	3	89
5	Fukui, T.[20]	2008	Japan	NSCLC	70	16%	Cytologic slides	HR-1	LCG1	Exon-21 L858R mutation/ -19 DEL	51–83	11	3	0	56
	Fukui, T.[20]	2008	Japan	NSCLC	276	16%	FFPE	HR-1	LCG1	Exon-21 L858R mutation/ -19 DEL	51–83	43	2	3	228
6	Do, H.[39]	2008	Australia	NSCLC	800	11%	FFPE	Rotor-Gene 6000	SYTO9	Exons 18–21	121–250	84	70	0	646
7	Fassina, A.[40]	2009	Italy	NSCLC	154	1.3%	FFPE	LightCycler 480	LCG1	Exon-21 L858R mutation/ -19 DEL	142, 190	2	0	1	151
8	Jacot, W.[41]	2011	France	TNBC	458	1%	Fresh frozen	Rotor-Gene 6000	SYTO9	Exon-21 L858R mutation/ -19 DEL	164, 182	3	0	0	455
9	Sriram, K.B.[42]	2011	Austrilia	NSCLC	194	5%	Fresh frozen	Rotor-Gene 6000	SYTO9	Exon-21 L858R mutation/ -19 DEL	250, 210	9	0	0	185
10	Gonzalez-Bosquet, J.[43]	2011	America	Breast cancer, endometrial cancer, ovarian cancer	216	2.8%	Fresh frozen	PTC 225 Thermal Cycler	LCG plus	Exon-23	213	6	0	0	210

(continued)

Table 1. Continued.

No.	Author	Year	Country	Diease	Number of amplicons	Prevalence	Specimen source	Instrument	Dye	Sequence analyzed	Amplicon size	TP*	FP	FN	TN
	Gonzalez-Bosquet, J.[43]	2011	America	Breast cancer, endometrial cancer, ovarian cancer	172	4%	FFPE	PTC 225 Thermal Cycler	LCG plus	Exon-23	213	7	19	0	146
11	Hu, C.[45]	2012	China	NSCLC	504	15%	FFPE	LightCycler 480	LCG1	Exons 18–21	183–236	74	4	0	426
	Hu, C.[45]	2012	China	NSCLC	188	12%	Fresh frozen	LightCycler 480	LCG1	Exons 18–21	183–236	23	2	0	163
12	Stigt, J.A.[46]	2013	Netherlands	NSCLC	488	4%	FFPE	LightCycler 480II	LCG1	Exons 18–21	247	15	0	5	468
13	Jing, C.-W.[11]	2013	China	NSCLC	480	9%	FFPE	LightCycler 480	LCG1	Exons 18–21	NA	45	3	0	432
	Jing, C.-W.[11]	2013	China	NSCLC	480	9%	Plasma	LightCycler 480	LCG1	Exons 18–21	NA	29	2	16	433
14	Lin, J.[47]	2014	China	NSCLC	144	8%	Supernatant	LightCycler 480	LCG plus	Exons 18–21	NA	12	6	0	126
	Lin, J.[47]	2014	China	NSCLC	144	13%	Cell pellets	LightCycler 480	LCG plus	Exons 18–21	NA	13	0	5	126
	Lin, J.[47]	2014	China	NSCLC	144	9%	FFPE	LightCycler 480	LCG plus	Exons 18–21	NA	13	0	0	131
15	Sun, H.[29]	2014	China	NSCLC	450	10.2%	FFPE	LightCycler 480II	LCG plus	L858R, T790M, 19DEL	NA	46	21	0	383
16	Clay, T.D.[48]	2014	Australia	Lung adenocarcinoma	400	3.5%	FFPE	Rotor-Gene 6000	SYTO 9	Exons 18–21	NA	14	0	0	386
17	Papadopoulou, E.[51]	2015	Greece	NSCLC	5888	4%	FFPE	Rotor-Gene 6000	SYTO 9	Exons 18–21	99–156	233	3	0	5652
18	Oyaert, M.[50]	2015	Belgium	NSCLC	1320	3%	FFPE	LightCycler 480	NA	Exons 18–21	NA	38	0	0	1282
19	Hinrichs, W.J.[49]	2015	Netherlands	NSCLC	100	7%	FFPE	LightCycler 480II	NA	Exons 19–21	NA	6	1	1	92
20	Wayhelova, M.[53]	2016	Czech Republic	Squamous cell carcinoma or adenocarcino-ma	96	0	Fresh frozen	Qubit 1.0	NA	Exons 18–21	NA	0	0	0	96
21	Clay, T.D.[52]	2016	Australia	Pulmonary adenocarcinoma	712	7%	FFPE	NA	NA	Exons 18–21	NA	53	0	0	659
22	Martínez-Carretero, C.[54]	2017	Spain	NSCLC	492	1.8%	FFPE	LightCycler 480	LCG1	Exons 18–21	183–238	7	0	2	483
23	Lu, H.-Y.[55]	2018	China	SCLC	2376	0.5%	Blood	LightCycler 480	LCG1	Exons 18–21	NA	12	0	0	2364
24	Frankel, D.[56]	2018	France	Lung adenocarcino-ma	28	21%	Cell pellets	LightCycler 480	LCG1	Exons 18–21	NA	5	0	1	22
25	Borràs, E.[44]	2011	Spain	Colorectal or lung cancer	108	4.6%	FFPE	LightCycler 480	NA	Exons 19–21	128–250	5	0	0	103
26	Joy, R.A.[57]	2020	India	NSCLC	232	24.4%	FFPE	LightCycler 480	NA	Exon 19, exon 21	NA	67	0	8	157

NSCLC: Non-small cell lung carcinoma; SCLC: Small cell lung cancer; TNBC: Triple-negative breast cancer; FFPE: Formalin-fixed and paraffin embedded; TP: True positive; FP: False positive; FN: False negative; TN: True negative; NA: Not available.

*: Outcome parameters were calculated on the basis of "PCR amplicons", not on the basis of tissue or blood samples.

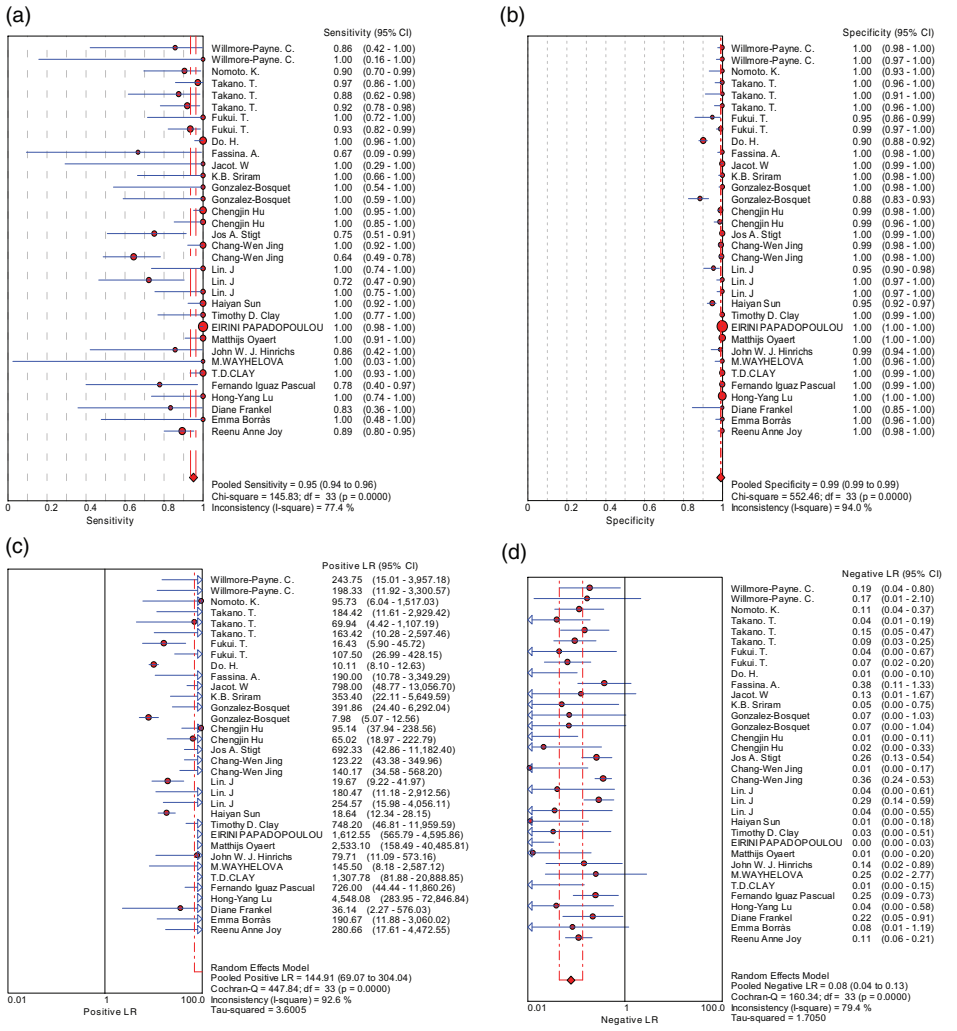

Figure 2. Forest plot estimates of the sensitivity (a), specificity (b), positive likelihood ratio (LR) (c), and negative LR (d) for high-resolution melting with 95% confidence intervals (CIs). Each solid circle represents a subset.

A funnel plot was applied to determine the presence of publication bias, which demonstrated that publication bias was not significant (Figure 4).

Meta-regression analysis and subgroup analysis

Multivariate meta-regression analysis with covariates, including instruments, dyes, specimen sources, and disease types, was performed to investigate the source of heterogeneity. The regression analysis results showed no statistical significance between studies, and subgroup analysis indicated no source of heterogeneity, but a threshold effect was present (data not shown).

Discussion

EGFR mutations predict TKI sensitivity, thus knowing this status could improve

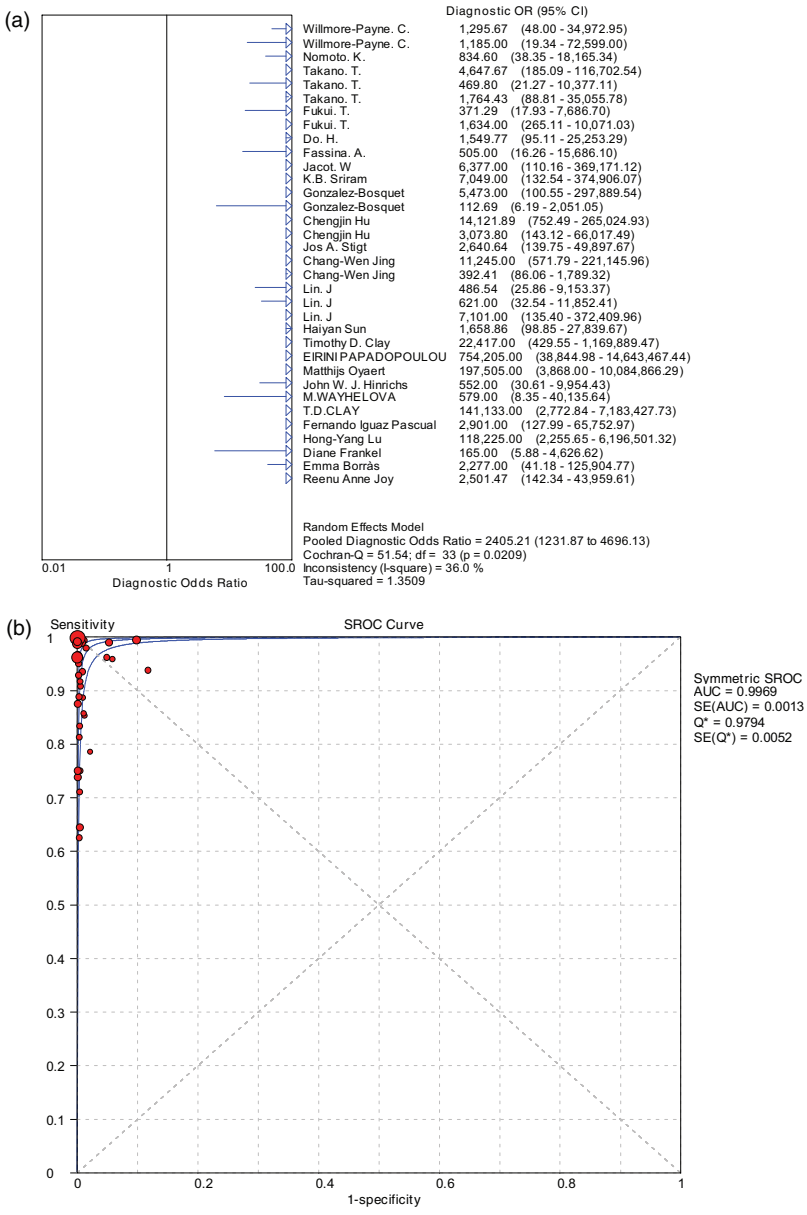

Figure 3. Forest plot estimates of the diagnostic odds ratio (a) with 95% confidence intervals (CIs) and summary receiver operating characteristic (SROC) curve (b) for high-resolution melting. Each solid circle represents a subset.

chemotherapy selection and patient outcome. Specific mutations in oncology-associated proteins respond to certain drugs and correlate with increased sensitivity, suggesting personalized therapeutics based on genotype.[24] Takano's group reported that advanced non-small cell lung cancer patients with *EGFR*

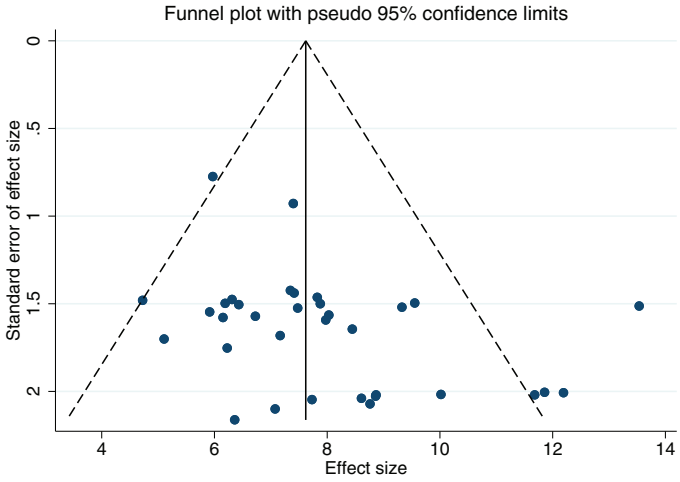

Figure 4. Funnel plot to assess potential publication bias. Each circle is a subset. Publication bias was not significant.

mutations had poorer overall survival, but gefitinib improved the treatment response.[58] Because it is part of the HER gene family, *EGFR,* known as HER-1, is relevant to breast cancer, and there has been ongoing interest in the *EGFR* gene-associated-oncology status. Therefore, a reliable method of screening *EGFR* mutations for therapeutic and prognostic triage may be needed to assess the accuracy in a range of tumor samples.

Thirty-four subsets from 26 published studies and 17,778 samples were assayed to evaluate the diagnostic accuracy of HRM analysis to identify *EGFR* mutations. Although the data show high overall diagnostic accuracy, there was substantial heterogeneity among eligible studies. Exploration of the reasons for heterogeneity rather than computation of a single summary measure has emerged as a main goal of meta-analyses. Thus, it is critical to investigate the sources of heterogeneity to determine whether they alter the appropriateness of statistical pooling of accuracy estimates. The threshold effect is a typical source of heterogeneity that arises when differences in specificities and sensitivities occur because different thresholds are used to define a positive (or negative) result.[59] A "shoulder-arm" shape of the points in the ROC curve indicates a threshold effect in our study, which may partially account for the heterogeneity observed. We performed meta-regression and subgroup analyses to explore further heterogeneity sources, including disease type, specimen source, distribution, instruments, and dye type. However, the heterogeneity source was not found. We also tried excluding the studies of Jacot and Gonzalez[41,43] on breast, endometrial, and ovarian cancer to reduce heterogeneity. However, the heterogeneities found before and after exclusion were similar, which may be the main reason for heterogeneities not resulting from the two studies. The DOR and SROC curve are considered when there is substantial heterogeneity,[60] as the DOR indicates accuracy when combining sensitivity and specificity data into a single ratio of a positive test result.[61] DOR values range from 0 to infinity, with higher values indicating better discriminatory test performances.[61] Our DOR was 2405.21 (95% CI: 1231.87–4696.13). As a

global indicator for assessing diagnostic performance, the SROC AUC also indicated a high accuracy of HRM analysis, with a Q value of 0.979 and an AUC close to 1 (0.997). The DOR and AUC data indicated high overall accuracy of HRM analysis for *EGFR* mutation screening. However, the accuracy of HRM analysis could be affected by sample types, amplicon length, dyes, instruments, PCR specificity, GC content, and melting analysis software.

Although formalin fixation and paraffin-embedding is a commonly used method in *EGFR* mutation detection, low yields of RNA/DNA are extracted from formalin-fixed paraffin-embedded tissues, and they are often degraded or may contain modifications that inhibit polymerase reactions, which can bias results. Additionally, the detection accuracy is critically dependent on the dye type, instrument resolution, PCR product length, and PCR specificity.[62,63] LCGreen Plus dye detects heterozygotes better than SYTO 9, which is better than EvaGreen.[64] Some of the latest real-time thermal cyclers modified to incorporate HRM can yield quality high-resolution data by melting 18 times slower than the HR-1 instrument.[24,65] Melting determination is performed immediately after PCR, and different heterozygotes may produce melting curves so similar that, although they vary from those of homozygous variants, they are not different.[66] Therefore, specific amplification of the target of interest is critical, requiring careful choices of primers and optimized temperature cycling.

HRM analysis has been used to discriminate many tumor variants, such as *BRAF* mutations in colorectal tumors, *KIT* (the c-kit gene) in gastrointestinal stromal tumors, *EGFR,* and *AKTI* in non-small cell lung cancer.[9,17] Driver oncogenes, including *EGFR*, *KRAS*, *and BRAF,* activated by deletion and/or missense/insertion mutations, drive the critical step toward developing non-small cell lung cancer. *EGFR*, *BRAF,* and *KRAS* mutation sensitivities in anti-EGF-receptor therapies are mutually exclusive. Recently published studies reported that HRM analysis is a specific and sensitive method for testing various samples, and a low quantity of DNA is needed for *BRAF* and *KRAS* mutation screening.[67,68] We noted that the SROC AUC was accurate for HRM scanning of the *EGFR* mutation. Therefore, HRM analysis may be a promising method to detect a series of driver oncogene mutations, including *EGFR, KRAS,* and *BRAF* mutations, but confirmation by direct sequencing or other methods is necessary, especially in a diagnostic context.

Our study has several limitations, such as substantial heterogeneity across all included studies. Although meta-regression and subgroup analyses were performed, the sources of heterogeneity were undetermined except for a threshold effect. Additionally, inherent discord was observed between HRM and DNA sequencing. There were 136 FPs and 51 FNs. Mutations found by HRM analysis should always be confirmed with DNA sequencing so that FPs are not an issue (they will be wild type afterward). FNs are relatively serious because they cannot be sequenced, and this may cause mutations in patients to be misclassified as wild type by HRM analysis. Therefore, these patients would be denied TKI therapy. However, the proportion of FNs is very low (approximately 0.29%). Thus, HRM analysis offers appropriate diagnostic performance for *EGFR* mutation screening in oncology-associated diseases and represents a method with high throughput, low labor, low cost, simplicity, and rapid turnaround, but positive results must be sequenced for diagnostic confirmation. Although our meta-analysis focused on the use of HRM analysis to detect *EGFR* gene exon mutations, we recognize the significant roles that *EGFR* gene methylation and epigenetic

regulation play in tumorigenesis. At present, there are relatively few studies on *EGFR* methylation status using HRM techniques, and these studies did not meet the criteria for our analysis. Therefore, our analysis does not encompass these areas. Future research may consider applying HRM analysis to detect *EGFR* methylation status, which could offer new insights into the role of *EGFR* in tumors.

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Author contributions

Shu Yu and Yue-Ping Liu conceptualized and designed the experiments. Yan Cheng analyzed the data. Shu Yu and Yue-Ping Liu wrote the manuscript. Chen-Cheng Tang conducted the investigation and revised the manuscript.

Declaration of conflicting interests

The authors declare that there is no conflict of interest.

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References

1. Ozanne B, Richards CS, Hendler F, et al. Over-expression of the EGF receptor is a hallmark of squamous cell carcinomas. *J Pathol* 1986; 149: 9–14.
2. Wirth LJ, Haddad RI, Lindeman NI, et al. Phase I study of gefitinib plus celecoxib in recurrent or metastatic squamous cell carcinoma of the head and neck. *J Clin Oncol* 2005; 23: 6976–6981.
3. Sharma SV, Bell DW, Settleman J, et al. Epidermal growth factor receptor mutations in lung cancer. *Nat Rev Cancer* 2007; 7: 169–181.
4. Mendelsohn J and Baselga J. The EGF receptor family as targets for cancer therapy. *Oncogene* 2000; 19: 6550–6565.
5. Scaltriti M and Baselga J. The epidermal growth factor receptor pathway: a model for targeted therapy. *Clin Cancer Res* 2006; 12: 5268–5272.
6. Pérez-Ramírez C, Cañadas-Garre M, Jiménez-Varo E, et al. MET: a new promising biomarker in non-small-cell lung carcinoma. *Pharmacogenomics* 2015; 16: 631–647.
7. Boeckx C, Weyn C, Vanden Bempt I, et al. Mutation analysis of genes in the EGFR pathway in head and neck cancer patients: implications for anti-EGFR treatment response. *BMC Res Notes* 2014; 7: 337.
8. Paez JG, Jänne PA, Lee JC, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* 2004; 304: 1497–1500.
9. Takano T, Ohe Y, Kusumoto M, et al. Risk factors for interstitial lung disease and predictive factors for tumor response in patients with advanced non-small cell lung cancer treated with gefitinib. *Lung Cancer* 2004; 45: 93–104.
10. Sequist LV, Joshi VA, Jänne PA, et al. Epidermal growth factor receptor mutation testing in the care of lung cancer patients. *Clin Cancer Res* 2006; 12: 4403s–4408s.
11. Jing CW, Wang Z, Cao HX, et al. High resolution melting analysis for epidermal growth factor receptor mutations in formalin-fixed paraffin-embedded tissue and plasma free DNA from non-small cell lung cancer patients. *Asian Pac J Cancer Prev* 2014; 14: 6619–6623.
12. Orita M, Iwahana H, Kanazawa H, et al. Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphisms. *Proc Natl Acad Sci U S A* 1989; 86: 2766–2770.
13. Chin TM, Anuar D, Soo R, et al. Detection of epidermal growth factor receptor

variations by partially denaturing HPLC. *Clin Chem* 2007; 53: 62–70.
14. Russell PA, Yu Y, Do H, et al. EGFR gene copy number alterations are not a useful screening tool for predicting EGFR mutation status in lung adenocarcinoma. *Pathology* 2014; 46: 32–36.
15. Sakairi Y, Sato K, Itoga S, et al. Transbronchial biopsy needle rinse solution used for comprehensive biomarker testing in patients with lung cancer. *J Thorac Oncol* 2014; 9: 26–32.
16. Nahta R, Trent S, Yang C, et al. Epidermal growth factor receptor expression is a candidate target of the synergistic combination of trastuzumab and flavopiridol in breast cancer. *Cancer Res* 2003; 63: 3626–3631.
17. Takano T, Ohe Y, Sakamoto H, et al. Epidermal growth factor receptor gene mutations and increased copy numbers predict gefitinib sensitivity in patients with recurrent non-small-cell lung cancer. *J Clin Oncol* 2005; 23: 6829–6837.
18. Willmore-Payne C, Holden JA and Layfield LJ. Detection of epidermal growth factor receptor and human epidermal growth factor receptor 2 activating mutations in lung adenocarcinoma by high-resolution melting amplicon analysis: correlation with gene copy number, protein expression, and hormone receptor expression. *Hum Pathol* 2006; 37: 755–763.
19. Wright GM, Do H, Weiss J, et al. Mapping of actionable mutations to histological subtype domains in lung adenocarcinoma: implications for precision medicine. *Oncotarget* 2014; 5: 2107–2115.
20. Fukui T, Ohe Y, Tsuta K, et al. Prospective study of the accuracy of EGFR mutational analysis by high-resolution melting analysis in small samples obtained from patients with non-small cell lung cancer. *Clin Cancer Res* 2008; 14: 4751–4757.
21. Yin X, Zheng L, Liu Q, et al. High-resolution melting curve analysis for rapid detection of rifampin resistance in Mycobacterium tuberculosis: a meta-analysis. *J Clin Microbiol* 2013; 51: 3294–3299.
22. Hamzehzadeh L, Khadangi F, Ghayoor Karimiani E, et al. Common KRAS and NRAS gene mutations in sporadic colorectal cancer in Northeastern Iranian patients. *Curr Probl Cancer* 2018; 42: 572–581.
23. Chambliss AB, Resnick M, Petrides AK, et al. Rapid screening for targeted genetic variants via high-resolution melting curve analysis. *Clin Chem Lab Med* 2017; 55: 507–516.
24. Montgomery JL, Sanford LN and Wittwer CT. High-resolution DNA melting analysis in clinical research and diagnostics. *Expert Rev Mol Diagn* 2010; 10: 219–240.
25. Taylor CF. Mutation scanning using high-resolution melting. *Biochem Soc Trans* 2009; 37: 433–437.
26. Erali M, Voelkerding KV and Wittwer CT. High resolution melting applications for clinical laboratory medicine. *Exp Mol Pathol* 2008; 85: 50–58.
27. Li BS, Wang XY, Ma FL, et al. Is high resolution melting analysis (HRMA) accurate for detection of human disease-associated mutations? A meta analysis. *PLoS One* 2011; 6: e28078.
28. Chevrier S, Arnould L, Ghiringhelli F, et al. Next-generation sequencing analysis of lung and colon carcinomas reveals a variety of genetic alterations. *Int J Oncol* 2014; 45: 1167–1174.
29. Sun H, Yang Y, Yang L, et al. Snapback primer mediated clamping PCR for detection of EGFR and KRAS mutations in NSCLC patients by high resolution melting analysis. *Biomed Res Int* 2014; 2014: 407537.
30. Bennani B, Gilles S, Fina F, et al. Mutation analysis of BRAF exon 15 and KRAS codons 12 and 13 in Moroccan patients with colorectal cancer. *Int J Biol Markers* 2010; 25: 179–184.
31. Obradovic J, Todosijevic J and Jurisic V. Application of the conventional and novel methods in testing EGFR variants for NSCLC patients in the last 10 years through different regions: a systematic review. *Mol Biol Rep* 2021; 48: 3593–3604.
32. Page MJ, Moher D, Bossuyt PM, et al. PRISMA 2020 explanation and elaboration: updated guidance and exemplars for reporting systematic reviews. *BMJ* 2021; 372: n160.
33. Whiting PF, Rutjes AW, Westwood ME; QUADAS-2 Group, et al. QUADAS-2: a

revised tool for the quality assessment of diagnostic accuracy studies. *Ann Intern Med* 2011; 155: 529–536.
34. Cheung AK, Greene T, Leypoldt JK; HEMO Study Group, et al. Association between serum 2-microglobulin level and infectious mortality in hemodialysis patients. *Clin J Am Soc Nephrol* 2008; 3: 69–77.
35. Moses LE, Shapiro D and Littenberg B. Combining independent studies of a diagnostic test into a summary ROC curve: data-analytic approaches and some additional considerations. *Stat Med* 1993; 12: 1293–1316.
36. Willmore-Payne C, Holden JA and Layfield LJ. Detection of EGFR- and HER2-activating mutations in squamous cell carcinoma involving the head and neck. *Mod Pathol* 2006; 19: 634–640.
37. Nomoto K, Tsuta K, Takano T, et al. Detection of EGFR mutations in archived cytologic specimens of non-small cell lung cancer using high-resolution melting analysis. *Am J Clin Pathol* 2006; 126: 608–615.
38. Takano T, Ohe Y, Tsuta K, et al. Epidermal growth factor receptor mutation detection using high-resolution melting analysis predicts outcomes in patients with advanced non small cell lung cancer treated with gefitinib. *Clin Cancer Res* 2007; 13: 5385–5390.
39. Do H, Krypuy M, Mitchell PL, et al. High resolution melting analysis for rapid and sensitive EGFR and KRAS mutation detection in formalin fixed paraffin embedded biopsies. *BMC Cancer* 2008; 8: 142.
40. Fassina A, Gazziero A, Zardo D, et al. Detection of EGFR and KRAS mutations on trans-thoracic needle aspiration of lung nodules by high resolution melting analysis. *J Clin Pathol* 2009; 62: 1096–1102.
41. Jacot W, Lopez-Crapez E, Thezenas S, et al. Lack of EGFR-activating mutations in European patients with triple-negative breast cancer could emphasise geographic and ethnic variations in breast cancer mutation profiles. *Breast Cancer Res* 2011; 13: R133.
42. Sriram KB, Tan ME, Savarimuthu SM, et al. Screening for activating EGFR mutations in surgically resected nonsmall cell lung cancer. *Eur Respir J* 2011; 38: 903–910.
43. Gonzalez-Bosquet J, Calcei J, Wei JS, et al. Detection of somatic mutations by high-resolution DNA melting (HRM) analysis in multiple cancers. *PLoS One* 2011; 6: e14522.
44. Borràs E, Jurado I, Hernan I, et al. Clinical pharmacogenomic testing of KRAS, BRAF and EGFR mutations by high resolution melting analysis and ultra-deep pyrosequencing. *BMC Cancer* 2011; 11: 406.
45. Hu C, Liu X, Chen Y, et al. Direct serum and tissue assay for EGFR mutation in non-small cell lung cancer by high-resolution melting analysis. *Oncol Rep* 2012; 28: 1815–1821.
46. Stigt JA, tHart NA, Knol AJ, et al. Pyrosequencing analysis of EGFR and KRAS mutations in EUS and EBUS-derived cytologic samples of adenocarcinomas of the lung. *J Thorac Oncol* 2013; 8: 1012–1018.
47. Lin J, Gu Y, Du R, et al. Detection of EGFR mutation in supernatant, cell pellets of pleural effusion and tumor tissues from non-small cell lung cancer patients by high resolution melting analysis and sequencing. *Int J Clin Exp Pathol* 2014; 7: 8813–8822.
48. Clay TD, Do H, Sundararajan V, et al. The clinical relevance of pathologic subtypes in metastatic lung adenocarcinoma. *J Thorac Oncol* 2014; 9: 654– 663.
49. Hinrichs JW, Van Blokland WT, Moons MJ, et al. Comparison of next-generation sequencing and mutation-specific platforms in clinical practice. *Am J Clin Pathol* 2015; 143: 573–578.
50. Oyaert M, Demedts I, Boone E, et al. Antitumoral activity of tyrosine kinase inhibitors in patients with non-small cell lung cancer harbouring rare epidermal growth factor receptor mutations. *Mol Diagn Ther* 2015; 19: 267–272.
51. Papadopoulou E, Tsoulos N, Tsirigoti A, et al. Determination of EGFR and KRAS mutational status in Greek non-small-cell lung cancer patients. *Oncol Lett* 2015; 10: 2176–2184.
52. Clay TD, Russell PA, Do H, et al. Associations between the IASLC/ATS/ERS lung adenocarcinoma classification and EGFR and KRAS mutations. *Pathology* 2016; 48: 17–24.

53. Wayhelova M, Mikulasova A, Smetana J, et al. Detection of oncogenic mutations in cervical carcinoma using method high resolution melting (HRM). *Neoplasma* 2016; 63: 779–788.
54. Martínez-Carretero C, Pascual FI, Rus A, et al. Detection of EGFR mutations in patients with non-small cell lung cancer by high resolution melting. Comparison with other methods. *Clin Chem Lab Med* 2017; 55: 1970–1978.
55. Lu HY, Qin J, Han N, et al. EGFR, KRAS, BRAF, PTEN, and PIK3CA mutation in plasma of small cell lung cancer patients. *Onco Targets Ther* 2018; 11: 2217–2226.
56. Frankel D, Nanni-Metellus I, Robaglia-Schlupp A, et al. Detection of EGFR, KRAS and BRAF mutations in metastatic cells from cerebrospinal fluid. *Clin Chem Lab Med* 2018; 56: 1970–1978.
57. Joy RA, Thelakkattusserry SK, Vikkath N, et al. Somatic mutation detection efficiency in EGFR: a comparison between high resolution melting analysis and Sanger sequencing. *BMC Cancer* 2020; 20: 902.
58. Takano T, Fukui T, Ohe Y, et al. EGFR mutations predict survival benefit from gefitinib in patients with advanced lung adenocarcinoma: a historical comparison of patients treated before and after gefitinib approval in Japan. *J Clin Oncol* 2008; 26: 5589–5595.
59. Zamora J, Abraira V, Muriel A, et al. Meta-DiSc: a software for meta-analysis of test accuracy data. *BMC Med Res Methodol* 2006; 6: 31.
60. Chen X, Li WL, Zhang YL, et al. Meta-analysis of quantitative diffusion-weighted MR imaging in the differential diagnosis of breast lesions. *BMC Cancer* 2010; 10: 693.
61. Cleophas TJ and Zwinderman AH. Meta-analyses of diagnostic studies. *Clin Chem Lab Med* 2009; 47: 1351–1354.
62. Herrmann MG, Durtschi JD, Bromley LK, et al. Amplicon DNA melting analysis for mutation scanning and genotyping: cross-platform comparison of instruments and dyes. *Clin Chem* 2006; 52: 494–503.
63. Herrmann MG, Durtschi JD, Bromley LK, et al. Instrument comparison for heterozygote scanning of single and double heterozygotes: a correction and extension of Herrmann et al., Clin Chem 2006;52:494-503. *Clin Chem* 2007; 53: 150–152.
64. Wittwer CT. High-resolution DNA melting analysis: advancements and limitations. *Hum Mutat* 2009; 30: 857–859.
65. Erali M and Wittwer CT. High resolution melting analysis for gene scanning. *Methods* 2010; 50: 250–261.
66. Von Ahsen N, Oellerich M and Schütz E. Limitations of genotyping based on amplicon melting temperature. *Clin Chem* 2001; 47: 1331–1332.
67. Chen D, Wang YY, Chuai ZR, et al. High-resolution melting analysis for accurate detection of BRAF mutations: a systematic review and meta-analysis. *Sci Rep* 2014; 4: 4168.
68. Liu YP, Wu HY, Yang X, et al. Diagnostic accuracy of high resolution melting analysis for detection of KRAS mutations: a systematic review and meta-analysis. *Sci Rep* 2014; 4: 7521.