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Review

Nucleic acid amplification-based HER2^{I655V} molecular detection for breast cancer

Bugi Ratno Budiarto [a, 1, *], Pimpin Utama Pohan [b], Desriani [a]

[a] Research Center for Biotechnology, Indonesian Institute of Sciences (LIPI), Jalan Raya Bogor Km. 46, Cibinong, 16911, Indonesia
[b] Faculty of Medicine, University of North Sumatra, Jalan Dr. T. Mansyur No.5, Padang Bulan, Medan Baru, Kota Medan, Sumatera Utara, Indonesia

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ABSTRACT

Single Nucleotide Polymorphism at codon 655 of HER2 gene has been extensively evaluated for its role as a susceptible biomarker for breast cancer development and the contradictive result of its role has been a debate among researchers as evidenced from case-control studies. Three platforms of molecular detection systems named PCR-RFLP, TaqMan assay, and AS-PCR have been used intensively in elucidating this important SNP with considering the affordability and simplicity of detection especially in research format which employs plenty of samples such as in the epidemiological study. Nevertheless, methodological related-bias generated from the association study between HER2^{I655V} SNP and breast cancer risk becomes primary drawback that must be addressed seriously in an attempt to obtain a solid conclusion. This review will discuss the application of nucleic acid amplification-based methods for HER2^{I655V} SNP detection, the potency of bias generated by these genotyping technologies, and strategies to improve their reliability of detection.

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1. Introduction

HER2 gene is member of the human epidermal growth factor receptor (HER) family located in chromosome 17q21. The product of this gene is a 185 kDa of membrane cells glycoprotein expressed from 27 coding exons with 1255 amino acid. This protein has significant biological role in cells homeostasis, growth and survival as dysregulated of this proto-oncogene product due to HER2 gene amplification and/or overexpression cause diverse tumorigenesis.[1] Immunohistochemistry (IHC) studies revealed that HER2 expression in breast, gastric, and ovarian cancer ranges from 10 to 30% of cancer, while in endometrial and esophageal cancer its number can be more than 80% cases.[2–6] The difference in HER2 protein expression level is well correlated with copy number of HER2 gene in the genome,[7] besides hormonal factors such as co-existing another hormonal-dependent signaling pathway that drives HER2-positive cancer more aggressive.[8] Specifically, HER2 gene plays very important role in normal breast epithelial cells function. HER2 protein dimerization with other HER family proteins (HER1, HER3, or HER4) as result of epidermal growth factor (EGF) or heregulin (HRG) ligand induction activates survival signaling pathways that instruct cells to proliferate, survive, or differentiate properly.[9] The net trafficking effect of elevated HER2 expression and the presence of HER3 protein are two essential components in altering the cell signaling and behavioral responses that transform breast cells into HER2-positive malignant cells. Upon EGF stimulation, overexpressed HER2 protein as result of HER2 gene amplification increases receptor lifetime and to shift the receptor distribution toward the surface, resulting in prolonged signaling.[10] This signal then further transmits into intracellular of breast cells through dimerization between HER2 protein with HER3 protein to promote cellular transformation as marked by sustained cells proliferation.[11] A recent finding has proven the role of overexpressed HER2 in neoplastic transformation of D492 breast cells line with stem cells properties through epithelial to mesenchyme transition (EMT) which is known as a hallmark of cancer.[12] In another study, mesenchymal characteristics of breast cancer as result of HER2 dysregulation were maintained TGFβ/SMAD signaling through the production of Tumor Growth Factor β (TGFβ) that ultimately repressed the production of E-cadherin which was essential for breast epithelial cells maintenance.[13] The important of E-cadherin role in EMT of breast cancer has been proven using Notch1 silencing on MCF-7 breast cancer cells line (HER2 positive type), showing the silenced cells exhibited reduced EMT process which was characterized by the increased expression of E-cadherin.

* Corresponding author. Research Center for Biotechnology, Indonesian Institute of Sciences-LIPI, Jalan Raya Bogor Km. 46, Cibinong, 16911, Bogor, Jawa Barat, Indonesia. Tel.: +62 021 8754587; fax: +62 021 8754588.
E-mail addresses: budiarto_bugie@yahoo.com, bugie1@lipi.go.id (B.R. Budiarto).
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[1] The main contributor.

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This result indicated that overexpressed HER2 can induce early breast carcinogenesis through EMT and this hallmark persisted even the breast cells have been established to advanced cancer.[14] In a clinical perspective, HER2 positive breast cancer exhibits a resistance to trastuzumab treatment.[15] Low respond to this therapy because HER2 positive breast cancer develops either de novo or acquired clinical resistance during trastuzumab therapy.[16] Evidence from in vitro study, gain-of-function mutation in PI3KCA genes causes HER2 positive breast cancer more resist toward trastuzumab therapy as a consequence of massive tumor growth under persistent activation of the PI3K–AKT–mTOR signaling pathway.[17,18] Meanwhile, acquired resistance to trastuzumab in HER2-overexpressed breast cancer is part of the result of gene upregulation under prolonged trastuzumab treatment. HER4 expression is upregulated following trastuzumab treatment in SKBR3 cells causing an ineffective response.[19] The overexpressed ADAM10, a member of ADAM family protease confers trastuzumab résistance by the mechanism of HER ligand shedding.[20]

Recent biological and epidemiological data suggest that overexpressed HER2 protein/or amplified HER2 gene is not the only one factor in breast carcinogenesis. Nonamplification of HER2 genomic alterations such as point mutation also plays an essential role in breast cancer risk. The number of HER2 somatic mutations recorded in The 62 Cancer Genome Atlas database until 2012 as many as eight type of point mutations that consist of S310F/Y, L755S, 63 D769H, G776S/V, V777L/M/A, V842I, and H878Y[21] and the overall HER2 mutation found in breast cancer itself is approximately 4.3% that is based on mutational analysis on its kinase domain[22] and this number may significantly increase due to the application of advanced molecular tool such as Next Generation Sequencing. Another variant mutant of HER2 gene named Q692*, P562S, P420fs, S413L, and S72A in transmembrane domain has been discovered by using NGS technology.[23] HER2 mutation can be found either in HER2-positive or HER2-negative breast cancer patients in which both types of cancers harbor a different type of point mutation. Breast cancer with overexpressed HER2 exhibits mutation at position S310F meanwhile breast cancer with no HER2 amplification shows mutation exclusively at kinase domain of HER2 at position I767 M and D769Y.[24,25] Those HER2 mutations are called activating mutation due to their specific role in driving the signaling HER2-derived cellular pathways by helping HER2 protein to interact structurally more actively with other cellular proteins which dictate cells to divide and grow rapidly.[26] Clinical evidence has underscored this activating mutation of HER2 especially those with no HER2 amplification have a poor survival rate.[27]

Another type of point mutation named Single Nucleotide Polymorphism (SNP) is also noted as a risk factor for breast neoplasia. SNP is defined as a variation in the DNA sequence that occurs in a population with a frequency of 1% or higher.[28,29] The number of SNP in HER2 gene that has been deposited in dbSNP database, NCBI so far is eight consist of Ser442Ser, Ser457Ser, Lys1177Lys, Trp452Cys, Ile654Val, Ile655Val, Pro927Arg, and Ala1170Pro. Among HER2 SNPs available only pathological SNP at Ile654Val, Ile655Val, and Ala1170Pro of HER2 has been proven epidemiologically contribute to breast risk.[30–32] Since polymorphic event at codon 655 of HER2 gene was first discovered in 1991 using DNA sequencing technique in tumors,[33] it has become a trigger for other researchers to further investigate its role in breast carcinogenesis. Formerly, the evidence of this gene polymorphism as a good candidate for tumor inducer came from Bargmann's study[34] in 1986 which stated that the transforming capacity of neu gene (a rat homolog of human HER2 gene) is elevated by the presence of point mutation at position V664E. Although at that time no any single mutation had been reported in HER2 gene, yet this finding highlighted that mutations, as well as genetic polymorphisms, reside along transmembrane domain of HER2 play important role in p185 function and may be considered as a susceptibility factor for cancer development. In fact, in vivo study has shown that animals injected with cells carrying the valine-encoding allele of HER2 resemble breast neoplastic characteristics.[34] Besides the potential role of HER2^{I655V} SNP as a biomarker for breast cancer development, this SNP can be used to predict the cardiotoxic effect of trastuzumab in HER2 positive breast cancer prior to therapy.[35] Clinical evidence shows that HER2 Ile/Val indeed is associated with a higher risk of cardiac toxicity. In a meta-analysis study, HER2 655 A > G polymorphism as a genetic marker of trastuzumab-induced cardiotoxicity in HER2-positive breast cancer patients.[37]

An interesting issue that comes from epidemiological studies of HER2^{I655V} SNP related to breast cancer risk showed inconsistency results among studies. Based on meta-analysis result conducted on 27 case–control studies with cases sample of 11,504 and control sample of 12,538 concluded that HER2^{I655V} SNP was associated with an increased breast cancer risk in overall population especially for Val Allele in African and Asian race.[38] In 2013, meta-analysis using 15,940 cases and 19,148 controls also showed Val allele would be a risk factor for breast cancer, emphasizing the influence of ethnicity on the power of susceptibility biomarkers of HER2^{I655V} SNP for breast cancer risk.[39] Moreover, updated meta analyses from 32 studies seem to further strengthen the association of HER2^{I655V} SNP with breast cancer risk.[40] Very recent finding concluded that HER2^{I655V} SNP can be used as a diagnostic marker for breast cancer in certain ethnic groups.[41] On the other meta-analysis study, the evidence for this association was insignificant.[42] The factors such as laboratory artifacts, lack of genotyping quality control or blinding and publication bias appear to have influenced these inconclusive results.[43] This finding indicates that reassessment on HER2^{I655V} SNP as a biomarker for breast cancer risk must be conducted using validated reliable molecular methods where practices for good laboratory are run properly. Interestingly, a study has confirmed that methodological errors approximately contribute to incorrect detection as much as 14 up to 58%.[44] This fact highlights that the application of molecular tools with low errors rate for this purpose should be a basic prerequisite.

2. Molecular methods for genotyping HER2^{I655V} gene

The first epidemiological study to elucidate the role of HER2^{I655V} SNP as a susceptible biomarker for breast cancer was come from Xie et al.'s study[30] in 2000 using Chinese population. Their study concluded that Val allele might be an indicator of early-onset breast cancer (women with age $\leq$ 45). Then several independent studies worldwide have been conducted to confirm this significant finding of HER2^{I655V} SNP as a risk factor for breast cancer development.[28,36,45–53] In term of molecular diagnostics perspective, interesting to note that such of HER2^{I655V} epidemiological studies mostly employing the nucleic acid amplification-based genotyping methods such as Allele-Specific PCR, Real-Time PCR, and PCR-RFLP to collect the data of HER2 genotypes from case and control samples.[54] These molecular tools offer the simplicity of detection and affordability in a research setting where research budget become a limiting factor without eliminating high accuracy of detection. Although DNA microarray for genotyping is now commercially available from Affymetrix technology and Illumina technology and this technology is very popular in recent years,[55–57] yet its application for HER2^{I655V} epidemiological study that uses hundreds of samples especially in developing countries is hampered mostly by research budget availability.

2.1. PCR combined with restriction fragment length polymorphism (PCR-RFLP)

PCR-RFLP is a molecular tool applied to detect single base difference within genome based on sequence-specific enzymatic cleavage of PCR products generating the different size and number of DNA fragments that result in characteristic banding patterns.[58] The application of PCR-RFLP to analyze HER2^{I655V} gene as a susceptibility biomarker for breast cancer risk in case and control study was first introduced by Xie et al.[30] In their study, the procedure for HER2 genotyping using PCR-RFLP was consist of four steps (DNA isolation, PCR amplification, PCR product digestion, and DNA electrophoresis) where *Bsm*AI was used as endonuclease enzyme to cut specific DNA sequence on HER2 gene amplicon of 148 bp, giving 116- and 32-bp fragments for the Val allele and a single 148-bp fragment for the Ile allele (Fig. 1). This developed method has been proven to have high accuracy and consistency result in genotyping for all the types of HER2 genotypes both in cases and control individuals. Modification on PCR-RFLP also has been reported by Keshava et al.[59] through shortening the number of primer bases from the previous study so that the fragment for the Ile and Val allele appeared as a single band of 130 bp and 106 bp, respectively. This strategy succeeded in eliminating by-product of enzyme digestion without reducing its detection accuracy. Nonetheless, the bands for heterozygote type (Ile/Val type) appeared in equal proportion in both studies where band for Ile dominated over the band for Val type, indicating there is a potency for genotyping error of PCR-RFLP. However, prolong digestion of HER2 amplicon over 16 h by *Bsm*AI seems to increase the proportion of Val band in heterozygote sample.[60] Until 2018, PCR-RFLP was still widely used for moderate to large-scale HER2^{I655V} genotyping study.[51–53,61–63]

2.2. TaqMan PCR

The principle of genotyping using TaqMan assay is the ability of 5′ nuclease or commercially named TaqMan® to release a probe

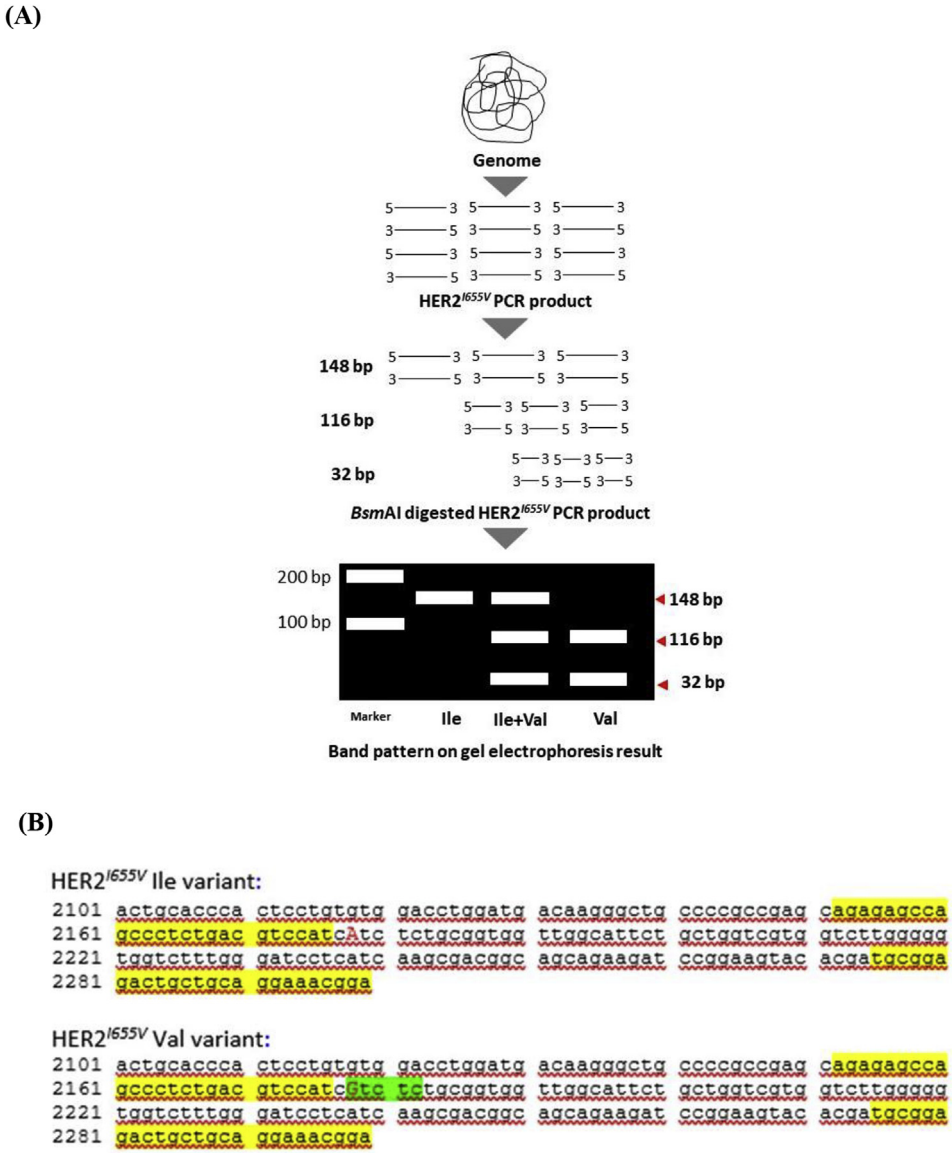

Fig. 1. PCR-RFLP procedure. (A) steps in performing HER2^{I655V} genotyping using PCR-RFLP as suggested by Xie et al.[30] (B) HER2 gene fragment (Gene Bank No. AK313683.1); DNA sequences with yellow color are primer binding sites for HER2^{I655V} variant amplification; Nucleotide with red color are SNP position of HER2^{I655V} gene; green color is specific DNA sequence for *Bsm*AI recognition.

that acts as fluorescence agent from the probe-attached PCR product during PCR amplification. This technology has been shown to have an ability in discriminating alleles that differ by single base substitution.[64,65] Real-Time PCR for HER2^{I655V} genotyping was first developed by Millikan et al.[66] in 2003 as illustrated in Fig. 2. TaqMan assay is initiated with mixing the assay components in PCR solution which consist of (1) forward and reverse primers to amplify the polymorphic sequence of interest, (2) two 5'site dye-labeled probes for allele-specific detection in which fluorescence is quenched by an Non-Flourescent Quencher (NFQ) at the 3′ site, (3) a minor groove binder at the 3′ end stabilizes the probe/template complex and (4) Taq Polymerase with 5′ → 3′ exonuclease activity. During PCR process, genome is separated into its single strand then at specific annealing temperature the primers and probes bind simultaneously into their specific target of the gene. Each uniquely labeled probe binds preferentially to one of the two alleles of the SNP of interest with different affinity. A fluorescent signal is generated as amplification proceeds due to the cleavage of the bound probe by TaqMan enzyme. Homozygosity for Allele G is read as fluorescent signal generated from VIC dye; homozygosity for Allele A is read as fluorescent signal generated from 6-carboxyfluorescein (FAM) dye, while the presence of both fluorescent signals indicates Allele G/Allele A heterozygosity.[67–69]

TaqMan assay gives 100% accuracy of detection in genotyping G2269A (rs3765534) that is responsible for thiopurine sensitivity in inflammatory bowel disease.[70] For HER2^{I655V} SNP study, the application of TaqMan assay exhibits 98% concordance result for each HER2^{I655V} allele variant between blinded quality control DNA and unblinded quality control DNA samples.[71] Moreover, 100% concordance result for HER2^{I655V} genotyping has also been

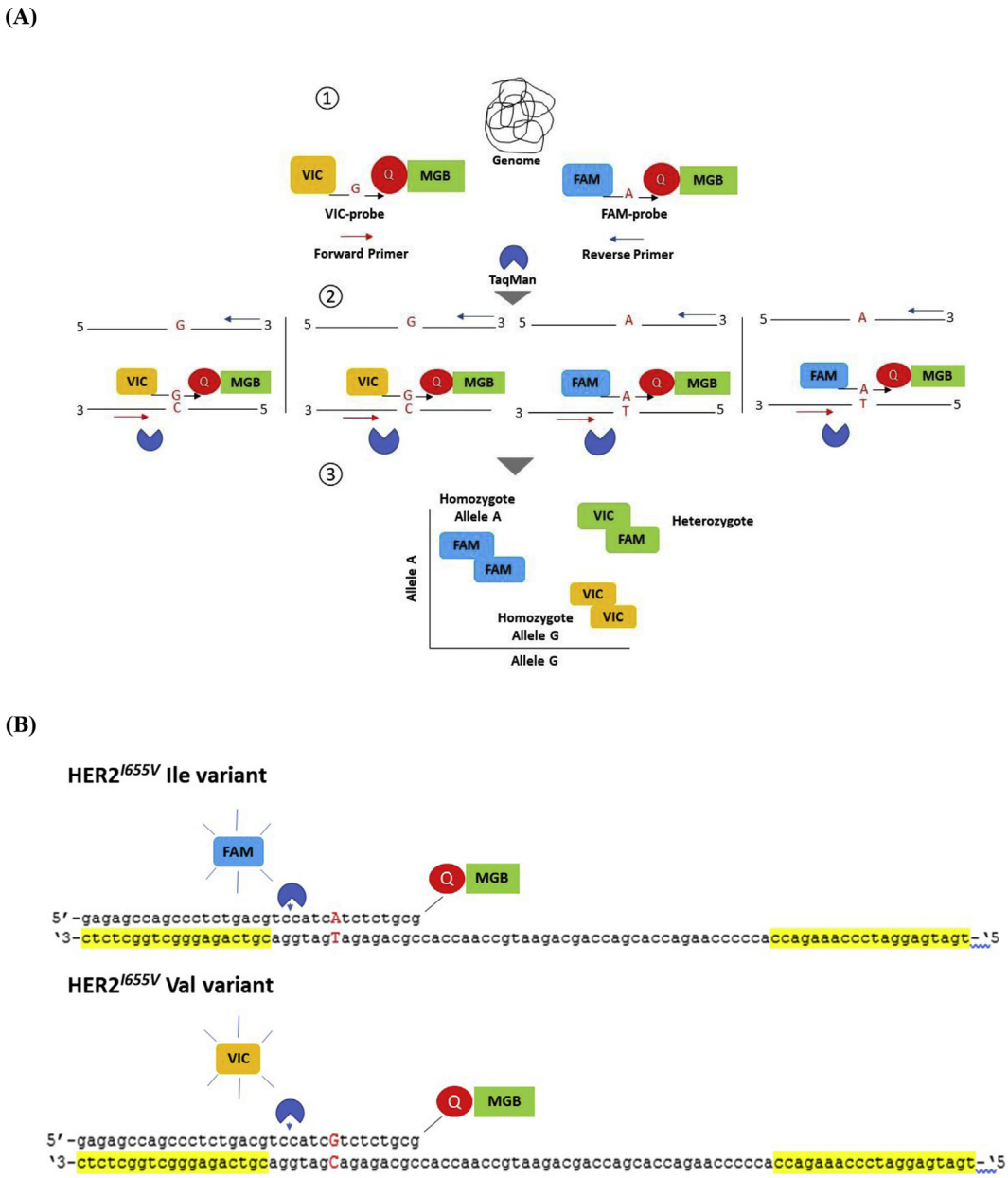

Fig. 2. TaqMan assay procedure. (A) Steps in performing HER2^{I655V} genotyping using TaqMan PCR. 1) PCR solution includes genome target as template, probe, primers and TaqMan solutions. 2) PCR reaction in which 5′nuclease-attached HER2^{I655V} sequence target with SNP occurs. 3) Pattern of HER2^{I655V} genotyping by TaqMan assay (B) detail of specific-probes binding on target HER2 SNP and releasing process of fluorescent reporter dye as result of specific-probe digestion by TaqMan enzyme during PCR process.

demonstrated by this technology.[36] Specific-probe designing is a pivotal point of TaqMan Assay to obtain high accuracy of HER2^{I655V} SNP detection. Besides it contains bases that match to corresponding HER2^{I655V} SNP, melting temperature and GC-content of specific-probe should be determined carefully. To genotype HER2^{I655V} gene, Millikan et al.[66] had designed probes with differing in their melting temperature by only 0.2 °C (65.3 °C for allele G and 65.1 °C for allele A) that specifically recognize and precisely differentiate each type of HER2 allele in TaqMan assay.

2.3. Allele-Specific PCR (AS-PCR)

AS-PCR is a DNA amplification-based molecular method used to amplify and discriminate simultaneously an SNP-harboring gene fragment from its wild-type DNA fragment when Taq polymerase lacking 5′−3′ exonuclease activity binds and extends duplex DNA sequence target-primer with match/mismatch at its 3′ end. Therefore, the formation of duplex DNA sequence target-mismatch primer will render Taq polymerase to amplify the specific PCR product. Dual-color based AS-PCR is the only PCR detection format for HER2^{I655V} SNP genotyping in epidemiological research setting which has been reported so far.[72–74] This technology is a modification of AS-PCR in which each specific primer labeled with fluorescence dye that representing HER2^{I655V} allelic types. FAM fluorescence dye attached at primer 5′ end recognizes allele G of HER2^{I655V} gene while HEX fluorescence dye attached at the same position as the first primer recognizes allele A of HER2^{I655V} gene. During PCR amplification, depending on allele type of HER2 in the genome, forward primer will anneal to match base and start extending from primer 3′ end, producing specific PCR amplicon. FAM-attached PCR amplicon shows blue color while HEX-attached PCR amplicon shows red color on agarose. Heterozygote type will produce mixed PCR amplicon bands with the same size at approximately 150 bp and shows purple color.[72,74] (Fig. 3). Dual color AS-PCR has simplified the traditional AS-PCR which uses 2-tube procedure to investigate both alleles separately.[75] Another cost-effective and less-laborious AS-PCR for HER2^{I655V} SNP detection, named amplicon-Shift Genotyping assay has also been developed. In spite of using dye-attached primers, a poly-GC sequence is added in one of specific forward primer at its 5' end so that SNP-containing PCR amplicon will have bigger band size than that of wild-type PCR amplicon on agarose. This assay shows a potency as a complementary method for large-scale genotyping purposes as proven when it was used for genotyping HER2^{I655V} gene in clinical breast cancer samples (61 samples) from Minang ethnic Indonesian to evaluate its genotype frequency.[76]

3. Genotyping errors may rise from HER2^{I655V} molecular methods

Nucleic acid amplification-based molecular methods employed for HER2^{I655V} genotyping study in the epidemiological setting have been proven to have high detection accuracy as mentioned above. PCR-RFLP uses endonuclease enzyme which specifically recognizes HER2^{I655V} SNP-containing bases sequence in PCR amplicon, allowing SNP detection with high specificity. Application of dye-attached probes together with 5′nuclease enzyme in TaqMan assay can accurately differential SNP type of HER2^{I655V} from non-SNP type of HER2^{I655V}. It is not only producing high specificity but also increasing the sensitivity of detection. Moreover, simplicity of HER2^{I655V} SNP detection while keeping its reliable result of genotyping has been shown by dual-color AS-PCR and Amplicon-Shift Genotyping based AS-PCR. Nonetheless, errors that attribute to the method application may still occur especially in research setting where practices for good laboratory is disobeyed. In fact, the genotyping data collected from a high-quality laboratory with skilled personnel using an established genotyping method and with quality control before association analysis is not always giving 100% guarantee free from errors.[77] In line with this statement, AS-PCR for HER2^{I655V} SNP detection also showed genotyping errors with the rate of 10%−50% depending on PCR condition applied.[78]

The association studies of HER2^{I655V} gene as a pathological SNP for breast cancer in a population collected from many published papers are conducted using case and control samples-based study design and this type of epidemiological research is assuming that dissimilarity in SNP prevalence between cases and control samples for certain status of disease is independent from any difference in genetic background so that the increased risk of developing a disease in case population is solely due to disease status.[79] Investigation on the power of case-control study in determining the SNP association to certain of diseases has risen the great concern on false conclusion due to genotyping errors.[80] Accordingly, Non-Differential Genotyping Error (NDGE) and Differential Genotyping Error (DGE) are the primary causes of this genotyping problem that ultimately rejects the incorrectly null hypothesis (type I error) or vice versa (type II error). NDGE is produced from misclassification of a true allele in genotyping either from case or control samples. NDGE occurs as a result of (1) Allele Drop Out (ADO) due to severe preferential amplification or detection one of the allele; a heterozygous subject is more likely to be misclassified as homozygote or homozygote subject is more likely to be coded as a missing subject, and (2) misclassifying or even missing a true genotype in samples. Suboptimal of method detection employed such as lack of sensitivity and specificity is the primary cause of this error.[77,81] Moreover, an error due to DGE such as in the genotyping process using the sample from a different institution or applying the instrumentation with different platform could worsen the bias.[82,83] This situation may partially explain why type I errors exhibited by a case-control study of HER2^{I655V} SNP in Chinese population.[30,84] Furthermore, these facts are also much of relevance to the current methodological approach for case-control studies of HER2^{I655V} SNP where the quality control of molecular methods is stated incompletely that impact on the contradictive result.[43] In fact, PCR-RFLP, TaqMan assay, and Allele-Specific PCR may contain a methodological error risk as proven from many studies.[30,59,78,85] In addition, molecular methods for HER2^{I655V} SNP genotyping basically employ PCR technology to enrich SNP-containing gene fragment either prior to genotyping processing as shown by PCR-RFLP or simultaneously with SNP detection as DNA amplification preceded as shown by TaqMan assay and AS-PCR. Generally, this kind of molecular technology consists of (1) pre-PCR process such as primer designing which includes SNP base into primer 3' end, DNA extraction from biological resources (blood, tissue, hair, or body fluids) to obtain sufficient DNA as a template, composition of PCR reagent such as DNA template, primers, $MgCl_2$, dNTPs, Taq Polymerase and buffer concentration to achieve optimum amplification, the choice of PCR reagents and attention for cleanliness of PCR workplace (2) PCR process includes optimizing each steps of cycle especially in denaturation and annealing step (temperature and time duration) in attempt to obtain high sensitivity and specificity of detection, and (3) post-PCR process which is the last step to detect PCR product either using agarose-based DNA electrophoresis for AS-PCR and software-based analysis for TaqMan assay genotyping. PCR product in PCR-RFLP must be first digested by endonuclease so that genotyping pattern can be detected on agarose-based DNA electrophoresis. Therefore, strict evaluation should be applied in each step of the procedure to avoid massive genotyping errors due to the complexity of this PCR process. Table 1 shows critical steps in which genotyping errors may possibly occur in HER2^{I655V} SNP genotyping using three detection platforms.

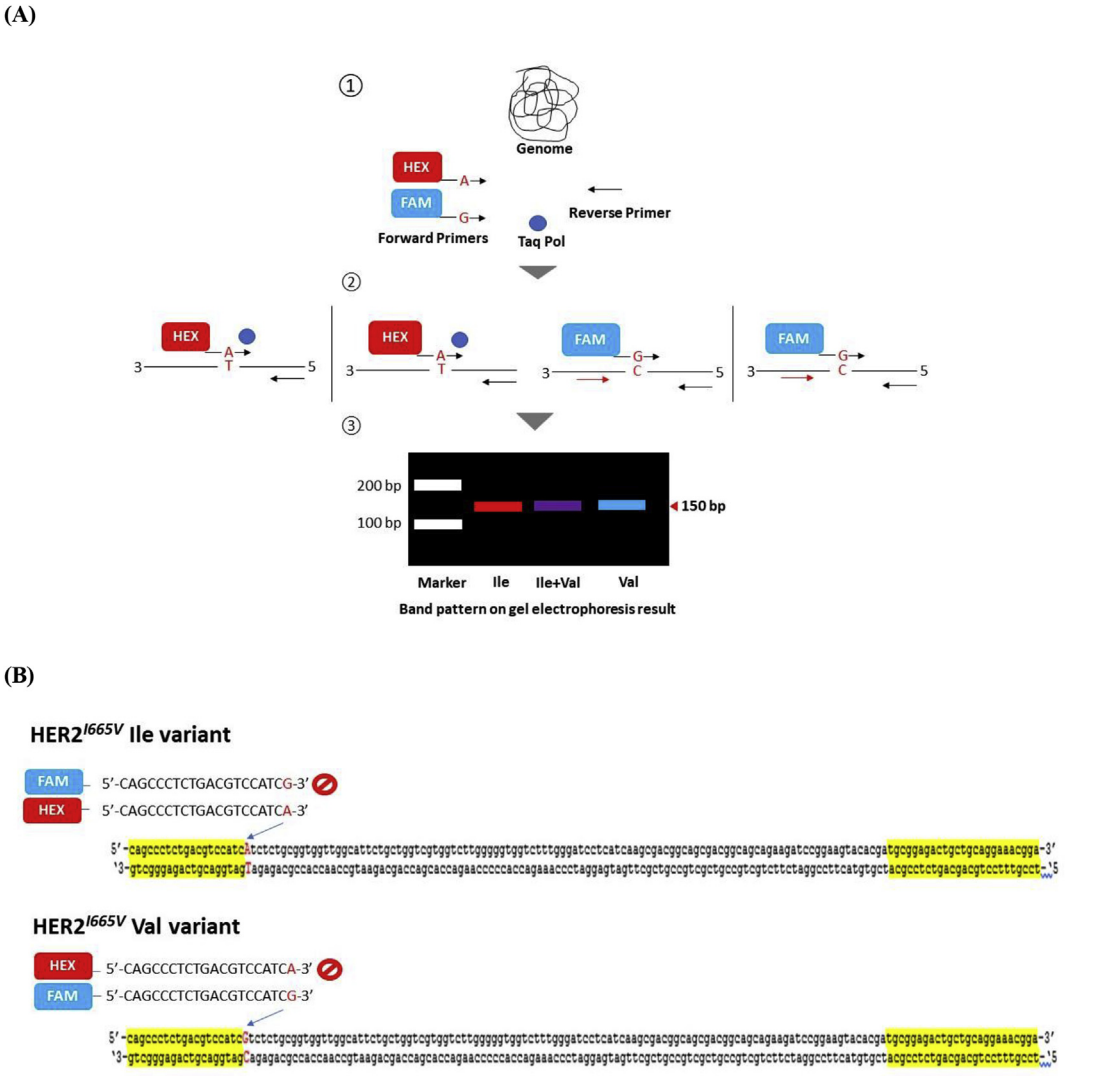

Fig. 3. Dual Color AS-PCR procedure. (A) Steps in performing HER2^{I655V} genotyping using Dual Color AS-PCR. 1) PCR solution will include genome target as template, dye-attached forward primers with match base for corresponding HER2^{I655V} allele types, common reverse primer, and Taq Polymerase solutions include $MgCl_2$ and dNTP. 2) PCR reaction in which Taq Polymerase extends duplex DNA-forward primers with matched HER2^{I655V} allele type. 3) Pattern of HER2^{I655V} genotyping by Dual Color AS-PCR. (B) detail of dye-attached forward primers binding on target HER2 SNP.

4. Performance of HER2^{I655V} molecular methods

To prevent genotyping errors in case-control study-based HER2^{I655V} SNP detection and to enhance quality of the genotyping data, a good reporting method as suggested by Strengthening the Reporting of Genetic Association Studies (STREGA) guideline is highly recommended. This guideline also encourages researchers for data transparency on research before publishing their finding, so that the assessment of the strengths and weaknesses of this evidence by third parties/other independent researchers could be performed in appropriate manner to obtain an unbiased conclusion.[86,87] Genotyping errors in the case-control study have been one of concern in STREGA guideline due to its effect on the power of statistical calculation such as Odd Ratios (OR) value. This fact is in line with population-based case and control study for HER2^{I655V} SNP as a biomarker candidate for breast cancer risk where the association between exposure and outcome is calculated based on this statistical value.[39] Because Val allele of HER2^{I655V} is a minor allele so that the proportion of this allele either in the case samples or in the control sample largely plays an important role in decision making whether the association of HER2^{I655V} SNP with breast cancer risk is true in population as stated by OR value, OR > 1 meaning that HER2^{I655V} SNP has significant association with breast cancer risk, while OR < 1 shows reverse association.[40] Therefore, genotype misclassification in samples from case or control subject due to lack of method reliability become a major drawback in HER2^{I655V} SNP studies, making HER2^{I655V} SNP as a universal candidate for susceptible biomarker of breast cancer development remains debatable.

Performance of nucleic acid amplification-based molecular methods in detecting the genetic polymorphisms of HER2^{I655V} gene in a population can be assessed by its assay reliability that reflects sensitivity, specificity, reproducibility, and consistency of method. Careful methodological application for the detection of HER2^{I655V} SNP using three platforms of assays (PCR-RFLP, TaqMan Assay and Allele-Specific PCR) has highlighted some important points regarding the reliable genotyping methods from which the trusted HER2^{I655V} genotyping data is generated. The first point is the method should have the capability in interrogating the all type of HER2^{I655V} genotypes in control sample to evaluate HER2^{I655V}

Table 1
The potential of genotyping errors in three platforms of molecular detection of HER2^{I655V} SNP.

PCR steps	Genotyping errors sources	Genotyping errors	Explanation	PCR platform
Pre-PCR	Quality of DNA is poor	Allele Drop Out either for AA genotype or GG genotype in AG genotype case	DNA is extracted from archival biological resources such as FFPE or blood	1,2 and 3
		Missing detection for all type of HER2^{I655V} genotypes	The extracted DNA still contains bio-contaminant as a result of suboptimal DNA extraction application Kit or method for DNA extraction is not suitable with biological matrix from which DNA extracted	
	Suboptimal primers design	Allele Drop Out either for GG genotype in AG genotype case	the quantity of PCR product is too low to be digested by endonuclease enzyme	1
		Missing detection for all type of HER2^{I655V} genotypes	Primers don't anneal on primer binding site of DNA target hence no DNA amplification occurs	1 and 2
	Suboptimal allele specific primers design	Misclassification among HER2^{I655V} genotype	It occurs when mismatching the SNP base at the position of primer 3 end with its target has mistaken	3
		Preferential amplification towards one of HER2 allele or even missing detection	The selection of penultimate base of allele-specific primer is not suitable	
	Suboptimal probes design	Missing detection for either AA or GG genotype in homozygous sample or both types in heterozygous sample	One of the probes or both probes does not anneal on probe binding site of DNA target due to significant Tm difference between probe The designed probes are too long or too short. Ideally, it should be 15–20 bp Probes form self- complementarity or complementary binding with primers	2
		Allele Drop Out either for AA genotype or GG genotype in AG genotype case	One of probe anneals ineffectively on binding site of DNA target so that the fluorescence produced from nuclease digestion doesn't reach the minimum base calling	
	PCR Workspaces	Misclassification among HER2^{I655V} genotype	Cross-contamination by foreign DNA from the air, contaminated reagents/ water or pipets	1,2 and 3
	Taq Polymerase characteristics in PCR reagent	Missing detection for all type of HER2^{I655V} genotypes	Taq Polymerase contains no 5 to 3 endonuclease activity	2
		Misclassification of homozygous HER2^{I655V} as heterozygous type	Taq Polymerase has no ability to discriminate SNP from its wild type	3
	$MgCl_2$ concentration	Missing detection for all type of HER2^{I655V} genotypes	Taq Polymerase doesn't work at the optimum activity	1,2 and 3
	Inappropriate primer ratio	Misclassification among HER2^{I655V} genotype	Over dominated of one of the allele-specific primers in binding the SNP/wild type-containing primer binding site	3
During PCR	Annealing temperature	Missing detection for all type of HER2^{I655V} genotypes	Inappropriately selected annealing temperature causes Primers to anneal to an unspecific region of the gene rather than their target	1
		Missing detection for all type of HER2^{I655V} genotypes	Inappropriately selected annealing temperature causes primers and robes anneal to unspecific region of gene rather that their target	2
		Preferential amplification towards one of HER2 allele or even missing detection	This event due to significant Tm difference between probes as a consequence of the difference in their GC content	
		Missing detection for all type of HER2^{I655V} genotypes	Allele-specific primer is not allowed to anneal on its target primer-binding site that contain base correspond to HER2 allele	3
		Allele Drop Out of one of HER2^{I655V} allele in case of heterozygous sample	Only one of the allele-specific primer can bind to primer-binding-site that contain base correspond to HER2 allele	
Post-PCR	Autocalling failure	Missing detection for all type of HER2^{I655V} genotypes	Software doesn't contain autocaller algorithm	2
	Period of time and quantity of *Bsm*AI used	Misclassification of AG or GG genotype as AA genotype	Incompletely digestion of PCR product, so that digested PCR product (GG genotype) doesn't appear on DNA electrophoresis result	1
	Inappropriate DNA agarose concentration*	heterozygous type of HER2^{I655V} misclassified as homozygous type	Too low concentration DNA agarose used in DNA electrophoresis analysis can make two band of PCR amplicon appears as one single band of PCR	3

1 refers to PCR-RFLP, 2 refers to TaqMan assay and 3 refers to Allele-Specific PCR.
*Refers to Amplicon shift-based AS-PCR.

genotype distribution whether it has departed from Hardy-Weinberg equilibrium. This also suggests that the methods should retain its sensitivity and specificity to avoid HER2^{I655V} genotypes misclassification. The second point is the method should show good reproducibility and consistency. This test is usually conducted by doing an independent test using control samples which are taken from the second tube of the sample. Then, genotype determination from this quality control samples is blindly identified. Complete agreement between genotyping result from quality control samples with study samples indicates high reproducibility and consistency of assays. Finally, the method showed no PCR amplification in blank sample to ensure no cross-contamination occurred.[30,69,88]

Interesting to note that 3 of 24 case-control studies of HER2^{I655V} SNP and breast cancer risk association using RFPL-PCR to genotype HER2^{I655V} SNP prevalence exhibited Hardy-Weinberg disequilibrium in control samples which are from Greek, Turkish and Tunisian ethnic group. Moreover, deviation from Hardy-Weinberg proportion has been shown by TaqMan assay in 2 studies using African-American and White ethnic group of 12 available publications. In other hands, none of Hardy-Weinberg disproportion has been detected when dual-color based AS-PCR is used to interrogate HER2^{I655V} SNP genotypes in control subjects.[41] Testing the departure of Hardy-Weinberg equilibrium from this finding may reflect there is a HER2^{I655V} genotyping bias as a result of either poor reliability of molecular assays or it is likely due to some errors in the phenotyping stage of sampling such as selection bias of participants, the usage of small number of samples, inadequate in the ratio of control to case sample, samples mislabeling, or samples retrieved from mixed population.[89–91]

Only a few from the published HER2^{I655V} association studies in which reporting on quality assurance of methods have adapted STREGA guideline. Accordingly, the source of DNA, the method of DNA extraction, and an approach how reproducibility, consistency,

and accuracy of the method established are essential elements in ensuring the method reliability in HER2^{I655V} SNP genotyping as adapted from this guideline. The source of DNA has been proven to interfere the genotyping result.[92] Moreover, treatments on the biological matrix such as long-term preservation using physical (iced storage) or chemical application (tissue fixation using formalin) from which genomic DNA retrieved further affect the quality of genotyping test due to DNA degradation.[75,93,94] In addition, genotype discordance as a result of the usage of DNA obtained from the different biological matrices (blood, buccal cells, biopsy tissue, and formalin-fixed tissue) which generated from the same platform assay is a common phenomenon in molecular diagnostic testing. The degree of genotype discordance seems likely to correlate to the choice of method of DNA extraction which plays a critical step in giving the DNA with high quality. In fact, eliminating of genotyping errors can be achieved by prior selecting the DNA extraction method that is compatible with biological matrix from which the DNA is obtained. More importantly, good quality of DNA can enhance reproducibility and consistency of genotyping result. Three molecular platforms of HER2^{I655V} SNP detection system have been applied to interrogate the type of HER2^{I655V} genotypes in DNA from blood, buccal cells, biopsy tissue, and even FFPE tissue. Kallel et al.[48] used PCR-RFLP to evaluate HER2^{I655V} in Tunician ethic group using frozen tissue from case subject and blood from control subject as DNA using DNA purification kit as reagent to isolate DNA. TaqMan assay has been applied in genotyping HER2^{I655V} SNP in blood-derived DNA from Sudan population.[95] DNA from the blood sample for HER2^{I655V} case and control study has also been evaluated for their genotype prevalence and its association with breast cancer development using Dual-Color AS-PCR in British population.[72] DNA isolated from non-invasive tissue such as from buccal cells for HER2^{I655V} SNP genotyping using TaqMan assay has also been reported.[70,96] Meanwhile, FFPE as source of DNA for the same purpose has been reported in case and control study in Korean population and Turkish population using PCR-RFLP.[97,98] The majority of HER2^{I655V} study in which three platform of diagnosis method employed used either FFPE, buccal cells or blood-derived DNA extracted with commercially available kits due to its simplicity in the DNA extraction process and standardized protocols.[51,68,70,96,98–101] In a certain condition when samples access is a primary barrier for the study, the usage of the different source of DNA in case and control subject is a solution.[48,70,97,98] Two independent studies using two different platforms of the assay (TaqMan assay vs PCR-RFLP) using blood as a source of DNA confirmed that no significant association between HER2^{I655V} SNP with breast cancer risk in Turkish population.[102,103] Furthermore, this insignificant association has been reported in the same population by using PCR-RFLP on DNA derived from FFPE for case subject and from blood for control subject.[98] No association was also noted in Greece population between HER2^{I655V} SNP and breast cancer risk when blood-derived DNA was genotyped using PCR-RFLP.[99,100] Unfortunately, lack of information regarding quality control test of the assay such as reproducibility test using these biological matrices in the majority of the association study between HER2^{I655V} SNP and breast cancer risk has reduced the reliability of the overall finding. Table 2 points the summarize of quality control test of assay using different biological matrices.

5. Strategies to improve reliability of HER2^{I655V} molecular methods

In an attempt to improve quality of the genotyping test of HER2^{I655V} SNP using three platforms of technologies (PCR-RFLP, TaqMan assay, and AS-PCR) it is reasonable to adhere to item number 8 and 9 of method section in STREGA recommendation. These two essential items particularly act as fundamental reference in performing and evaluating the quality test of genotyping methods on DNA retrieved from various biological matrices before commencing the real testing on case and control subjects, and consequently increases in confidence of the generated genotyping data. Therefore, HER2^{I655V} genotyping methods must be assessed for (1) the potency of bias due to technical aspects of method itself or the usage of DNA samples from different biological matrices, and (2) for consistency and accuracy of genotyping results either it is tested in intra-laboratory or inter-laboratory comparison.[86,87]

The potency of genotyping bias due to the technical aspect of HER2^{I655V} genotyping methods such as PCR condition or the usage of DNA extracted from different biological matrices hampers the validity of the genotyping data. Therefore, it is suggestible to quantitatively evaluate genotyping errors as suggested by Taberlet et al.[104] to know the rate of errors of each method. This technique counts the errors rate as a percentage based on the total number of amplicon with correct genotype divided with amplicon with incorrect genotype or no amplification in multiple tube assay. This technique has been applied successfully in evaluating the rate of genotyping errors due to several PCR conditions such as annealing temperature of PCR, the amount of DNA template, and the ratio of DNA template in AS-PCR[78] and this similar approach can be applied to other platforms of HER2^{I655V} SNP detection such as PCR-RFLP and TaqMan assay. Unfortunately, no information is available recently regarding the rate of genotyping errors for these two HER2^{I655V} SNP detection system so that the evaluation study is warranted to be conducted.

The reproducibility of the genotyping data obtained from a platform of molecular detection either conducted by the different lab worker in the same laboratory or by others laboratory worker in different laboratory can have repercussions on the strength of data integrity.[104] The reproducibility test of the genotyping methods will point directly both consistency and accuracy of the genotyping data which gives essential information on how good the assay works. This can be achieved by doing concordance test of the same samples in several replicate samples of testing through intra-laboratory or inter-laboratory comparison procedure. Ideally, genotyping assays show good performance if the value of concordance test in both procedures of comparison is around 99%.[105] The employment of PCR-RFLP in evaluating HER2^{I655V} SNP as breast cancer risk is a good example of how the reproducibility test of this method is conducted where intra-laboratory comparison of the genotyping data yields perfect concordance.[30] Inter-laboratory assay of TaqMan assay in genotyping variant of HER2^{I655V} allele showed 98% concordance, while perfect match result from intra-laboratory assay is obtained by this method.[70] Although these two genotyping assay become popular procedure for the most of association study between HER2^{I655V} SNP and breast cancer risk, yet their reproducibility test in new study must be performed prior to genotyping the real samples due to the diversity in the usage of biological matrices and DNA extraction methods. In addition, reproducibility test is also prerequisite for AS-PCR as no information for this test is available in previous study.[72] Such reproducibility test as part of genotyping quality control test is an essential component in most of the association study of HER2^{I655V} SNP and its establishment which is in line with the currently available guideline in every new study will enhance the confidence of the finding.

6. Conclusion

Three platforms of molecular detection systems have been employed in finding the evidence of HER2^{I655V} SNP in breast carcinogenesis where PCR-RFLP and TaqMan Assay has become two

Table 2
Genotyping quality control test of three platforms of HER2^{I655V} genotyping assay using DNA from different biological matrices.

Author	Ethic	Year	Assay	Genotyping quality control test	Biological matrix	DNA isolation method	Case-control study finding
Xie et al.[30]	Chinese	2000	PCR-RFLP	Mentioned	Blood (in case and control subject)	Salting out	+
Baxter and Campbell[73]	Caucasian	2001	AS-PCR	Mentioned	Not mentioned	Not mentioned	–
Keshava et al.[59]	Caucasian, African-American and Latinas	2001	PCR-RFLP	Not mentioned	Blood (in case and control subject)	Not mentioned	–
Wang-Gohrke and Chang-Claude[106]	Caucasian	2001	PCR-RFLP	Mentioned	Blood (in case and control subject)	Not mentioned	–
Hishida[107]	Japanese	2002	Not mentioned	Mentioned	Not mentioned	Not mentioned	–
Millikan et al.[66]	African-American and Caucasian	2003	TaqMan	Not mentioned	Blood (in case and control subject)	DNA extractor	–
Montgomery et al.[75]	Australian	2003	AS-PCR	Not mentioned	Blood (in case and control subject)	Not mentioned	+
Akisik and Dalay[103]	Turkish	2004	PCR-RFLP	Not mentioned	Blood (in case and control subject)	Phenol-chloroform	–
Kamali-Sarvestani[60]	Iranian	2004	PCR-RFLP	Not mentioned	Blood (in case and control subject)	Phenol-chloroform	–
Pinto et al.[108]	Portuguese	2004	PCR-RFLP	Mentioned	Blood (in case and control subject)	Salting out	+
An et al.[98]	Korean	2005	PCR-RFLP	Mentioned	FPPE (case subject)-Blood (control subject)	Not mentioned	–
Kalemi et al.[100]	Greek	2005	PCR-RFLP	Not mentioned	Blood (in case and control subject)	E.Z.N.A DNA isolation kit	–
Nelson et al.[97]	European	2005	TaqMan	Not mentioned	Buccal Cells (in case and control subject)	Gentra system DNA extraction	–
Benusiglio et al.[89]	British	2006	TaqMan	Mentioned	Blood (in case and control subject)	Not mentioned	–
Zubor et al.[109]	Slovak Republican	2006	PCR-RFLP	Not mentioned	Blood (in case and control subject)	Salting out	+
Papadopoulou et al.[101]	Greek	2007	PCR-RFLP	Not mentioned	Blood (in case and control subject)	Puregene-DNA Purification kit of Gentra System	+
Tommasi et al.[68]	Caucasian	2007	TaqMan	Not mentioned	Blood (in case and control subject)	Wizard genomic DNA isolation Kit	+
Lee et al.[110]	Taiwan	2008	PCR-RFLP	Not mentioned	Blood (in case and control subject)	Salting out	+
Mutluhan et al.[45]	Turkish	2008	PCR-RFLP	Not mentioned	Blood (in case and control subject)	Phenol-chloroform	+
Naidu et al.[46]	Malaysian	2008	PCR-RFLP	Not mentioned	Blood (in case and control subject)	PUREGENE Genomic DNA Purification kit	–
Qu et al.[71]	Chinese	2008	TaqMan	Mentioned	Buccal Cells (control subject)-Blood (case subject)	PUREGENE Genomic DNA Purification kit	–
Rajkumar et al.[111]	South Indian	2008	TaqMan	Mentioned	Blood (in case and control subject)	Not mentioned	–
Siddig et al.[96]	Sudan	2008	TaqMan	Mentioned	Blood (in case and control subject)	QiaAmp DNA Blood Mini kit	+
Kallel et al.[48]	Tunisian	2010	PCR-RFLP	Not mentioned	Biopsy (case subject)-Blood (control subject)	Wizard genomic DNA extraction kit	–
Kara et al.[49]	Turkish	2010	PCR-RFLP	Not mentioned	Blood (in case and control subject)	Salting out	–
Sezgin et al.[99]	Turkish	2011	PCR-RFLP	Not mentioned	FPPE (case subject and control subject)	Nucleospin Tissue Kit	–
Abdraboh et al.[51]	Egyptian	2013	PCR-RFLP	Not mentioned	Blood (in case and control subject)	Wizard DNA Purification kit	+
Ozturk et al.[52]	Turkish	2013	PCR-RFLP	Not mentioned	Blood (in case and control subject)	Salting out	+
Al-janabi et al.[102]	Iraqi	2015	PCR-RFLP	Not mentioned	Frozen Tissue (case subject)-Blood (control subject)	ReliaPrep™ Blood gDNA Miniprep kit	+
Carrillo-Moreno et al.[62]	Mexican	2016	PCR-RFLP	Mentioned	Not mentioned	Salting out	+

+ meaning that an association between HER2^{I655V} SNP with breast cancer risk was observed.
- meaning that no association between HER2^{I655V} SNP with breast cancer risk was observed.

molecular assays that widely used for this purpose. It is apparent that genotyping errors of methods is the factor that may hamper significantly on the validity of the genotyping data of the study. Therefore, appropriate steps in eliminating the source of errors become an integral part of this research activity. Furthermore, to increase the performance of HER2^{I655V} molecular detection in every new study, it is suggestible to conduct the reproducibility test of the methods before commencing the genotyping test in the real sample. This test not only will improve the method reliability but also increase the confidence of the genotyping data presented.

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References

1. Iqbal N, Iqbal N. Human epidermal growth factor receptor 2 (HER2) in cancers: overexpression and therapeutic implications. *Mol Biol Int*. 2014;2014:1–9.
2. Burstein HJ. The distinctive nature of HER2-positive breast cancers. *N Engl J Med*. 2005;353:1652–1654.
3. Sakai K, Mori S, Kawamoto T, et al. Expression of epidermal growth factor receptors on normal human gastric epithelia and gastric carcinomas. *J Natl Cancer Inst (Bethesda)*. 1986;77:1047–1052.
4. Flejou JF, Paraf F, Muzeau F, et al. Expression of c-erbB-2 oncogene product in Barrett's adenocarcinoma: pathological and prognostic correlations. *J Clin Pathol*. 1994;47:23–26.
5. Berchuck A, Kamel A, Whitaker R, et al. Overexpression of HER-2/neu is associated with poor survival in advanced epithelial ovarian cancer. Cancer Res. 50:4087–4091.
6. Rolitsky CD, Theil KS, McGaughy VR, Copeland LJ, & Niemann TH. HER-2/neu amplification and overexpression in endometrial carcinoma. Int J Gynecol Pathol, 18:138–143.
7. Lin WL, Kuo WH, Chen FL, et al. Identification of the coexisting HER2 gene amplification and novel mutations in the HER2 protein-overexpressed mucinous epithelial ovarian cancer. *Ann Oncol*. 2011;18:2388–2394.
8. Arpino G, Wiechmann L, Osborne CK, & Schiff R. Crosstalk between the estrogen receptor and the HER tyrosine kinase receptor family: molecular mechanism and clinical implications for endocrine therapy resistance. Endocr Rev. 29:217–233.
9. Birtwistle MR, Hatakeyama M, Yumoto N, Ogunnaike BA, Hoek JB, Kholodenko BN. Ligand-dependent responses of the ErbB signaling network: experimental and modeling analyses. *Mol Syst Biol*. 2007;3:1–16.
10. Hendriks BS, Opresko LK, Wiley HS, Lauffenburger D. Coregulation of epidermal growth factor receptor/human epidermal growth factor receptor 2 (HER2) levels and locations: quantitative analysis of HER2 overexpression effects. *Cancer Res*. 2003;63(5):1130–1137.
11. Holbro T, Beerli RR, Maurer F, Koziczak M, Barbas CF, Hynes NE. The ErbB2/ErbB3 heterodimer functions as an oncogenic unit: ErbB2 requires ErbB3 to drive breast tumor cell proliferation. *Proc Natl Acad Sci Unit States Am*. 2003;100(15):8933–8938.
12. Ingthorsson S, Andersen K, Hilmarsdóttir B, Mælandsmo GM, Magnusson MK, Gudjonsson T. HER2 induced EMT and tumorigenicity in breast epithelial progenitor cells is inhibited by coexpression of EGFR. *Oncogene*. 2016;35:4244.
13. Gupta P, Srivastava SK. HER2 mediated de novo production of TGFβ leads to SNAIL driven epithelial-to-mesenchymal transition and metastasis of breast cancer. *Mol Oncol*. 2014;8:1532–1547.
14. Shao S, Zhao X, Zhang X, et al. Notch1 signaling regulates the epithelial–mesenchymal transition and invasion of breast cancer in a Slug-dependent manner. *Mol Canc*. 2015;14:28.
15. Lavaud P, Andre F. Strategies to overcome trastuzumab resistance in HER2-overexpressing breast cancers: focus on new data from clinical trials. *BMC Med*. 2014;12(1):1–10.
16. Chandarlapaty DG and S. HER2-amplified breast cancer: mechanisms of trastuzumab resistance and novel targeted therapies. *Expert Rev Anticancer Ther*. 2011;11(1):263–275.
17. Hennessy BT, Smith DL, Ram PT, Lu Y, Mills GB. Exploiting the PI3K/AKT pathway for cancer drug discovery. *Nat Rev Drug Discov*. 2005;4:988–1004.
18. Kataoka Y, Mukohara T, Shimada H, Saijo N, Hirai M, Minami H. Association between gain-of-function mutations in PIK3CA and resistance to HER2-targeted agents in HER2-amplified breast cancer cell lines. *Ann Oncol*. 2010;21(2):255–262.
19. Norasikin S, Nafi M, Generali D, Kramer-marek G, Strina C, Cappelletti M. Nuclear HER4 mediates acquired resistance to trastuzumab and is associated with poor outcome in HER2 positive breast cancer. *Oncotarget*. 2014;5(15):5934–5949.
20. Feldinger K, Generali D, Kramer-marek G, Gijsen M, Ng TB, Wong JH. ADAM10 mediates trastuzumab resistance and is correlated with survival in HER2 positive breast cancer. *Oncotarget*. 2014;5(16):6633–6646.
21. Cerami E, Gao J, Dogrusoz U, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov*. 2012;2:401–404.
22. Lee JW, Soung YH, Seo SH, Kim SY, Park CH, Wang YP. Somatic mutations of ERBB2 kinase domain in gastric, colorectal, and breast carcinomas. *Clin Canc Res*. 2006;12(1):57–61.
23. Park YH, Shin H, Jung HH, Choi Y, Ahn T, Park K. Role of HER2 mutations in refractory metastatic breast cancers: targeted sequencing results in patients with refractory breast cancer. *Oncotarget*. 2015;6(31):32027–32038.
24. Endo Y, Dong Y, Yoshimoto N, et al. HER2 mutation status in Japanese HER2-negative breast cancer patients. *Jpn J Clin Oncol*. 2014;44:619–623.
25. Endo Y, Dong Y, Kondo N, et al. HER2 mutation status in Japanese HER2-positive breast cancer patients. *Breast Canc*. 2016;23:902–907.
26. Sun Z, Shi Y, Shen Y, Cao L, Zhang W, Guan X. Analysis of different HER-2 mutations in breast cancer progression and drug resistance. *J Cell Mol Med*. 2015;19(12):2691–2701.
27. Wang T, Xu Y, Sheng S, et al. HER2 somatic mutations are associated with poor survival in HER2-negative breast cancers. *Cancer Sci*. 2017;108:671–677.
28. Brookes AJ. The essence of SNPs. *Gene*. 1999;234:177–186.
29. Karki R, Pandya D, Elston RC, Ferlini C. Defining "mutation" and "polymorphism" in the era of personal genomics. *BMC Med Genomics*. 2015;8(1):1–7.
30. Xie D, Shu XO, Deng Z, Wen WQ, Creek KE, Dai Q. Population-based, case–control study of HER2 genetic polymorphism and breast cancer risk. *J Natl Cancer Inst*. 2000;92(5):412–417.
31. Frank B, Hemminki K, Wirtenberger M, Bermejo JL, Bugert P, Klaes R. The rare ERBB2 variant Ile654Val is associated with an increased familial breast cancer risk. *Carcinogenesis*. 2005;26(3):643–647.
32. Si P, Xu Y, Ouyang T, Li J, Wang T, Fan Z. HER2 Pro1170Ala polymorphism is associated with decreased survival rate in HER2-negative breast cancer. *Oncol Lett*. 2017;13(5):3793–3798.
33. Papewalis J, Nikitin AY, Rajewsky MF. G to A polymorphism at amino acid codon 655 of the human erbB-2/HER2 gene. *Nucleic Acids Res*. 1991;19:5452.
34. Bargmann CI, Hung MC, Weinberg RA. The neu oncogene encodes an epidermal growth factor receptor-related protein. *Nature*. 1986;319:226–230.
35. Beauclair S, Formento P, Fischel JL, Lescaut W, Largillier R, Chamorey E. Role of the HER2 [Ile655Val] genetic polymorphism in tumorogenesis and in the risk of trastuzumab-related cardiotoxicity. *Ann Oncol*. 2007;18(8):1335–1341.
36. Lemieux J, Diorio C, Cŏté MA, Provencher L, Barabé F, Jacob S. Alcohol and HER2 polymorphisms as risk factor for cardiotoxicity in breast cancer treated with trastuzumab. *Anticancer Res*. 2013;33(6):2569–2576.
37. Gómez Penã C, Dávila-Fajardo CL, Martínez-González LJ, Carmona-Saéz P, Soto Pino MJ, Sánchez Ramos J. Influence of the HER2 Ile655Val polymorphism on trastuzumab-induced cardiotoxicity in HER2-positive breast cancer patients: a meta-analysis. *Pharmacogenetics Genom*. 2015;25(8):388–393.
38. Lu S, Wang Z, Liu H, Hao X. HER2 Ile655Val polymorphism contributes to breast cancer risk: evidence from 27 case-control studies. *Breast Canc Res Treat*. 2010;124(3):771–778.
39. Wang H, Liu L, Lang Z, Guo S, Gong H, Guan H. Polymorphisms of ERBB2 and breast cancer risk: a meta-analysis of 26 studies involving 35,088 subjects. *J Surg Oncol*. 2013;108(6):337–341.
40. Chen W, Yang H, Tang WR, Feng SJ, Wei YL. Updated meta-analysis on HER2 polymorphisms and risk of breast cancer: evidence from 32 studies. *Asian Pac J Cancer Prev APJCP*. 2014;15(22):9643–9647.
41. Krishna BM, Chaudhary S, Panda AK, Mishra DR, Mishra SK. Her2-Ile655Valpolymorphism and its association with breast cancer risk: an updated meta-analysis of case-control studies. *Sci Rep*. 2018;8(1):1–19.
42. Ma Y, Yang J, Zhang P, Liu Z, Yang Z, Qin H. Lack of association between HER2 codon 655 polymorphism and breast cancer susceptibility: meta-analysis of 22 studies involving 19,341 subjects. *Breast Canc Res Treat*. 2011;125(1):237–241.
43. Dahabreh IJ, Murray S. Lack of replication for the association between HER2 I655V polymorphism and breast cancer risk: a systematic review and meta-analysis. *Cancer Epidemiol*. 2011;35(6):503–509.
44. Hosking L, Lumsden S, Lewis K, et al. Detection of genotyping errors by Hardy–Weinberg equilibrium testing. *Eur J Hum Genet*. 2004;12:395.
45. Mutluhan H, Akbas E, Erdogan NE, Soylemez F, Senli MS, Polat A. The influence of HER2 genotypes as molecular markers on breast cancer outcome. *DNA Cell Biol*. 2008;27(10):575–579.
46. Naidu R, Yip CH, Taib NA. Polymorphisms of HER2 Ile655Val and cyclin D1 (CCND1) G870A are not associated with breast cancer risk but polymorphic allele of HER2 is associated with nodal metastases. *Neoplasma*. 2008;55(2):87–95.
47. Tao W, Wang C, Han R, Jiang H. HER2 codon 655 polymorphism and breast cancer risk: a meta-analysis. *Breast Canc Res Treat*. 2009;114(2):371–376.
48. Kallel I, Kharrat N. HER2 polymorphisms and breast cancer in Tunisian women. *Genet Test Mol Biomarkers*. 2010;14(1):29–35.
49. Kara N, Karakus N, Ulusoy AN, Ozaslan C, Gungor B, Bagci H. P53 codon 72 and HER2 codon 655 polymorphisms in Turkish breast cancer patients. *DNA Cell Biol*. 2010;29(7):387–392.
50. Zhang M, Guo LL, Cheng Z, et al. A functional polymorphism of TGFBR2 is associated with risk of breast cancer with ER+, PR+, ER+ PR+ and HER2-expression in women. *Oncol Lett*. 2011;2:653–658.
51. AbdRaboh NR, Shehata HH, Ahmed MB, Bayoumi FA. HER1 R497K and HER2 I655V polymorphisms are linked to development of breast cancer. *Dis Markers*. 2013;34:407–417.
52. Ozturk O, Canbay E, Kahraman OT, Fatih Seyhan M, Aydogan F, Celik V. HER2 Ile655Val and PTEN IVS4 polymorphisms in patients with breast cancer. *Mol Biol Rep*. 2013;40(2):1813–1818.
53. Roca L, Diéras V, Roché H, Lappartient E, Kerbrat P, Cany L. Correlation of HER2, FCGR2A, and FCGR3A gene polymorphisms with trastuzumab related cardiac toxicity and efficacy in a subgroup of patients from UNICANCER-PACS04 trial. *Breast Canc Res Treat*. 2013;139(3):789–800.
54. Riaz SK, Rashid MM, Kayani MA, Malik MFA. Role of HER-2 Ile655Val polymorphism as universal cancer susceptibility marker among different cancers. *Arch Iran Med*. 2016;19(6):430–438.
55. Trevino V, Falciani F, Barrera-Saldaña HA. DNA microarrays: a powerful genomic tool for biomedical and clinical research. *Mol Med*. 2007;13:527.
56. Teumer A, Ernst FD, Wiechert A, et al. Comparison of genotyping using pooled DNA samples (allelotyping) and individual genotyping using the affymetrix genome-wide human SNP array 6.0. *BMC Genomics*. 2013;14:506.
57. Bumgarner R. Overview of DNA microarrays: types, applications, and their

future. *Curr Protoc Mol Biol*. 2013;101(1), 22-1.
58. Rohit A, Maiti B, Shenoy S, Karunasagar I. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) for rapid diagnosis of neonatal sepsis. *Indian J Med Res*. 2016;143:72–78.
59. Keshava C, McCanlies EC, Keshava N, Wolff MS, Weston A. Distribution of HER2(V655) genotypes in breast cancer cases and controls in the United States. *Cancer Lett*. 2001;173(1):37–41.
60. Kamali-Sarvestani E, Talei AR, Merat A. Ile to Val polymorphism at codon 655 of HER-2 gene and breast cancer risk in Iranian women. *Cancer Lett*. 2004;215(1):83–87.
61. Haghshenas L, Khandouzi M. HER2 Ile655Val SNP and risk of breast cancer. *IJMER*. 2014:1–10.
62. Carrillo-Moreno DI, Figuera LE, Magaña-Torres MT, Zúñiga-González G, Puebla-Pérez AM, Gallegos-Arreola MP. Association of a HER2 Ile655Val (Rs1136201) polymorphism in breast cancer in A Mexican population. *W J Res Rev*. 2016:15–20.
63. de Almeida FC, Banin Hirata BK, Ariza CB, Losi Guembarovski R, de Oliveira KB, Suzuki KM. HER2 Ile655Val polymorphism is negatively associated with breast cancer susceptibility. *J Clin Lab Anal*. 2018;32(6):1–6.
64. Livak KJ. Allelic discrimination using fluorogenic probes and the 5′ nuclease assay. *Genet Anal*. 1999;14:143–149.
65. McGuigan FEA, Ralston SH. Single nucleotide polymorphism detection: allelic discrimination using TaqMan. *Psychiatr Genet*. 2002;12:133–136.
66. Millikan R, Eaton A, Worley K, Biscocho L, Hodgson E, Huang WY. HER2 codon 655 polymorphism and risk of breast cancer in African Americans and whites. *Breast Canc Res Treat*. 2003;79(3):355–364.
67. Malkki M, Petersdorf EW. Genotyping of single nucleotide polymorphisms by 5′ nuclease allelic discrimination. *Immunogenetics*. 2012:173–182.
68. Lee S, Fedele V, Lacalamita R, Bruno M, Schittulli F, Ginzinger D. 655Val and 1170Pro ERBB2 SNPs in familial breast cancer risk and BRCA1 alterations. *Cell Oncol*. 2007;29(3):241–248.
69. Schleinitz D, DiStefano JK, Kovacs P. Targeted SNP genotyping using the TaqMan® assay. In: *Disease Gene Identification*. 2011:77–87.
70. Osaki R, Imaeda H, Ban H, Aomatsu T, Bamba S, Tsujikawa T. Accuracy of genotyping using the TaqMan PCR assay for single nucleotide polymorphisms responsible for thiopurine sensitivity in Japanese patients with inflammatory bowel disease. *Exp Ther Med*. 2011;2(5):783–786.
71. Qu S, Cai Q, Gao YT, Lu W, Cai H, Su Y. ERBB2 genetic polymorphism and breast cancer risk in Chinese women: a population-based case-control study. *Breast Canc Res Treat*. 2008;110(1):169–176.
72. Wu M, Du ZW, Liu JN, Song Y, Wang YL, Zhang GZ. Improved allele-specific polymerase chain reaction for single nucleotide polymorphism genotyping. *Chem Res Chin Univ*. 2010;26:259–262.
73. Baxter SW, Campbell IG. Re: population-based, case–control study of HER2 genetic polymorphism and breast cancer risk. *J Natl Cancer Inst (Bethesda)*. 2001;93:557–558.
74. Hauptmann M, Sigurdson AJ, Chatterjee N, Rutter JL, Hill DA, Doody MM. Re: population-based case-control study of HER2 genetic polymorphism and breast cancer risk. *J Natl Cancer Inst*. 2003;95(16):1251–1252.
75. Montgomery GW, Campbell MJ, Dickson P, Herbert S, Siemering K, Ewen-White KR. Estimation of the rate of SNP genotyping errors from DNA extracted from different tissues. *Twin Res Hum Genet*. 2005;8(4):346–352.
76. Suemizu H, Ohnishi Y, Maruyama C, Tamaoki N. Two-color allele-specific polymerase chain reaction (PCR-SSP) assay of the leptin receptor gene (Leprdb) for genotyping mouse diabetes mutation. *Exp Anim*. 2001;50:435–439.
77. Budiarto BR, Desriani. Dataset reporting detection of breast cancer-related HER2I655Vpolymorphism using allele-specific polymerase chain reaction. *Data Br*. 2016;9:689–695.
78. Heid IM, Lamina C, Küchenhoff H, Fischer G, Klopp N, Kolz M. Estimating the single nucleotide polymorphism genotype misclassification from routine double measurements in a large epidemiologic sample. *Am J Epidemiol*. 2008;168(8):878–889.
79. Budiarto BR, Azamris, Desriani. Modified allele-specific PCR improves HER2 Ile655Val detection by reducing genotyping errors. *Appl Cancer Res*. 2017;37(1):36.
80. Lewis C, Knight J. Genetic association studies: design, analysis and interpretation. *Briefings Bioinf*. 2012;3(2):146–153.
81. Schork NJ, Fallin D, Thiel B, Xu X, Broeckel U, Jacob HJCD. The future of genetic case-control studies. *Adv Genet*. 2001;42:191–212.
82. Baena IA, Garcés-Palacio IC Iii H, Grisales I. The effect of misclassification error on risk estimation in case-control studies Efeito da má classificação na estimativa de risco em estudo caso-controle. *Rev Bras Epidemiol*. 2015;18(182):341–356.
83. Sinnott JA, Kraft P. Artifact due to differential error when cases and controls are imputed from different platforms. *Hum Genet*. 2012;131(1):111–119.
84. Johnson EO, Hancock DB, Levy JL, et al. Imputation across genotyping arrays for genome-wide association studies: assessment of bias and a correction strategy. *Hum Genet*. 2013;132:509–522.
85. Zheng W, Kataoka N, Xie D, Young SR. RESPONSE: Re: population-based, case-control study of HER2 genetic polymorphism and breast cancer risk. *J Natl Cancer Inst (Bethesda)*. 2001;93:558–559.
86. Gaedigk A, Freeman N, Hartshorne T, et al. SNP genotyping using TaqMan® technology: the CYP2D6* 17 assay conundrum. *Sci Rep*. 2015;5:9257.
87. Little J, Higgins JPT, Ioannidis JPA, Moher D, Gagnon F, Von Elm E. STrengthening the REporting of genetic association studies (STREGA)- an extension of the STROBE statement. *Genet Epidemiol*. 2009;33(7):581–598.
88. Thelle DS. STROBE and STREGA: instruments for improving transparency and quality of reporting scientific results. *Eur J Epidemiol*. 2009;24(1):7–8.
89. Benusiglio PR, Lesueur F, Luccarini C, Conroy DM, Shah M, Easton DF. Common ERBB2 polymorphisms and risk of breast cancer in a white British population: a case-control study. *Breast Cancer Res*. 2005;7(2):204–209.
90. Leal SM. Detection of genotyping errors and pseudo-SNPs via deviations from Hardy-Weinberg equilibrium. *Genet Epidemiol*. 2005;29(3):204–214.
91. Salanti G, Amountza G, Ntzani EE, Ioannidis JPA. Hardy-Weinberg equilibrium in genetic association studies: an empirical evaluation of reporting, deviations, and power. *Eur J Hum Genet*. 2005;13(7):840–848.
92. Chen B, Cole JW, Grond-Ginsbach C. Departure from Hardy weinberg equilibrium and genotyping error. *Front Genet*. 2017;8:1–6.
93. Nedel F, André DDA, Oliveira IOD, Tarquinio SBC, Demarco FF. Buccal cells submitted to three different storage conditions before DNA extraction. *J Appl Oral Sci*. 2009;17:113–115.
94. van Huis-Tanja L, Kweekel D, Gelderblom H, et al. Concordance of genotype for polymorphisms in DNA isolated from peripheral blood and colorectal cancer tumor samples. *Pharmacogenomics*. 2013;14:2005–2012.
95. Marisi G, Passardi A, Calistri D, Zoli W, Amadori D, Ulivi P. Discrepancies between VEGF– 1154 G> a polymorphism analysis performed in peripheral blood samples and FFPE tissue. *Int J Mol Sci*. 2014;15:13333–13343.
96. Siddig A, Mohamed AO, Kamal H, Awad S, Hassan AH, Zilahi E. HER-2/neu Ile655Val polymorphism and the risk of breast cancer: a case-control study. *Ann N Y Acad Sci*. 2008;1138:84–94.
97. Nelson SE, Gould MN, Hampton JM, Trentham-Dietz A. A case-control study of the HER2 Ile655Val polymorphism in relation to risk of invasive breast cancer. *Breast Cancer Res*. 2005;7(3):R357–R364.
98. An HJ, Kim NK, Oh D, Kim SH, Park MJ, Jung MY. Her2V655 genotype and breast cancer progression in Korean women. *Pathol Int*. 2005;55(2):48–52.
99. Sezgin E, Sahin FI, Yagmurdur MC, Demirhan B. *HER-2/neu* gene codon 655 (Ile/Val) polymorphism in breast carcinoma patients. *Genet Test Mol Biomarkers*. 2011;15(3):143–146.
100. Kalemi TG, Lambropoulos AF, Gueorguiev M, Chrisafi S, Papazisis KT, Kotsis A. The association of p53 mutations and p53 codon 72, Her 2 codon 655 and MTHFR C677T polymorphisms with breast cancer in Northern Greece. *Cancer Lett*. 2005;222(1):57–65.
101. Tommasi E, Simopoulos K, Tripsianis G, Tentes I, Anagnostopoulos K, Sivridis E. Allelic imbalance of HER-2 codon 655 polymorphism among different religious/ethnic populations of northern Greece and its association with the development and the malignant phenotype of breast cancer. *Neoplasma*. 2007;54(5):365–373.
102. Al-janabi AM, Algenabi AHA, Alkhafaji SM, Alsabri I. Association of TP53 [Arg72Pro] gene polymorphism and breast cancer risk in Iraqi female patients. *Exp Mol Pathol*. 2015;6(12):1128–1138.
103. Akisik E, Dalay N. Estrogen receptor codon 594 and HER2 codon 655 polymorphisms and breast cancer risk. *Exp Mol Pathol*. 2004;76(3):260–263.
104. Taberlet P. Reliable genotyping of samples with very low DNA quantities using PCR. *Nucleic Acids Res*. 1996;24(16):3189–3194.
105. Berry DP, O'Brien A, Wall E, McDermott K, Randles S, Flynn P. Inter- and intra-reproducibility of genotypes from sheep technical replicates on Illumina and Affymetrix platforms. *Genet Sel Evol*. 2016;48(1):1–5.
106. Wang-Gohrke S, Chang-Claude. J. Re: population-based, case-control study of HER2 genetic polymorphism and breast cancer risk. *J Natl Cancer Inst*. 2001;93(21):1657.
107. Hishida A. Re: population-based, case-control study of HER2 genetic polymorphism and breast cancer risk. *J Natl Cancer Inst*. 2002;94(23):1807–1808.
108. Pinto D, Vasconcelos A, Costa S, et al. HER2 polymorphism and breast cancer risk in Portugal. *Eur J Cancer Prev*. 2004;13(3):177–181.
109. Zubor P, Vojvodova A, Danko J, et al. HER-2 [Ile655Val] polymorphism in association with breast cancer risk: a population-based case-control study in Slovakia. *Neoplasma*. 2006;53(1):49, 1.
110. Lee SC, Hou MF, Hsieh PC, et al. A case–control study of the HER2 Ile655Val polymorphism and risk of breast cancer in Taiwan. *Clin Biochem*. 2008;41(3):121–125.
111. Rajkumar T, Samson M, Rama R, et al. TGFβ1 (Leu10Pro), p53 (Arg72Pro) can predict for increased risk for breast cancer in south Indian women and TGFβ1 Pro (Leu10Pro) allele predicts response to neo-adjuvant chemo-radiotherapy. *Breast Canc Res Treat*. 2008;112(1):81–87.