Methodology

ALLELE-SPECIFIC POLYMERASE CHAIN REACTION (AS-PCR) AS A TOOL FOR GENOTYPING *KCNQ1* RS2237892 POLYMORPHISM

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ABSTRACT

Aims: To develop and validate a protocol of allele-specific polymerase chain reaction (AS-PCR) for detecting the *KCNQ1* rs2237892 polymorphism.

Methods: Genomic human DNA was extracted from peripheral blood samples of a population-based cohort in Ha Nam province, Vietnam. The AS-PCR protocol was optimized by adjusting annealing temperatures (Ta) and primer concentrations. To ensure accuracy, the genotyping results obtained from AS-PCR were validated against restriction fragment length polymorphism (RFLP) using the *Pvu*II enzyme and direct Sanger sequencing in a subset of samples.

Results: The optimal annealing temperature for the AS-PCR assay was determined to be 59°C, yielding a clear 392-bp diagnostic band. The AS-PCR method demonstrated 100% concordance with both RFLP and Sanger sequencing results across all tested genotypes (CC, CT, and TT). The protocol effectively discriminated between the risk allele (C) and the alternative allele (T) with high sensitivity and specificity.

Conclusion: The established AS-PCR protocol provides a reliable and high-throughput tool for genotyping the *KCNQ1* rs2237892 variant. Given its minimal equipment requirements and high accuracy, this method is well-suited for large-scale genetic epidemiological studies and clinical screening in resource-limited settings like Vietnam.

Key words: *type 2 diabetes mellitus, genotyping, rs2237892, KCNQ1 gene, AS-PCR.*

I. INTRODUCTION

Type 2 diabetes mellitus (T2DM) is becoming an increasingly important global public health challenge, with a rapidly rising prevalence. According to the International Diabetes Federation, approximately 537 million adults were living with diabetes in 2021, and this number is projected to reach 783 million by 2045, representing nearly one in ten adults worldwide [1]. In Vietnam, the burden of T2DM has also increased substantially, with nearly 5 million cases reported in 2022, reflecting a concerning upward trend [2]. T2DM is not only highly prevalent but also associated with serious chronic complications affecting the cardiovascular system, eyes, nervous system, and kidneys, thereby contributing significantly to morbidity, mortality, and healthcare costs [3].

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T2DM is a multifactorial metabolic disorder resulting from the interaction between genetic predisposition and environmental factors. Epidemiological studies have estimated the heritability of T2DM to range from 30% to 70%, and individuals with a first-degree relative affected by T2DM have a significantly increased risk of developing the disease [4, 5]. Furthermore, population-based studies have demonstrated that T2DM tends to occur at lower body mass index levels in Asian populations, highlighting the important contribution of genetic susceptibility [6].

The advent of Genome-wide association studies has enabled the identification of more than 40 genetic loci associated with T2DM susceptibility. Many of these loci, including *TCF7L2, CDKAL1, HNF4A*, and *HNF1B*, are involved in pancreatic β-cell function and insulin secretion [7, 8]. However, each genetic variant confers only modest effects, and the cumulative and interactive effects of multiple variants, together with environmental and epigenetic factors, ultimately determine disease susceptibility.

Among the identified susceptibility genes, *KCNQ1* has emerged as a key genetic determinant, particularly in Asian populations. This gene encodes a voltage-gated potassium channel that plays an essential role in regulating insulin secretion in pancreatic β-cells. Several common variants, including rs2237892, rs2237895, and rs2237897, have been consistently associated with increased risk of T2DM and impaired insulin secretion across diverse populations [9, 10]. In addition, *KCNQ1* variants have been linked to other diabetes-related conditions, such as gestational diabetes mellitus and diabetic nephropathy [11]. These findings underscore the critical role of genetic factors, particularly *KCNQ1* variants, in the pathogenesis and clinical manifestations of T2DM. Nevertheless, the magnitude of these effects and their interactions with environmental factors remain incompletely understood, especially in understudied populations such as the Vietnamese population. Further research in this context is essential to provide scientific evidence for improved prevention strategies, early diagnosis, and personalized treatment of T2DM.

To investigate the role of genetic variants in T2DM, particularly polymorphisms in *KCNQ1*, the selection of an appropriate genotyping method is crucial. In this study, Allele-specific polymerase chain reaction (AS-PCR) was employed as a precise and efficient technique for detecting genetic variants. AS-PCR is a modification of the Polymerase chain reaction method that enables direct identification of specific alleles based on selective primer binding to target DNA sequences.

The principle of AS-PCR relies on the design of oligonucleotide primers with allele-specific nucleotides at the 3′ end, corresponding to each variant of interest. This subtle difference ensures that amplification occurs only when the primer perfectly matches the DNA template, allowing accurate discrimination of genotypes. Due to its high specificity, AS-PCR provides a rapid, reliable, and cost-effective approach for detecting single-nucleotide polymorphisms (SNPs) compared with more complex sequencing methods.

In this study, AS-PCR was applied to develop a genotyping protocol for the rs2237892 variant of the *KCNQ1* gene in

the Vietnamese population. The development of a highly accurate and simple method not only enhances the efficiency of genetic epidemiological studies but also lays the foundation for future applications in risk screening and personalized medicine for T2DM.

II. METHODS

2.1. Subjects and samples

Study participants were recruited from a previous cross-sectional study conducted in Ha Nam province, located in the southwest of the Red River Delta, Vietnam. The study protocol was approved by the Ethics Committee of the National Institute of Hygiene and Epidemiology (Approval No. IRB-VN01057-34/2016). Participants included patients diagnosed with T2DM and healthy control subjects. In addition, a total of 55 blood samples were selected and used specifically for method development and optimization of the genotyping procedure prior to formal analysis. Fasting venous blood samples were collected in the morning after at least 8 hours of overnight fasting. Blood samples were immediately centrifuged to separate plasma and cellular components.

Genomic DNA was extracted from peripheral blood leukocytes using Wizard® Genomic DNA Purification Kit (Promega Corporation, USA). The purity and concentration of the extracted DNA were assessed using a Nanodrop spectrophotometer by measuring the optical density ratio (OD260/280). Then the DNA samples were subsequently normalized to a final concentration of at least 15ng/µL for ready-to-use.

2.2. Genotyping using AS-PCR

Genotyping of the *KCNQ1* rs2237892 polymorphism was performed using the allele-specific polymerase chain reaction (AS-PCR) method, based on previously described protocols. The principle of AS-PCR relies on the selective amplification of target alleles using primers designed to match specific nucleotide variants at the 3′-end, allowing precise discrimination of genotypes. This approach has been widely applied for single nucleotide polymorphism (SNP) detection due to its simplicity and high specificity. The methodology was adapted from the amplification refractory mutation system (ARMS) originally described by Newton CR et al. (1989), with subsequent refinements reported by Ye S et al. (2001) [12, 13].

In this study, AS-PCR was applied to detect the SNP at the rs2237892 locus of the *KCNQ1* gene. Two allele-specific primer sets were designed for genotyping. The first set consisted of a wild-type-specific forward primer paired with a common reverse primer, while the second set included a mutant-specific forward primer combined with the same common reverse primer.

The reference sequence for the *KCNQ1* rs2237892 polymorphism (GenBank accession No. NC_000011.10:2818520:C>T) was obtained from the National Center for Biotechnology Information. Primer design was carried out using Primer3Plus and WASP. To enhance allele specificity, deliberate mismatches were introduced at the 3′-end of the forward primers. In

addition, primer sequences were modified to create a restriction site specific to the mutant allele, enabling further validation of genotyping results. The following primers were used Wildtype Forward Primer 5'-CTGTCACAGGACTTTGCCACAC-3′ and Common Reverse Primer 5′-TCCTAGATGTGATTCTGCTGGG-3′ for detect allele C and used Mutant Forward Primer 5'-CTGTCACAGGACTTTGCCACAT-3′ and Common Reverse Primer 5′-TCCTAGATGTGATTCTGCTGGG-3′ for detect allele T (Fig. 1). The melting temperatures (Tm) of all primers were approximately 59°C, as calculated using the OligoAnalyzer™ tool. A gradient PCR was performed to optimize the annealing temperature, testing 55°C, 57°C, and 59°C.

Figure 1. *Schematic representation of the AS-PCR primer design.*

Schematic representation of primer binding sites within the KCNQ1 gene sequence. The wild-type forward primer (KCNQ1_FW_W) and mutant forward primer (KCNQ1_FW_M) differ at the 3′-terminal nucleotides (C→T), enabling allele-specific amplification. The common reverse primer (KCNQ1rs2237892R) anneals downstream of the SNP site. Arrows indicate the direction of primer extension. The polymorphic site (rs2237892, C>T) is highlighted, demonstrating the nucleotide discrimination at the 3'-end of allele-specific primers.

PCR amplification was carried out under the following conditions: initial denaturation at 94°C for 3 minutes; 35 cycles of denaturation at 94°C for 30 seconds, primer annealing at 55°C, 57°C, or 59°C for 40 seconds, and extension at 72°C for 40 seconds; followed by a final extension at 72°C for 8 minutes and cooling at 25°C. Each genotype was determined using two independent PCR reactions, with a total reaction volume of 10µL containing 3.5µL nuclease-free water, 0.75µL forward primer, 0.75µL reverse primer (10pmol of each primer), 2µL genomic DNA, and 3.0µL GoTaq® Green PCR Master Mix (2X) (Promega Corporation, USA), which includes GoTaq® DNA polymerase, reaction buffer (pH 8.5), 400µM each dNTP, and 3mM $MgCl_2$. PCR products were separated by electrophoresis on a 2.5% agarose gel in 0.5X TBE buffer at 100 V. DNA bands were visualized using a Geldoc-It™ gel documentation system.

2.3. Validation of genotyping by RFLP and sequencing

To validate the genotyping results obtained by AS-PCR, approximately 10% of the samples were randomly selected and re-analyzed using the Restriction fragment length polymorphism method, as previously described by Nguyen Thi Yen for the *KCNQ1* rs2237892 polymorphism. This approach was used as a reference method for comparison [14]. Briefly, the rs2237892 polymorphism was genotyped by PCR on genomic DNA with primer 5'-GCATCCTAAGGTTCAG-3' (forward) and 5'-TGGGTCATCAGACTAGGGTA-3' (reverse). PCR amplification of the target region was performed, followed by restriction enzyme digestion using *Pvu*II to differentiate alleles based on fragment length patterns. A gel imaging system was used to determine genotype results to detect results. Genotypes were determined according to fragment patterns as follows: CC genotype (217 bp and 122 bp), TT genotype (339 bp), and CT genotype (339 bp, 217 bp, and 122 bp). In this study, 10% of samples were genotyped using RFLP-PCR analysis, and then 90% rest of the samples used the allele-specific primer (ASP) genotyping method to observe results. Three randomly selected samples representing each genotype (CC, CT, and TT) were validated by direct DNA sequencing. The concordance between AS-PCR, RFLP, and sequencing results demonstrated the reliability of the genotyping approach used in this study.

III. RESULTS

3.1. Optimization of AS-PCR conditions

This section looked for the SNP *KCNQ1* rs2237892 C→T via AS-PCR. Firstly, the optimal annealing temperature (Ta) of two designed primer sets was examined by Gradient PCR through a range of Ta with different annealing temperatures (55°C, 57°C, and 59°C). PCR products were separated by electrophoresis on a 2% agarose gel. At 55°C, amplification of the expected 392 bp fragment was observed; however, the bands appeared relatively diffused and were accompanied by weak non-specific background signals, suggesting suboptimal primer specificity at this temperature. Increasing the annealing temperature to 57°C improved band clarity, with reduced background noise and more distinct amplification products, although minor non-specific amplification was still detectable in some lanes. At 59°C, the amplification showed the highest specificity and efficiency. The target bands at 392 bp were sharp, well-defined, and consistently intense across samples, with minimal background noise and no observable non-specific bands. Importantly, allele discrimination was clearly achieved, as amplification patterns corresponded accurately to the expected genotypes (CC, CT, and TT). No amplification was detected in the negative control (–) and blank (B) lanes, confirming the absence of contamination and primer-dimer formation.

Based on these results, 59°C was selected as the optimal annealing temperature for subsequent genotyping experiments. Under these optimized conditions (Table 1), PCR amplification was performed for 38 cycles, including an initial denaturation at 94°C for 3 minutes,

followed by denaturation at 94°C for 30 seconds, annealing at 59°C for 40 seconds, and extension at 72°C for 40 seconds. A final extension step was carried out at 72°C for 8 minutes, and the reaction was terminated by cooling to 25°C.

Figure 2. *Optimization of annealing temperature for AS-PCR amplification of KCNQ1 rs2237892.*

Agarose gel electrophoresis (2%) showing PCR products amplified at different annealing temperatures (55°C, 57°C, and 59°C). The expected amplicon size was 392 bp. The strongest and most specific amplification was observed at 59°C, which was selected as the optimal annealing temperature for subsequent genotyping. The DNA ladder (L) is shown on the right. Ta: annealing temperature. (a) Amplification using the wild-type primer set (KCNQ1_FW_W and KCNQ1_CMN_R); (b) amplification using the mutant primer set (KCNQ1_FW_M and KCNQ1_CMN_R). From left to right, the gel is divided into three sections corresponding to annealing temperatures of 55°C, 57°C, and 59°C. In each section, lanes 1–5 represent: genotype CT (lane 1), genotype CC (lane 2), genotype TT (lane 3), negative control (–, lane 4; no DNA template), and blank (B, lane 5; no reaction components). This pattern is repeated across all three temperature conditions (lanes 6–10 and 11–15). Lane 16 corresponds to the DNA ladder.

Table 1. *The components for the AS- PCR reaction.*

PCR 1: Components for detecting allele C	Volume (µl)	PCR 2: Components for detecting allele T	Volume (µl)
Nuclease-free water	3.5 µl	Nuclease-free water	3.5 µl
PCR Master mix	3.0 µl	PCR Master mix	3.0 µl
KCNQ1_FW_W	0.75 µl	KCNQ1_FW_M	0.75 µl
KCNQ1_CMN_R	0.75 µl	KCNQ1_CMN_R	0.75 µl
DNA template	2.00 µl	DNA template	2.00 µl
Total volume	10.0 µl	Total volume	10.0 µl

3.2. Genotype *KCNQ1* rs2237892 by AS-PCR

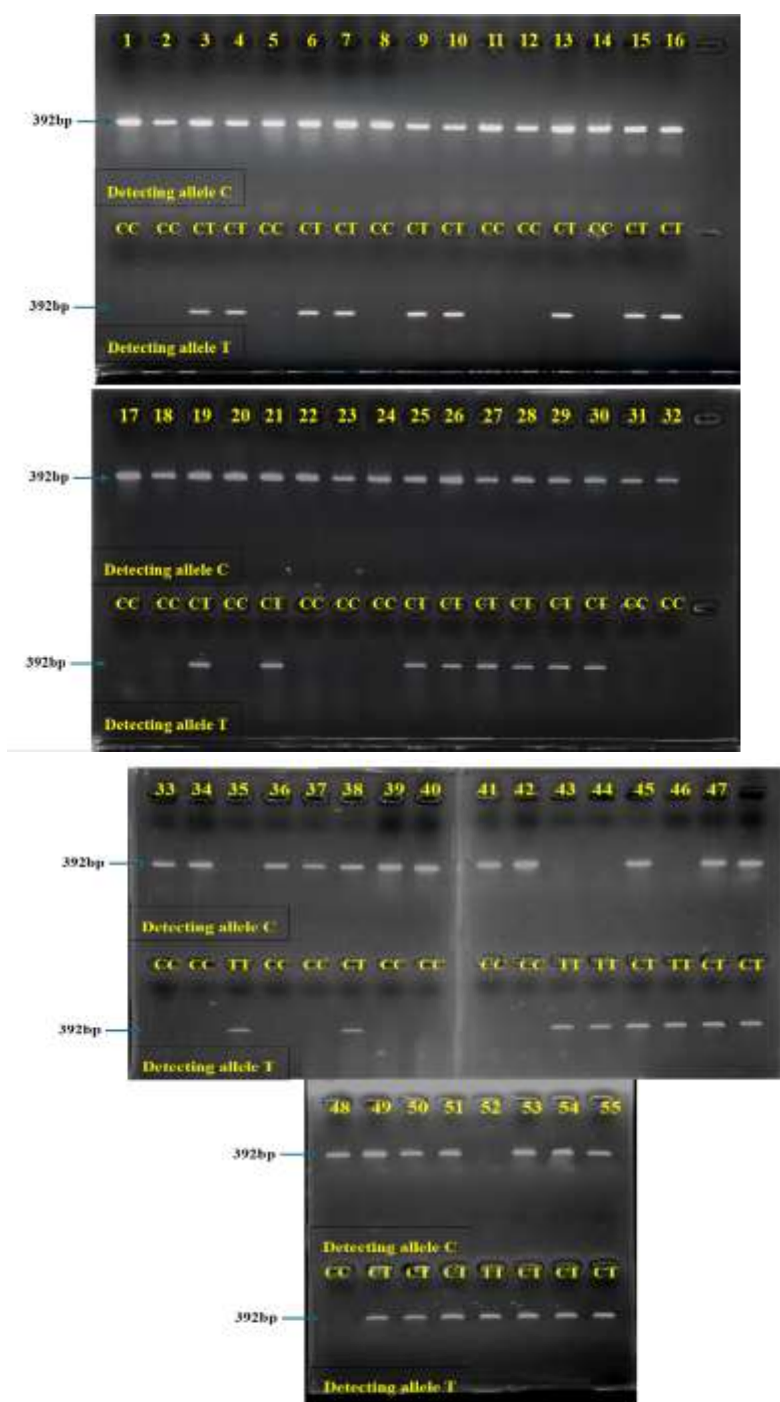

Figure 3*. Genotyping result of the KCNQ1 rs2237892 using AS-PCR.*

Agarose gel electrophoresis (2%) shows allele-specific amplification of the *KCNQ1* rs2237892 (C→T) polymorphism in 55 samples. Two separate reactions were performed for each sample to detect allele C and allele T, respectively. The expected amplicon size was 392 bp. Samples showing amplification only in the allele C reaction were classified as homozygous wild type (CC), those showing amplification only in the allele T reaction were classified as homozygous mutant (TT), and samples showing amplification in both reactions were identified as heterozygous (CT). The genotype assignments are indicated above each lane.

The AS-PCR successfully discriminated against the three genotypes (CC, CT, and TT) based on allele-specific amplification patterns. Homozygous CC samples produced bands exclusively in the allele C reaction, while TT samples showed amplification only in the allele T reaction. Heterozygous CT samples exhibited bands in both reactions, consistent with the presence of both alleles. The observed band intensity was generally uniform across samples, indicating stable amplification efficiency. No amplification was detected in control lanes, supporting the specificity of the assay. Overall, the results demonstrate that the optimized AS-PCR protocol provides a robust and accurate approach for genotyping the *KCNQ1* rs2237892 polymorphism.

3.2. Genotyping of KCNQ1 rs2237892 by PCR–RFLP and validation of AS-PCR results

The *KCNQ1* rs2237892 polymorphism was further genotyped using the PCR–restriction fragment length polymorphism (PCR–RFLP) method to validate the AS-PCR results. The PCR amplicon (339 bp) was digested using the *Pvu*II restriction enzyme in a total reaction volume of 20 µL, containing 10 µL of PCR product, 1× reaction buffer, 5 U of *Pvu*II enzyme, and nuclease-free water. The digestion was performed at 37°C for 2–3 hours, followed by enzyme inactivation at 65°C for 20 minutes. The digested products were separated on a 2% agarose gel and visualized under UV illumination. Genotypes were determined based on fragment patterns: the CC genotype produced two fragments (217 bp and 122 bp), the TT genotype remained undigested (339 bp), and the CT genotype displayed three bands (339 bp, 217 bp, and 122 bp) (Fig. 4).

To assess the reliability of the AS-PCR method, 30% of the total samples (n = 16/55) were randomly selected and re-genotyped using PCR–RFLP. The results obtained from PCR–RFLP were fully concordant with those from AS-PCR, yielding a 100% concordance rate, indicating high accuracy and reproducibility of the allele-specific assay. In addition, representative samples of each genotype (CC, CT, and TT) were further validated by direct DNA sequencing, which confirmed the genotyping results.

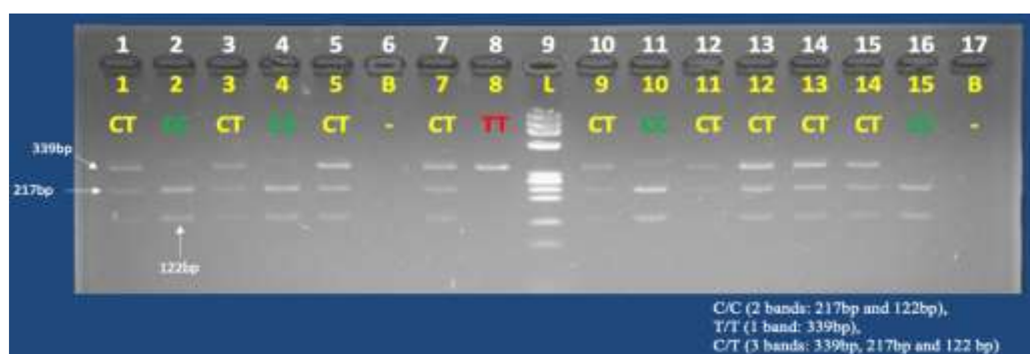

Figure 4. *PCR product of rs2237892 of KCNQ1 digested by restriction enzyme and electrophoresed on 2% agarose gel.*

Separated PCR-RFLP products of the targeted gene, which were digested by *Pvu*II. Three genotypes of *KCNQ1* rs2237892 were identified according to the length size of digested products: C/C (2 bands: 217bp and 122bp), T/T (1 band: 339bp), and C/T (3 bands: 339bp, 217bp, and 122 bp); L: Ladder, B: Blank, respectively.

3.3. Sequencing validation

To further validate the genotyping results obtained by RFLP-PCR, Sanger sequencing was performed on selected samples with known genotypes. Representative samples corresponding to all three genotypes—CC (Fig. 5A), CT (Fig. 5B), and TT (Fig. 5C)—as identified by the RFLP and AS-PCR method, were analyzed. The sequencing results showed complete concordance with the genotypes determined by PCR-RFLP, confirming the accuracy of the method. Overall, the genotyping success rate was 100%, as indicated by the presence of clear and interpretable banding patterns in agarose gel electrophoresis. These findings demonstrate the reliability and robustness of the genotyping approach used in this study.

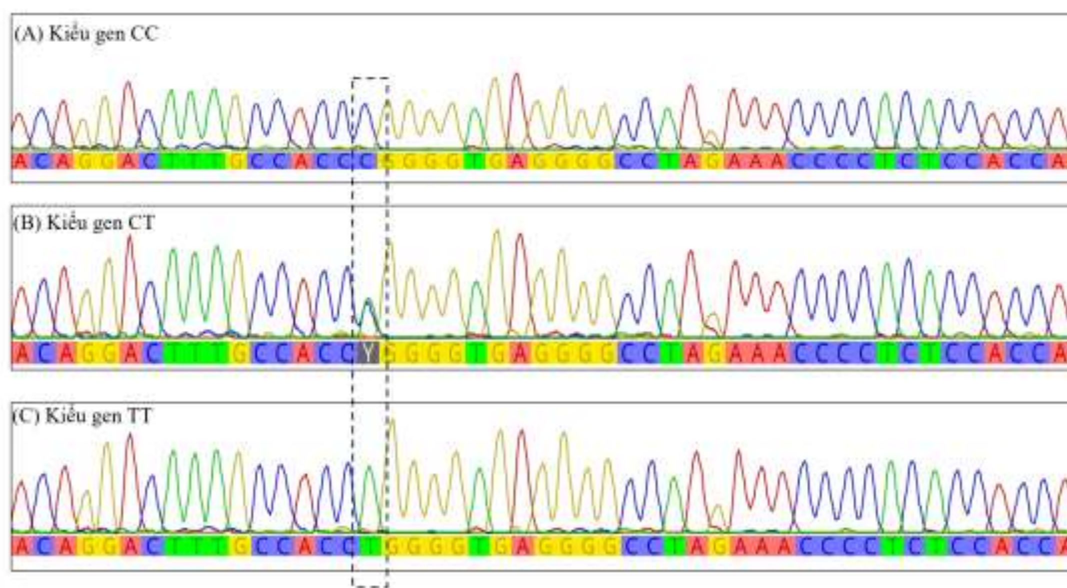

Figure 5. *Validation of KCNQ1 rs2237892 genotypes by Sanger sequencing.*

Representative chromatograms of *KCNQ1* rs2237892 genotypes confirmed by Sanger sequencing. (A) Homozygous CC genotype showing a single peak of cytosine (C); (B) Heterozygous CT genotype showing overlapping peaks of cytosine (C) and thymine (T); (C) Homozygous TT genotype showing a single peak of thymine (T) respectively.

IV. DISCUSSION

Potassium Voltage-Gated Channel Subfamily Q Member 1 (*KCNQ1*, also known as KV7.1 or KVLQT1), within the critical region for long QT syndrome-1 locus [15]. By genomic sequence analysis, the *KCNQ1* gene contains 19 exons and spans more than 400 kb. The exon sizes range from 47 to 1,122 bp. *KCNQ1* is responsible for encoding a type of protein known as KvLQT1, which functions as a voltage-gated potassium channel [16]. Common genetic variants in *KCNQ1*, particularly single-nucleotide polymorphisms rs2237892, have been strongly associated with β-cell insulin secretion dysfunction and an increased risk of type 2 diabetes mellitus, as demonstrated in both Asian and European populations using oral glucose tolerance tests [17]. GWAS first established

KCNQ1 as a type 2 diabetes susceptibility locus in East Asian and European cohorts, with significant associations for variants including rs2237892 and rs2237895 [18]. Subsequent meta-analyses confirmed that the rs2237892 C allele significantly increases T2DM risk in Asian populations, especially in Chinese, Korean, and Malaysian groups, while results in South Asians have been less consistent. Individual case–control studies in Chinese and Korean cohorts further support the association of *KCNQ1* variation with impaired β-cell function and elevated diabetes risk. Some aggregated analyses, however, have reported nonsignificant associations, highlighting the need for larger, well-powered studies.

Recent genome-wide association studies have consistently shown that *KCNQ1* SNPs are more prevalent in Asian populations than in European populations [19], underscoring the gene's important role in metabolic diseases among Asians. Several studies conducted in Japan, China, and Korea have reported strong associations between *KCNQ1* variants and impaired β-cell function as well as increased risk of type 2 diabetes mellitus, whereas these associations appear weaker or less consistent in European cohorts. In Vietnam, although large-scale GWAS data remain limited, emerging genetic studies on metabolic disorders have similarly highlighted the relevance of Asian-specific risk alleles [20], emphasizing the need for affordable and reliable genotyping approaches tailored to local research settings [18], [21].

In this study, the present study aimed to develop and validate a cost-effective and accurate genotyping protocol for *KCNQ1* polymorphisms, considering laboratory workflow, analytical capacity, and financial constraints commonly encountered in Vietnamese laboratories. Various SNP genotyping techniques have been employed in previous Asian studies, including TaqMan assays and high-throughput genotyping platforms, particularly in Japan and China, where access to advanced infrastructure is more widespread. However, such approaches remain costly and less feasible for routine use in many Vietnamese research institutions. Therefore, alternative methods such as PCR-RFLP, allele-specific PCR (AS-PCR), and CTPP-PCR represent more practical solutions.

Consistent with reports from studies in China and Thailand, our findings demonstrate that PCR-RFLP and AS-PCR yield highly concordant genotyping results, confirming their reliability for detecting *KCNQ1* SNPs. Importantly, the genotypes obtained using these two methods were fully consistent with DNA sequencing results, which served as the reference standard, thereby validating the accuracy of the proposed protocol. Similar validation strategies have been employed in several Asian studies, where sequencing was used selectively to confirm PCR-based genotyping results, ensuring both cost efficiency and data reliability.

From a methodological perspective, PCR-RFLP has been extensively utilized in genetic studies in Vietnam due to its straightforward assay design and high specificity, particularly for SNPs that alter restriction enzyme recognition sites. However, consistent with findings from our study and previous reports across Vietnam and other Southeast Asian countries, PCR-RFLP is labor-intensive and less suitable for large-scale studies. Although RFLP-based approaches provide reliable genotyping for diagnostic markers and possess strong

discriminatory power, they are associated with several limitations. Notably, the procedure is time-consuming, technically demanding, and often requires processing many samples, which can increase overall costs. While DNA sequencing offers comprehensive genetic information, it is considerably more expensive and requires substantial computational resources. In contrast, alternative techniques such as PCR-RFLP, allele-specific PCR (AS-PCR), and TaqMan PCR represent more cost-effective options, allowing the detection of genetic variation at a slightly lower resolution.

In contrast, AS-PCR offers a faster and more scalable alternative, making it advantageous for population-based studies. Nevertheless, similar to observations in studies from India and China, AS-PCR in our study required careful primer design and extensive optimization to avoid false-positive amplification, especially when unmodified allele-specific primers were used.

AS-PCR is an incredibly useful method that enables the identification of alleles with subtle variations in their nucleotide sequences through PCR amplification. This innovative approach involves the conduction of two PCR reactions simultaneously, resulting in significant time and cost savings. While using this method, ASPCR utilizes the Taq DNA polymerase's inability to extend a primer in the absence of a complementary 3' nucleotide on the DNA template, making it a reliable method for allele discrimination. However, using unmodified, mutation-specific primers in ASPCR can sometimes lead to false-positive results, even with optimal conditions. This is especially problematic when testing clinical samples where accuracy is crucial. To ensure accurate detection of mutations, it is necessary to invest time and resources in designing and optimizing allele-specific DNA primers. Furthermore, the complex task of generating primers and templates, combined with the need to finely tune PCR conditions for multiple objectives, greatly contributes to the intricate nature of establishing dependable multiplex reactions. Therefore, the development of a new method capable of amplifying all alleles effectively would greatly enhance the credibility of ASPCR as a reliable technique.

Overall, the strong agreement between PCR-RFLP, AS-PCR, and sequencing in this study reinforces the credibility of PCR-based genotyping approaches for *KCNQ1* SNP analysis. Given their low cost, minimal equipment requirements, and validated accuracy, these methods are particularly well-suited for application in Vietnam and other developing Asian countries, where financial and technical limitations may restrict access to high-throughput genotyping platforms. The combined use of AS-PCR or CTPP-PCR for initial large-scale screening, followed by PCR-RFLP or sequencing for confirmation, represents a balanced and sustainable strategy for genetic epidemiology studies in Asian populations [22]. PCR-RFLP is an efficient technique to determine fragment length polymorphism.

In this study, allele-specific polymerase chain reaction (AS-PCR) was employed to genotype the KCNQ1 single-nucleotide polymorphism rs2237892. Genotyping was performed in a population-based cohort consisting of more than 1.500 individuals aged 40–64 years who were recruited from Ha Nam province, Vietnam.

The optimization focused on the simultaneous adjustment of allele-specific and common primer concentrations within each reaction to improve amplification specificity and to minimize competitive interactions among primers. Following optimization, the protocol was applied uniformly to all samples under standardized conditions, and genotyping results were recorded for subsequent analysis. This optimized AS-PCR protocol enabled stable amplification patterns and consistent genotype assignment throughout the study population, supporting its application for SNP genotyping in population-based genetic studies.

V. CONCLUSION

In conclusion, the results of the present study show that AS-PCR is indeed a powerful technique, a rapid, accurate, and cost-effective solution for genotyping SNPs, which can be easily customized. We reported that the ASPCR protocol was highly efficient and sensitive in simultaneously amplifying and clearly discriminating between two alleles, C and T, to genotype SNP rs2237892 in the *KCNQ1* gene. It has been successfully utilized in Vietnamese individuals, so we hope this protocol can be used in other Asian populations sharing similar genetic characteristics.

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