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Rapid Detection of *SLCO1B1* Polymorphisms Using Duplex Fluorescence Melting Curve Analysis: Implications for Personalized Drug Dosing in Clinical Settings

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Objective: The polymorphism of the solute carrier organic anion transporter family member *1B1 (SLCO1B1)* gene exerts a marked influence on drug transport, thus playing a pivotal role in personalized drug dosing. This study endeavours to establish a rapid, precise, and straightforward method for detecting *SLCO1B1* genetic variants utilizing Duplex Fluorescence Melting Curve Analysis (DFMCA).

Methods: Whole blood samples were collected from 54 individuals from Meizhou People's Hospital (2023.01–2023.03), with a mean age of 58.90 years (SD = 7.86), including 28 men and 26 women. DNA was extracted from these samples and subjected to PCR amplification targeting two allelic regions. Primers, fluorescent probes, and corresponding allelic target sequences were designed specifically for two common *SLCO1B1* polymorphisms (rs2306283 and rs4149056). The functionality of the fluorescent probes in binding to their respective allelic targets was verified using melting curve analysis, enabling the identification of distinct melting temperatures for different genotypes. Subsequently, DFMCA was employed to differentiate genotypes based on the melting temperature shifts of the corresponding fluorescent probes. The sensitivity, accuracy, and consistency of the method were evaluated, with sequencing validation performed on a subset of samples.

Results: DFMCA facilitated the concurrent detection and accurate genotyping of both polymorphisms within 2 hours, demonstrating concordance with sequencing results from randomly selected samples. Importantly, stable detection performance was achieved for human genomic DNA at concentrations ≥ 3.125 ng. In a cohort comprising Han Chinese individuals from southern China, the allele frequencies for rs2306283 (A: 28.7%, G: 71.3%) and rs4149056 (T: 88.89%, C: 11.11%) concurred well with previous studies in the Han Chinese population.

Conclusion: The SNP typing system utilizing DFMCA technology presents advantages in terms of speed, ease of use, accuracy, and cost-effectiveness, making it a suitable tool.

Keywords: *SLCO1B1*, melting curve analysis, gene polymorphism, drug delivery

Introduction

The organic anion-transporting polypeptide 1B1 (OATP1B1), also known as OATP-C, OATP2, or LST1, is encoded by the *SLCO1B1* gene and is specifically localized to the basolateral membrane of hepatocytes.[1] It plays a pivotal role in the uptake and elimination of both endogenous and exogenous substances by hepatocytes, including bile acids, unconjugated

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bilirubin, thyroxine, statins, repaglinide, enalaprilat, temocapril, valsartan, olmesartan, methotrexate, and the active metabolite 7-ethyl-10-hydroxycamptothecin (SN-38) of irinotecan.[2] The Hakka people are an ethnic group formed by the integration of the people from the central plains of China with different ethnic groups during the southward migration, and Meizhou city is one of the main gathering places of Hakka people.[3] In the southern Chinese Hakka population with cardiovascular and cerebrovascular diseases, two major single nucleotide polymorphisms (SNPs) in the *SLCO1B1* gene, rs2306283 (c.388A>G) and rs4149056 (c.521T>C), are prevalent.[4] Remarkably, the rs4149056 polymorphism significantly compromises the transport function of OATP1B1 for its substrates, leading to elevated plasma concentrations of statins (e.g, pravastatin, atorvastatin, rosuvastatin) and an elevated risk of myopathy in patients harbouring the 521C allele during treatment with simvastatin and cerivastatin.[5] Conversely, the rs2306283 polymorphism does not directly influence OATP1B1 function but is crucial for the haplotype resolution of the gene.[6] Given the potential for severe adverse reactions to statins, including liver injury and rhabdomyolysis, variants in *SLCO1B1* markedly influence patients' responses to statin therapy. To mitigate the risk of adverse effects, expert consensus recommends personalized statin selection based on the *SLCO1B1* genotype.[7] Currently, various methods are available for detecting genetic polymorphisms, including Restriction Fragment Length Polymorphism (RFLP),[8] gene chip- also known as DNA microarrays, gene chips allow for the simultaneous detection of multiple polymorphisms,[9] duplex pyrosequencing assay- this is a sequencing-by-synthesis technique that provides real-time sequence data,[10] allele-specific PCR- method uses primers that are specific to the alleles being investigated,[11] TaqMan real-time PCR- fluorescent probes use to detect specific DNA sequences during PCR amplification,[12] high-resolution melting-curve (HRM) analysis- it involves amplifying DNA in the presence of a fluorescent dye, followed by slowly melting the DNA duplexes.[13] Nevertheless, these methods are often hindered by operational intricacies, prolonged processing times, reliance on expensive equipment, the requirement for post-PCR manipulations, and an inability to concurrently detect multiple polymorphisms. Consequently, their widespread adoption in clinical diagnostics remains limited. Duplex Fluorescence Melting Curve Analysis (DFMCA) emerges as an efficient and potent method for mutation detection in the molecular biological detection of personalized medicine.[13] By leveraging the differential melting temperatures exhibited by probe-target hybrids during thermal denaturation, DFMCA utilizes dual-labeling technology to facilitate rapid, straightforward, and high-throughput genotyping.[14] The detection principle of this technology is that the DNA sample is amplified by asymmetric PCR to produce a large number of single-stranded DNA products that complement the probe. During the melting curve analysis, the probe hybridized with different single-stranded DNA products to form different heteroduplex hybrids, which had different stability degrees and different melting temperatures (Tm). The difference in Tm values could realize the detection and typing of gene variation. This approach has proven successful in screening for mutations associated with various genetic disorders.[15,16] The present study aims to utilize DFMCA technology to concurrently detect and genotype two critical polymorphic loci (rs2306283 and rs4149056) within the *SLCO1B1* gene in a single PCR reaction, thereby enhancing both detection efficiency and clinical convenience.

Materials and Methods

The study was approved by the hospital's Research Ethics Board (approval number 2020-C-89) and was conducted in accordance with the principles outlined in the Declaration of Helsinki.

Sample Collection and DNA Extraction

A Fifty-four whole blood specimen (10mL each, non-duplicated) were collected from individuals (mean age 58.90, S ±D=7.86; 28 males' year, 26 females' year) undergoing routine health exams at Meizhou People's Hospital between January 1, 2023-March 13, 2023. The specimens were obtained by trained nursing staff under aseptic conditions. The timing of sample collection was not influenced by specific environmental conditions. The blood samples were first mixed thoroughly to prevent cell clumping and ensure an even distribution of nucleated cells. Blood specimens were then aliquoted to prevent cross-contamination and stored at 4°C until processed further. Nucleic acid extraction from the whole blood samples was performed utilizing Promotor ® automated nucleic acid extractor (ACON Biotech Co., Ltd., Hangzhou, China) along with its dedicated nucleic acid extraction kit. This system ensured consistent and efficient isolation of high-quality genomic DNA. The extraction followed by the quality and concentration of the extracted DNA

was assessed using an ND-1000 full-wavelength UV-Vis spectrophotometer (NanoDrop, USA) to ensure a final DNA concentration within the range of 5–100 ng/mL, facilitating direct application in subsequent experiments or storage at −20°C for future use.

Plasmid Standard Construction and Genotyping Assay

Genotyping assays followed, and standard plasmids containing both wild-type and homozygous mutant sequences of the *SLCO1B1* gene were custom-synthesized by Sangon Biotech (Shanghai, China). These plasmids served as controls in the experiments. Plasmid DNA was extracted using the DNA extraction kit (TIANGEN Biotech, China), following the manufacturer's protocol, which included steps for lysing cells, binding plasmid DNA to a silica column, washing to remove contaminants, and eluting the purified DNA. The concentration and purity of the plasmid DNA were verified, ensuring their readiness for use in the validation and calibration of the genotyping assays.

Design of Primers and Probes

The full sequence of the *SLCO1B1* gene (NCBI Reference Sequence: NG_011745.1) was retrieved from the NCBI database (https://www.ncbi.nlm.nih.gov/) (Last ascension-25/09/2023) to identify the locations of the rs4149056 and rs2306283 polymorphisms. Based on these locations, primers and probes were designed to target the specific polymorphic sites. For each polymorphism, two self-quenching probes and corresponding amplification primer pairs were designed using Oligo7.0 software and the melting temperature prediction tool available at https://store.sangon.com/base. Initially, the probes were designed to specifically bind to the targeted loci of interest. Subsequently, amplification primers were designed around these probes to ensure optimal hybridization. The melting temperature (Tm) of the probes was carefully adjusted to be slightly higher or lower than that of the primers to achieve optimal conditions for hybridization and amplification. GC content was carefully considered during the design process. For both primers and probes, the GC content was maintained within the optimal range of 40% to 60% to enhance stability and specificity. This range supports effective binding and minimizes the risk of non-specific interactions or secondary structures. To confirm the specificity of the designed primers and probes, a homology search was conducted using the NCBI database to ensure no cross-reactivity with non-target sequences. The final sequences of the probes and primers were synthesized and provided by Shanghai Bioengineering Co. Ltd (Table 1).

Strategy of Duplex Fluorescence Melting Curve Analysis

This study designed a technical strategy for self-quenching fluorescence probes with double-labelled end and asymmetric PCR melting curve analysis (Figure 1A-B). A systematic approach was undertaken to develop and optimize genotyping for the *SLCO1B* gene polymorphisms rs4149056 and rs2306283, following the designed strategy depicted in Figure 1A. To detect two genetic polymorphism sites, a pair of primers and a self-quenching fluorescent probe were designed specifically for each polymorphism site. Each fluorescent probe used for polymorphism detection was labelled with a unique fluorophore, corresponding to distinct detection channels, such as carboxy fluorescein (FAM) and hexachloro fluorescein (HEX), in the real-time quantitative PCR instrument. This process involved incorporating primers and probes specific to each polymorphic site into PCR reaction tubes, followed by asymmetric PCR amplification. Subsequently, PCR amplification generated abundant single-stranded products complementary to the corresponding fluorescent probes.

Table 1 Primers and Probes Designed for Detection of SLCO1B1 Polymorphisms rs4149056 and rs2306283

***SLCO1B1* Polymorphic Variants**	**Oligonucleotide**	**Sequence**	**Size (bp)**
rs2306283 (c.388A>G)	Forward primer (5'-3')-F1	CCCACTATCTCAGGTGATGC	134
	Reverse primer (5'-3')-R1	GCAGTGATGTTCTTACAGTTACAGG	
	Probe primer (5'-3')-Q1	CTGATGAATCGATATTAGTTTCTTCAG	
rs4149056 (c.521T>C)	Forward primer (5'-3')-F2	GAGTCTCCCCTATTCCACG	235
	Reverse primer (5'-3')-R1	CGACACCATATTGTCAAAGTTTGCA	
	Probe primer (5'-3')-Q2	CCCATGAACACATATATCCACATGGG	

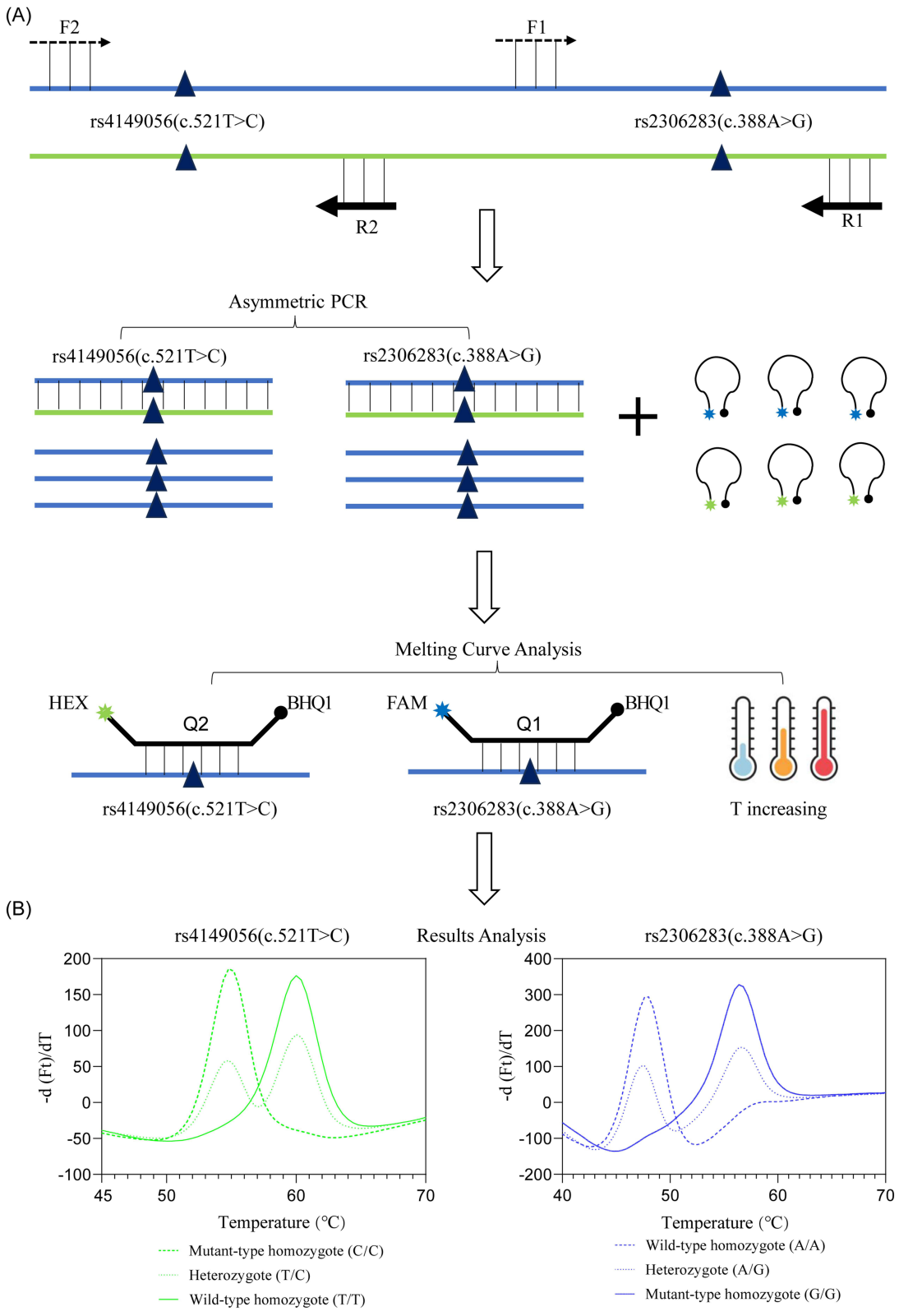

Figure 1 Flowchart of the assay for gene polymorphism detection (**A**), and the typical genotyping results with plasmid of each polymorphism site (**B**).
Notes: Limiting primer (F1 and F2); Abundant primer (R1 and R2); Probes: Q1 and Q2; (T) temperature.

A melting curve analysis was performed on these products, spanning from low to high temperatures, during which the fluorescence intensity across all channels was recorded continuously. Ultimately, the accompanying software of the real-time fluorescent quantitative PCR instrument was utilized for result analysis, extracting melting peaks from each curve. The genotype of each polymorphic site was determined by analyzing the number of melting peaks and their corresponding Tm values. Finally, the detection method was established using plasmid DNA standards of diverse genotypes and subsequently evaluated methodologically (Figure 1B). The melting temperature of the primers designed for *SLCO1B1* gene amplification is slightly lower, and asymmetric PCR is applied to amplify the single-stranded DNA that binds to the probe in a large amount. Specifically, the ratio of limiting primer to non-limiting primer is between 1/5 and 1/50, and here we chose 1/10. 2) Probes Q1 and Q2, designed for melting curve analysis were designed with Shared-stem molecular beacons and the 5 and 3 terminals were labelled with fluorophore and quencher, respectively. This assay was a one-step closed-tube genotyping method that involved asymmetric PCR and melting curve analysis running on a SLAN-96S Real-Time PCR Platform. The optimized reaction system was a total volume of 25mL that contains 10 to 100 ng gDNA, 2.4 mmol/L $MgCl_2$, 0.16 mmol/L dNTPs, 40 nmol/L *SLCO1B1* gene primers (F1/F2), 400 nmol/L asymmetric primers (R1/R2), 80 nmol/L self-quenched probes (Q1/Q2), and 0.25U Mighty Taq HS DNA Polymerase with 1× Mighty Taq HS Buffer (Takara Bio Co Ltd, Da Lian, China). The protocol began with an initial denaturation for 5 minutes at 95 °C, 50 cycles of 15 seconds at 95°C, 30 seconds at 52°C and 30 seconds at 72°C, followed by a melting curve analysis procedure consisting of denaturation for 1 minute at 95°C, hybridization for 3 minutes at 40 °C, and a continuous temperature increase from 40 °C to 70 °C. Fluorescence data of carboxyfluorescein (FAM) and hexachloro fluorescein (HEX) channels were recorded during the melting analysis procedure by instrument-matched software. The mutation types and sample genotypes were identified based on Tm values of melting peaks.

Sensitivity, Specificity, and Accuracy Evaluation

To assess analytical sensitivity, we conducted an analysis utilizing this assay on a series of twofold serially diluted gDNA samples, with concentrations spanning from 50 ng to 3.125ng. To evaluate specificity and accuracy, we performed random population sampling of human genomic DNA, focusing on samples with concentrations at the initially determined detection limit. Subsequently, we statistically analyzed the Tm values of both wild-type and mutant alleles for each polymorphism. The Tm difference (ΔTm) between the wild-type and mutant alleles was calculated. Subsequently, we randomly selected products from each polymorphism for sequencing verification, which was conducted by Sangon Biotech Co., Ltd (Shanghai, China).

Statistical Analysis

The data were analyzed using SPSS statistical software version 26.0 and Snap Gene 2021. The allelic and genotypic frequency distributions of the polymorphisms rs4149056 and rs2306283 were assessed for compliance with Hardy-Weinberg equilibrium (HWE) utilizing chi-square (χ^2) tests. A p-value <0.05 indicated a statistically significant deviation.

Results

Analytical Sensitivity Evaluation of the Method

The yield of extracted genomic DNA (gDNA) varied based on the source and processing of blood samples. This variability can affect the sensitivity and reliability of the assay. To address this, we evaluated the analytical sensitivity of our method by testing gDNA concentrations ranging from 3.125 to 50 ng per 25 μL reaction, with water as the negative control. Our findings demonstrated that accurate analysis was achievable throughout this entire dosage spectrum. Specifically, the melting temperatures of 56.4°C and 59.9°C were observed for the 388G/G and 521T/T genotypes, respectively (Figure 2). These results confirm the method's effectiveness in detecting different genotypes and its robustness across the tested gDNA concentrations.

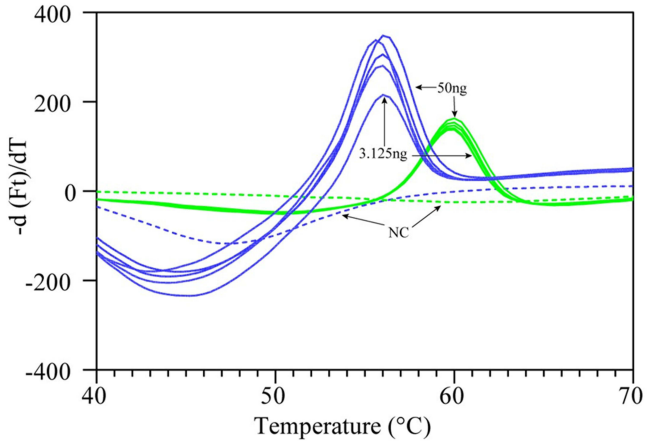

Figure 2 Melting Temperatures for 388G/G and 521T/T Genotypes Across a Range of gDNA Concentrations.
Notes: Blue and Green dotted line were negative control without DNA.

Analytical Accuracy Evaluation of the Method

The detection system discriminates between various polymorphisms through the identification of unique Tm values, whereby two distinct polymorphic sites are independently allocated to separate detection channels, each capable of accommodating up to two discernible melting peaks. To mitigate the risk of misinterpretation stemming from excessive fluctuations in the Tm values, a rigorous evaluation of the repeatability of these values was undertaken. Consequently, we performed genotyping of two polymorphic sites within the *SLCO1B1* gene across fifty-four collected human genomic DNA samples. As evident from Table 2, the results demonstrate a high degree of stability, with all melting peaks exhibiting 3-fold standard deviations (3SDs) below 0.52°C, ensuring no overlap between adjacent peaks and excellent discrimination. Moreover, the differences in Tm values (ΔTm) between distinct alleles at each polymorphic site demonstrate remarkable consistency, with all ΔTm values substantially surpassing 5.40°C and their respective 3SDs remaining below 0.39°C. These data strongly suggest that consistent and stable detection outcomes can be achieved across different laboratory settings, by various operators, using assay systems prepared in different batches, and applied to multiple samples. Consequently, this detection system exhibits exceptional repeatability and reproducibility in genetic polymorphism analysis.

Genotype and Allele Frequency Analysis

In the analysis of 54 samples, the genotypes AA, AG, and GG at the rs2306283 locus were observed in 3, 25, and 26 individuals, respectively, comprising 5.55%, 46.30%, and 48.15% of the total samples. Furthermore, the allele frequencies of A and G were 31 and 77 respectively, corresponding to 28.7% and 71.3% of the total alleles. Regarding the rs4149056 polymorphism, the genotypes TT, TC, and CC were observed in 44, 8, and 2 samples, respectively, accounting for 81.48%, 14.81%, and 3.71% of the study population. The allele frequencies for T and C totalled 96% and 12, % respectively, which constituted 88.89% and 11.11% of the total alleles (Table 3). To validate the genotyping accuracy for the two loci, rs2306283 and rs4149056, 22 samples, each possessing distinct genotypes, underwent sequencing utilizing

Table 2 Stability and Repeatability of Melting Peaks and Δtm Values for SLCO1B1 Gene Polymorphisms

SLCO1B1 Polymorphic Variants	Allele	Tm ±3SDs/°C	ΔTm ±3SDs/°C
rs2306283(c.388A>G)	A	47.451±0.516	− 9.160±0.143
	G	56.455±0.336	
rs4149056(c.521T>C)	T	59.936±0.237	5.410±0.165
	C	54.705±0.288	

Notes: Tm is the average value; ΔTm =Tm(wild-type)-Tm(mutant-type).

Table 3 Distribution of Genotypes and Allele Frequencies for rs2306283 and rs4149056 Polymorphisms in the Study Population

SLCO1B1 Polymorphic Variants	Genotypes	Sample Size (n)	Genotype Frequency (%)	Alleles	Sample Size (n)	Genotype Frequency (%)
rs2306283(c.388A>G)	AA	3	5.55	A	31	28.7
	AG	25	46.3	G	77	71.3
	GG	26	48.15			
rs4149056(c.521T>C)	TT	44	81.48	T	96	88.89
	TC	8	14.81	C	12	11.11
	CC	2	3.71			

primer pairs F1-R1 and F2-R2, specifically designed to target polymorphisms. The sequencing results demonstrated full congruence with the initial genotyping outcomes, thereby attesting to the high specificity and accuracy of the employed assay (Figure 3). Chi-square (χ^2) tests were conducted to evaluate the adherence of observed allele and genotype frequencies at rs2306283 and rs4149056 to the Hardy-Weinberg equilibrium within the study population. The results indicating that rs2306283 (χ^2=0.928, p=0.335) and rs4149056 (χ^2=3.375, p=0.066) polymorphisms conform to the theoretical equilibrium, thereby corroborating the lack of substantial deviation from random mating patterns in the sampled population.

Discussion

Statins are recognized as one of the most effective drugs for lowering low-density lipoprotein cholesterol (LDL-C) levels and are widely used in the prevention and treatment of cardiovascular diseases. Despite their overall safety profile and minimal side effects, statins are considered to be underutilized in medical practice, partly attributed to the incidence of statin-induced muscle toxicities.[17] SNPs within the SLCO1B1 gene, which encodes for the human hepatic OATP1B1, result in modifications to the transporter's function and altered pharmacokinetics of various statin drugs, pravastatin among them.[18] This study focuses on two frequently occurring single nucleotide polymorphisms (SNPs) within the *SLCO1B1* gene in the Chinese population.[19] DFMCA technology was utilized to facilitate concurrent detection and genotyping of these two SNPs (Figure 1B). Performance assessment demonstrated exceptional repeatability and reproducibility, with 3SDs of Tm values for each polymorphism remaining below 0.52°C (Table 2). Furthermore, the system demonstrated high sensitivity, accurately analyzing human genomic DNA down to a concentration of 3.125 ng/mL (Figure 2), thus fulfilling the requirements for clinical DNA sample testing. DFMCA,[20] an innovative technology, harnesses duplex-color fluorescence coupled with melting curve analysis to facilitate simultaneous, high-throughput target identification within a single PCR cycle. Post-PCR, a streamlined melting curve analysis suffices for rapid genotype determination, eliminating the need for complex post-processing steps. This approach excels in real-time monitoring of fluorescence intensity changes during temperature variation, yielding a temperature-dependent fluorescence intensity curve for precise genotyping. DFMCA has demonstrated success in genetic disease screening, drug metabolism gene profiling, and microbial molecular diagnostics. Currently, SNP detection methods for the *SLCO1B1* gene encompass two primary categories: those requiring post-PCR processing (RFLP, gene chips, pyrosequencing, Sanger) and real-time qPCR (qPCR & HRM) that forgo post-PCR steps. The former, though effective, necessitates multi-step procedures prone to contamination, time-consuming, and limiting throughput, thereby hindering widespread clinical adoption. Conversely, qPCR methods streamline the process, offering speed and convenience, yet their capacity for multi-locus detection within a single reaction is limited, constraining their clinical applicability to some extent. Compared with the gene chip method, DFMCA has the advantages of simple operation, shorter time, lower cost, higher sensitivity, and repeatability. Compared with the gold standard detection method, PCR-Sanger sequencing, DFMCA technology simplifies the cumbersome process of DNA extraction, PCR amplification, purification, capillary electrophoresis, and sequencing down to two major steps: DNA extraction, PCR amplification, and melting curve analysis, thereby significantly streamlining the operational workflow. For two SNP loci in the SLCO1B1 gene, using primers and

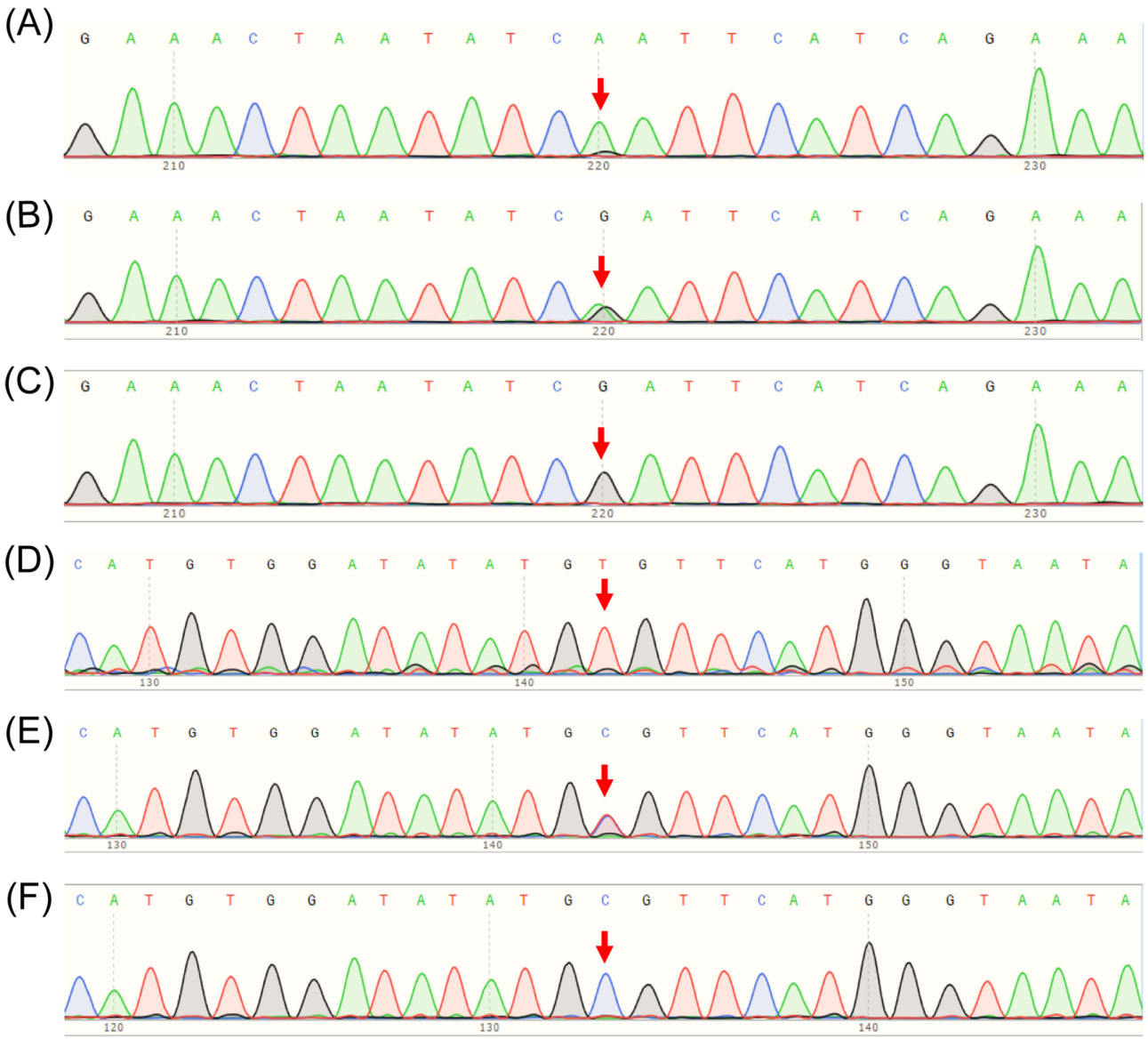

Figure 3 Sequence analysis verifies the presence of nucleotide changes.
Notes: (**A**) A388G wild-type homozygote A/A. (**B**) A388G Heterozygous A/G. (**C**) A388G mutant-type homozygote G/G. (**D**) T521C wild-type homozygote T/T. (**E**) T521C Heterozygous T/G. (**F**) T521C mutant-type homozygote C/C.

probes with approximately 5 nmol of DNA, DFMCA can efficiently detect over 200 samples, effectively reducing the detection costs. However, the main shortcoming of DFMCA cannot be overlooked, that is, the design of the probe. Some SNP sites are difficult to design probes for due to the GC content nearby and the hairpin structure. The position of the probe and the combination of bases need to be constantly adjusted to optimize the Tm value, GC content, and secondary structure of the probe. Notably, TaqMan qPCR probes may exhibit fluorescence quenching unless hybridized, whereas DFMCA, upon hybridization, emits intensified fluorescence due to fluorophore-quencher separation, enhancing detection sensitivity. Additionally, genotyping of 54 randomly selected human genomic DNA samples and subsequent comparison with sequencing results from a subset of these samples confirmed complete concordance, underscoring the high specificity and accuracy of DFMCA technology. A genotyping analysis was conducted on two SNPs (rs2306283 and rs4149056) within the *SLCO1B1* gene using 54 randomly selected genomic DNA samples from the Hakka population in southern Chinese. Table 3 summarizes the results, indicating that for rs2306283 (c.388A>G), the allele frequencies of A and G were 28.70% and 71.30%, respectively. Meanwhile, for rs4149056 (c.521T>C), the allele frequencies of T and

C were 88.89% and 11.11%, respectively. These distribution frequencies in the Han Chinese population in southern China align with previous studies on the Han Chinese population. Remarkably, the allele mutation rate for 388G>A among healthy Han Chinese males attains 71.3%, markedly surpassing that in Caucasians (30.0%) while displaying a minor divergence from Africans (74.0%) and Japanese (62.9%). Furthermore, the allele mutation rate for 521T>C amounts to 11.11%, notably surpassing that of Africans (2.0%) while subtly varying from Caucasians (14.0%) and Japanese (15.8%).[21,22] These observations reinforce the substantial geographical and ethnic variations in the mutation rates of 388G>A and 521T>C. Furthermore, the polymorphism of the *SLCO1B1* gene is extensively implicated in the metabolism of diverse drugs, encompassing anticancer agents,[23] antimicrobials,[24] and statins.[25] Therefore, the polymorphism of the *SLCO1B1* gene represents a crucial factor influencing individual drug responses in the Chinese population. By integrating genetic testing for this polymorphism with exhaustive clinical data before drug administration, it is envisioned that treatment efficacy and safety will be optimized, leading to a substantial reduction in the occurrence of severe adverse reactions. The rapid advancements in molecular biotechnology have enabled humanity to delve into the molecular intricacies of diseases, species, and drug mechanisms, fostering precision diagnostics and prevention through the detection of specific DNA base variations within the genome. This, in turn, propels the personalization of drug therapies.[26] The detection method based on DFMCA technology is not only suitable for pharmacogenomic gene polymorphism analysis, but also can be applied to various fields of clinical detection, including the screening and detection of tumor gene mutations, genotyping of pathogenic microorganisms, and methylation detection. However there are some shortcomings in this study, such as this study only focused on the two most common polymorphisms of the *SLCO1B1* gene, and no other polymorphisms of this gene were included in the detection system established in this study. Moreover, there was no methodological comparison between the detection method of *SLCO1B1* gene polymorphism established in this study and some test kits on the market. However, the entire process of this DFMCA method, from amplification to detection, is completed within two hours, offering unparalleled convenience, speed, and a notable enhancement in throughput. Compared to existing methodologies, our technology exhibits remarkable advantages, making it an ideal candidate for widespread clinical adoption and a potent enabler of precision medicine.

Conclusion

The Duplex Fluorescence Melting Curve Analysis (DFMCA) technology demonstrated in this study offers a rapid, precise, and cost-effective method for genotyping two significant *SLCO1B1* polymorphisms (rs2306283 and rs4149056) in the Han Chinese population. This technology's high sensitivity and specificity, combined with its ability to concurrently detect multiple SNPs in a single, closed-tube system, highlight its potential as a valuable tool for clinical genetic testing. The allele frequencies observed in this study align with previous research, reinforcing the importance of understanding ethnic and geographical variations in *SLCO1B1* polymorphisms, which have profound implications for drug metabolism and personalized medicine. Despite certain limitations, such as focusing solely on two polymorphisms and lacking comparison with other detection methods, DFMCA presents significant advantages over existing techniques. Its simplicity, speed, and reduced risk of contamination, along with the elimination of post-PCR handling steps, position DFMCA as a promising alternative for routine clinical use. By facilitating rapid and accurate genotyping, this method could play a crucial role in optimizing drug therapy, minimizing adverse drug reactions, and advancing the practice of precision medicine, particularly in populations with distinct genetic backgrounds.

Data Sharing Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics Approval

This study adhered strictly to the ethical principles outlined in the Declaration of Helsinki and received approval from the Institutional Ethics Committee of Meizhou People's Hospital, with reference number 2020-C-89, ensuring compliance with international ethical standards.

Concept for Publication

Written informed consent was obtained from all participants prior to specimen collection. All individuals agreed to the use of their data for research and publication purposes.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no competing interests.

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