

Establishment of a Rapid TaqMan-qPCR Assay for GLP-1R rs6923761 Genotyping and Consistency Analysis with Sanger Sequencing

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Objective: To establish a rapid TaqMan probe-based qPCR assay for genotyping the *GLP1R* rs6923761 polymorphism and to determine its concordance with Sanger sequencing.

Methods: Genomic DNA was isolated from buccal swabs collected from 22 adolescent volunteers. Each sample was genotyped by TaqMan qPCR and Sanger sequencing. DNA concentration and purity were assessed before amplification. Genotype and allele frequencies, Hardy-Weinberg equilibrium (HWE), overall concordance, Cohen's kappa, and the exact 95% confidence interval (CI) for concordance were calculated.

Results: TaqMan qPCR identified 11 GG and 11 GA samples; no AA homozygote was observed. The G and A allele frequencies were 75.0% and 25.0%, respectively. Sanger sequencing gave identical genotype calls for all 22 samples. Concordance was 100% (22/22; exact 95% CI, 84.6%-100%), with a Cohen's kappa of 1.00. Genotype distribution did not deviate significantly from HWE (chi-square = 2.44, $p = 0.118$).

Conclusions: In this initial sample set, the TaqMan qPCR assay distinguished the GG and GA genotypes of *GLP1R* rs6923761 with complete concordance to Sanger sequencing. The closed-tube workflow is suitable for further evaluation as a rapid assay for known-SNP genotyping. Because AA samples were unavailable and analytical sensitivity and reproducibility were not formally tested, additional validation is required before clinical or pharmacogenetic use.

Keywords: *GLP1R*; rs6923761; TaqMan qPCR; Sanger sequencing; SNP genotyping

Introduction

Overweight and obesity are increasingly common among children and adolescents in China, accompanied by a rising burden of metabolic disease and early-onset type 2 diabetes¹⁻³. Lifestyle, nutrition, and physical activity remain major determinants, but genetic variation may also contribute to differences in metabolic traits and in response to incretin-based therapies⁴⁻⁶.

The glucagon-like peptide-1 receptor gene (*GLP1R*) encodes the GLP-1 receptor (GLP-1R), a 463-amino-acid class B G-protein-coupled receptor⁷. rs6923761 is a common missense variant in *GLP1R* in which a G₁A substitution results in p.Gly168Ser^{8,9}. The variant has been examined in relation to endogenous GLP-1 responses, insulin secretion, gastric emptying, obesity-related traits, type 2 diabetes, gestational diabetes, and response to GLP-1 receptor agonists (GLP-1RAs)⁸⁻¹⁴.

The clinical significance of rs6923761 remains unset-

tled. Several small studies have reported associations with metabolic traits or responses to liraglutide and exenatide, including gastric emptying and glycemic outcomes, whereas larger pharmacogenomic analyses suggest that treatment effects may vary across phenotypes and populations¹⁰⁻¹⁴.

At present, rs6923761 is better considered a candidate pharmacogenetic marker than a validated predictor of GLP-1RA efficacy, weight loss, glycemic response, or dose.

Sanger sequencing provides direct sequence confirmation but requires PCR product processing and is relatively labor-intensive when large numbers of samples are tested. TaqMan allelic discrimination uses allele-specific fluorescent probes in a closed-tube PCR format and is well suited to rapid genotyping of known SNPs¹⁵⁻¹⁹.

We therefore established a TaqMan qPCR assay for rs6923761 using buccal swab-derived DNA and compared the resulting genotype calls with Sanger sequencing in a preliminary cohort of adolescents. The study was designed to evaluate genotyping concordance and workflow feasibility; it was not designed to test associations with obesity, diabetes, or response to GLP-1RAs.

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Table 1 Comparison of two typical SNP detection technologies.

Feature	Sanger Sequencing	TaqMan-qPCR SNP Genotyping
Principle	Chain-termination sequencing; reads the target sequence	Allele-specific fluorescent probe hybridization and cleavage
Throughput per run	Typically, 24-96 samples	Typically, 96-384 samples
Turnaround time	Approximately 8-24 hours depending on workflow	Approximately 1.5-3 hours depending on workflow
Cost per sample	Relatively high	Relatively low after assay setup
Contamination risk	Higher due to multiple open-tube steps	Lower due to closed-tube amplification and detection
Key characteristic	Sequence confirmation and discovery of unexpected variants	Rapid genotyping of known SNPs
Application context	Variant validation and sequence-level confirmation	Large-scale known-SNP genotyping and epidemiological studies

Methods

Study Participants

Twenty-two adolescent volunteers (11 male and 11 female; 12-18 years of age) were recruited through a school-based research activity. Buccal swab samples from all participants were used for rs6923761 genotyping. Exclusion criteria were self-reported diabetes, major chronic disease, acute oral inflammation, and antibiotic use within the previous 2 weeks. These criteria were used to avoid acute illness or oral conditions that could interfere with sample collection or DNA quality.

Ethics Approval and Informed Consent

The protocol was approved by the Institutional Ethics Committee of Beijing Zhenguo Integrative Chinese and Western Medicine Hospital and was conducted in accordance with the Declaration of Helsinki. Because all participants were minors, written informed consent was obtained from a legal guardian and assent was obtained from each participant before sampling. Samples and data were anonymized before laboratory analysis. Genotyping was performed for research purposes only and was not used to guide treatment.

DNA Extraction and Quality Control

Participants rinsed their mouths with water and refrained from eating or drinking for 30 min before sampling. Buccal mucosa from both cheeks was swabbed for approximately 30 s per side using disposable medical swabs²⁰. Swab heads were immediately placed into 2-mL tubes containing DNA preservation solution (Swab DNA Storage Tube, catalog No. WE0400, Baiao

Leibo Technology Co., Ltd.) and transported to the laboratory at room temperature within 48 h.

Genomic DNA was extracted with the MagPure Swab DNA KF Kit (catalog No. D6317-01, Huayunbio) on a KingFisher Flex automated magnetic-bead platform (Thermo Fisher Scientific, Waltham, MA, USA). The workflow included lysis, Proteinase K digestion, magnetic-bead capture, and ethanol-based washing. DNA concentration and A260/A280 and A260/A230 ratios were measured before PCR. All 22 DNA extracts provided sufficient material for both genotyping methods.

TaqMan qPCR Genotyping

TaqMan qPCR was performed in accordance with the relevant MIQE reporting principles²¹. Reactions used Hieff Unicon Universal TaqMan Probe U+ qPCR Mix (Yeasen Biotechnology, Shanghai, China; catalog No. 16710ES60). Primers and probes were designed from the genomic sequence flanking rs6923761 and checked for specificity with Primer-BLAST before synthesis by Generay Biotechnology.

The G-allele probe was labeled with VIC and the A-allele probe with 6-FAM; both probes carried a 3' MGB non-fluorescent quencher. Final concentrations were 400 nM for each primer and 200 nM for each probe.

Each run included a no-template control (NTC) containing nuclease-free water. Amplification and endpoint fluorescence measurements were performed on an Archimed 384 high-throughput real-time PCR system (Kunpeng Gene), and genotype calls were generated with Archimed Analyzer software.

Allelic discrimination was based on endpoint FAM/VIC clustering: VIC-only signal was called GG, FAM-only signal

Table 2 Primers and probes used for GLP-1R rs6923761 genotyping.

Name	Sequence (5-prime to 3-prime)	Modification
rs6923761-Forward	CTCCTTCTCTGCTCTGGTTATCG	None
rs6923761-Reverse	GTCCGGGGCCACCTTACCT	None
rs6923761-probe-G	CGATCCTCCTCGGCT	5' VIC, 3' MGB/NFQ
rs6923761-probe-A	CGATCCTCCTCAGC	5' 6-FAM, 3' MGB/NFQ

Table 3 Reaction systems for TaqMan-qPCR and Sanger PCR.

Component	qPCR volume (μL)	Final concentration	Sanger PCR volume (μL)
2x qPCR or PCR master mix	5	1x	25
Forward primer	0.4	400 nM	2
Reverse primer	0.4	400 nM	2
G probe	0.2	200 nM	/
A probe	0.2	200 nM	/
Sample DNA	1	Approximately 10-100 ng input	1
Nuclease-free water	2.8	/	20
Total	10	/	50

Table 4 Amplification program for qPCR. Fluorescence was collected in FAM and VIC channels.

Step	Temperature	Time	Cycles
Initial background fluorescence collection	60 °C	30 sec	1
Pre-denaturation	95 °C	10 min	1
Denaturation	95 °C	15 sec	40*
Annealing/extension and fluorescence detection	60 °C	1 min	
Final fluorescence collection	60 °C	30 sec	1

*Fluorescence collection, detection channels: FAM, VIC

AA, and combined FAM/VIC signal GA. Samples without a clear cluster or with discordant replicate results were repeated.

Sanger Sequencing

The region containing rs6923761 was amplified using the Sanger PCR reaction mixture shown in Table 3. The expected product was 214 bp, and amplification products were examined by agarose gel electrophoresis before sequencing. Sanger sequencing was performed on an ABI-3000-Px platform according to the laboratory protocol. Chromatograms were inspected at rs6923761: a single G peak was called GG, overlapping G/A peaks GA, and a single A peak AA.

Samples with high background or ambiguous base calls were re-amplified and re-sequenced. The amplification primers were 5'-GCTACGCACTCTCCTTCTC-3' (forward) and 5'-CAACCTCATATTCTACGGTCAG-3' (reverse).

Statistical Analysis

Genotype counts and allele frequencies were calculated by direct counting. HWE was assessed by the chi-square test²³. Concordance was defined as the proportion of samples with identical TaqMan qPCR and Sanger genotype calls. An exact binomial (Clopper-Pearson) 95% CI was calculated for the concordance rate²⁴, and Cohen's kappa was used to assess

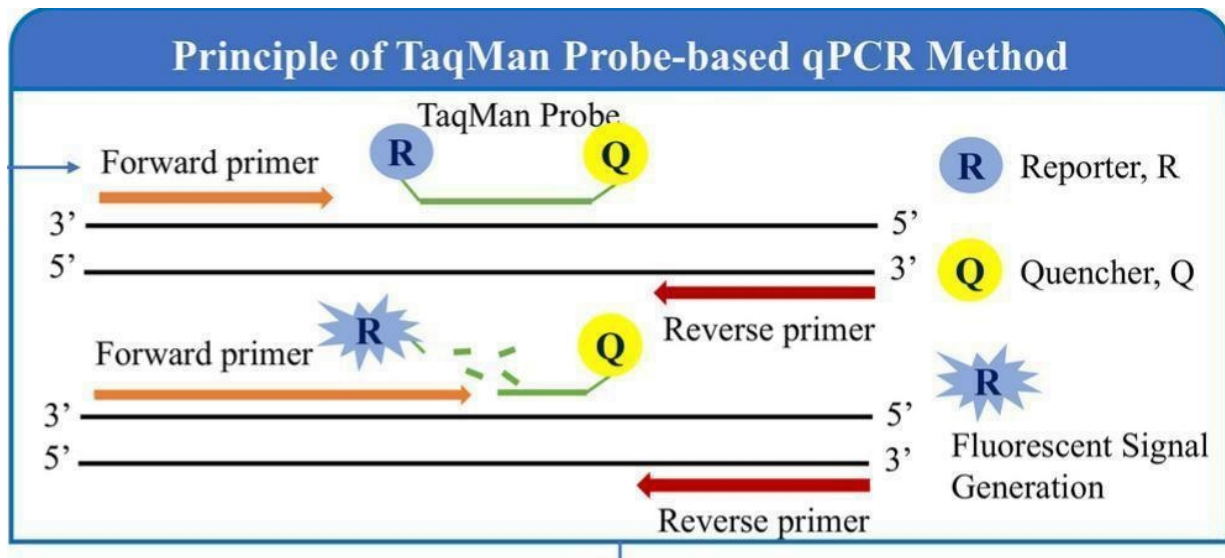


Figure. 1 Principle of TaqMan-qPCR allelic discrimination²² A perfectly matched allele-specific probe hybridizes to the target and is cleaved by the 5' nuclease activity of DNA polymerase during extension, separating reporter and quencher and generating allele-specific fluorescence.

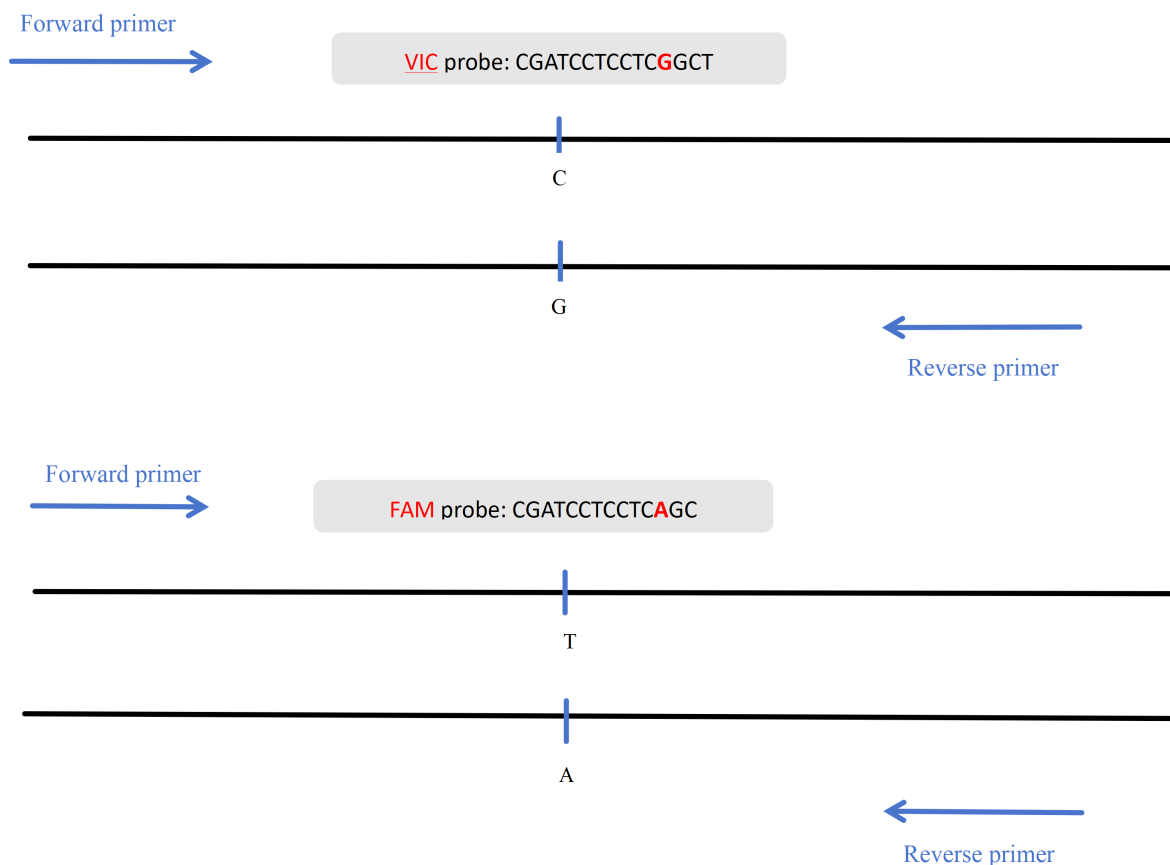


Figure. 2 Schematic of the rs6923761 amplicon and positions of the primers and allele-specific probes. The expected amplicon is 214 bp, and the polymorphic site is interrogated by VIC- and FAM-labeled probes.

agreement beyond chance²⁵. A two-sided p value <0.05 was considered statistically significant.

Results

DNA Yield and Purity

All 22 buccal swab samples yielded DNA that could be analyzed by both methods. DNA concentrations ranged from 18.6 to 76.4 ng/μL (mean, 42.8 ng/μL). A260/A280 ratios ranged from 1.78 to 1.93 and A260/A230 ratios from 1.86 to 2.21 (Table 5). No sample was excluded because of insufficient DNA yield or purity.

Table 5 DNA concentration and purity of the 22 oral swab samples.

Sample ID	DNA concentration (ng/μL)	A260/A280	A260/A230
1	35.2	1.84	2.03
2	41.7	1.86	2.08
3	28.9	1.81	1.96
4	52.4	1.89	2.12
5	46.8	1.87	2.05
6	31.5	1.82	1.91
7	58.6	1.90	2.16
8	33.7	1.79	1.88
9	39.4	1.85	2.01
10	44.1	1.88	2.09
11	63.2	1.91	2.18
12	38.6	1.78	1.86
13	37.9	1.84	2.00
14	49.5	1.87	2.07
15	55.3	1.90	2.15
16	33.8	1.83	1.94
17	72.1	1.92	2.20
18	26.4	1.80	1.89
19	47.6	1.86	2.06
20	76.4	1.93	2.21
21	40.3	1.85	2.02
22	60.7	1.91	2.17

Genotyping by TaqMan qPCR

Endpoint fluorescence separated the samples into two genotype clusters. Eleven samples showed predominantly VIC fluorescence and were called GG, while 11 showed both VIC and

FAM signals and were called GA. No FAM-only AA cluster was observed. The NTC remained outside the genotype clusters and showed no detectable allele-specific signal (Figure 3). The resulting genotype frequencies were 50.0% GG, 50.0% GA, and 0% AA; the G and A allele frequencies were 75.0% and 25.0%, respectively.

Table 6 Concordance between TaqMan qPCR and Sanger sequencing for *GLP1R* rs6923761. No AA homozygous sample was available in this cohort; direct validation therefore included GG and GA genotypes only.

Genotype	TaqMan-qPCR (n)	Sanger sequencing (n)
GG	11	11
GA	11	11
AA	0	0
Total	22	22

The observed genotype distribution did not differ significantly from HWE (expected counts: GG, 12.38; GA, 8.25; AA, 1.38; chi-square = 2.44, p = 0.118). TaqMan qPCR and Sanger sequencing agreed in all 22 samples, giving a concordance rate of 100% (22/22; exact 95% CI, 84.6%-100%) and a Cohen’s kappa of 1.00.

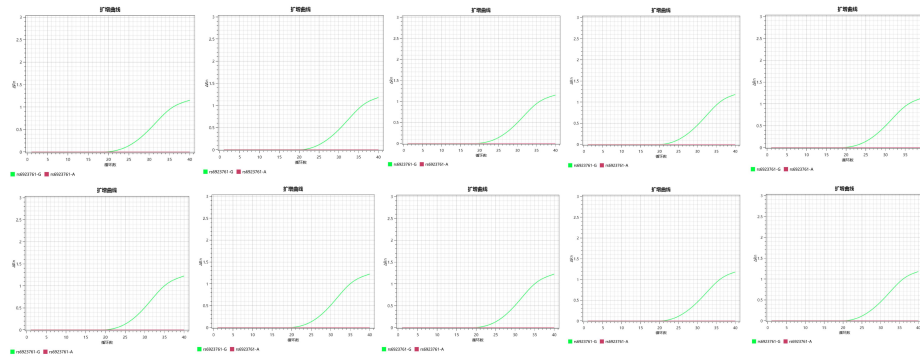
Concordance with Sanger Sequencing

Sanger sequencing confirmed the same 11 GG and 11 GA genotypes identified by TaqMan qPCR. GG samples showed a single G peak at rs6923761, whereas GA samples showed overlapping G and A peaks (Figure ??). One sample had substantial background in the initial sequencing run; repeat amplification and sequencing produced an interpretable chromatogram and confirmed the GA genotype. No discordant genotype call was observed between the two methods.

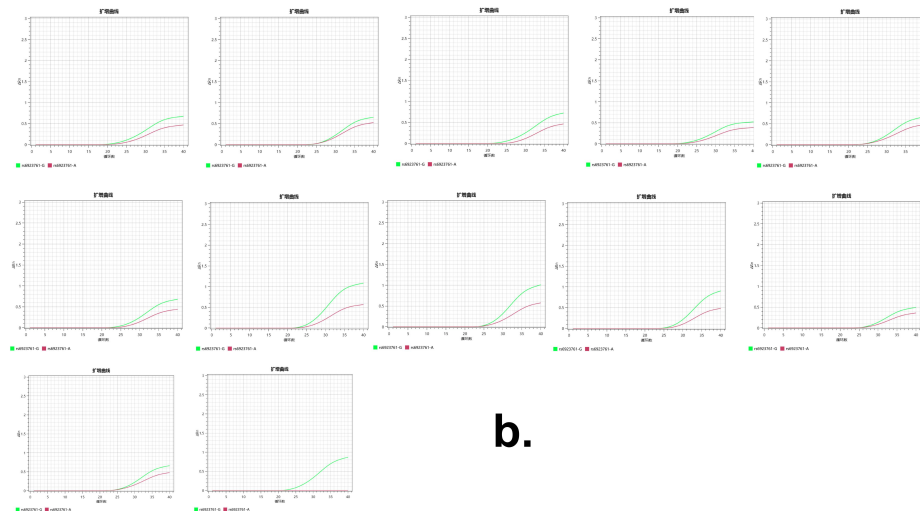
Discussion

Assay Performance and Concordance

The main finding of this study is the complete agreement between TaqMan qPCR and Sanger sequencing for the 22 samples tested. The two methods assigned the same 11 samples as GG and the same 11 as GA, and the qPCR NTC remained negative. For a known biallelic SNP, the TaqMan workflow has a practical advantage because amplification and genotype discrimination are completed in a closed tube without a separate sequencing step. The present data support the feasibility of the assay for rs6923761 genotyping, but they should not be interpreted as a complete analytical validation.



a.



b.

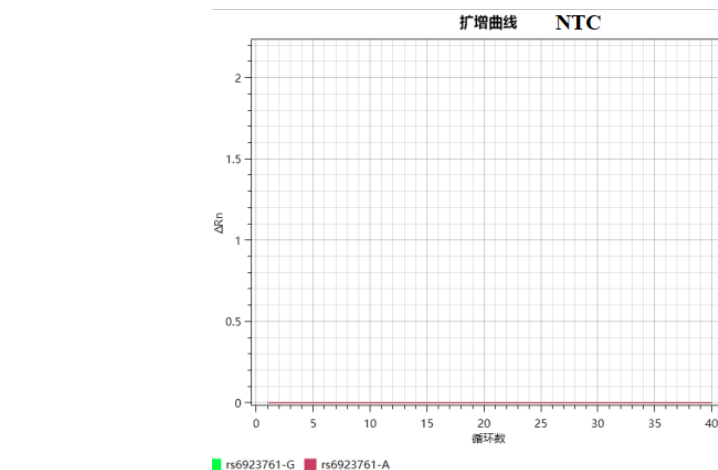


Figure. 3 TaqMan qPCR genotyping of *GLPIR* rs6923761. (A) Representative GG samples show a predominant VIC signal. (B) Representative GA samples show both VIC and FAM signals. The no-template control (NTC) showed no allele-specific amplification and remained outside the genotype clusters.

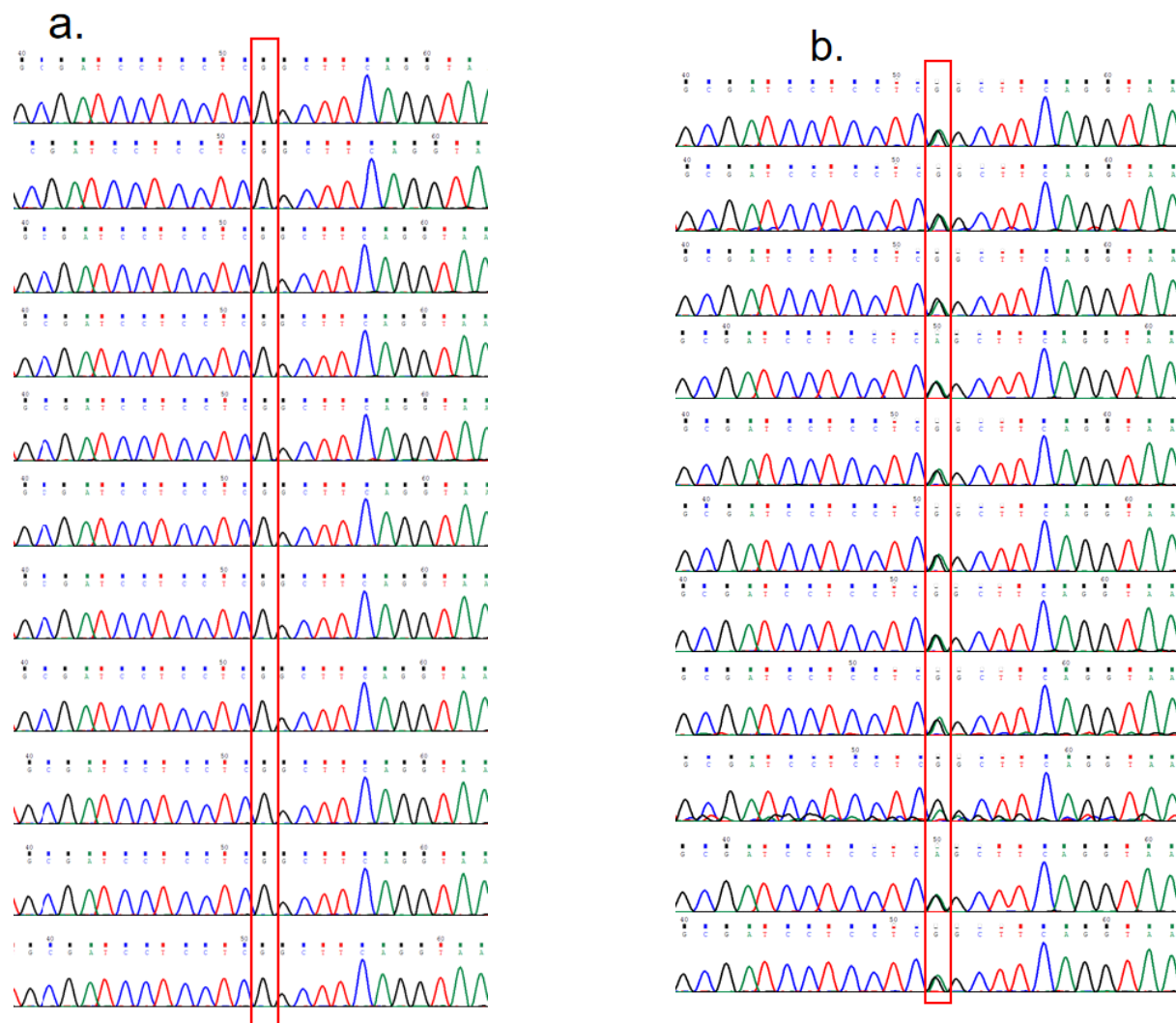


Figure. 4 Sanger sequencing chromatograms for *GLPIR* rs6923761. (A) A GG homozygous sample shows a single G peak at the polymorphic site. (B) A GA heterozygous sample shows overlapping G and A peaks.



Figure. 5 Agarose gel electrophoresis of the *GLPIR* rs6923761 PCR products. All 22 samples showed a single band consistent with the expected 214-bp amplicon.

Interpretation of rs6923761 and Study Scope

The A-allele frequency in this small cohort was 25.0%, and no AA homozygote was observed. Given the sample size, the absence of an AA sample limits the direct evidence for the FAM-only genotype cluster and is more important for assay validation than the observed HWE result. The rs6923761 variant has been associated in previous studies with metabolic phenotypes and with responses to GLP-1RAs, but the direction and magnitude of these associations have not been consistent across studies²⁶. Because no obesity phenotype, glycemic measurements, or treatment-response data were collected here, the present study cannot address the clinical relevance of the variant.

Limitations

Several limitations should be considered. First, the cohort was small, and the exact 95% CI around the observed 100% concordance extended down to 84.6%. Second, no AA homozygous sample was available, so all three possible genotype classes were not directly verified. Third, the study did not include serial template dilution, intra- and inter-run reproducibility testing, robustness experiments, or a formal limit-of-detection analysis. The small expected AA count also limits the usefulness of a chi-square HWE test in this cohort. Finally, participants were recruited from a single school-based population, and the genotype frequencies should not be generalized to the broader adolescent population. Future validation should include confirmed AA-positive material, replicate testing across runs, and a larger independent sample set.

Conclusion

A TaqMan qPCR assay for *GLP1R* rs6923761 produced the same genotype calls as Sanger sequencing in 22 buccal swab samples containing GG and GA genotypes. The assay provides a rapid closed-tube approach for genotyping this known SNP. Validation with confirmed AA samples and formal analytical performance testing is still required before the method is used for clinical interpretation or pharmacogenetic decision-making.

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