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A capillary electrophoresis method for genotyping the 9-bp exon 1 insertion/deletion in *BDKRB2*

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Abstract

Aim—To develop and apply a novel genotyping method for the 9-bp exon 1 insertion/deletion polymorphism in *BDKRB2*.

Materials & methods—DNA from 718 patients with heart failure was extracted using standard methods and a region containing exon 1 of *BDKRB2* was amplified with PCR. The PCR product was separated using the Qiagen QIAxcel[®] capillary electrophoresis system. The bp size of the PCR product was calculated and the genotypes determined using Qiagen BioCalculator[®] software.

Results—Capillary electrophoresis accurately genotyped samples with >99% call rate and 700 s run time per row of a 96-well plate (i.e., less than 1 min per sample). The frequency of the deletion was 49% in the Caucasian patients (n = 441) and 45% in the African-American (n = 277).

Conclusion—Capillary electrophoresis is a rapid, accurate and sensitive method for genotyping the 9-bp exon 1 insertion/deletion polymorphism in *BDKRB2*.

Keywords

9-bp exon 1 insertion/deletion; *BDKRB2*; bradykinin; capillary gel electrophoresis; heart failure; polymorphism; QIAxcel[®]

Cardiovascular disease (CVD) is a major public health problem. Each day >2200 Americans die from CVD, which accounts for one out of every three deaths in the USA [1]. Current research has focused on identifying genetic polymorphisms associated with the predisposition, prognosis and response to drug therapy in CVD. One such CVD-relevant gene encodes the bradykinin receptor B2 (*BDKRB2*). Bradykinin, through its B2 receptor, exerts many vascular protective effects: the stimulation of microangiogenesis, inhibition of smooth-muscle cell growth, coronary vasodilatation, increased local nitric oxide synthesis and antithrombotic actions [2]. A common, 9-bp insertion/deletion (indel) polymorphism exists in exon 1 of *BDKRB2* (no rs identifier in the National Center for Biotechnology

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Information database of SNPs [dbSNPs]) [101]. The frequency of the deletion allele is approximately 50% and approximately 40% in healthy Caucasians and African-Americans, respectively [3]. The presence of the deletion results in increased gene transcription [4] and mRNA expression [5]. The *BDKRB2* indel has been associated with CVD-relevant phenotypes such as blood pressure [3], left ventricular growth [6] and response to antihypertensive treatment [7].

The most studied CVD-relevant indel is a 287-bp deletion in the *ACE* gene, and well-established genotyping methods are available in the literature [8–10]. However because of the small (9 bp) size of the *BDKRB2* indel, rapid and accurate genotyping of this polymorphism for large-scale CVD association studies is challenging. One currently used method for genotyping the *BDKRB2* indel is a two-step PCR followed by gel electrophoresis; however this method is time intensive [11]. Single-step PCR followed by gel electrophoresis has also been performed, but very long separation times are required to reach a sensitivity which allows for the differentiation between alleles [12]. An alternative method is PCR followed by sequencing [5]; however, sequencing indels often does not discriminate heterozygotes, and therefore a preparation step with gel electrophoresis is additionally required. Genotyping small indels in hundreds to thousands of patients in large-scale CVD association studies requires rapid, automated, sensitive and accurate methods. Unlike the methods reported in the literature, capillary electrophoresis satisfies these requirements. Herein we report a capillary electrophoresis method for genotyping the 9-bp indel in exon 1 of *BDKRB2*, and, for the first time, the frequency of this polymorphism in Caucasian and African-American patients with heart failure.

Materials & methods

Patients

Patients with heart failure ($n = 718$; 441 Caucasians and 277 African-Americans) from the United Investigators to Evaluate Heart Failure (UNITE-HF) [13] multicenter DNA repository (UNITE-DNA) were genotyped. Patients were enrolled in UNITE-DNA from 13 outpatient specialty heart failure clinics across the USA if they had a history of heart failure defined as dyspnea on exertion or edema due to cardiac causes. There was no left ventricular ejection fraction requirement. The University of North Carolina at Chapel Hill (NC, USA) has served as the coordinating center for the UNITE-DNA network since its founding in 2000. The study was approved by the institutional review boards at each of the study sites, and informed consent was obtained from all participants prior to enrollment.

DNA sample preparation

Peripheral blood (25 ml) was drawn from each patient using ethylenediaminetetraacetic acid tubes as an anticoagulant. Genomic DNA was extracted from peripheral blood samples using standard methods [14]. The stock DNA concentration was determined using the Quant-iTTM PicoGreen[®] dsDNA kit (Invitrogen, Molecular Probes, OR, USA). Working stock DNA (1 ng/ μ l) was made by normalizing aliquots of stock DNA in molecular-grade water (Mediatech, VA, USA) using a Biomek[®] 3000 laboratory automated workstation (Beckman Coulter, CA, USA).

PCR

Based on the exon 1 9-bp indel polymorphism in *BDKRB2* reported by Braun *et al.* [15], the following forward and reverse primers were used: 5'-GCCCTTGAAAGATGAGCTG-3' and 5'-AACTCCCCACGACCACAG-3'. The expected PCR product size with the insertion allele was 275 bp and the deletion allele was 266 bp. The PCR mixture contained 10 μ l of HotStarTaq[®] Plus Master Mix (2 $\times$) (Qiagen, Hilden, Germany), 4 μ l of Q solution (5 $\times$)

(Qiagen), 2 µl of each primer (10 µM) and 2 µl of working stock DNA (1 ng/µl) in a final volume of 20 µl. A DNA Engine Tetrad[®] 2 Peltier thermal cycler (BioRad, CA, USA) was used with the following conditions based on Braun *et al.* [15]: initial incubation at 95°C for 5 min followed by 40 amplification cycles (1 min at 94°C, 1 min at 53°C and 1 min at 72°C) and a final elongation at 72°C for 10 min.

Capillary electrophoresis

High-resolution capillary electrophoresis was performed using a QIAxcel[®] DNA high-resolution gel cartridge (Qiagen) on a QIAxcel system (Qiagen), as per the manufacturer's instructions. A QX DNA Size Marker (Qiagen) with 17 fragment sizes ranging in size from 25 to 450 bp was used to size PCR products. A QX Alignment Marker (Qiagen), which consisted of 15 bp and 500 bp fragments, was injected onto the cartridge with each sample. The OM700 method in the BioCalculator[®] software (Qiagen) was used for all analyses; this corresponds to a 10 s sample injection time at 5 kV, and 700 s separation time at 3 kV. The QIAxcel system injected 0.1 µl of 20 µl PCR products onto a cartridge for analysis. The retention time of the PCR fragments relative to the 15-bp and 500-bp QX Alignment Marker fragments was calculated using the BioCalculator software (Qiagen). The PCR product sizes were then determined by comparing the retention time with the QX DNA Size Marker. The BioCalculator software produces a digital gel image and an electropherogram for fragment analysis.

Quality control

To ensure genotyping accuracy, the following quality control measures were performed: one negative control per 96-well plate (i.e., water used in place of DNA); all samples without an initial genotype call were rerun; comparison of UNITE-DNA allele frequencies to published frequencies of the same race; repeat genotyping of 10% of samples, randomly chosen and verification of concordance between calls; visual inspection of gel images by one individual; visual inspection of gel images for 10% of samples selected at random by a separate individual; and the χ^2 test for deviation from the Hardy–Weinberg equilibrium. To confirm the accuracy of our genotyping method, we obtained six DNA samples of known genotype, which included two samples of each genotype, from an independent laboratory. These samples were genotyped using PCR amplification followed by DNA sequencing [3].

Results

The QIAxcel (Qiagen) capillary electrophoresis system was used to genotype the 9-bp exon 1 indel in *BDKRB2* in 718 patients with heart failure and genotyped in an independent laboratory. As shown in Figure 1, the expected PCR product sizes of 275 bp for the insertion and 266 bp for the deletion were detected, and there was 100% genotype concordance between the six samples genotyped by an independent laboratory and our genotyping results, indicating that our method is accurate. As shown in electropherograms produced with the standard BioCalculator software of the QIAxcel system (Figure 2), the peaks were readily detectable when injecting 0.1 µl of PCR product. The genotype calls were output as a .csv file (Figure 3). This method was rapid, in which the total run time for each row (12 samples) of a 96-well plate was 700 s (i.e., less than 1 min per sample). The initial genotype call rate was 95.5%, and after the original failed samples were repeated, the final call rate was 99.3%. There was 100% concordance between the initial sample runs and the 10% randomly chosen samples run a second time, indicating that the method is reproducible. The frequency of the deletion was 49% in the Caucasian patients and 45% in the African–American (n = 441 Caucasians and n = 277 African–Americans). The genotype frequencies for each race were in agreement with Hardy–Weinberg equilibrium (for Caucasians, χ^2 : 0.5, p = 0.48; for African–Americans, χ^2 : 2.5, p = 0.12).

Discussion

CVD is a major public health problem, and genetic polymorphisms may contribute to the predisposition, prognosis and response to drug therapy in CVD. Indeed, the 9-bp insertion/deletion polymorphism in exon 1 of *BDKRB2* has been associated with CVD-relevant phenotypes in small studies [3,6,7]; however, large clinical trials are needed to confirm the utility of this polymorphism. Current genotyping methods for this small indel are time and labor intensive, making large-scale genotyping a challenge. However, capillary electrophoresis is a rapid, automated, sensitive and accurate method for genotyping small indels [16], and this has been demonstrated here for the 9-bp indel polymorphism in exon 1 of *BDKRB2*. Capillary electrophoresis has several advantages over currently used methods such as gel electrophoresis: no gel-casting or staining is required; samples are loaded automatically; minute sample volumes are required; detection of the PCR products is automated when the fragments pass the detector in the capillary and analyzed with software (as opposed to visual inspection of gels upon exposure to UV light); avoids handling of hazardous materials such as ethidium bromide; and high resolution separation is achieved in minutes [10]. These advantages greatly facilitate the use of this method for genotyping in large-scale CVD association studies. However, convenience comes at a cost. After the upfront cost of the QIAxcel unit, it cost approximately US\$0.75 to run each sample on the QIAxcel after DNA extraction and PCR. Therefore, as with any genotyping method, cost is an important consideration when selecting QIAxcel capillary electrophoresis.

In addition, this is the first report of the allele frequencies for the exon 1 9-bp indel of *BDKRB2* in both Caucasian and African-American patients with heart failure. Both races had similar frequencies (49% in the Caucasian heart failure patients and 45% in the African-American heart failure patients), and the frequencies were similar to those reported previously in healthy subjects (~50% and ~40% in healthy Caucasians and African-Americans, respectively) [3].

Future perspective

In the far future, a complete genetic profile of every patient may be available to healthcare practitioners in order to individualize each patient's care. This eventually will be possible because of decreasing costs, increasing coverage and improved bioinformatics capacity for genome-wide association studies and sequencing. However, in the near future, targeted genotyping is required for researchers to elucidate the association between functionally implicated polymorphisms (such as indels) and clinical outcomes in complex diseases, which require large-scale genotyping of clinical trials and cohorts. Like genome-wide association studies and sequencing, the cost of capillary electrophoresis may also decrease, and at that point it would completely replace traditional gel-slab electrophoresis for genotyping indels. Currently, the only disadvantage of capillary electrophoresis over traditional gel electrophoresis is cost.

Executive summary

Cardiovascular disease & the 9-bp insertion/deletion in exon 1 of *BDKRB2*

- Cardiovascular disease is a major public health problem.
- Bradykinin, through its B2 receptor (*BDKRB2*), exerts many vascular protective effects.
- Current methods for genotyping the 9-bp insertion/deletion (indel) in exon 1 of *BDKRB2* are time and labor intensive, which limits its application to genotyping samples in large cardiovascular clinical trials.

A novel method for genotyping the 9-bp indel in exon 1 of *BDKRB2*

- We developed and applied a robust method for genotyping the 9-bp indel in exon 1 of *BDKRB2* using the Qiagen QIAxcel® capillary electrophoresis system in 718 patients with heart failure.
- This method displayed 100% genotype concordance with samples sequenced in an independent laboratory and 10% with randomly repeated samples.
- A minute volume of PCR product was necessary, and the final call rate was >99%.

Conclusion

- Capillary electrophoresis with a QIAxcel system is an automated, accurate, reliable and rapid method for genotyping the *BDKRB2* exon 1 indel.
- The frequency of the *BDKRB2* exon 1 indel is similar between Caucasian and African-American patients with heart failure and healthy subjects of matched race.

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Figure 1. Gel image displaying expected PCR product sizes and concordance with six samples genotyped in an independent laboratory

Lanes E01 and E02 are homozygous for the insertion, lanes E03 and E04 are heterozygous and lanes E05 and E06 are deletion homozygotes.

†Alignment markers.

Figure 2. Electropherograms produced by standard BioCalculator software
 Electropherograms for lanes (A) E01 (homozygous for insertion), (B) E03 (heterozygous) and (C) E05 (homozygous for deletion) are presented.
 †Alignment markers.

	A	B	C
1	WELL	DEL	INS
2	E01	0	1
3	E02	0	1
4	E03	1	1
5	E04	1	1
6	E05	1	0
7	E06	1	0

Figure 3. Genotype calling output

Genotype calling for lanes E01–E06 is output as a .csv file, which is displayed in Microsoft Excel[®] 2010.