Analysis of SCN10A Gene Mutation in Northeastern Thai Patients with Nephrolithiasis by High Resolution Melting Method

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ABSTRACT

SCN10A gene, encoding voltage-gated sodium channel 1.8 (Na\(_{1.8}\), has been suggested as a candidate gene for nephrolithiasis in Northeastern (NE) Thai patients. To analyze mutations of SCN10A gene, polymerase chain reaction-high resolution melting (PCR-HRM) analysis, a powerful method for detection of sequence variations by generating melting curve pattern, was performed in 180 NE patients with nephrolithiasis. A total of 28 variations with 11 novel variations of 8 exonic variations (6 non-synonymous and 2 synonymous) and 3 intronic variations were identified by PCR-HRM and were confirmed by DNA sequencing. All of novel variations have impact on structure and function of the Na\(_{1.8}\) protein and mRNA splicing process as predicted by 5 web-based programs. These results indicate PCR-HRM provides a simple, rapid, and cost-effective method of medium-throughput method that is successfully used for scanning SCN10A gene mutations. Some of the identified mutations in this study may cause nephrolithiasis in NE Thai patients. In order to validate their potential pathogenic effects, therefore, segregation testing in the affected families and functional study should be further investigated.

ค่าสำคัญ: นั่นไอ, ยี่, SCN10A, พีซีอาร์, เฮิร์ม, การกลายพันธุ์

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INTRODUCTION

Nephrolithiasis or kidney stone, a morbidity and occasional mortality clinical disorder, is a common worldwide public health problem. In Thailand, the highest prevalence was observed in the Northeastern (NE) area. The etiology of nephrolithiasis is generally multifactorial, both genetic and environmental factors play role in disease. To date, genetic contribution to nephrolithiasis has been recognized and a number of the causative genes have been reported but they are varying among populations. Our previous studies have suggested a genetic contribution to the risk of nephrolithiasis in NE Thai population and found that genetic variation in prothrombin (F2) gene is associated with nephrolithiasis in female patients (Sritippayawan et al., 2009; Rungrojo et al., 2011; Rungrojo et al., 2012). Subsequently, genome-wide linkage analysis and exome sequencing were performed to investigate gene(s) involving in nephrolithiasis in multiple families. The results revealed that mutation of SCN10A gene, encoding the voltage-gated sodium channel 1.8 (Na\textsubscript{v}1.8), is a possible cause of nephrolithiasis in one extended family (unpublished data). The SCN10A is a very large gene comprising 27 exons which code for a 1,956 amino acid of Na\textsubscript{v}1.8 protein. The Na\textsubscript{v}1.8 has a central role in pain signaling, thermal-induced inflammation and cold stimulation (Akopian et al., 1996) and SCN10A gene mutations has been reported to be a cause of altered pain sensitivity and painful neuropathy (Faber et al., 2012). However, it has not been reported to be involved in nephrolithiasis.

Since a mutation of SCN10A gene is initially observed as a cause of nephrolithiasis in only one family and complete validation of SCN10A gene has not been determined, it is possible that SCN10A mutation may be a cause of nephrolithiasis in other families. Therefore, in this study high resolution melting (HRM) analysis, a simple method that widely uses for detection of sequence variations by examining melting curves of amplicons (Zhou et al., 2005; Nettuwakul et al., 2010), was performed to investigate mutations of SCN10A gene in NE Thai patients with nephrolithiasis. Novel mutations identified in this study may a cause of disease leading to the better understanding in the pathogenesis of nephrolithiasis in the NE Thai patients.

MATERIALS AND METHODS

Genomic DNA of 180 patients with nephrolithiasis were examined for mutations of SCN10A by PCR-HRM. Thirty-six primers pairs were designed for amplifying the 36 fragments that cover 27 exons and intron-exon boundaries of SCN10A. PCR-HRM analysis was performed in a single run on a LightCycler 480 II machine (Roche Diagnostics, Germany) with addition of Resolight dye (Roche Diagnostics, Germany) in PCR reaction. The PCR condition included an initial denaturation at 95 °C for 10 min, followed by 40 cycles of 95 °C for 20 sec, 57-61 °C for 20 sec (depends on each fragments), and 72 °C for 20 sec. Before the HRM step, the product was heated to 96 °C for 1 min and then cooled to 40 °C. Melting curves were obtained by increasing the temperature to 96 °C with 25 acquisitions of continuous florescence detection. The melting curves were normalized, temperature-shifted and converted to difference plots by Gene Scanning 1.5.0 (Roche Diagnostics, Germany). The detected variations were confirmed by DNA sequencing and were predicted for their possible impact on structure and function of protein and mRNA splicing process using web-based programs (Mutation Taster, VarioWatch, PolyPhen2, SIFT, and ESEfinder).
RESULTS AND DISCUSSION

PCR-HRM analysis of 36 fragments of the \textit{SCN10A} gene showed that there were different melting curve patterns in the analysis of 23 fragments and a total of 28 variations were identified. The examples of melting curves, different plots, and nucleotide sequences of identified variations are shown in Figure 1. Seventeen variations are known SNPs that have not been reported to be involved in nephrolithiasis. Eleven variations are novel that can be classified into two groups based on their location in the gene, including eight exonic variations (six non-synonymous and two synonymous) and three intronic variations. All of these novel variations were predicted to have impact on structure and function of the Na$_{\text{v}}$1.8 protein and mRNA splicing process (data not shown).

![Figure 1](image)

\textbf{Figure 1} Melting curves, different plots, and nucleotide sequence analyses of \textit{SCN10A} gene variations in (A) exon 2 and (B) exon 5

Our results demonstrated that HRM analysis was able to detect the variations of \textit{SCN10A} gene in 23 PCR fragments from their melting curve patterns that had been confirmed by DNA sequencing. However, some studied PCR fragments were not shown any different melting curve pattern, suggesting there is no variation or the variation may not be detected by HRM method. Nevertheless, this technique is a useful screening method to detect gene mutation as it has been reported to have high sensitivity (95%) and specificity (99%) (Reed and Wittwer, 2004).

CONCLUSION

PCR-HRM is a simple, rapid, and cost-effective method that is efficiently used for scanning \textit{SCN10A} gene mutations. Some of the mutations identified in this study may cause nephrolithiasis in NE Thai patients. However, segregation analysis within affected families and functional study should be further investigated in order to validate their potential pathogenic effects.
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