

The Mutation Spectrum and Two Novel Point Mutations in the APC Gene in Vietnamese Patients with Familial Adenomatous Polyposis

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Abstract

Background: Familial adenomatous polyposis (FAP) is a hereditary disorder primarily caused by germline mutations in the *APC* gene. The most common type of mutation in the *APC* gene is point mutation, while deletion mutation is much less frequent. The current study was conducted to investigate the mutation spectrum of the *APC* gene in Vietnamese FAP patients. **Methods:** Patients with the clinical diagnosis of FAP on colorectal endoscopy were screened for mutations in the *APC* gene using Sanger sequencing. Those who exhibited no point mutation subsequently underwent MLPA assay to detect deletion and duplication mutations. Besides, the relatives of patients with mutated *APC* genes were recruited for detecting carrier status. **Results:** Sixty-three patients with clinical colorectal polyposis were recruited. Mutations in the *APC* gene were detected in 26/63 patients (41.3%). Genetic analysis of 105 asymptomatic relatives of these 26 patients found mutations in the *APC* gene in 55 individuals (52.4%). **Conclusion:** We successfully established the *APC* gene mutation spectrum in Vietnamese FAP patients for the first time. Of importance, we discovered two novel point mutations in the *APC* gene. The high prevalence of carrier status in asymptomatic family members of patients with mutation emphasizes the crucial role of appropriate genetic screening for early diagnosis, surveillance, and preventive measurements.

Keywords: Familial adenomatous polyposis- *APC* gene- point mutation- sequencing- MLPA

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Introduction

Familial Adenomatous Polyposis (FAP) is a rare precancerous condition inherited in an autosomal dominant manner. This condition was first described in 1925 (Lockart-Mummary, 1925). Without appropriate early treatment, almost 100% FAP patients would develop colorectal cancer before 40 (Sheng et al., 2010). It is estimated that FAP affects 1 in 8,000 to 10,000 individuals and accounts for 0.94% of all colorectal cancer cases in China (Yang et al., 2016), a neighboring country of Vietnam. In Vietnam, there is no documentation of the prevalence of FAP. Clinically, FAP is characterized by the occurrence of hundreds to thousands of colorectal adenomatous polyps. Besides, FAP is also associated with an increased risk of cancer development in other organs, including the small intestine, thyroid gland, pancreas, liver, central nervous system, and bile ducts. However, these manifestations are typically encountered in less than 10% of affected individuals (Jasperson et al., 2010).

In 1991, the link between Adenomatous Polyposis Coli (*APC*) gene and FAP was established (Plawski et al., 2013). Various mutations in the *APC* gene in germline cells can cause FAP. Mutations in the *APC* gene occur in 85% of patients with severe FAP and between 30 to 60% of attenuated FAP patients (Gómez-Fernández et al., 2009). The *APC* gene is located on the long arm of chromosome 5 (5q21) and consists of 8,535 base pairs spanning 15 exons. Exon 15 comprises more than 75% of the *APC* gene's coding sequence and is the most common target for deleterious germline and somatic mutations. The *APC* gene encodes a 2843-amino acid protein, a tumor suppressor regulating cell proliferation and chromosome segregation (Zhang and Shay, 2017).

Identifying mutations in the *APC* gene plays an essential role in preventing, early detecting, and managing colorectal cancer. Family members of FAP patients with confirmed mutations in the *APC* gene should also be screened for these mutations. The genetic screening results will dictate the planning of appropriate genetic counseling

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and preventive measures. The majority of mutations in the *APC* gene are point mutations in different locations; hence the Sanger sequencing is the method of choice in detecting these mutations. There has been no study using DNA sequencing in detecting *APC* gene mutations in patients and their family members in Vietnam.

In this study, we investigated the mutational spectrum of the *APC* gene in Vietnamese AFP patients. We also performed genetic screening for the asymptomatic family members of FAP patients with confirmed *APC* gene mutations.

Materials and Methods

Patients

We recruited 63 FAP patients who exhibited more than 100 adenomatous polyps in the colon and rectum or displayed more than 20 adenomatous polyps with a positive family history of FAP. Positive family history of FAP was defined as colorectal cancer diagnosed in at least two first-degree relatives.

Among these 63 FAP patients, 25 patients showed point mutations in the *APC* gene on Sanger sequencing. To identify carrier status in asymptomatic relatives of FAP patients, we also performed Sanger sequencing in 101 family members (brothers, sisters, and children) of the 25 patients mentioned above.

We further performed Multiplex Ligation-dependent Probe Amplification (MLPA) in 38 FAP patients who exhibited no point mutation on Sanger sequencing to detect deletion mutations in the *APC* gene. Deletion mutation was detected in only one patient. We then carried out an MLPA assay for deletion mutation in 4 asymptomatic relatives of this patient (Figure 1).

The study protocol was approved by the Ethics Board in Biomedical Research, University of Medicine and Pharmacy at Ho Chi Minh City. Written informed consent was obtained from all adult patients and asymptomatic participants. For children under 18 years of age, the written consent was signed by their parents.

Extraction of genomic DNA from collected blood samples

For DNA isolation, 2ml of EDTA anticoagulated venous blood was collected and DNA was extracted within 24 hours. Genomic DNA extraction from peripheral blood was performed using QIAamp DNA Blood Mini Kit according to recommendations by the manufacturer (Qiagen). The concentration and purification of extracted DNA were checked. Only DNA samples with the absorbance ratio at 260 nm and absorbance at 280 nm (A260/A280) from 1.8 to 2.0 were accepted for downstream PCR and sequencing.

Sequencing technique and result interpretation

PCR was performed for amplification of 15 exons in the *APC* gene. PCR product was purified before Sanger sequencing using BigDye® Terminators V3.1 Cycle Sequencing kit (Applied Biosystems) (Cat. No. 4337457). Sequencing results were analyzed using the software CLC Main Workbench v5.5 (Qiagen).

MLPA analysis

In addition to point mutations in the *APC* gene, which are detected on sequencing, deletion and duplication mutations can be further identified by MLPA in around 12% FAP patients. Therefore, we performed MLPA analysis using the SALSA MLPA Kit P043-APC Probemix Kit in conforming to recommendations by the manufacturer. The amplicons were separated by capillary electrophoresis and automatically analyzed to identify the mutations based on GeneMarker ver 1.6 (Softgenetics).

Results

Patient characteristics

We recruited into the study 63 AFP patients in whom there were 16 patients (25.4%) already diagnosed with colorectal cancer. The sequence of genetic testing is presented schematically in Figure 1. The majority of patients were from 30 to 60 years old. Nearly two-thirds (63.6%) were males (Table 1). There were two children of 5 years of age. These two children were initially diagnosed with juvenile polyposis syndrome, but detailed family history revealed suspected manifestations of FAP. Hence, we performed the genetic investigation, and both children showed nonsense mutation on exon 15 of the *APC* gene. Among 25 FAP patients with identified point mutations, there were seven patients (28.0%) with colorectal cancer.

Point mutations identified in AFP patients

Using Sanger sequencing, we identified point mutations in the *APC* gene in 25 patients (39.7%) who were clinically diagnosed with FAP. Nineteen among these 25 patients (70.1%) showed point mutations in exon 15. In two patients, we could identify two novel point mutations. These novel point mutations comprised 8% of all point mutations identified. All two point mutations were located on exon 15. One novel point mutations identified in this study was missense mutations, and the another was frameshift mutation.

Characterization of the two novel mutations identified in the current study

Table 2 summarizes the main clinical manifestations and genetic findings of the two FAP patients with novel point mutations. These two patients were referred to us for the presence of multiple colorectal polyps on colonoscopy.

Family members of the first patients (FAP 18) refused to undergo a genetic investigation. Patient FAP33 had a strong family history of gastrointestinal cancer. His mother and one of his maternal aunts died in their forties

Table 1. Sex and Age of Patients Recruited in the Study

Age (yrs)	Male		Female		Both sexes	
	n	%	n	%	n	%
Under 30	7	11.1	4	6.3	11	17.4
From 30 to 60	25	39.7	19	30.1	44	69.8
Above 60	8	12.7	0	0	8	12.7
Total	40	63.5	23	36.5	63	100

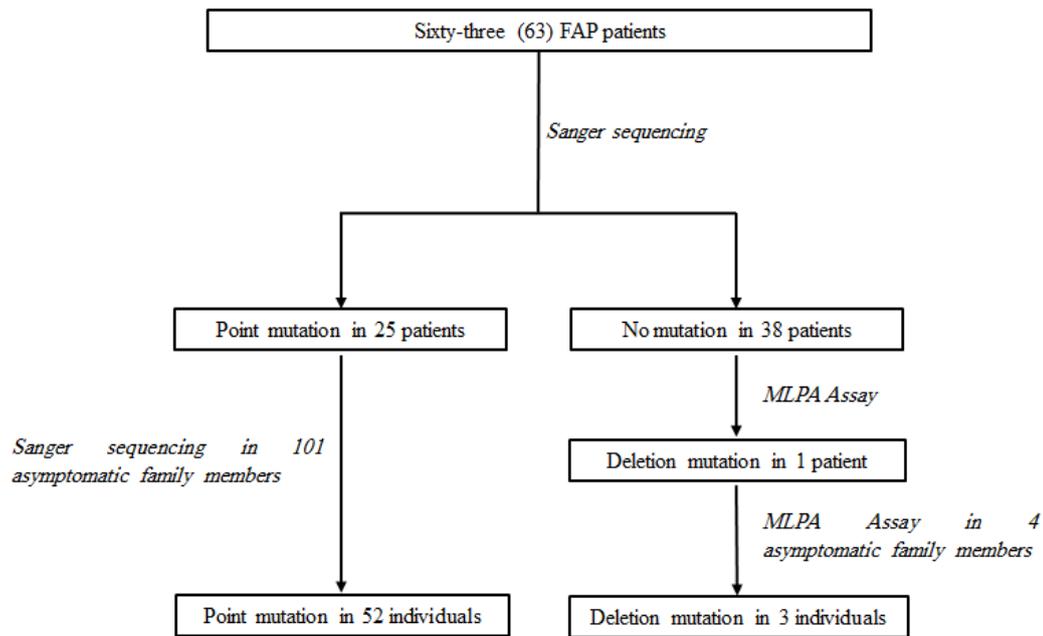


Figure 1. Study Flowchart. Sixty-three recruited FAP patients underwent Sanger sequencing. Left arm: 25 patients showed point mutation in the APC gene. Sanger sequencing was subsequently performed in 101 asymptomatic family members of these 25 patients, and 52 individuals displayed point mutation. Right arm: MLPA assay was performed in 38 patients without point mutation, and deletion mutation was found in 1 patient. MLPA assay was then carried out in 4 asymptomatic family members of this patient, and three individuals exhibited similar deletion mutation.

Table 2. Clinical and Genetic Characteristics of Two Patients with Novel Point Mutations

Patient ID	Age	Phenotype	exon	Mutation	Consequence	Cancer
FAP18	21	100-1000 colorectal polyps, multiple jaw osteomas	15	c.4550delA	p.Gln1517ArgfsX6	No
FAP33	31	100-200 colorectal polyps	15	c.5766G>C	p.Gln1922His	No

after episodes of apparent lower gastrointestinal bleeding and allegedly colorectal tumors. However, we could not obtain any written medical document on their diagnosis and treatment. Sanger sequencing was performed for

the father, and no mutation was detected. The impact of these two point mutations on APC protein is schematized in Figure 2.

To illustrate the impact of the novel mutations on

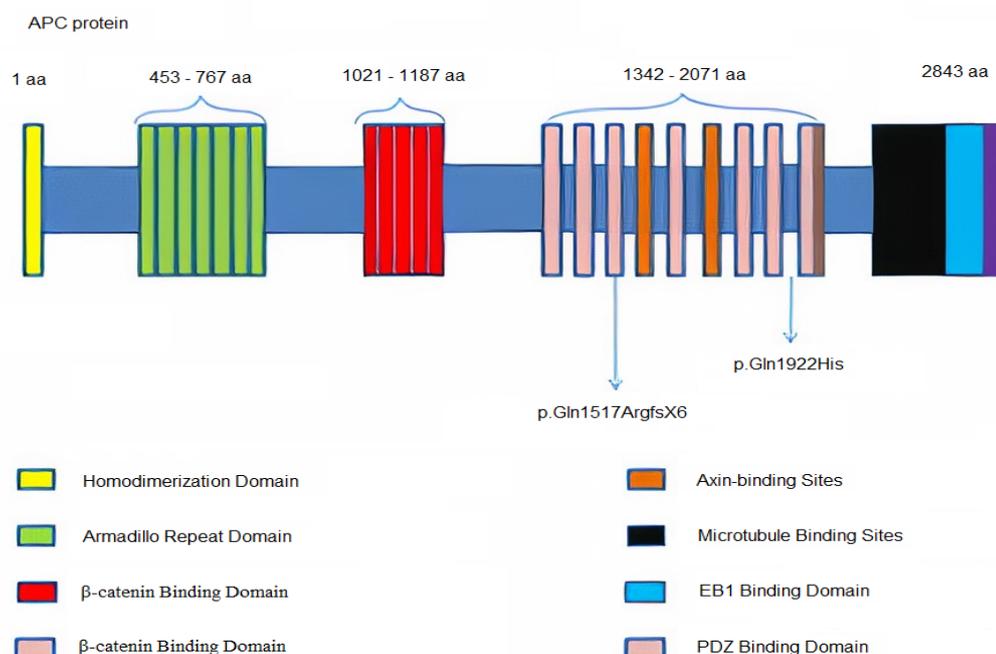


Figure 2. Schematic Diagram of the APC Protein Structure with Functional Domains and Sites of Two Mutations

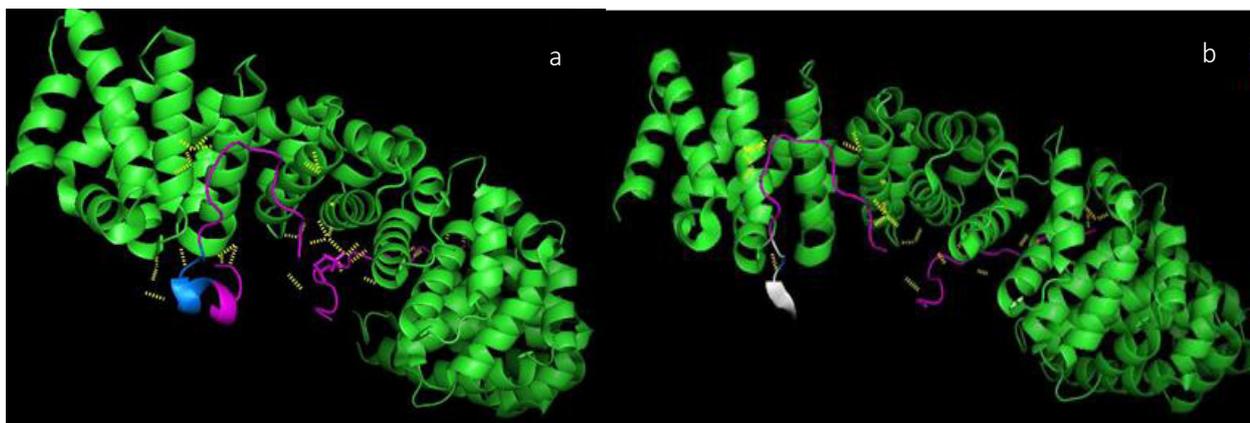


Figure 3. Structure Modeling of Wild Type and p.Gln1517ArgfsX6 mutation of the APC Gene with PyMOL. Compared with the wild type, the mutant leads to premature termination and potential loss of ability to bind with β -catenin. a: Wild-type APC protein and β -catenin protein. b: p.Gln1517ArgfsX6 mutation of APC protein and β -catenin protein.

APC protein, we used the molecular visualization system PyMOL 2.3 for Windows to build the wild type and mutated APC protein and β -catenin protein (Figure 3)

Deletion mutations in APC gene

We also performed MLPA analysis on samples from 38 FAP patients who did not show any point mutation in the APC gene on Sanger sequencing to detect any potential deletion mutation. We identified only one patient with a large fragment deletion in the middle of exon 15 (Figure 4). The other 37 FAP patients showed neither deletion nor duplication in the APC gene.

Mutation spectrum of APC gene in the Vietnamese population

Point mutations could be divided into two groups:

- Truncating mutations: These mutations include nonsense and frameshift mutations resulting in a premature stop codon that signals translation termination. In our study, 72% of patients showed truncating mutations.
 - Missense mutations: These mutations lead to a change of amino acid of the protein. In our study, there were seven patients (28%) showing missense mutations.
- In addition to point mutations, we could identify one patient with a deletion mutation in the middle of exon 15 of the APC gene. We would initially establish the APC gene's mutation spectrum from Vietnamese FAP patients (Figure 4).

For carrier status screening, Sanger sequencing and MLPA analysis were performed in 105 asymptomatic family members of these 26 patients with mutations in the APC gene. We detected mutations in the APC gene

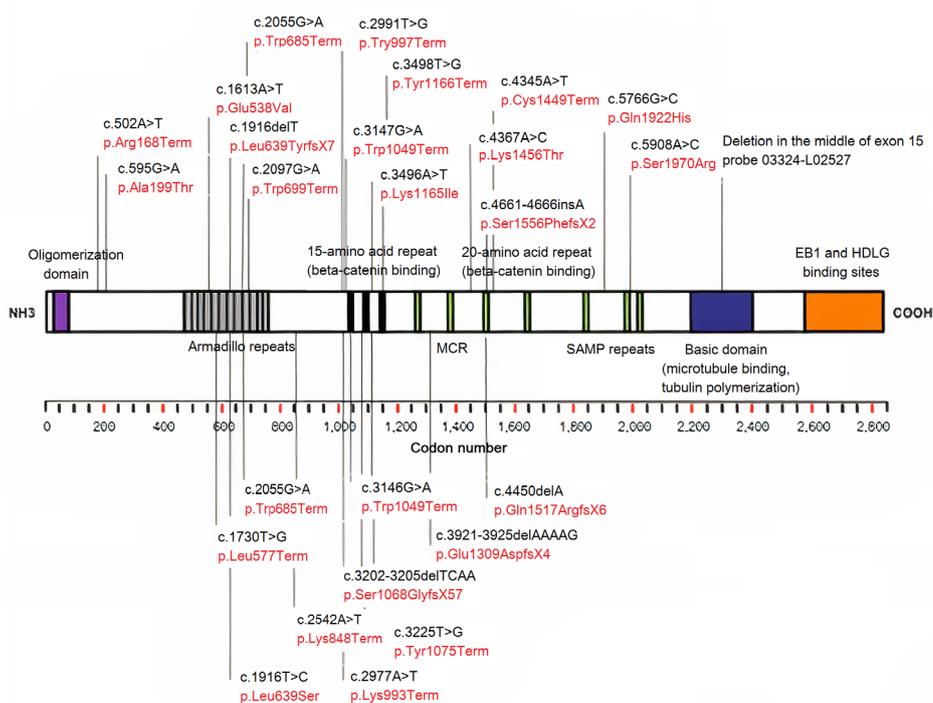


Figure 4. Mutation Spectrum of APC Gene in the Vietnamese Population based on the Current Study. Most of the mutations are located in exon 15, and a majority of mutations are point mutations. The only deletion mutation identified is found in the mid of exon 15.

in 55 individuals (52.4%). Devoted GI surgeons and geneticists confidentially educated all these carriers on the risk of cancerization, appropriate surveillance, surgical management, and prophylactic measurement.

Discussion

The first time in Vietnam, the Sanger sequencing for genetic diagnosis of FAP was successfully established. This achievement was expected to contribute to the improvement of prompt diagnosis, appropriate treatment, and prophylactic measurements in FAP patients and their asymptomatic relatives.

In this study, we were able to recruit only individuals who agreed to participate in the investigation. Therefore, the study population might be not a true reflection of FAP patients in Vietnam. Although most patients were from 30 to 60 years old, 12.7% were older than 60, and two were five years old. This wide range of ages of our patients made the comparison with other populations difficult. However, the colorectal cancerization rate in our FAP patients seemed lower compared to that in previous studies where almost 100% FAP patients will develop colorectal cancer before the age of 40 years (Sheng et al., 2010). Otherwise, 16 out of 63 FAP patients (25.4%) in the current study were diagnosed with colorectal cancer, indicating the critical role of FAP in the development of colorectal cancer in Vietnam patients.

Among 25 patients who exhibited point mutation in the *APC* gene, seven patients (28.0%) were diagnosed with colorectal upon presentation. This percentage was comparable to that of the whole FAP population (with and without mutation). The youngest FAP patient with cancerization was 41, and the oldest was 66 years of age.

In our study, the prevalence of point mutations in the *APC* gene in patients with FAP was 39.7%, lower than that in investigations carried out in China, Brazil, and Japan. Jin and coworkers identified 9 in 14 Chinese FAP patients (64.3%) with *APC* gene mutations (Jin et al., 2010). Torrezan & coworkers analyzing 23 Brazilian FAP patients and identified 14 patients (60.9%) with mutations (Torrezan et al., 2013). Miyoshi & coworkers investigated 79 Japanese FAP patients and found out the prevalence of mutations in the *APC* gene was 67.1% (Miyoshi et al., 2013). This difference in mutation prevalence might be partially explained by the ethnic and geographical genetic variation (Yang et al., 2016). In our study, 52.4% of asymptomatic individuals from families of patients with point mutation carried mutations in the *APC* gene. This rate was comparable to that of a Polish population survey (Plawski and Slomski, 2008).

The most critical finding in the current study was that we successfully discovered two point mutations in the *APC* gene. These novel point mutations accounted for 16% of all mutations identified in our FAP patients. Both two de novo identified point mutations were on exon 15. It could be explained that exon 15 is the biggest exon comprising 75% of the *APC* gene's coding sequence. Many studies have confirmed this finding in the region where most point mutations in the *APC* gene occur (Bérout and Soussi, 2008). More than 1,000 mutations in the *APC* gene have

been shown to have a close correlation to the development of FAP. The number of mutations will increase when more studies are about to be carried out in new populations. These two novel point mutations discovered in this study would enrich the *APC* gene database worldwide.

The two novel point mutations are located in the *APC* regions where mutations have been associated with the classic FAP phenotype. Both patients showed the classic FAP phenotype, characterized by hundreds to thousands of colorectal adenomatous polyps. The youngest patient (FAP18) exhibited extracolonic involvement, namely multiple jaw osteomas, which are usually encountered in Gardner syndrome, a subtype of FAP. The prevalence of osteomas is approximately 20% in FAP patients, which is much higher than that in the general population (1–2%). Mutations are found in codons 767 to 1,578 (Groen et al., 2008).

The present study had several limitations. In an important proportion of recruited FAP patients, the first symptom presentation's time point was vaguely recalled. Patients with socio-economic restraint were not sufficiently aware of first and minor signs and symptoms of disease. Many patients sought medical help only at the advanced stage of disease. This inaccurate recording of the first symptoms hindered us from establishing the genotype-phenotype correlations with confidence. The current study focused in a bias manner on colorectal polyps and cancer and did not exhaustively record extracolonic manifestations. This shortage prevented us from describing the complete picture of disease in this population.

In conclusion, by applying Sanger sequencing and MLPA assay to detect point, deletion, and duplication mutations in the *APC* gene in Vietnamese FAP patients, our study has initially established the mutational spectrum in this population. The total prevalence of mutations in the *APC* gene was 41.3%, in which 39.9% of FAP patients showed point mutations, and 1.6% exhibited deletion mutation. Importantly, our study discovered two point mutations in the *APC* gene and presented genotype-phenotype correlations of these mutations. These results would contribute to the database of *APC* gene mutations in the world population. The carrier status rate in families of patients with the mutation was also significantly high, at 52.4%, underlining the critical role of genetic screening and strict clinical surveillance of these individuals for early identification, prophylactic measurements, and prompt intervention. A nationwide registry on the *APC* gene's mutation spectrum and detailed genotype-phenotype correlations should be strongly encouraged.

Author Contribution Statement

NHB: Designing and supervising the study; writing the first manuscript. NTBS, NHH, NTT: Performing the genetic analysis; collecting and interpreting the data. LMK: supervising the study, interpreting the data and writing the final manuscript. All the authors have read and approved the final manuscript.

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Study approval

The current study was examined and approved by the Scientific Board, Department of Science and Technology, Ho Chi Minh City, Vietnam [308/QD-SKHCN].

Ethical Approval

The ethical issue of the current study was handled and approved by the IRB, University of Medicine and Pharmacy at Ho Chi Minh city.

Availability of data

The clinical dataset used in this study is available from the corresponding author on reasonable request.

Conflict of interest

The authors declare no conflict of interest.

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