NUCLEOTIDE SEQUENCE OF MITOCHONDRIAL CO I AND RIBOSOMAL ITS II GENES OF *OPISTHORCHIS VIVERRINI* IN NORTHEAST THAILAND

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Abstract. The mitochondrial cytochrome c oxidase subunit I (CO I) gene and the second internal transcribed spacer region (ITS II) gene of *Opisthorchis viverrini* were compared among *O. viverrini* from various areas in northeast Thailand. The nucleotide sequences of partial CO I gene (417bp) of *O. viverrini* differed among *O. viverrini* originated from Ubon Ratana, Leongpleuy, Ban Phai, Maha Sarakham, and Chatturat. These intraspecific variations were classified into 5 patterns but no area-specific pattern was observed. Amino acid sequence deduced from the nucleotide sequences of these genes was identical. Nucleotide sequences of a region of the *O. viverrini* ITS II gene (296 bp) from different areas were identical. However, they were different from those of *Clonorchis sinensis*, *Haplorchis taichui*, *H. pumilio*, *Fasciola gigantica*, *Echinostoma malayanum* and *Centrocestus* sp.

INTRODUCTION

The liver fluke, *Opisthorchis viverrini*, is a major parasitic infection in Thailand. The infection causes hepathobiliary diseases and has been implicated in development of cholangiocarcinoma (Haswell-Elkins *et al*, 1992; Sithithaworn *et al*, 1994; Satarug *et al*, 1998). Currently the morphological characteristics of either the metacercariae recovered from various intermediate hosts and adult worms from human are indistinguishable and limited information on genetic studies is available.

The structure of ribosomal gene of *O. viverrini* has partially been clarified (Korbsrisate *et al*, 1991; 1992) but little is known on the existence of genetic variation. Recently, ribosomal second internal transcribed spacer region II (ITS II) gene (Hoste *et al*, 1995; Ramachandran *et al*, 1997) and cytochrome c oxidase subunit I (CO I) gene of mitochondrial DNA (Okamoto *et al*, 1995; Hashimoto *et al*, 1997) have been used to analyze genetic variations of several parasites.

In this study, nucleotide sequence of mitochondrial CO I gene was analyzed to explore the genetic variation among *O. viverrini* isolated from different areas in northeast Thailand and ITS II gene of *O. viverrini* was compared with various kind of trematodes.

MATERIALS AND METHODS

Origin of parasites

The metacercariae of *O. viverrini* and other trematodes were obtained from various intermediate hosts in northeast Thailand (Table 1). They developed to adult worms in hamsters. Some adult worms were recovered from autopsy of a patient. *Clonorchis sinensis* from Korea were kindly supplied by Dr Sung-Tae Hong, Seoul National University.

Isolation of DNA and PCR

Adult worm or sometimes, metacercariae were homogenized on ice in a microcentrifuge tube using a hand made glass pestle. DNA was extracted by phenol extraction technique. One metacercaria was heated in 10 μl distilled water at 95˚C for 5 minutes to extract DNA. Total DNA was used for PCR amplification without isolation of mitochondrial DNA. The PCR conditions for ITS II gene analysis were as follows: 94˚C for 1 minute, 52˚C for 1 minute and 72˚C for 3 minutes for 30 cycles. Primers used were 5´-CGAGTATCGATGAAGAACGCAGC-3´ (LC1 primer) as a forward primer and 5´-ATATGCTTAAGTTCAGCGGG-3´ (HC2 primer) as a reverse primer (Navajas *et al*, 1994).

Table 1
Geographical origin of *Opisthorchis viverrini* in northeast Thailand.

Area/Province	Source of DNA
Ubon Ratana/Khon Kaen	Adult
Leongpleuy/Khon Kaen	Adult
Ban Phai/Khon Kaen	Adult
Maha Sarakham district/ Maha Sarakham	Adult
Chatturat/Chaiyaphum	Adult
Autopsy/Khon Kaen	Adult
Phimai/Nakhon Ratchasima	Metacercaria
Buri Ram district/Buri Ram	Metacercaria

The PCR conditions for CO I gene were as follows: 95˚C for 1 minute, 40˚C for 1 minute and 72˚C for 2 minutes for 30 cycles. Primers used were 5´-TTTTTTGGGCATCCTGAGGTTTA-3´ (MCO1-A primer) as a forward primer and 5´-TAAAGAAAGAACATAATGAAAATGAGC-3´ (MCO1-B primer) as a reverse primer. The PCR products were purified with QIA quick gel extraction kit (Qiagen, Germany) and used as template for cycle sequencing. The sequence was analyzed using an ABI sequencer (ABI 310).

RESULTS

Mitochondrial CO I gene of *O. viverrini*

Nucleotide sequences (417bp) of mitochondrial partial CO I gene of *O. viverrini* originated from various area and worms from patient were compared (Fig 1). Intraspecific variations were found at 4 alignment positions, positions at 18, 165, 192 and 330, in specimens from the same area as well as different area. These variations were classified into 5 patterns (Table 2) but did not show specificity according to area.

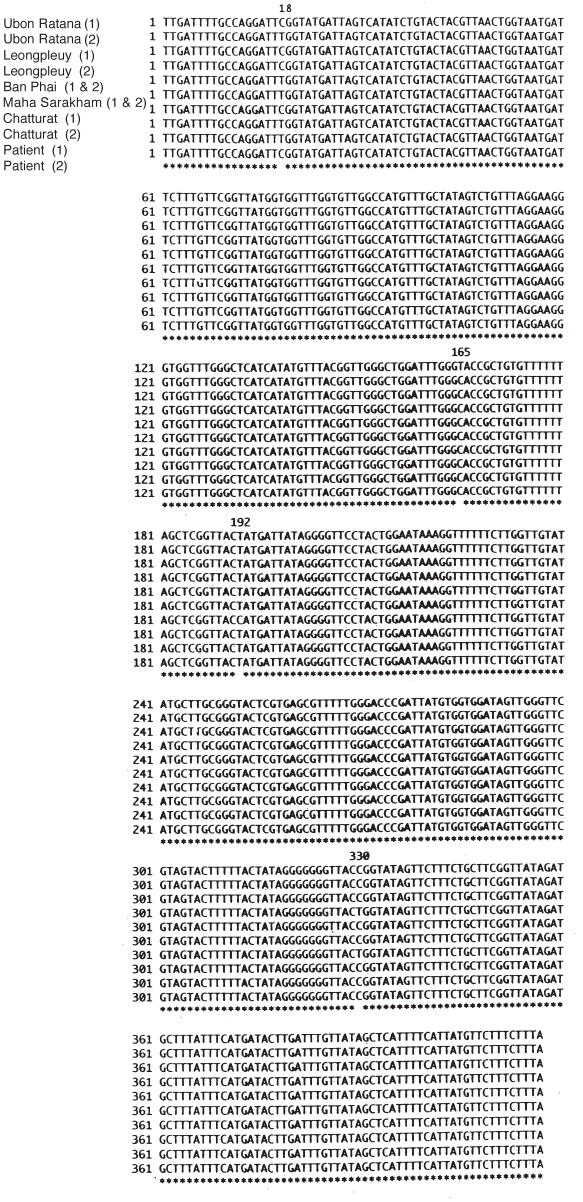

Fig 1- Nucleotide sequences of a region of the CO I gene of mitochondrial DNA of *O. viverrini*. Number on alignment shows the position of intraspecific variation.

Table 2
Summary of genetic variations of CO1 gene of mitochondrial DNA of *Opisthorchis veverrini*.

Origin		Pattern mark	Alignment position			
			18	165	192	330
Ubon Ratana	(1)	E	C	T	T	C
Ubon Ratana	(2)	A	T	C	T	C
Leongpleuy	(1)	B	C	C	T	C
Leongpleuy	(2)	D	C	C	T	T
Ban Phai	(1 & 2)	A	T	C	T	C
Maha Sarakham	(1 & 2)	A	T	C	T	C
Chatturat	(1)	C	C	C	C	T
Chatturat	(2)	A	T	C	T	C
Patient[a]	(1)	A	T	C	T	C
Patient[a]	(2)	B	C	C	T	C

[a] Different worm from same patient.

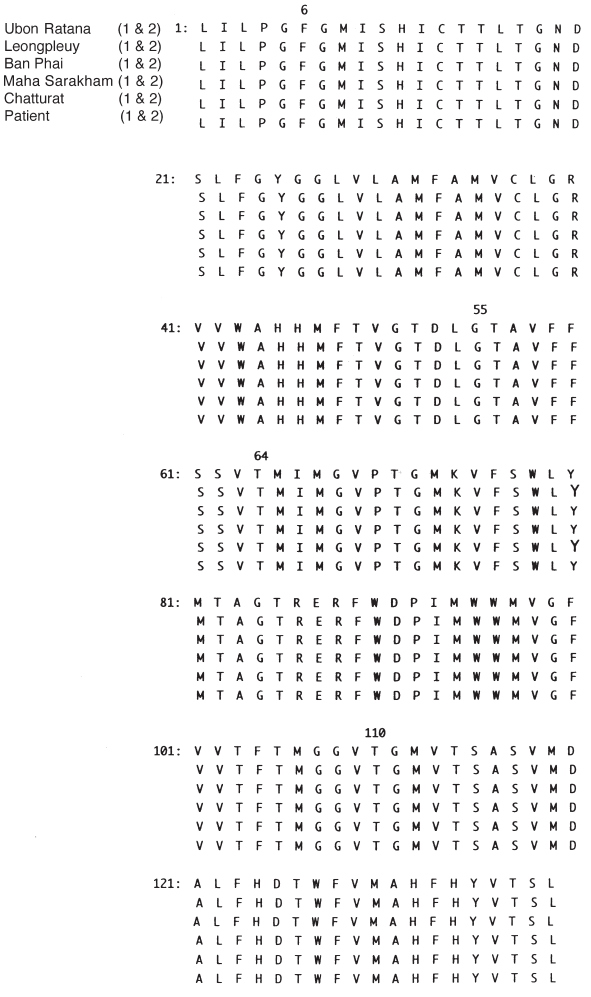

Fig 2- Amino acid sequences of a region of the CO I gene of mitochondrial DNA of *O. viverrini* deduced from nucleotide sequences. Alignment positions at 6, 55, 64, and 110 correspond the alignment position at 18, 165, 192 and 330 of Fig 1.

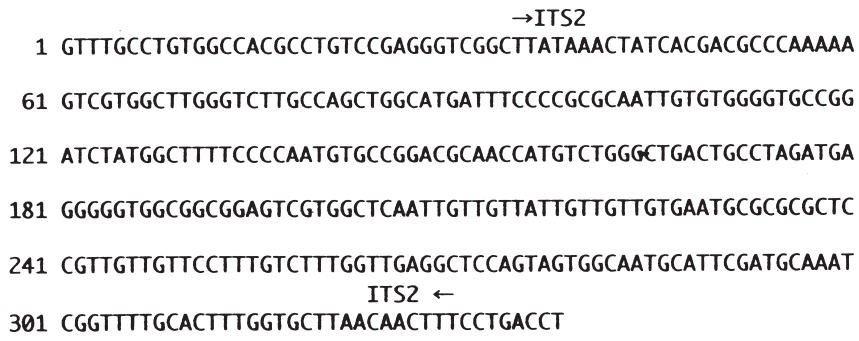

Fig 3- Nucleotide sequences of a region of the ITS II gene (296 bp) of ribosomal DNA of *O. viverrini*. from Ubon Ratana, Ban Pai, Phimai, Buri Ram and patient (one is presented).

Amino acid deduced from the nucleotide sequences contained alignment position 18, 165,192 and 330 was phenylalanine (F), glycine (G), threonine (T) and valine (V) respectively. Therefore, amino acid sequence of the CO I gene of *O. viverrini* originated from various area was identical (Fig 2).

Ribosomal ITS II gene

The 5´ and 3´ ends of ITS II sequence were determined by comparative alignment with *Echinostoma revolutum* 5.8S gene and *O. viverrini* 28S gene, respectively (Sorensen *et al*, 1998; Korbsrisate *et al*, 1992). Nucleotide sequences (296 bp) of a region of *O. viverrini* complete ITS II gene from Ubon Ratana (adult), Ban Phai (adult), Phimai (metacercaria), Buri Ram (metacercaria) and patient (adult) were identical (one is presented in Fig 3) but they were different from those of *C. sinensis* (302 bp), *Haplorchis taichui* (446 bp), *H. pumilio* (290 bp), *Fasciola gigantica* (361 bp), *E. malayanum* (429 bp) and *Centrocestus* sp (320 bp) (Fig 4). The reverse primer specific for *O. viverrini* (OVSP primer) was designed from the differences of these sequences. The combination of forward primer LC1 and reverse primer HC2 amplified ITS II genes of all trematodes tested through positions of bands of *H. taichui* and *E. inalayanum* were a little higher than others, whereas the combination of forward primer LC1 and reverse primer OVSP amplified only *O. viverini* (Fig 5).

DISCUSSION

The complete CO I gene of animals consist of about 1.5 kbp. We examined the existence of genetic variation of *O.viverrini* originated from 5 areas in 3 provinces and specimens from autopsy of a patient in northeast Thailand by amplifying about 420 bp in the central region of CO I gene by PCR. We found the intraspecific variations in CO I gene of *O. viverrini* for the first time. However, amino acid sequence deduced from the nucleotide sequences of these genes was identical. Therefore, it is considered that there is no differences of function as CO I gene.

Korbsrisate *et al* (1991, 1992) clarified the whole structure of the ribosomal DNA and nucleotide sequences of 28S and 18S regions. We examined *O. viverrini* ITS II gene originated from various area but there was no difference among them. This is the first report of *O. viverrini* ITS II gene.

The nucleotide sequence of ITS II gene of *O. viverrini* was different from those of other trematodes. The sample *C. sinensis* we used was from Korea and the nucleotide sequence of ITS II gene of that was identical with those from Korea (AF217094) and China (AF217099) registered in Gene Bank. The nucleotide sequence of ITS II gene of *F. gigantica* from Thailand was identical with those of *F. gigantica* from Japan and Malaysia (Hashimoto *et al*, 1997). The nucleotide sequence of ITS II gene of *E. malayanum* was a little different from *E. trivolvis* (AF067852) and *E. revolutum* (AF067850) registered in Gene Bank.

It is often difficult to identify species of trematodes based on the egg morphology (Tesana *et al*, 1991) or metacercariae so that ELISA system and DNA probe were developed (Sirisinha *et al*, 1991; Sermswan *et al*, 1991). We are trying to design *O. viverrini* specific primer from the differences of ITS II gene among trematodes.

Although there are small differences of CO I gene among *O. viverrini* isolates in this study, the results suggests that there is genetic variation among *O. viverrini* population in northeast Thailand which deserve further investigation covering wider geographic area in Thailand and other Southeast Asian countries.

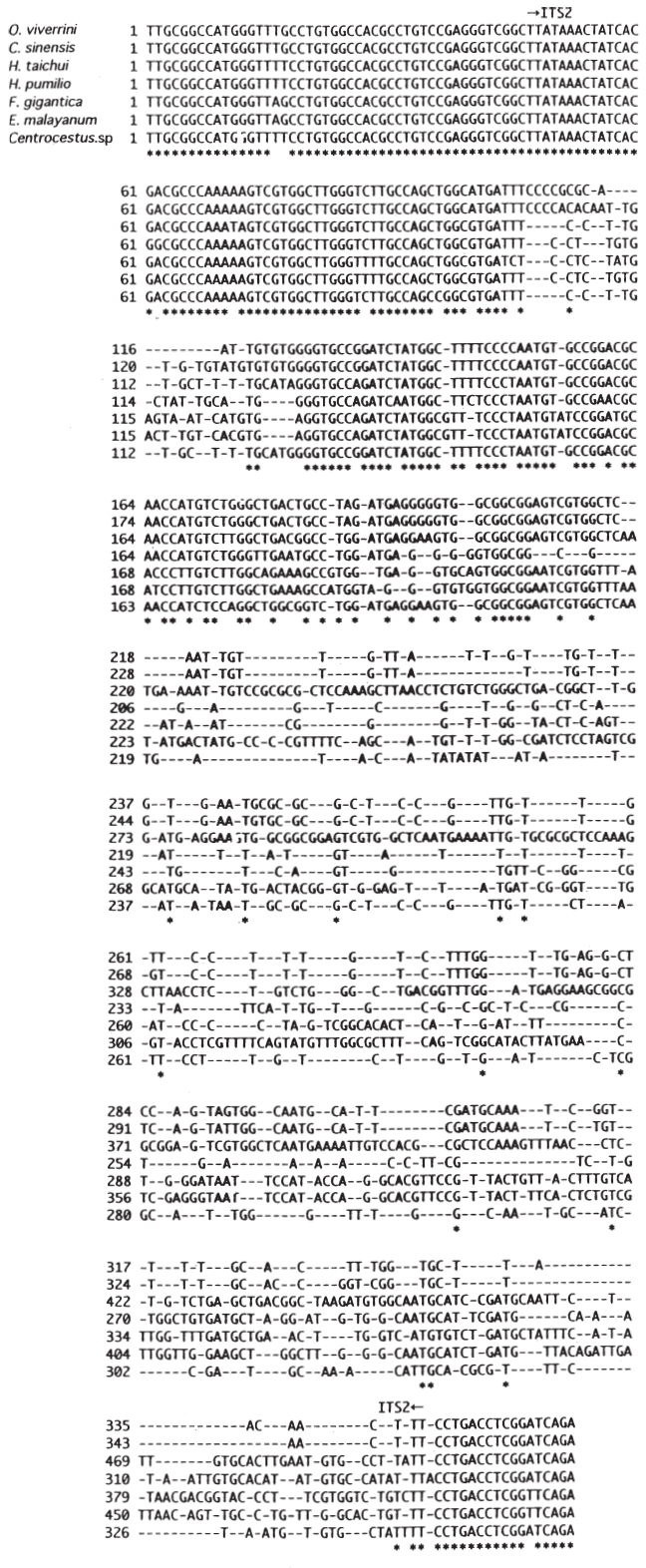

Fig 4- Nucleotide sequences of a region of the ITS II gene of ribosomal DNA of trematodes.

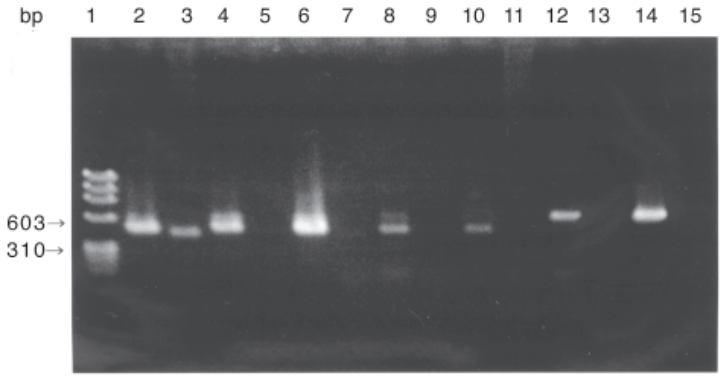

Fig 5- Result of PCR of ITS II genes from trematodes.

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