

CYP3A4

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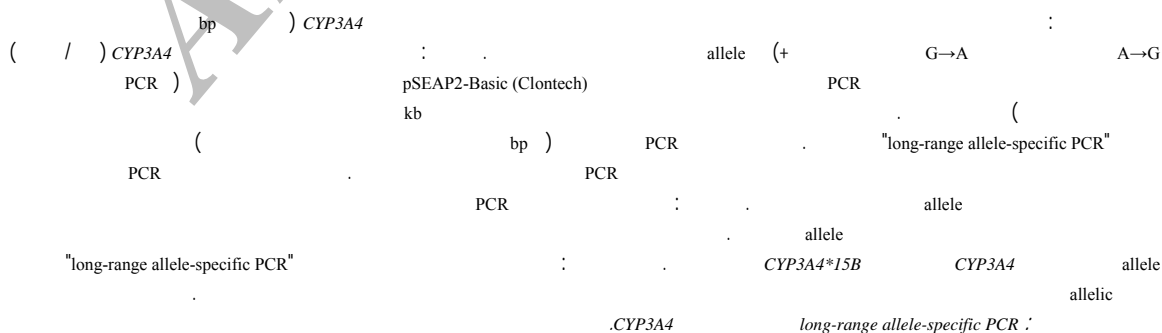
Linkage analysis of three heterozygous mutations found in human CYP3A4 gene separated by long distances

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OBJECTIVES: In order to determine whether the three heterozygous *CYP3A4* gene mutations (a 9 bp insertion at -845, an A→G transition at -392 and a G→A transition at +14269) found in genomic DNA amplification from one subject were in the same allele, linkage analysis was performed. **Methods:** An initial amplification and cloning (using pSEAP-Basic plasmid, Clontech) of the 5'-proximal promoter region (-1201/-61) was used to separate the alleles and determine if the two mutations in this region are linked. Using the recombinant plasmid DNAs as template, PCR amplification of 592 bp (to cover both mutations) of the *CYP3A4* promoter (-929/-337) was performed and sent directly for sequencing. Following this, "long-range (-858/+14536) allele-specific PCR" was performed using specific primers discriminating the two alleles. Resulting PCR products were then used as templates after gel extraction and purification to amplify each corresponding exon 6. The exon 6 PCR products were then sequenced to identify on which allele the exon 6 mutation was present. **Results:** The results of cloning and sequencing experiments on promoter region revealed that both mutations are in the same allele. The results obtained from "long-range allele-specific PCR" again showed that all three mutations found in *CYP3A4* gene are in fact present on the same allele introducing a novel *CYP3A4* allele "*CYP3A4*15B*". **Conclusion:** This work has revealed successful application of carefully designed cloning and "long-range allele-specific PCR" experiments to linkage analysis of mutations found in different locations of a gene.

Key words: Long-range allele-specific PCR, Cloning, *CYP3A4* gene.



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CYP3A4*15B allele P450 CYP3A4

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()

CYP3A4 :

(Clontech) pSEAP2-Basic

bp PCR () 7q22.1 kb ()

EcoRI HindIII () CYP3A4

5'-cccaagcttGACCACTGCCCCATCATTGC-3' (Forward) mRNA ()

5'-ccggaattcTGCTGGGCTATGTGCATGGAGC-3' (Reverse)

quick PCR PCR (QIAGENE) A→G) CYP3A4*1B allele (

pSEAP2-Basic (EcoRI HindIII Sata () CYP3A4*2 (Ser222Pro) allele ()

pX-SEAP2 () CYP3A4 () Hsieh CYP3A4 /

PCR (/) bp PCR CYP3A4 ()

bp) ()

G→A A→G (+

5'-AGAATCACAGAGGACCAGCC-3' (Forward) :

5'-CTTATCAGAAACTCAAGTGG -3' (Reverse)

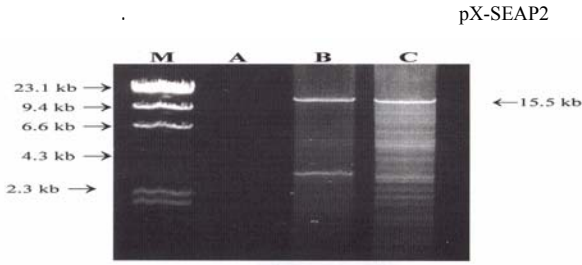
PCR

long-range "

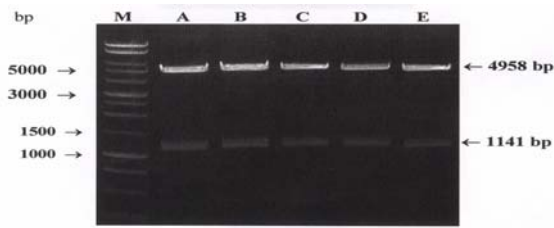
PCR : bp) "allele-specific PCR (

PCR

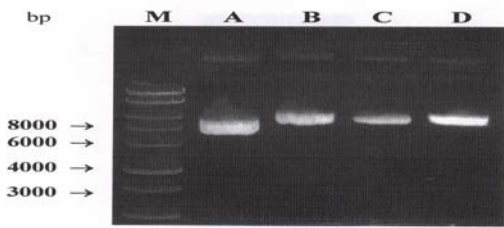
allele



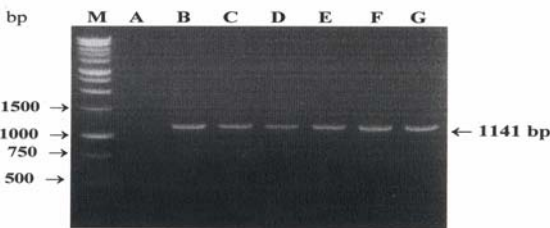
(M) .CYP3A4 PCR :
 allele PCR (B) . (A) .DNA/HindIII
 allele PCR (C)



pX-SEAP2 :
 (B,C,D) .pSEAP2-Basic (A) . kb DNA (M)
 .(CYP3A4 bp)



pX-SEAP2 :
 .CYP3A4 bp HindIII EcoRI
 (A,B,C,D,E) . kb DNA (M)



CYP3A4 PCR :
 (A) . kb DNA (M) .genomic DNA PCR
 (C,D,E,F,G) .genomic DNA PCR (B) .
 PCR

bp)

(CYP3A4

(Eppendorf) Triplemaster® PCR

PCR

() PCR

bp) / kb

(CYP3A4

: () allele

5'-AATGACCTAAGAAGTCACCAGAA-3' (wild type) or

5'-AATGACCTAAGAAGATGGAGTAG-3' (insertion-mutant)

5'-TGGATATGTAAACCCTGGCCC-3'

μl genomic DNA ng (1 μl)

(pmol)

μl

* μl

() / μl dNTP*

μl

μl TripleMaster®

PCR

()

94 °C for 2 minutes (initial denaturation) 1 cycle

94 °C for 25 seconds (denaturation)

62 °C for 30 seconds (annealing)

68 °C for 12 minutes (extension)

68 °C for 10 minute (final extension)

28 cycles

1 cycle

/ kb

Ultra Clean™ 15 DNA (MO BIO, USA)

PCR

PCR

5'-TGTCTTCTGGGACTAGAGTC-3' (forward)

5'-GGGAGAAGATCCTTTTCCTCC-3' reverse)

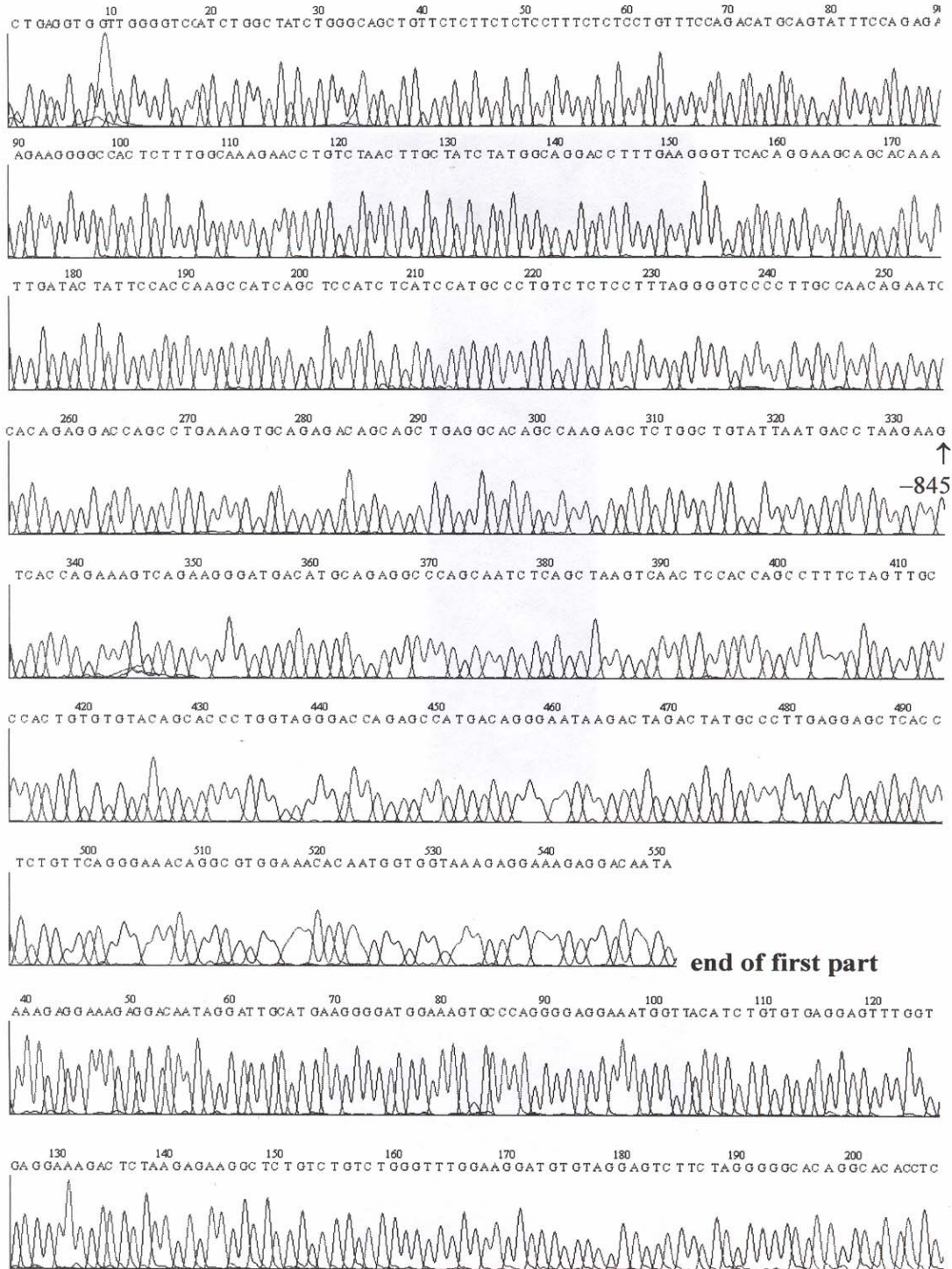
() PCR

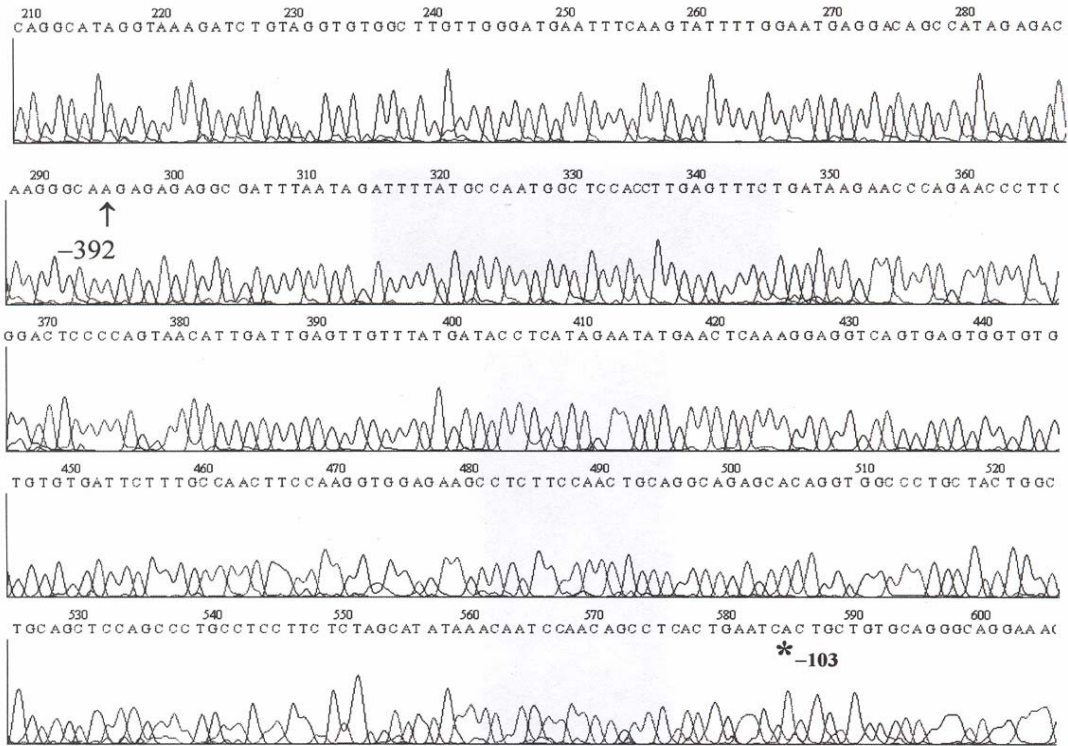
CYP3A4

CYP3A4 () ()

PCR

allele

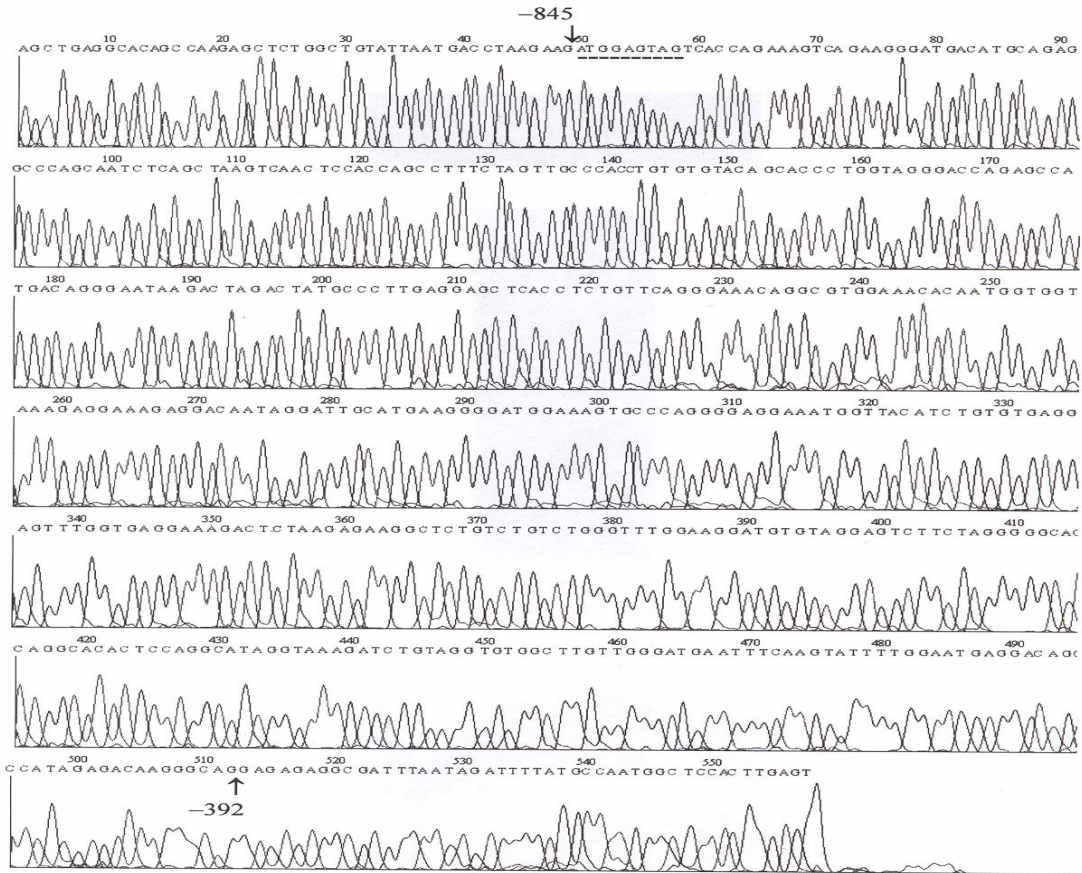




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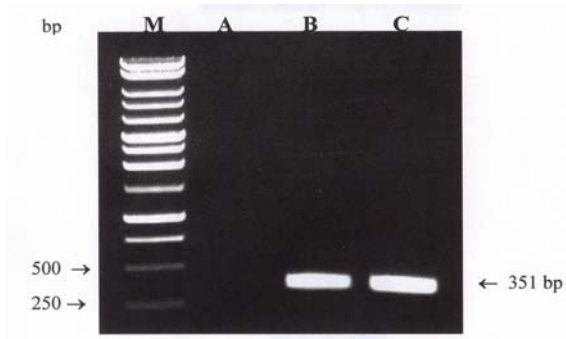
CYP3A4



allele

pX-SEAP2

CYP3A4



allele () PCR PCR

(allele)

allele PCR

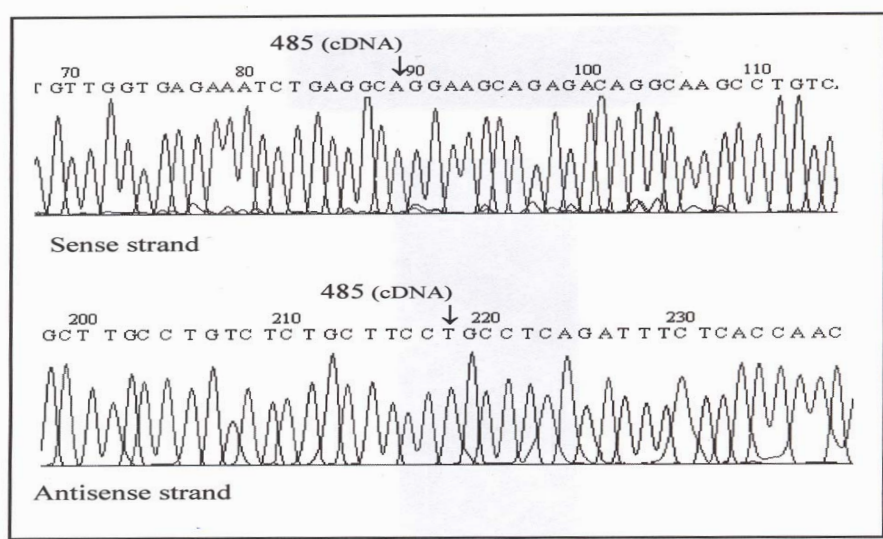
CYP3A4 allele

G→A CYP3A4*15A

allele

PCR : (A) kb DNA (M) PCR (B) (C) allele allele

"CYP3A4*15B" (http://www.imm.ki.se/CYPalleles/cyp3a4.htm)



allele PCR DNA :

DNA pH PCR (bp) PCR PCR allele "genomic" DNA kb PCR allele PCR allele . CYP3A4*15B Taq) (insertion)) "3'-5' proofreading" DNA (human fetal liver) 'HFL-a' (Pwo Pfu

CYP3A4 (CYP3A7
 CYP3A7 CYP3A7 CYP3A4
 'HFL-a'
 CYP3A4 CYP3A7
 CYP3A4
 CYP3A4
 (CYP3A4*15B) allele
 PCR cloning A→G allele
 (CYP3A4*1B)
 ()

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