Molecular Analysis of Esterase D Polymorphism

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INTRODUCTION

Since the polymorphism of Esterase D(EsD) was described initially by Hopkinson et al. (1973), a number of population studies were reported. EsD polymorphism has been one of the most useful markers in genetic studies. Three common phenotypes, EsD 1, EsD 2-1 and EsD 2, are identified and determined by two autosomal codominant alleles, EsD^1 and EsD^2. Although the complete EsD cDNA have been sequenced, there are no information about the phenotypes of EsD. In this study, we make clear the relationship between EsD isozyme phenotypes detected by electrophoresis and DNA sequences. Moreover, a RFLP related to EsD polymorphism is presented and applied to the EsD typing by DNA samples.

MATERIALS AND METHODS

EsD phenotypes were determined by the method described by Hopkinson et al (1973) and isoelectric focussing electrophoresis. Peripheral blood samples were obtained from each three healthy individuals exhibiting three common EsD phenotypes, EsD 1, 2-1 and 2. Total RNA was extracted from the peripheral blood samples by guanidinium / hot phenol method (Maniatis et al.1982). Genomic DNA was isolated from peripheral blood samples using SDS-proteinase K treatment and phenol / chloroform extraction. Primers for the amplification and sequencing were constructed according to the DNA sequence presented by Young et al.(1988). Reverse transcription was carried out using Gene Amp™ RNA PCR Kit (Perkin Elmer-Cetus). The amplification of a DNA fragment including the coding region of EsD cDNA was carried out under the following conditions : denaturation, 1 min at 94 ℃ ; annealing, 1 min at 55 ℃ and extension, 3 min at 72 ℃; 30 cycles. After the generation of single-strand DNA fragments by an asymmetric PCR using inner primers, direct sequencing was carried out by employing a Sequenase™ Kit version 2.0 (United State Biochemical Co.). RFLP analysis was carried out using restriction enzyme *Ssp* I. An amplified EsD cDNA was used as a probe for detecting the RFLP patterns from genomic DNA samples.

RESULTS

The DNA fragments with a same length were amplified using total RNA samples from individuals exhibiting the different EsD phenotypes. Sequence analysis of the amplified EsD cDNA fragments demonstrated a single difference among three common EsD phenotypes. A guanine(G)-adenine(A) substitution was observed in the position of nucleotide 698 of EsD cDNA described previously (Young et al.1988)(This position was 569th nucleotide from the first nucleotide at starting codon)(Fig. 1). The nucleotides in the cDNAs of EsD 1 and EsD 2 phenotypes were G and A, respectively. In EsD 2-1 phenotype, there were two bands, G and A, at the same position of the autoradiogram. The G-to-A point mutation led to a substitution of the amino acid at residues 190. The predicted amino acids were glycine(Gly) and glutamic

acid(Glu) in the cDNAs from EsD 1 and EsD 2 phenotypes, respectively. The G-to-A substitution between the cDNAs exhibiting EsD 1 and EsD 2 phenotypes led to the change of the digestion site with a restriction enzyme *Ssp* I. A new digestion site occurred in the cause of a point mutation in EsD 2-type cDNA. The cDNA from EsD 2-1 phenotype was heterozygote both of EsD 1 and EsD 2-type cDNAs. When genomic DNA samples from EsD 1, EsD 2-1 and EsD 2 phenotypes were digested with *Ssp* I and applied to southern hybridization analysis using the amplified EsD cDNA as a probe, two polymorphic fragments, which were approximately 1.5kbp and 0.8kbp in size, were observed(Fig. 2). In the individuals exhibiting EsD 1 phenotype, only the 1.5kbp-polymorphic fragment was detected. On the other hand, the samples from EsD 2 phenotype showed the 0.8kbp-polymorphic fragment instead of the 1.5kbp-fragment. Both of them were detectable in the individuals exhibiting EsD 2-1 phenotype.

DISCUSSION

We present here the sequences of EsD cDNAs from common three phenotypes and made clear the differences of EsD cDNA and the predicted amino acid sequences among different EsD phenotypes. When the pI values of EsD proteins predicted by cDNA sequences were calculated, although there were some differences between pI values observed by IEF and estimated by the predicted amino acid sequences, EsD 1 protein had higher pI value than EsD 2 protein, that was in accordance to that observed by IEF. The G-to-A substitution between cDNAs from EsD 1 and EsD 2 phenotypes makes the change of not only a predicted animo acid sequence but also a digestion site with restriction enzyme *Ssp* I. The difference of the digestion site makes it possibly and easily to determine the EsD phenotypes using DNA samples. EsD is present abundantly and ubiquitously in almost human tissues. EsD typing has been carried out using the lysate of red blood cells by some kinds of electrophoresis and enzyme activity using methylunbelliferyl esters as a substance. In the samples except red blood cells and in the specific condition, for instance, old samples which has lost EsD activity or the tiny samples which have no detectable EsD activity, EsD typing is difficult. However, using the RFLP of EsD gene with *Ssp* I , it is possible to determine the EsD phenotype from the samples without EsD activity.

REFERENCES

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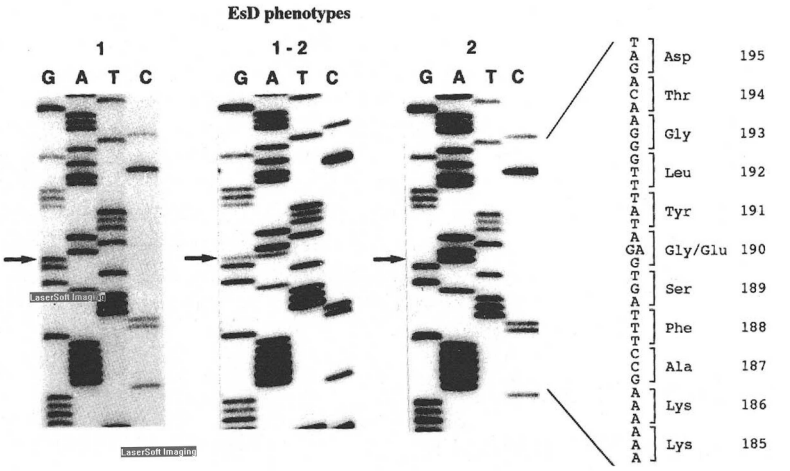

Fig. 1 Representation of the characteristic sequence difference among EsD phenotypes EsD 1, EsD 2-1 and EsD 2. Arrow heads indicate the polymorphic positions. A G-to-A point mutation is observed among EsD 1 and EsD 2 phenotypes. There are two bands(G and A) at the same level of the autoradiogram, which indicate that EsD 2-1 phenotype is heterozygous for the G-to-A mutation. The codon numbers and the corresponding amino acids are listed to the right.

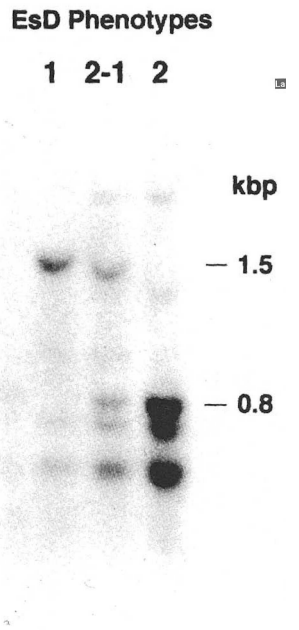

Fig. 2 Southern blot analysis of the polymorphic *Ssp* I digestion site on the genomic DNA caused by the G-to-A point mutation by the amplified EsD cDNA as a probe. Total DNAs from individuals exhibiting EsD 1 show a 1.5 kbp-band and lack a 0.8 kbp-band. Those from EsD 2 phenotype show a 0.8 kbp-band and lack a 1.5 kbp-band, indicating that a *Ssp* I restriction site occurs in the 1.5 kbp-band caused by G-to-A point mutation. Both of them are observed in EsD 2-1 phenotype. Approximately size is shown in the right.

3. Quality control and quality assurance