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Restriction Fragment Length Polymorphisms in the 5′ End Region of the Human Argininosuccinate Synthetase Gene

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Argininosuccinate synthetase (AS) (L-citrulline: L-aspartate ligase, EC 6.3.4.5) is a urea cycle enzyme which is deficient in patients with citrullinaemia (McKusick 21570). The majority of Japanese patients with citrullinaemia have a distinctive variant with an AS deficiency only in the liver, and the onset of clinical symptoms is usually in adolescence or sometimes later (Saheki *et al.*, 1981; Akaboshi *et al.*, 1983). The gene coding for AS is contained within 63 kilobase (kb) of human genomic DNA (Freytag *et al.*, 1984) and fourteen 'processed' type pseudogenes are known to be dispersed on the human genome (Beaudet *et al.*, 1982). DNA analysis in cases of citrullinaemia, using an AS cDNA (Su *et al.*, 1981) as a probe, was expected to be extremely difficult, because of the presence of numerous pseudogenes (Daiger *et al.*, 1984).

As a first step to clarify the molecular basis for the Japanese late-onset type citrullinaemia, we have isolated the human active AS gene and characterized its 5′ end region by restriction mapping, Southern blot procedures and nucleotide sequence analysis (Jinno *et al.*, 1985). We now report the usefulness of the DNA fragments derived from the 5′ end region of the AS gene for studying the restriction fragment length polymorphisms (RFLPs) in the AS gene region.

MATERIALS AND METHODS

Restriction enzymes were purchased from Takara Shuzo and *E. coli* DNA polymerase I was from Boehringer (Mannheim). [α-^{32}P]dCTP (3000 Ci mmol^{-1}) was obtained from Amersham. We prepared three different DNA fragments from the previously cloned 5′ end region of the AS gene (Jinno *et al.*, 1985) and used as hybridization probes in this experiment: (1) 0.65 kb *Pvu*II/*Sma*I fragment derived from the 5′ flanking region; (2) 1.5 kb *Bam*HI/*Eco*RI fragment from the first intron; and (3) 0.8 kb *Eco*RI fragment from the second intron (Figure 1(a)). All these DNA fragments contain neither the exon regions of the AS gene nor the human nuclear highly repetitive DNA sequences. Human genomic DNAs isolated from peripheral white blood cells of 20 unrelated healthy Japanese were analysed by

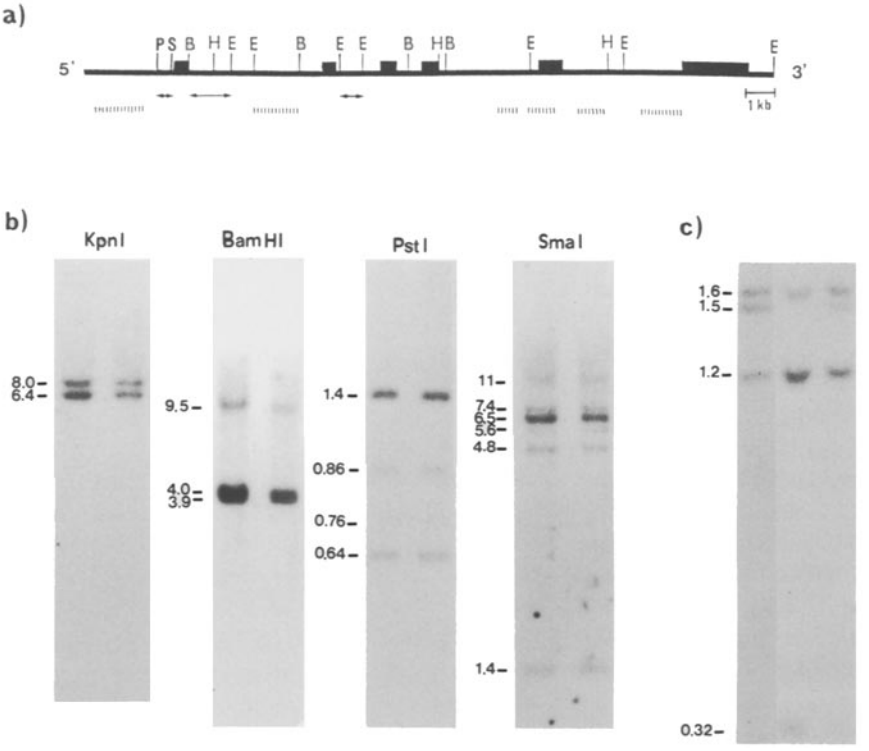

Figure 1 (a) Structure of the 5′ end region of the human AS gene (Jinno *et al.*, 1985). Exons or restriction fragments hybridizing with the As cDNA (Su *et al.*, 1981) are denoted by thick lines. Arrows and striped thick lines below the restriction map indicate the DNA fragments used as probes and the restriction fragments in which nuclear highly repetitive sequences are detected, respectively. Only relevant restriction sites are shown and the symbols are as follows: *Eco*RI (E), *Bam*HI (B) *Hind*III (H), *Pvu*II (P) and *Sma*I (S). (b) Southern blot analysis of RFLPs in the As gene region. Six-base restriction enzymes used are indicated on the top of each panel and two of each representative results of 10 individuals are shown. The sizes (kb) of bands are indicated at the left side of each panel. (c) Southern blot analysis of RFLPs in the AS gene region. A four-base enzyme, *Alu*I, was used in this experiment. Three representative findings in 20 individuals are shown and their genotypes from left to right are *a/a*, *b/b* and *a/b*. Sizes of the bands are indicated at the left of the panel

Southern blot procedures, after digestion with one of the following restriction enzymes: *Bam*HI, *Kpn*I, *Pst*I, *Sma*I and *Alu*I. The general procedures for DNA isolation, nick-translation and DNA blot hybridization were performed as previously described (Jinno *et al.*, 1985).

RESULTS AND DISCUSSION

We first analysed 10 different human genomic DNAs by Southern blot procedures, using a mixture of the three DNA fragments derived from the 5′ end region of the

AS gene as a probe (Figure 1(a)). The results obtained with four kinds of six-base enzymes are shown in Figure 1(b) and revealed that the *Kpn*I, *Bam*HI and *Pst*I digests all generate two to four distinct hybridization bands ranging from 0.64 kb to 9.5 kb (Figure 1(b), panels *Kpn*I, *Bam*HI and *Pst*I). The *Sma*I digests, which might be incomplete digests, showed numerous hybridization bands (Figure 1(b), panel *Sma*I). All these six-base enzymes produced no apparent RFLP among 10 individuals. However, the mixture of the DNA fragments derived from the 5′ end region of the AS gene is a useful probe for studying RFLPs in the AS gene region because, of four six-base enzymes tested, three produced excellent digest patterns. Accordingly, we further analysed 20 different human genomic DNAs by Southern blot procedures, after digestion with a four-base enzyme, *Alu*I. Here we used a mixture of the 0.65 kb *Pvu*II/*Sma*I fragment and the 0.8 kb *Eco*RI fragment as a hybridization probe, and detected a high-frequency RFLP (Figure 1(c)). Apparently the *Alu*I digests produced three constant bands, 1.6 kb, 1.2 kb and 0.32 kb, and one variable band, 1.5 kb (Figure 1(c)). From the size and density, we concluded that the 1.5 kb variable band contains one polymorphic *Alu*I site and is cleaved into 1.2 kb and 0.32 kb bands by *Alu*I digestion. Our results indicate that the allele frequency studied in 20 Japanese is as follows: 1.5 kb allele (allele *a*) 0.6, and 1.2 kb and 0.32 kb allele (allele *b*), 0.4. The 20 Japanese could be classified into three genotypes by this RFLP; *a*/*a*, 8/20, *b*/*b*, 4/20 and *a*/*b*, 8/20.

Since a deficiency of AS activity in the late-onset type citrullinaemia is limited to the liver, neither fibroblasts nor leukocytes can be used for the diagnosis. There is no technique available to make a prenatal or presymptomatic diagnosis of this type of citrullinaemia. There has been a report on RFLPs of AS-related genes (Daiger *et al.*, 1984). However, as this work was performed using as AS cDNA prepared from the pAS-1 (Su *et al.*, 1981) as a probe, numerous bands derived from pseudogenes were detected, in addition to the bands derived from the functional AS gene, and the detected RFLPs are not necessarily related to the active AS gene. In our present work, we used DNA fragments prepared from the 5′ end region of the AS gene as probes. These fragments do not share DNA homology with the AS pseudogenes. Thus the results obtained are clear, and the detected RFLPs are expected to be directly related to the active AS gene.

Using this system, we looked for other restriction enzymes producing RFLPs and found that *Hin*fI produces another RFLP (data not shown). Studies along this line should facilitate DNA analysis of the late-onset type citrullinaemia.

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