Restriction fragment length polymorphisms in the *ETS-1* proto-oncogene. Comparison of Saudi and Western populations

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Summary. We have cloned part of the *ETS 1* proto-oncogene and demonstrated the presence of two polymorphic Sst I restriction sites. A probe derived from one of our clones revealed the presence of 8.3 kb, 9.5 kb and/or 11.5 kb fragments on Southern blots of human DNA samples. The relative frequencies of these alleles appear to be significantly different between Saudi and Western populations, but there are no apparent differences in these frequencies between Saudi non-leukemic and leukemic individuals.
Key words. *ETS 1* proto-oncogene; RFLP; Saudi; leukemia.

The concept of a genetic basis for cancers and leukemias has been greatly strengthened by the discovery of a group of genes (proto-oncogenes), each of which when altered (to become an oncogene) has been implicated in transforming normal cells into neoplastic cells[1]. It has been postulated that the *ETS 1* proto-oncogene may be important in acute lymphoblastic leukemia (ALL) because it is expressed primarily in lymphoblasts[2], and because of its localization to human chromosome 11 band q23[3], a region associated with ALL-specific chromosome abnormalities[4]. So far, however, only one case of a rearrangement of *ETS 1* in ALL has been reported[5]. One reason for this lack of association may be that the available *ETS 1* probes may recognize a region of *ETS 1* which is some distance from the putative region of rearrangement. Therefore, we are currently cloning all of *ETS 1* and isolating probes for different regions of this gene. In the course of this work, we have discovered two restriction fragment length polymorphisms (RFLP), which we report in this paper. We have examined the frequencies of these RFLPs in both Saudi and Western groups and have further compared their frequencies between Saudi normal and leukemic individuals.

Materials and methods. We isolated a 800 bp Hind III fragment (probe A) from the 5.4 kb human *ETS 1*-H3 clone[3], and used this to screen three different genomic libraries constructed from Mbol-partial digest fragments of human leukocyte DNA, cloned into EMBL 3. We isolated 2 positive clones after screening 10^6 pfu from the first library, 8 positive clones from the second library and 3 positive clones from the third library[6]. Each clone was amplified, DNA prepared from it, and restriction site mapped[7]. A 4.3 kb EcoRI fragment of clone Dla was isolated after digestion with EcoRI (probe B), and purified by gel electrophoresis. This probe was used in the detection of the RFLPs.

To detect RFLPs, high molecular weight DNA was isolated from peripheral blood leukocytes as previously described[8]. 10 µg of each DNA sample was digested to completion with the relevant restriction enzyme, separated on a 1% (w/v) agarose gel and blotted onto Amersham nylon hybond filters. The filters were then hybridized to a nick translated ^{32}P-probe (probe B) and washed in accordance with the Amersham booklet. The filters were exposed to Kodak XAR film with intensifying screens at −70 °C for 1–5 days to visualize the hybridization fragments.

Results and discussion. We are currently isolating a series of overlapping clones, representing the *ETS 1* proto-oncogene, from a human genomic library. Initially, we isolated two clones (Dla and Dlb) after screening 10^6 pfu of a human genomic library. After restriction site mapping, these clones appeared to be essentially the same. We isolated a 2.2 kb EcoRI fragment from the 5′-end of the Dla clone as well as a 4.3 kb EcoRI fragment from the 3′-end (probe B). Hybridization of the 2.2 kb fragment to Southern blots of human DNA resulted in the appearance of smears so that no distinct bands were discernable; indicating the presence of a repetitive sequence(s) in this probe. However, when probe B was hybridized to Southern blots of human DNA that had been digested with Sst I, two fragments of 9.5 and/or 11.5 kb were observed in most samples (fig. 1), and fragments of 8.3 and 9.5 kb in two individuals. The frequency of occurrence of these fragments varied between individuals, indicating the presence of two polymorphic Sst I sites, and that these fragments represented different alleles. Co-dominant segregation of the 9.5 and 11.5 kb alleles was demonstrated in 6 Saudi families (fig. 2). No RFLPs were observed when the DNA samples were digested with Bgl II, EcoRI, Hind III or Pvu II.

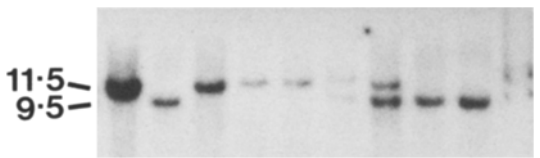

Figure 1. Southern blot analysis of peripheral blood leukocyte DNA samples from 10 Saudi individuals. The DNA was digested with Sst I, and the blot hybridized with the *ETS 1* probe B.

We subsequently isolated 8 clones from a second library, of which clones D2a, D4a and D5a were restriction site mapped. Finally, we isolated a further 3 clones from a third genomic library. All of the latter clones (LA4, LB4, LG4) also had their restriction site maps determined. We combined the maps from all of these clones and produced a map covering approximately 26 kb of the human *ETS 1* gene (fig. 3). The positions of almost all of the identified restriction sites were identical between different clones where they had re-

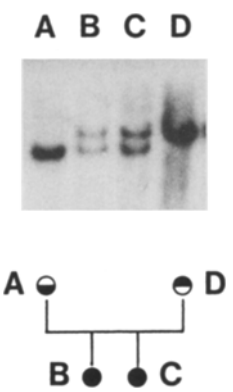

Figure 2. Southern blot analysis of peripheral blood leukocyte DNA samples from a Saudi family, comprising father (A), mother (D) and two children (B, C). The DNA was digested with Sst I, and the blot hybridized with the *ETS 1* probe B.

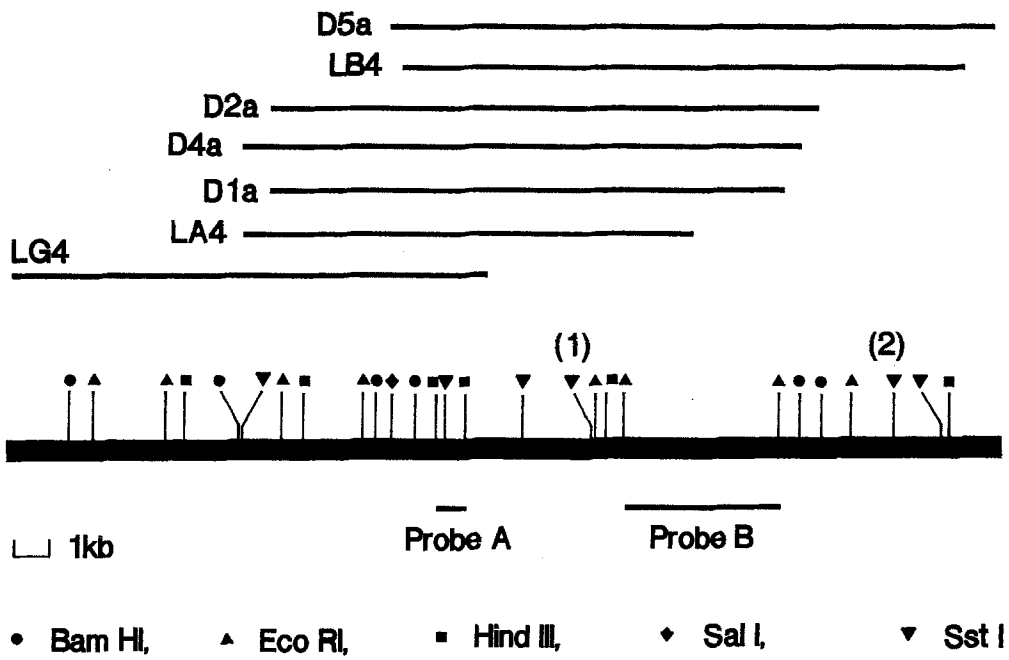

Figure 3. Restriction site map of part of the human *ETS 1* gene. The relative positions of the isolated clones (D1a, D2a, D4a, D5a, LA4, LB4, LG5) are shown above the map. The regions represented by probe A (which was used for screening the genomic libraries) and probe (which was used for probing Southern blots) are shown below the map.

gions of overlap. However, two Sst I sites (Sst I (1) and Sst I (2)) were found to be present in some clones and missing in others. It is clear from studying the positions of these two sites relative to the probe B region that these are the polymorphic sites responsible for generating the different sized fragments that we have detected in our Southern blot analyses. If both sites are absent then a 11.5 kb fragment is generated by Sst I digestion of the DNA. If the Sst I (1) site is present (see fig. 3), as in clone LA4, then a fragment of 9.5 kb is generated. Furthermore, if both Sst I (1) and Sst I (2) sites are present, as in clone LB4, then a fragment of 8.3 kb is generated after Sst I digestion. A clone which contains only the Sst I (2) site would theoretically generate a 10.3 kb fragment, but we have never detected such a fragment in any of our studies.

We examined DNA samples from 41 unrelated Saudi individuals and observed that the frequencies of the 8.3, 9.5 and 11.5 kb alleles were 0.01, 0.45 and 0.54 respectively. These data were compared to data obtained from a Western group, consisting of American and European caucasian expatriates working in Riyadh. The frequencies for the Sst I RFLPs in these two groups are shown in the table. Chi-squared analysis of these data (applying the Yates correction factor) indicate a significant difference in the 9.5 and 11.5 kb allelic frequencies between the two groups ($p < 0.01$).

Recently, RFLPs have gained increasing importance as possible markers for predisposition to genetic disease. In particular, proto-oncogene RFLPs have been investigated for their association with neoplastic disease[9,10] and, as *ETS 1* has been implicated in ALL, we investigated whether there is any evidence for the association of any of the *ETS 1* RFLPs that we have identified, with ALL. In total, 14 Saudi patients were studied but no significant difference was observed between them and the non-leukemic Saudi population ($p > 0.1$). It appears therefore that there exists a Sst I – RFLP in the *ETS 1* proto-oncogene which occurs at a much higher frequency in the Saudi population than it does in Western individuals. There is, however, no evidence that such RFLPs are associated with the incidence of ALL in this population. These data also demonstrate however that when correlations between RFLP frequencies and disease incidence are investigated it is important to consider the racial backgrounds of both the patients and the individuals in the control group.

Table 1. *ETS 1* RFLP frequencies in Saudi and Western samples

Sst I fragment (kb)	RFLP frequencies		
	Western	Saudi	Saudi ALL patients
11.5	0.72	0.54	0.54
9.5	0.26	0.45	0.46
8.3	0.02	0.01	0.00

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