

**Fig. 4** Protective effect of JM8-36 immunization. LOU rats immunized as described in Fig. 2 legend, giving positive immunofluorescence reactions and significant eosinophil-dependent cytotoxicity, were infected with 800 *S. mansoni* cercariae. Parasite burdens were measured 3 weeks later by liver perfusion<sup>16</sup>. The number of worms obtained from rats immunized with JM8-36 AB<sub>2</sub> antibodies (■) was compared with those obtained from control groups, that is LOU rats injected with physiological saline (▨) or with IgM purified from normal rat serum (□). The percentage of protection was calculated by the formula  $(a - b)/a \times 100$ , where  $a$  = the number of worms obtained from the saline-injected control group and  $b$  = the number of worms recovered from AB<sub>2</sub>-immunized rats.

Thus, immunization with anti-idiotypic antibodies represents an alternative approach to immunization against pathogens. Although recent studies have produced encouraging results<sup>8-12</sup> concerning the potential substitution of conventional vaccines by anti-idiotypic antibodies, this strategy is only in its early stages<sup>13</sup>. In the context of schistosomiasis, idiotypic vaccines could be of particular use when relevant protective epitopes cannot easily be produced by the modern tools of molecular biology. Although the rat is a non-permissive host, there is now clear evidence<sup>14</sup> that all the specific effector mechanisms of immunity described in this model also occur in human infection. The possibility of using such immunization procedures in humans remains unexplored and cannot be directly extrapolated from experimental infections. However, work in progress in our laboratory, indicating the existence of cross-reacting idiotypes in human schistosome infection, is encouraging.

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## Individual-specific 'fingerprints' of human DNA

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Simple tandem-repetitive regions of DNA (or 'minisatellites') which are dispersed in the human genome frequently show substantial length polymorphism arising from unequal exchanges which alter the number of short tandem repeats in a minisatellite<sup>1-4</sup>. We have shown previously that the repeat elements in a subset of human minisatellites share a common 10-15-base-pair (bp) 'core' sequence which might act as a recombination signal in the generation of these hypervariable regions<sup>5</sup>. A hybridization probe consisting of the core repeated in tandem can detect many highly polymorphic minisatellites simultaneously to provide a set of genetic markers of general use in human linkage analysis<sup>5</sup>. We now show that other variant (core)<sub>n</sub> probes can detect additional sets of hypervariable minisatellites to produce somatically stable DNA 'fingerprints' which are completely specific to an individual (or to his or her identical twin) and can be applied directly to problems of human identification, including parenthood testing.

Three human minisatellites, termed 33.5, 33.6 and 33.15, each comprised of tandem repeats of various versions of the core sequence, have been cloned previously and characterized<sup>5</sup> (Fig.

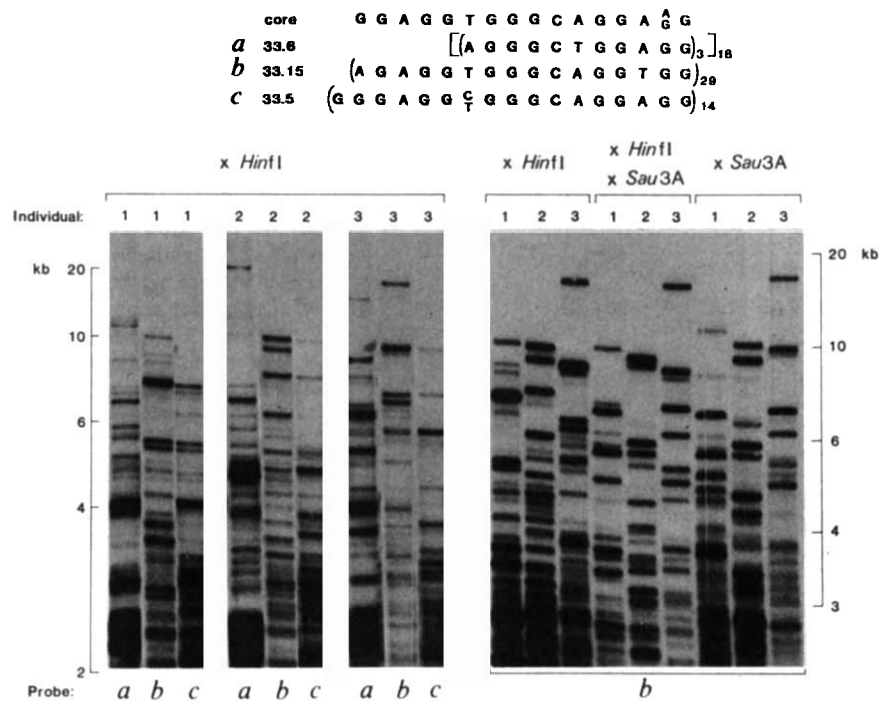
**Table 1** Similarities of DNA fingerprints between random pairs of individuals

Probe	DNA fragment size (kb)	No. of fragments per individual ±s.d.	Probability x that fragment in A is present in B	Maximum mean allelic frequency/homozygosity
33.6	10-20	2.8 ± 1.0	0.11	0.06
	6-10	5.1 ± 1.3	0.18	0.09
	4-6	5.9 ± 1.6	0.28	0.14
33.15	10-20	2.9 ± 1.0	0.08	0.04
	6-10	5.1 ± 1.1	0.20	0.10
	4-6	6.7 ± 1.2	0.27	0.14

Samples (8 µg) of blood DNA<sup>6</sup> taken from a random sample of 20 unrelated British caucasians were digested with *Hinf*I and Southern blot hybridized with minisatellite probes 33.6 or 33.15 as described in Fig. 1 legend. Each DNA fingerprint (individual A) was compared with the pattern in the adjacent gel track (individual B), and the number of bands in A which were clearly absent from B, plus those which had a co-migrating counterpart of roughly similar autoradiographic intensity in B, were scored. The data shown are averages for all pairwise comparisons. A small proportion (~6%) of additional weakly hybridizing fragments in A were matched by strongly hybridizing fragments in B, and because in such cases it was not possible to decide whether the band in A was also present in B, such fragments were ignored. If co-migrating bands in A and B are always identical alleles of the same minisatellite locus, then the probability  $x$  that an allele in A is also present in B is related to the frequency  $q$  of that allele by  $x = 2q - q^2$ . As the allele frequency is low, then  $q^2 \ll q$  and therefore the mean probability  $\bar{x}$  is approximately related to the mean allele frequency  $\bar{q}$  by  $\bar{x} \approx 2\bar{q}$ . Furthermore, assuming that there is little variance in  $q$  between alleles, then the number of alleles  $n \approx 1/\bar{q}$  and the mean homozygosity is therefore approximately given by  $\sum_1^n q_i^2 \approx n\bar{q}^2 = \bar{q}$ . In practice, an (unknown) proportion of co-migrating bands in A and B will be derived by chance from different minisatellite loci, and thus the estimates of mean allele frequency and homozygosity are maximal and depend on the electrophoretic resolution of minisatellite fragments. Probability estimates: the mean probability that all fragments detected by probe 33.15 in individual A are also present in B is  $0.08^{2.9} \times 0.20^{5.1} \times 0.27^{6.7} = 3 \times 10^{-11}$ .

**Fig. 1** Hypervariable fingerprints of human DNA. DNA samples prepared from three individual placentae (1-3) were digested with *Hinf*I and/or *Sau*3A and Southern blot hybridized with <sup>32</sup>P-labelled single-stranded DNA probes prepared from M13 recombinants 33.5, 33.6 or 33.15, each of which contains a human minisatellite consisting of tandem repeats of closely related variants of the consensus sequences shown; the repeat unit in 33.6 is in turn a diverged trimer<sup>5</sup>. Each probe produces a different fragment pattern whose complexity is largely independent of the tetranucleotide restriction endonuclease used. Resolution of polymorphic fragments less than 4 kb long is improved in double digests with *Hinf*I plus *Sau*3A, due to the elimination of background hybridization caused presumably by relatively diverged and invariant *Hinf*I minisatellite fragments<sup>5</sup> which have accumulated *Sau*3A cleavage sites within one or more repeat units. In double digests, the number of resolvable polymorphic fragments detected by probe 33.15 can be increased from ~15 to ~23 per individual, at the expense of losing ~20% of long single-digest minisatellite fragments which presumably contain a *Sau*3A cleavage site in most or all repeat units.

**Methods.** DNA was isolated from fresh human placentae as described elsewhere<sup>5</sup>. Samples (8 µg) of DNA were digested with *Hinf*I and/or *Sau*3A, in the presence of 4 mM spermidine trichloride to aid complete digestion, recovered after phenol extraction by ethanol precipitation, and electrophoresed through a 20-cm long 0.6% agarose gel at 30 V for ~24 h, until all DNA fragments <1.5 kb long had electrophoresed off the gel. DNA was then transferred by blotting to a Sartorius nitrocellulose filter<sup>7</sup>. High specific activity (>10<sup>9</sup> c.p.m. <sup>32</sup>P per µg DNA) single-stranded M13 probes were prepared as described previously<sup>5</sup>. The precise probes used were: 33.5, a 220-nucleotide *Hae*III fragment containing the minisatellite plus 60 nucleotides of flanking human DNA, subcloned into the *Sma*I site of M13mp8 (ref. 8); 33.6, a 720-nucleotide *Hae*III fragment containing the minisatellite plus 50 nucleotides of flanking human DNA subcloned into the *Sma*I site of M13mp8; 33.15, a 592-nucleotide *Pst*I/*Aha*III fragment containing the minisatellite plus 128 nucleotides of flanking human DNA subcloned into M13mp19 DNA digested with *Pst*I plus *Sma*I. Southern blot hybridization and washing were performed in 1×SSC at 65 °C as described previously<sup>5</sup>. Filters were autoradiographed at room temperature without an intensifier screen for 4 days.



1). Probe 33.15 has been shown to hybridize to multiple hypervariable fragments in *Hinf*I digests of human DNA<sup>5</sup>. Probes 33.5 and 33.6 also detect a complex set of hypervariable regions in human DNA (Fig. 1). Probes 33.5 and 33.15 contain repeats of a similar version of the complete core sequence and consequently produce similar, but not identical, DNA fingerprints. In contrast, probe 33.6 is comprised of a shortened derivative of the core and hybridizes to a largely novel set of hypervariable fragments. Probe 33.15 detects ~15 resolvable hypervariable fragments per individual in the 4–20-kilobase size range, whereas probe 33.6 detects ~11 additional fragments of this size which are not detected by probe 33.15. Probe 33.5 hybridizes on average to about two further large fragments not detected by probes 33.6 or 33.15.

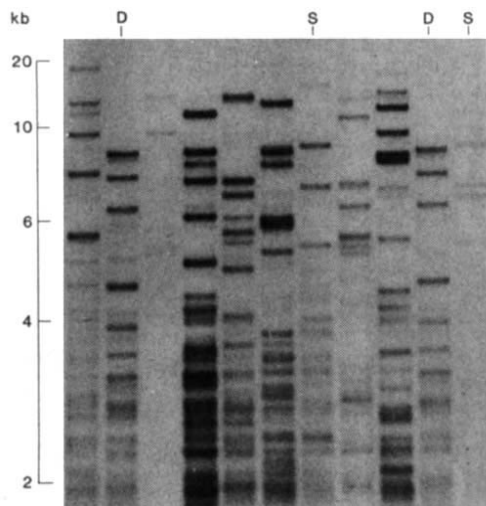
The DNA fingerprint pattern for the longest hypervariable fragments is largely independent of the 4-bp recognition restriction endonuclease used (Fig. 1). This strongly suggests that these large fragments are not derived from longer minisatellites, but that each contains a complete long homogeneous minisatellite devoid of restriction endonuclease cleavage sites and flanked by human DNA containing the normal high density of 4-bp cleavage sites. This is in agreement with previous data showing that most of these large minisatellite fragments are unlinked and segregate independently in pedigrees<sup>5</sup>.

To determine the variability of DNA fingerprints, DNA samples from a panel of 20 unrelated British caucasians were digested with *Hinf*I and fingerprinted using probes 33.6 and 33.15 (Table 1). Pairwise comparisons of the DNA fingerprints showed that the minisatellite patterns were highly specific to an individual, and that few fragments are shared between two randomly selected individuals. The probability of shared bands increases for smaller minisatellite fragments, probably resulting from lower genetic variability (higher allele frequencies) of these loci<sup>5</sup> combined with the fortuitous co-migration of unrelated

minisatellite fragments. From the degree of band sharing, one can obtain maximal approximate estimates of mean allele frequency and homozygosity (Table 1). For the longest minisatellite fragments in particular, the mean allele frequency is very low (<0.04) and the mean heterozygosity rises to >96%. This is consistent with previous pedigree analysis which has shown that most of these large hypervariable DNA fragments are present in the heterozygous state<sup>5</sup>.

The data in Table 1 allow us to estimate the individual specificity of a DNA fingerprint. For probe 33.15, the probability that all 15 resolved fragments in the 4–20-kb size range in an average individual A are also present in a second unrelated individual B is  $3 \times 10^{-11}$  (see Table 1 for details of probability estimates); the probability that the fingerprints of A and B are identical, that is, that all fragments less than 4 kb also match and that B does not possess any additional 4–20-kb fragments, is therefore  $\ll 3 \times 10^{-11}$ . Similarly, the probability that A and B have identical fingerprints for both probes 33.15 and 33.6 is  $\ll 5 \times 10^{-19}$ . If individuals A and B are related, the chance of fingerprint identity is increased, to a maximum for parents/offspring and sibs. For nonconsanguineous marriages, as the heterozygosity for each fragment is very high, the probability that a hypervariable fragment in sib A is present in sib B is ~1/2, and thus the probability that all 15 resolved bands detected by probe 33.15 in sib A are present in sib B is  $\sim 2^{-15} = 3 \times 10^{-5}$  ( $\sim 10^{-8}$  for both probes 33.15 and 33.6). These DNA fingerprints are therefore almost totally individual-specific, even within a single family (except for identical twins, see below).

The DNA fingerprints obtained using these core minisatellite probes are reproducible and are suitable for individual identification. In addition, sufficient DNA (0.5–5 µg) can be isolated rapidly from a single drop of human blood for DNA fingerprinting. This is illustrated in Fig. 2, in which DNA fingerprints were produced from a randomized panel of individuals, including

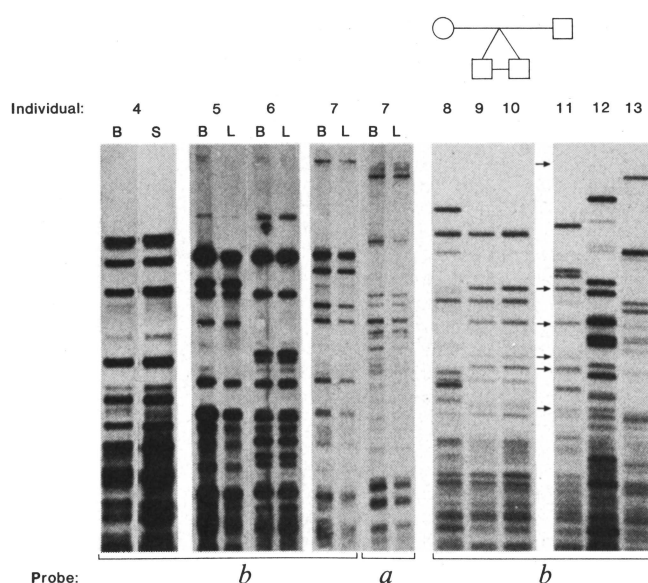


**Fig. 2** Individual identification using DNA fingerprints from small samples of blood. DNA was prepared by a rapid procedure using one or two drops of blood from a panel of 11 individuals and digested with *Hinf*I; DNA fingerprints were prepared as described in Fig. 1 legend, using 33.15 as a probe. The panel consisted of nine unrelated individuals, two of whom had been previously DNA fingerprinted, plus two sisters. The autoradiograph was inspected by a colleague who was unaware of the order of samples on the autoradiograph. He correctly identified the two previously fingerprinted individuals on the basis of pattern identity, as well as identifying the two sisters, who have several bands in common (tracks S). He also correctly deduced the fact that duplicate samples had been taken from another individual (tracks D). **Methods.** One or two drops (30–100  $\mu$ l) of blood were collected from a fingerprick into a 1 ml 1  $\times$  SSC in an Eppendorf centrifuge tube. Cells were pelleted in an Eppendorf microfuge for 1 min, haemolysed by rapid suspension in 1 ml water and immediately made isotonic by the addition of 0.25 ml 5  $\times$  SSC. White blood cells, nuclei and red cell ghosts were pelleted by centrifugation for 3 min, resuspended in 0.2 ml 0.2 M Na-acetate pH 5.6 and lysed by the addition of SDS to 1%. The lysate was extracted twice with phenol/chloroform and DNA collected by two rounds of ethanol precipitation at room temperature. DNA was dissolved in 20  $\mu$ l water and digested with *Hinf*I in the presence of 4 mM spermidine trichloride at 37°C for 1 h. Electrophoresis and hybridization were performed as described for Fig. 1.

two people whose DNA had been fingerprinted previously, and two sisters. The two previously characterized individuals could be readily and unambiguously identified on the basis of DNA fingerprint comparisons, as could the two sisters, who have a substantial number of minisatellite fragments in common.

We have shown previously that these hypervariable minisatellite fragments are stably inherited in a mendelian fashion, and that the mutation rate to a new length allele is low (of the order of 0.001–0.004 per locus per gamete for the longest minisatellite fragments)<sup>5</sup>. Several experiments show that these DNA fingerprints are also somatically stable, an essential prerequisite for identification purposes (Fig. 3). Thus, the DNA fingerprints for sperm and blood DNA are indistinguishable, as are the patterns of monozygous twins. Furthermore, the patterns appear to be stably maintained in cultured cells, as shown by comparing the DNA fingerprints of blood DNA with DNA isolated from Epstein-Barr virus-transformed lymphoblastoid cell lines derived from the same individual.

The DNA fingerprints produced by minisatellite probes 33.6 and 33.15 are therefore sufficiently stable and individual-specific for use in human identification in, for example, forensic medicine, and could be used for the routine identification and authentication of human cell lines in culture. They also provide a reliable method for paternity testing (see Fig. 3). Approximately half of the polymorphic minisatellite fragments in an offspring are derived from the father, and these paternal frag-



**Fig. 3** Somatic stability of DNA fingerprints and their use in paternity testing. Patterns of hypervariable DNA fragments are compared between blood (B) and sperm DNA (S) of individual 4, and between blood DNA (B) and DNA isolated from transformed lymphoblastoid cell lines (L) derived from related individuals 5–7 (6 is the daughter and 7 is the maternal aunt of woman 5; the kinship is evident in the number of fragments shared by these individuals). DNA fingerprints are also shown for blood DNA from identical twins (9, 10) and are compared with their mother (8), father (11) and two unrelated men (12, 13). Resolved paternal DNA fragments in the twins (arrowed) were identified by eliminating maternal bands, and are all present in the father but not in individuals 12 or 13.

**Methods.** Lymphoblastoid cell lines transformed by Epstein-Barr virus<sup>9</sup> and stored in liquid nitrogen were re-established in liquid culture after 2 yr. These cultured lymphocytes were washed twice in normal saline; DNA from the lymphocyte pellet and from white blood cells was prepared as described elsewhere<sup>6</sup>. Sperm DNA was prepared similarly, except that sperm collected from semen were treated with 1 M 2-mercaptoethanol for 5 min at room temperature before lysis with SDS. DNA fingerprints were prepared as described for Fig. 1, using 5- $\mu$ g samples of DNA digested with *Hinf*I and hybridized with probe 33.6 (a) or 33.15 (b).

ments can be identified by comparison of the mother's and offspring's DNA fingerprints. All of these paternal fragments must be present in the father (allowing for a possible rare new mutation). Because, in practice, probe 33.15 will detect approximately six resolved paternal fragments in the 4–20-kb range (Table 1, Fig. 3), we can use the data in Table 1 to estimate the probability that incorrect paternity will not be detected, that is, that the putative father will by chance possess all six paternal-specific DNA fragments. This probability is low ( $\sim 5 \times 10^{-5}$ ) if the putative father is not related to the true father, but increases to  $\sim 2^{-6} = \sim 0.016$  if they are closely related (brothers, father/son). If both probes 33.6 and 33.15 are used, these probabilities are reduced to  $\sim 4 \times 10^{-8}$  and  $\sim 8 \times 10^{-4}$ , respectively, although the precise probabilities will depend on the exact resolution and complexity of the DNA fingerprint patterns obtained, and will be improved if additional paternal fragments <4 kb long can be identified (for example, by using double digests, Fig. 1). We conclude that in the vast majority of cases, the combined use of probes 33.6 and 33.15 will be sufficient to identify cases of incorrect parenthood. An interesting corollary is that these DNA fingerprints could be used with an equal level of reliability to establish true biological parentage.

This work is the subject of a UK patent application. Enquiries should be addressed to the NRDC. We thank John F. Y. Brookfield for helpful discussions, Dr R. S. Pereira for help and advice with lymphoblastoid cell lines, and Dr G. Corney and many

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## DNA restriction fragments associated with $\alpha_1$ -antitrypsin indicate a single origin for deficiency allele *PIZ*

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The  $\alpha_1$ -protease inhibitor, or  $\alpha$ -antitrypsin (AAT), a major plasma inhibitor of leukocyte elastase and bacterial proteases, is encoded at the *PI* locus on chromosome 14 (14q24.3-q32.1)<sup>1</sup>. A deficiency of AAT in individuals homozygous for the *PIZ* allele occurs in about 1 in 2,000-8,000 caucasians<sup>2</sup> and is associated with an increased risk of early adult onset emphysema<sup>3</sup> and liver disease in childhood<sup>4</sup>. We have now used DNA polymorphisms associated with the AAT gene to investigate the origin of the *PIZ* allele. Using two genomic probes<sup>5</sup> extending into the 5' and 3' flanking regions, respectively, we have identified eight polymorphic restriction sites. Extensive linkage disequilibrium occurs throughout the probed region with the *PIZ* allele, but not with normal *PIM* alleles. The *Z* allele occurs mainly with one haplotype, indicating a single, relatively recent, origin in caucasians.

Venous blood samples were collected in EDTA from 32 normal unrelated controls, 26 patients with AAT deficiency (PI type ZZ), and 15 pairs of parents and 16 sibs of patients with AAT deficiency. PI type ZZ patients included 12 adults with emphysema, 3 adults with liver disease, 9 children with liver disease and 2 healthy adults. Relatives of 16 normal controls were studied: members of 2 normal two-generation families with a total of 7 children, and 53 members of a three-generation family of a PI ZZ proband which included 12 unrelated spouses. The genetic type of AAT (PI type) was determined by isoelectric focusing of plasma in acrylamide gels, pH 3.5-6 (ref. 6). Blot hybridization of leukocyte DNA digested with specific enzymes

**Table 1** Allele frequencies for 5' DNA polymorphisms (4.6-kb probe)

Restriction enzyme	Alleles (kb)	Allele frequency		PIC*
		+	-	
<i>SstI</i>	1.8, 1.9	0.649	0.351	0.35
<i>MspI</i>	0.95, 0.98	0.506	0.494	0.38
<i>AvaII</i>	0.9, 1.1	0.636	0.364	0.36

\* Polymorphism information content, calculated according to the formula of Botstein *et al.*<sup>21</sup>, expressing the extent of variability at each locus.

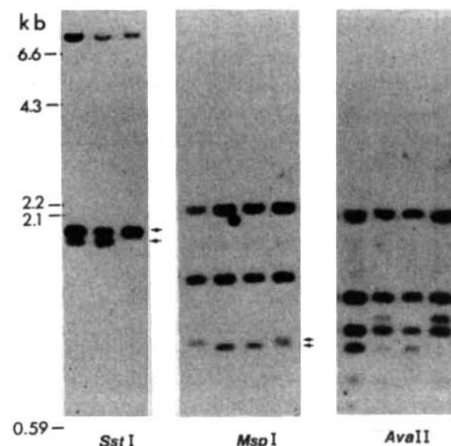
is described in Fig. 2 legend. The 5' probe (4.6 kilobases, kb) and 3' probe (6.5 kb) have been described elsewhere<sup>7</sup> (Fig. 1), both having been subcloned from phage clone  $\alpha$ AT35<sup>5</sup>.

Of 10 restriction enzymes tested with the 5' 4.6-kb probe, 3 showed restriction site polymorphisms; these were *SstI*, *MspI* and *AvaII*. For each enzyme, one or both of two possible restriction fragments were observed. When a particular restriction site was present (designated +), a shorter DNA fragment was observed than when the restriction site was absent (designated -). Family studies indicated that the pair of DNA fragments for each enzyme were alleles. Among 32 unrelated normal controls, the allele frequencies were as shown in Table 1. The 4.6-kb genomic probe has been completely sequenced<sup>8</sup>, so the positions of the restriction sites were identified. Fragments of the expected sizes were observed. The distances between restriction sites were 2 kb between *SstI* and *MspI* sites, and 0.2 kb between *MspI* and *AvaII* sites.

A  $\chi^2$  analysis of 2x2 tables, or exact calculation of probabilities where required<sup>9</sup>, was used to examine the data for possible association between a specific allele at one site and a specific allele at each of the other two sites. No significant association between alleles was observed, indicating free recombination between the three restriction sites, with no evidence



**Fig. 1** The  $\alpha_1$ -antitrypsin gene and adjacent flanking regions, described previously<sup>4,17</sup>. Solid areas are coding regions, dots indicate untranslated regions, open areas are introns. Restriction sites for the enzymes *EcoRI* and *BamHI* (B) are indicated. \*, Polymorphic restriction sites for *SstI* (S), *MspI* (M) and *AvaII* (A). The two genomic probes used in this study are shown above the gene.



**Fig. 2** Patterns of DNA fragments are shown for the three enzymes *SstI*, *MspI* and *AvaII*, using the 4.6-kb 5' probe. DNA extracted from leukocytes was completely digested with specific restriction enzymes according to conditions recommended by the manufacturer. Fragments were separated by size in 0.8% agarose gels. DNA fragments were transferred to Biodyne membranes (Pall). Probes were labelled with <sup>32</sup>P using a nick-translation kit (Amersham). After prehybridization, <sup>32</sup>P-labelled probe (3.0x10<sup>7</sup> counts per filter) was hybridized overnight. Filters were rinsed twice in 2xSSC (SSC: 1.5x10<sup>-4</sup> M NaCl, 1.5x10<sup>-5</sup> M Na-citrate). Membranes were washed in 2xSSC at 60 °C for 45 min, then twice in 0.1% SDS in 0.1xSSC at 60 °C for 45 min, rinsed briefly in SSC, blotted, then exposed to X-ray film for 2-3 days. Fragment sizes in kb are indicated on the left. Anode is at the bottom. Arrows mark the polymorphic fragments. From left to right, lanes show the following allele designations: +-, ++, --, --, ++, ++, +-; ++, +-, ++, --.