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ORIGINAL ARTICLE

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Population genetic and family data for the human minisatellite locus D16S309 (MS205) in Germans

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Abstract The distribution of restriction fragments at the DNA minisatellite locus D16S309 was estimated by investigating blood samples from 2617 unrelated West German Caucasians and 1269 offspring. Furthermore segregation of fragments was studied in a large family and in trios. Altogether 2296 meioses were studied, revealing 7 paternal and 3 maternal mutations. Inspection of "phenotypes" did not reveal any remarkable deviation from Hardy-Weinberg equilibrium.

Key words DNA · D16S309 · Population genetic data

Introduction

The extreme polymorphism and the mendelian inheritance of variable number of tandem repeat (VNTR) regions make them well suited for parentage casework. The value and impact of investigations of the polymorphic VNTR regions in paternity testing has been documented by many authors [2–4, 10–13, 15, 18, 19].

This paper aims at reporting population genetic key notes, such as "allele" frequency distribution, heterozygozity rate, power of exclusion of non-fathers, mutation rate, and Hardy-Weinberg equilibrium analysed in the Hinf I-polymorphism of locus D16S309 [17].

Materials and methods

We have tested single individuals, a multi-member family, and people involved in paternity cases. All of them were German Caucasians. In parentage cases, paternity was established by conventional hemogenetic means and by means of DNA analyses [5, 9–12]. Exclusions from paternity were based on at least 2 contradictions to the general accepted genetic pathways.

Genomic DNA was isolated from peripheral white blood cells according to the non-toxic extraction method described by Miller et al. [14]. DNA samples (5 µg) were digested with the restriction enzyme Hinf I at a concentration of 6U/µg DNA according to the manufacturer's recommendations.

Separation of DNA fragments was achieved by electrophoresis in 0,7% agarose gels (20 × 20 cm) in TBE buffer (0.134 M Tris-base, 74.9 mM boric acid, 2.55 mM EDTA-Na, pH 8.8) for approx. 18 h at 0.9 V/cm. Electrophoresis was stopped when the 2.0 kb marker fragments had migrated 12 cm.

Probe MS205 detects a VNTR polymorphism at locus D16S309 [1, 17]. Meaningful polymorphisms can be detected in genomic DNA samples digested with MboI and HinfI but cannot be detected in HaeIII, RsaI or HpaII genomic digests as these enzymes cut within the minisatellite repeat unit [17]. Probe MS205 was purchased from Zeneca Bio Products. Hybridization and detection of fragments was carried out according to manufacturer's specifications using the chemiluminescence technique. Fragments were visualized by luminescence at +37° C on X-ray films.

The migration of fragments was measured by means of a digitizing tablet (DNASYS) with a 0.5 mm resolution. Measurements were carried out twice on two different lumigraphs independently by two persons. The fragment sizes in kilobase pairs (kb) were calculated by local approximation [6]. Sizes of restriction fragments measured on at least 2 different lumigraphs were taken as basis of the determination of the standard deviation [9]. The s.d. varied from 1–3%. A genomic DNA was used as an internal blot to blot control.

Hardy-Weinberg equilibrium was scrutinized by means of a computer programme [16].

Results

Segregation of fragments

The multi-member family ST. kindly provided blood samples enabling the study of the segregation of restriction fragments (Fig. 1). As can be seen, the inheritance of fragments follows an autosomal, codominant genetic pathway.

Distribution of restriction fragments in the West German population and a comparison between adults and children

Figure 2 shows the size distribution of restriction fragments in 2617 adult random Germans and in 1269 children. Kb size measurements were carried out as described in the methods section. For practical reasons the scale was subdivided into steps of 100bp. The raw data are available on request. Figure 2 shows, that the fragments range in size from about 700bp to 5.9kb, however the majority of fragments, range from approx. 2kb to 4kb, with frequen-

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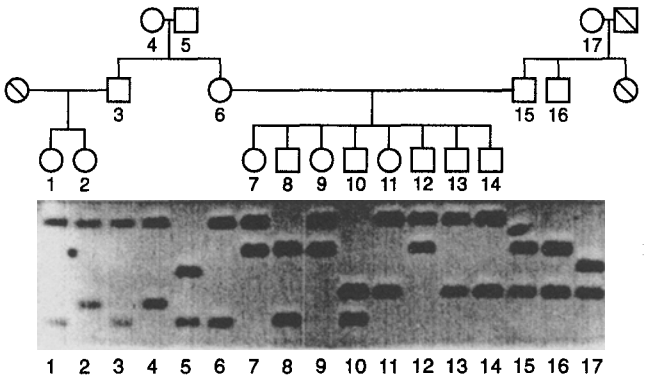

Fig. 1 Segregation of DNA fragments in the multi-member family ST

cies from about 2% to about 8%. No statistical differences in the frequency distributions between the adult group and the offspring group could be found.

Analysis of DNA samples with fragments smaller than 1 kb showed that the detection of those fragments may become difficult due to the fuzziness of the bands (data not shown).

Mutations

Our definition of a mutation is given elsewhere [8]. In 1010 paternal and 1286 maternal meioses studied we found 7 paternal and 3 maternal mutations at this locus which indicates again that the maternal chromosomes are more prone to mutations than maternal ones [8].

Heterozygosity

Heterozygosity reflects the degree of polymorphism and in parentage testing it indicates the power to exclude non-fathers from paternity. In the group of 2617 unrelated individuals (adult group) tested, 95 persons appeared to be homozygous by revealing only one fragment. All 95 DNA samples were additionally investigated in a short-time (7 h) electrophoresis in order to detect fragments smaller than 700 bp. (This step, however, cannot solve the problem of so called "close heterozygotes"). The observed heterozygozity rate at locus D16S309 was 96.4%. We compared the heterozygozity rate of the adult group with the group of offsprings from which both parental fragments were known. If the parental fragments showed small size differences, we called a child a heterozygote even if we were not able to discriminate between the two fragments. Thus it turned out that the heterozygosity rate in the offspring group was 97.6% which in not a significant difference ($\chi^2 = 0.02 < 2.706 = \chi^2_{0.005}$) to the adult group. It should be kept in mind, however, that the "true heterozygosity" is approximately 99.7% in Caucasians [1].

Exclusions and false inclusion rates

The observed exclusion rate was approximately 94%. 19 out of 311 non-fathers could not be excluded from paternity.

Hardy-Weinberg equilibrium (HWE)

HWE follows rigorously under three conditions:

1. a Mendelian pattern of inheritance (no mutations and alleles segregate independently),
2. no selection (the expected number of fertile progeny from a mating reaching maturity does not depend on the genotypes of the mates),
3. an infinite, unstructured population (i.e. mating and genotypes are uncorrelated in an infinite population).

If a population is in HW equilibrium, then the allele frequencies remain constant from one generation to another.

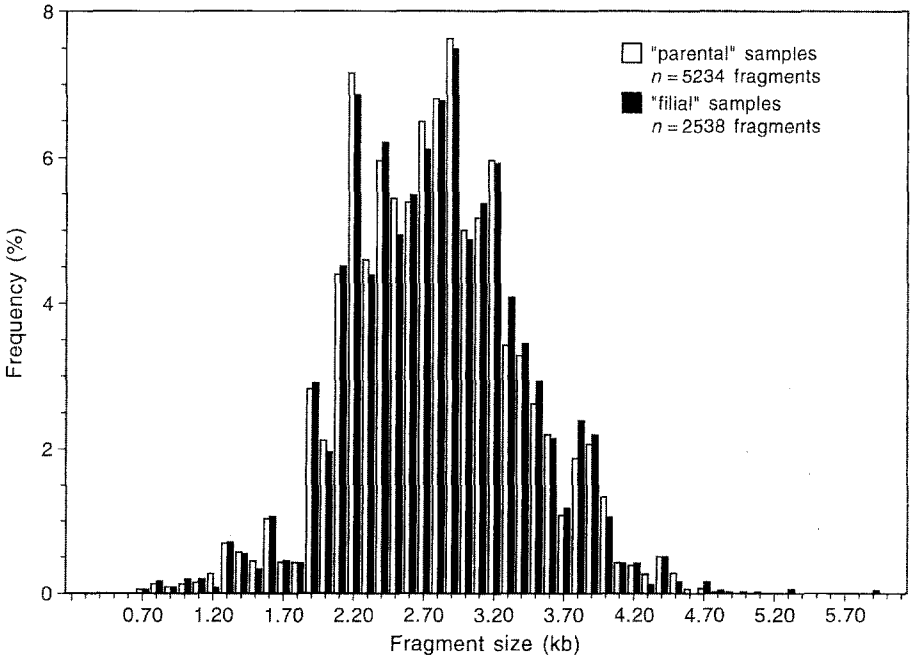

Fig. 2 Probe MS205, distribution in West Germans. Size distribution of Hinf I/D 16S309 fragments observed in 2617 adults and 1269 children

Table 1 Binning of the fragments in arbitrary bins

Bin	Kb range	Number of fragments of unrelated adults		Number of fragments of filial generation	
		observed	expected	observed	expected
1	< 1.7	196	193.3	91	93.7
2	> 1.7 ≤ 2.0	243	243.8	119	118.2
3	> 2.0 ≤ 2.1	144	144.8	71	70.2
4	> 2.1 ≤ 2.2	308	306.4	147	148.6
5	> 2.2 ≤ 2.3	328	323.9	153	157.1
6	> 2.3 ≤ 2.4	293	292.9	142	142.1
7	> 2.4 ≤ 2.5	301	284.9	122	138.1
8	> 2.5 ≤ 2.6	296	302.4	153	146.6
9	> 2.6 ≤ 2.7	267	268.0	131	130.0
10	> 2.7 ≤ 2.8	397	386.6	177	187.4
11	> 2.8 ≤ 2.9	354	361.0	182	175.0
12	> 2.9 ≤ 3.0	338	338.1	164	163.9
13	> 3.0 ≤ 3.1	261	260.6	126	126.4
14	> 3.1 ≤ 3.2	275	269.4	125	130.6
15	> 3.2 ≤ 3.3	275	286.2	150	138.8
16	> 3.3 ≤ 3.4	161	163.6	82	79.4
17	> 3.4 ≤ 3.5	178	179.8	89	87.2
18	> 3.5 ≤ 3.7	194	202.0	106	98.0
19	> 3.7 ≤ 3.9	205	215.5	115	104.5
20	> 3.9	220	210.8	93	102.8

The converse can be expected. We have thus performed a generation to generation comparison, and did not find significantly differing "allele" frequencies (Fig. 2). To test for HWE the observed "alleles" were subdivided into 20 approx. equally sized classes (Table 1) according to the empirical distribution of fragment sizes. Due to this approach we avoided classes with low allele frequencies. In order to scrutinize HWE we employed a computer programme (GENEPOP) [16], which allows an unbiased estimation of exact HW probability using the Markov chain method described by Guo and Thompson [7].

The resulting χ^2 of 215.2 with a p value of 0.101 (190 df) does not indicate any deviation from HWE. From simulations it is known that this procedure has the power to detect at least serious deviations.

Discussion

D16S309 is another DNA minisatellite locus that can meaningfully be used in parentage testing. Probe MS205 detects Hinf I fragments ranging from 0.7kb to 5.9kb with the vast majority of fragments in the range from 2kb to 4kb. This means that the majority of fragments does not overlap with fragments detected by probes MS31 and MS43A in simultaneous hybridizations.

The data on fragment frequencies and mutation rate provide a sound basis for the application of this single-locus minisatellite polymorphism to paternity testing [5].

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