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Mutation Rate in Human Microsatellites: Influence of the Structure and Length of the Tandem Repeat

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Summary

In 10,844 parent/child allelic transfers at nine short-tandem-repeat (STR) loci, 23 isolated STR mismatches were observed. The parenthood in each of these cases was highly validated (probability >99.97%). The event was always repeat related, owing to either a single-step mutation ($n = 22$) or a double-step mutation ($n = 1$). The mutation rate was between 0 and 7×10^{-3} per locus per gamete per generation. No mutations were observed in three of the nine loci. Mutation events in the male germ line were five to six times more frequent than in the female germ line. A positive exponential correlation between the geometric mean of the number of uninterrupted repeats and the mutation rate was observed. Our data demonstrate that mutation rates of different loci can differ by several orders of magnitude and that different alleles at one locus exhibit different mutation rates.

Introduction

Short tandem-repeat (STR) polymorphisms (Edwards et al. 1991*a;* Tautz and Schlotterer 1994) have become a powerful tool for human identification (Gill et al. 1994), chromosome mapping and linkage analysis (Hearn et al. 1992), and the study of molecular evolution (Meyer et al. 1995*b*) and population genetics (Bowcock et al. 1994; Brinkmann et al. 1996*a,* 1996*b*). Microsatellite instability and/or generation of new alleles can be disease related (Richards and Sutherland 1996). With regard to microsatellites, several studies exist dealing with mutation events in eukaryotes (Petes et al. 1997; Wierdl et al. 1997), but there are limited data concerning human genetics (Jin et al. 1996). Weber and Wong (1993) have typed mutation events in cell lines from CEPH families and have validated ~20 such events in di-, tri-, and tetranucleotide loci with an average mutation rate of 1.2×10^{-3}. Heyer et al. (1997) estimated Y-chromosome STR mutation rates from the analysis of pedigrees spanning several generations. They found a mean mutation rate of 0.2%, which is close to the rate found by Weber and Wong (1993). Others have performed computer simulations under several assumptions, compared these simulations to actual data, and formed conclusions regarding mutation rates and mechanisms (Valdes et al. 1993; DiRienzo et al. 1994). Several other species also have been investigated (Glenn et al. 1996). From the analysis of such data, it is obvious that single (repeat)–step mutations account for ~90% of STR mutation events, followed by double-step mutations and a very limited number of multistep mutations. The induction of mutations in minisatellites, by radiation, has been studied in the Hiroshima and Nagasaki areas (Kodaira et al. 1995) and in the Chernobyl area (Dubrova et al. 1996). Whereas the former study found no evidence for an increase in the mutation rate, the latter study detected a twofold increase. In this study, we have investigated in depth ~11,000 meioses in one pentameric and eight tetrameric loci.

Material and Methods

Approximately 16 informative systems including four or five STRs were applied in routine paternity testing. In cases of isolated STR mismatches between parent(s) and child, further in-depth analysis was performed. The first step was to include another five STRs. If the mismatch remained isolated, statistical analysis of the probability of the respective parenthood was performed. Since the discrepant system was always included in the calculation (Fimmers et al. 1992), the overall parenthood probability decreased significantly after this step, by two or more orders of magnitude. The assumption of a new mutation was made only if the final figure—that is, after inclusion of four or five additional STRs—attained or exceeded 99.97%. For cases of con-

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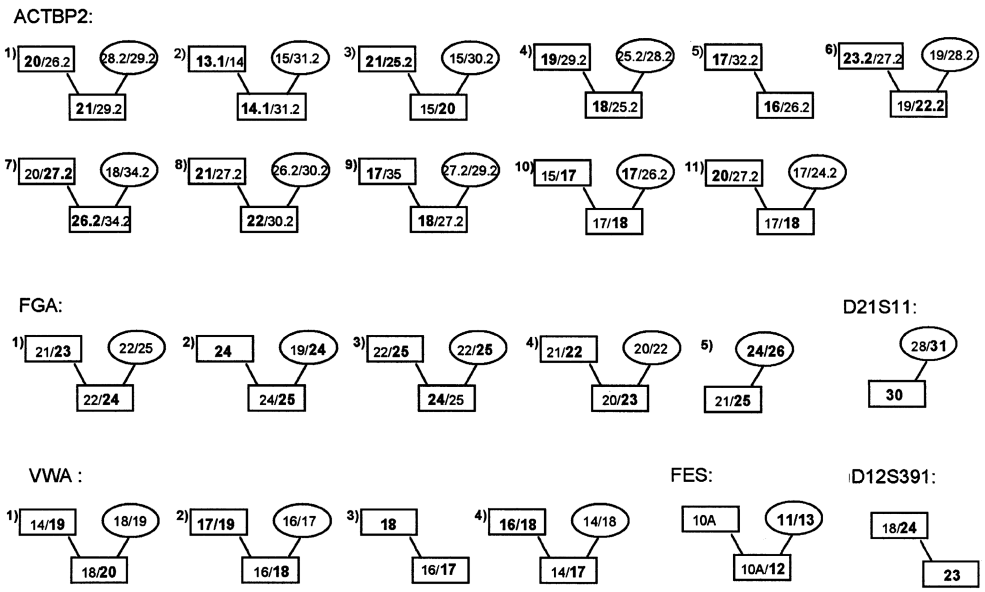


Figure 1 Pedigrees and allelic situations in 23 parent-child pairs with mutations. Numbers in boldface indicate mutation events. An oval symbol indicates the mother. For ACTBP2, example 1, the first step was to exclude the filial allele that matches one parent—for example, 29.2. The mutation event is 20 to 21. The alternative would be 26.2 to 21, 5.5 steps plus a point mutation. In example 2, one repeat gain was much more likely than a 3-bp loss, because 13.1 and 14.1 have the same basic structure (AAAG AAAAG $[AAAG]_{11-12}$). This sequence has been observed only once among >300 alleles sequenced. All other alternatives to one-step (examples 1–10) or two-step (example 11) events would be gross changes, together with hexamer insertions/losses. If both parents' allele numbers are in boldface, the origin of the mutation was unknown. If one parent has two alleles in boldface, the direction of the mutation was unknown.

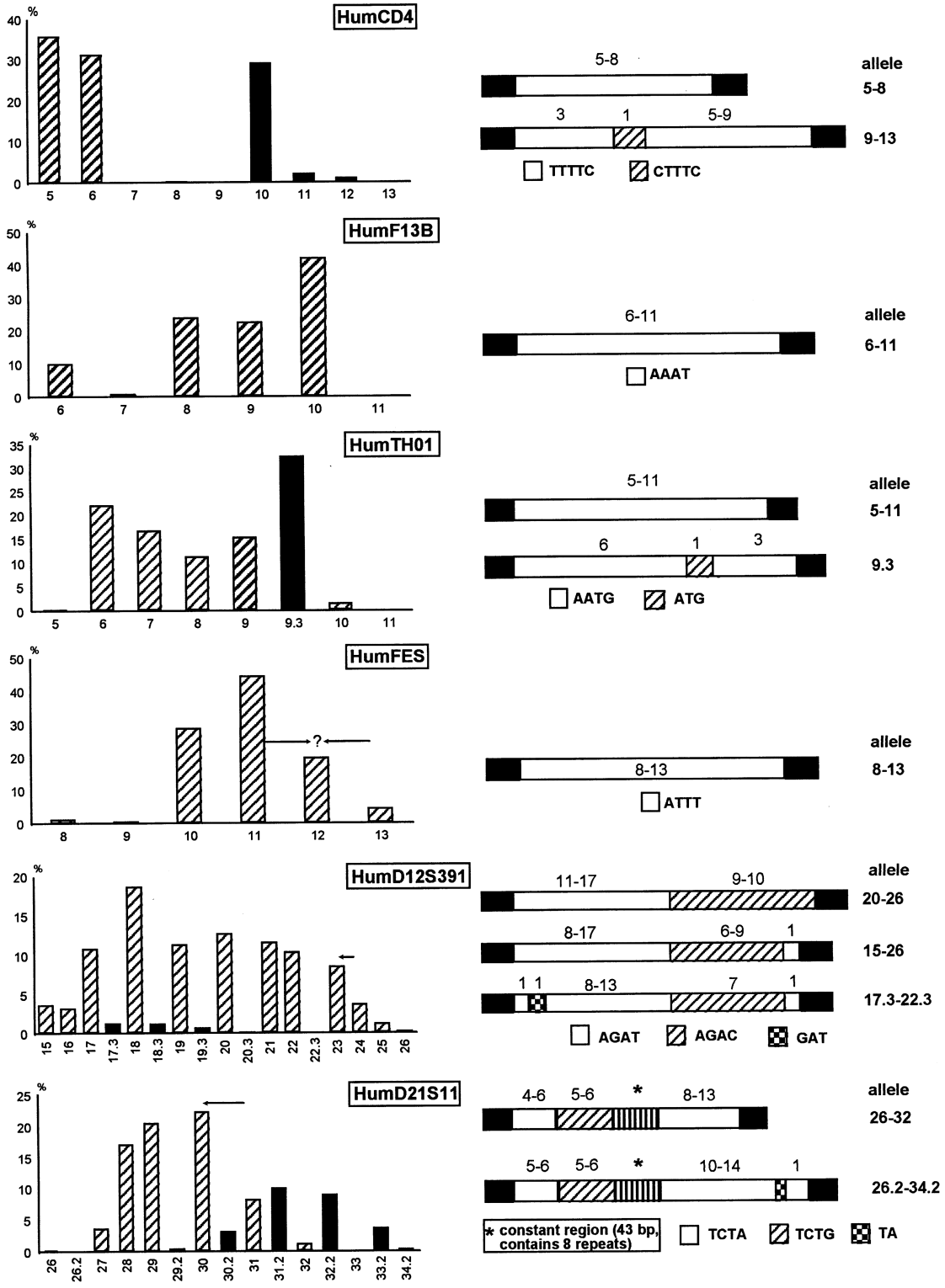
firmed new mutations, all alleles in the father, mother, and child, at the locus involved, were sequenced.

Origin

In accordance with the literature (e.g., Weber and Wong 1993) we have defined the origin of the "new" allele as follows: If there were two possibilities, the shortest mutational step was considered to be the actual one. For example, if one parental allele differed by one repeat and the other by two repeats, when compared with that of the child, we inferred a one-step mutation. Also, if for structural reasons one alternative was far more likely than the other, this step was considered to be the actual one (fig. 1). If two parental alleles exhibited the same difference when compared with the new one, the origin was declared unknown. If exclusion was possible for both parents, then the origin was declared uncertain. For further details, see figure 1.

DNA Methodology

DNA was extracted from venous-blood samples and quantified (Miller et al. 1988; Waye et al. 1989). PCR reactions were performed in accordance with the following literature: for HumTH01 (GenBank accession number D00269), Edwards et al. (1991*a*), as modified by Wiegand et al. (1993); for HumVWA (GenBank accession number M25716), Kimpton et al. (1992), as modified by Möller et al. (1994); for HumF13B (GenBank accession number M64554), Nishimura and Murray (1992), as modified by Alper et al. (1995); for HumD21S11 (GenBank accession number M84567), Sharma and Litt (1992), as modified by Möller et al. (1994); for HumCD4 (GenBank accession number M86525), Edwards et al. (1991*b*); for HumACTBP2 (GenBank accession number V00481), Polymeropoulos et al. (1992), as modified by Möller and Brinkmann (1994); for HumD12S391 (GenBank accession number G08921), Lareu et al. (1996); for HumFGA (GenBank accession number G33478), Barber et al. (1996), as modified by Rolf et al. (1997*a*); and, for HumFES (GenBank accession number M14589), Polymeropoulos et al. (1991) and Möller et al. (1994). Separation of alleles was achieved under either native gel conditions (Allen et al. 1989) or denaturing conditions (ACTBP2, D21S11, D12S391, and FGA), by application of fluorescence detection (AlfExpress, Pharmacia). Alleles were always identified by direct comparisons with sequenced allelic ladders, either visually (side by side) or by use of a computer. The measured length always exactly corresponded to the sequenced length, the precision being better than ± 0.5 bp (variation width). Thus, length differences, between alleles, of 1 bp were routinely resolved,


HumCD4
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* constant region (43 bp, contains 8 repeats)
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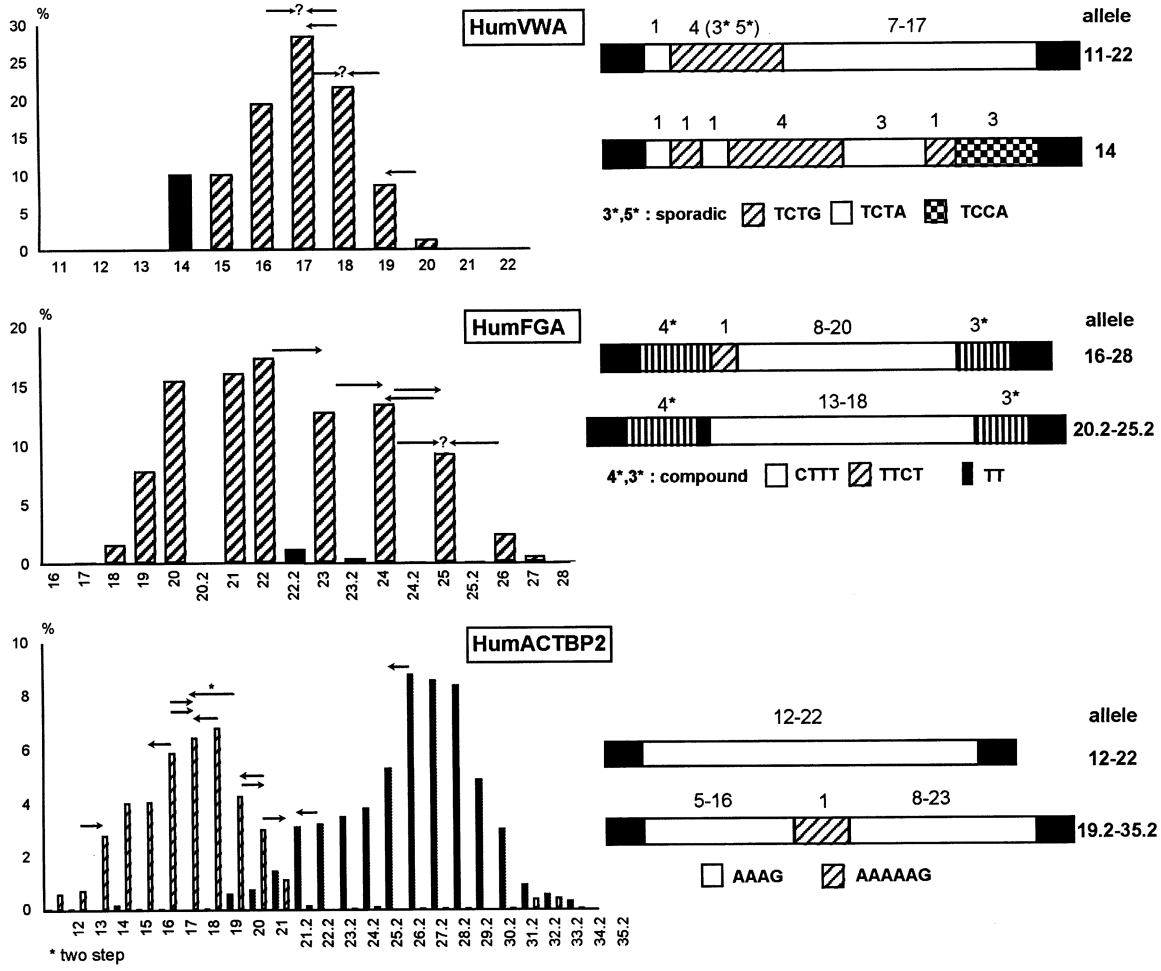

Figure 2 Allele frequencies (*left panels*) and sequences (*right panels*) for nine STRs in the German population. Regular alleles are indicated by hatched bars; and irregular alleles are indicated by blackened bars. Arrows indicate one mutation event. For D12S391, the sequences of alleles 20–26 and 15–26 were different and are represented separately, whereas their frequencies are combined in the hatched bars of the histogram. For ACTBP2, besides the insertion of one hexamer, there existed two hexamer insertions in very long alleles and also pentamer insertions. The frequencies of irregular alleles are combined in the blackened bars of the histogram. Allele frequencies are based on the following numbers of individuals: for FES, 616; for CD4, 988; for TH01, 1,712; for FGA, 1,072; for D21S11, 600; for F13B, 604; for VWA, 1,714; for D12S391, 972; and for ACTBP2, 948.

and even point mutations were detected, if native conditions were applied (Möller et al. 1994).

Sequencing

All amplified alleles of the relevant system were excised from silver-stained gels, were extracted, and subsequently were reamplified (Möller and Brinkmann 1994). Sequencing was performed by use of Taq Cycle sequencing with commercially available kits and equipment (ABI 373 Sequencer and ABI sequencing Kit, Applied Biosystems). Both strands were sequenced for confirmation, with the exception of ACTBP2, for which one strand was sequenced twice.

Results

Among 10,844 parent/child allele transfers at nine loci, we observed 23 mutations at six of the loci (table 1 and fig. 1). The pedigrees and the alleles present in the parents and the child are shown in figure 1. Approximately 98% of the paternity cases were Caucasoids living in Germany or Austria; all father-mother-child triplets showing mutations were from this major ethnic group. In figure 2, the allele frequencies and the sequence of the alleles present in the general Caucasoid population are shown. The allele frequencies are updates of previously published data sets (Meyer et al. 1995*a;* Rolf et

Table 1

Number of Meioses and Mutations, Compared with the Mutation Rate and the Heterozygosity Rate of the Nine STR Loci

Locus	Mean Length[a]	No. of Meioses[b]	No. of Mutations	Mutation Rate (%)	Heterozygosity Rate[c]
CD4	5.66	969	0	.0	.67
F13B	8.74	1,033	0	.0	.67
TH01	7.78	2,008	0	.0	.78
FES	10.79	850	1	.117	.65
VWA	10.8	2,013	4	.199	.81
D12S391	11.47	562	1	.178	.87
D21S11	11.0	557	1	.18	.86
FGA	13.84	1,246	5	.401	.86
ACTBP2	15.21	1,608	11	.684	.93

[a] The geometric mean of the number of uninterrupted repeats in the variable stretch.
[b] Total paternal and maternal allelic transfers.
[c] With regard to the German population sample in this study.

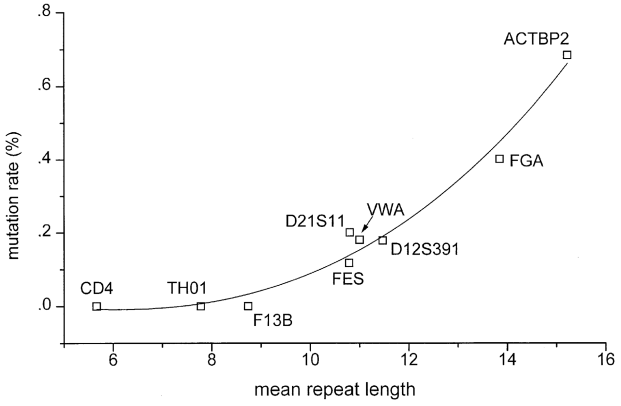

Figure 3 Correlation of mutation rate and geometric mean of the repeat lengths.

al. 1997*a;* Brinkmann et al. 1998). In addition to the length variation due to the number of repeats, sequence differences due to point mutations and insertions/deletions that interrupt the homogeneous repeat stretch are quite common at microsatellite loci (fig. 2). Sequence differences between the alleles at one locus are indicated by the shading of the bars in the figures showing frequency versus size (fig. 2). All mutations observed were either repeat losses or repeat gains. Twenty-two were single-step mutations, and one was a double-step mutation (figs. 1 and 2 [indicated by arrows]). None of the mutations observed was an insertion or deletion of an incomplete repeat. If all the repeat gains and losses are summarized, the result is the loss of two repeats; that is, in ~11,000 transfers, a small DNA loss occurred.

The mutation rate showed a positive correlation to the geometric mean of the number of variable uninterrupted repeats. The latter relation can be expressed as an exponential function (fig. 3).

In several cases, the mother or the "father" was missing from the triplet, but the overall ratio of maternal to paternal meioses was ~1, for all loci examined, with only a minor deviation ($\sim \pm 1\%$). However, the ratio of paternal versus maternal mutations was 17:3, whereas for three additional cases the origin remained unclear. At three loci, we observed 20 of the mutations (table 1 and figs. 1 and 3). The major mutational activity at these three loci was shifted slightly to the longer alleles in the unimodal distribution.

Discussion

It is now generally accepted that replication slippage (Levinson and Gutman 1987) is the major mechanism causing new mutations in microsatellites. In our 23 observations, no indication of other possible mechanisms was found. Also, in accordance with the literature, there seemed to exist a strong correlation with the number of homogeneous repeats (Wierdl et al. 1997). It has been concluded from studies of yeast that interspersed irregular repeats have an inhibitory effect on mutation events (Petes et al. 1997). This was confirmed in our study. The fact that the majority of the mutation events in our study were shifted to the longer alleles in the (unimodal) distribution profile(s) in the population examined supports the finding of the higher susceptibility of long (homogeneous) stretches to mutation events.

Structure/Length Relation

In a subgroup of three systems (fig. 2), we observed no mutations. In these systems, the allele lengths versus frequency distributions were not unimodal, and the geometric means of the number of homogeneous repeats were $\leqslant 9$. In all the other systems, we observed mutations. Their repetitive regions were longer, the geometric means of the number of repeats of homogeneous stretches being >10. In addition, their allele lengths versus frequency distributions were approximately unimodal. In some cases, bimodal distributions of alleles are caused by two subgroups of alleles with differences in the sequence and arrangement of repeats. For example, this was the case at the loci D21S11 and ACTBP2.

On the basis of the aforementioned relationships, a number of population-genetics findings are readily interpreted: The multimodal distributions (fig. 2) of the systems with low mutation rates can be explained by genetic drift and founder effects. Homogeneous repeat regions with repeat numbers <10 seem to mutate extremely seldom. Therefore, these systems can be used for ethnic-affiliation estimations (Meyer et al. 1995*a;* Shriver et al. 1997), since differences in allele frequencies

between populations are not obscured by new mutations.

Interrupted alleles (i.e., alleles with interspersed irregular units) mutate less often than uninterrupted alleles: the best example is ACTBP2, for which we observed eight mutations (rate 1.2%) in the uninterrupted alleles (40% of all alleles; depicted by the left peak in fig. 2). The geometric mean of the lengths of these alleles is 18 repeats. In contrast, only three mutations (rate 0.3%) were observed in the interrupted alleles (60% of all alleles; depicted by the right peak in fig. 2). The geometric mean of the lengths of these alleles is 28 repeats. Although the interrupted alleles are longer, they are less susceptible to mutation, evidently because their homogeneous repeat stretches have a mean length of only 14 repeats, owing to the interspersed repeats. For the same reason, VWA allele 14, which has only 3 regular repeats on either side of a variant repeat (fig. 2), hardly ever mutates. So far, no "daughter allele" of VWA allele 14 (e.g., VWA allele 13* or 15*) has been observed, even though a mutated allele 14* would migrate strikingly differently under native electrophoretic conditions and even though thousands of alleles 13 and 15 have been investigated, so far, and large numbers also have been sequenced. Similarly, no regular allele 14 has been observed; thus, repeat losses nearly never occur at allele 15, although the mutation rates of alleles 16 and 17 are rather high. Interestingly, Africans have a third group of alleles (Evett et al. 1997) with consensus structures, but with lengths <14, that are separated from the longer alleles by allele 14*. These alleles might have been lost by bottleneck effects in Europeans/Asians and have not been reconstructed by mutations. Again, this indicates that the short fragments in this STR are nearly free of mutations. Similarly, daughter alleles of TH01 9.3—namely, 6.3, 7.3, 8.3, 10.3, etc.—have been detected only sporadically (Evett et al. 1997; Klintschar et al. 1998). The same considerations apply to allele CD4 10 in Caucasoids. On the other hand, irregular units in the flanking region of the proper repeat region or close to it (e.g., FGA and D21S11 alleles denoted by the suffix ".2") do not appear to have a strong inhibitory effect on mutations, as can be derived from the distribution profiles for which significant numbers of daughter alleles were observable.

Compound Repetitive Structures

Three loci in our survey (FGA, VWA, and ACTBP2) have multiple repeat-like structures, in both flanking regions, that do not exhibit length variability (Möller et al. 1994; Barber et al. 1996; Rolf et al. 1997*b*). The loci D12S391 and D21S11 have two and three regions, respectively, that express length variability. Interestingly, in our study, these five loci had the highest mutation rates and the longest homogeneous (uninterrupted) repeat regions. Thus, a question arises: does the repetition surrounding the so-called compound structure of the repeat region influence the mutation rate as well? Within one system, the mutation rate strongly correlates to the number of repeats, since longer alleles mutate more often. Thus, it is possible that compound structures are only an accompanying or side phenomenon without any triggering function for new mutations. However, we cannot rule out completely the possibility that these compound structures can (over long evolutionary periods) trigger, for example, the growth of homogeneous stretches, to an extent that makes them more susceptible to repeat slippage.

We observed a small overall DNA loss in our study, although this observation may have been due to the limited sample size. The major mutation activity was found in the longer alleles and could have led to some distortion of the pure Gaussian shape of the allele length versus frequency distribution, with flatter foothills mainly on the right-hand side(s), if these alleles mutate in both directions with the same probability. This would coincide with the observation that the average number of repeats present in various primate species is lower than that present in humans and correlates negatively with their divergence time from humans (Meyer et al. 1995*b*). However, the conservation of a Gaussian shape for the distribution for humans would require an excess of gains over losses on the left-hand side of the profile and an excess of losses over gains on the right-hand side. Interestingly, of the 13 mutations with known direction, on the right-hand side of the profile, 8 are repeat losses, and only 5 are repeat gains. Our observation seems to apply mainly to autosomal tetrameric (and one pentameric) microsatellites with high AT and AG proportions. We suggest that such events in Y-chromosome STRs should be evaluated more thoroughly before analogies can be established.

Sex and Age Influence

There obviously exists a sex difference for mutations, at a ratio of ~17:3 between males and females. In addition, the father's age seems to influence the mutation rate: the mean age ($\pm$SD) of all the fathers in our data set was 28.7 $\pm$ 7.6 years, whereas the mean age of the fathers who were involved in the mutation events was 32.5 $\pm$ 7.8 years. This difference is significant, with $P = .01$ in a *t*-test.

The difference is associated with different numbers and types of cell divisions in germ-cell genesis; oogonia undergo ~22 divisions before meiosis starts and oocytes are generated. Spermatogonia renew continuously by mitosis, and some continue through meioses, before they become sperm cells; thus, the sperm cells of older men

have undergone more divisions than the cells of younger men. For a 29-year-old man, one can assume ~350 divisions (Vogel and Motulsky 1997). Therefore, the DNA in a "typical" sperm cell from a father in our study was replicated ~16 times more often than the DNA in an oocyte. Consequently, the mutation rate, expressed per mitosis, seems to be somewhat lower in males. However, owing to the small number of mutation events observed in the females in our study, this difference is not very significant.

Taken together, most of the variation at microsatellite loci can be assumed to be caused by males. This is in accordance with observations of other species in which higher rates for males also have been described (Shimmin et al. 1993). Interestingly, in a recent study, Ellgreen and Fridolfsson (1997) were able to demonstrate that very similar sex relationships also have been found in birds, although the females are heterogametic and the males are homogametic, for the W and Z sex chromosomes, respectively. Weber and Wong (1993) also found a very similar relationship (~5:1 to 6:1) during their study of CEPH reference families.

Acknowledgments

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

GenBank, http://www.ncbi.nlm.nih.gov/Web/Genbank (for the following loci: HumTH01 [D00269], HumVWA [M25716], HumF13B [M64554], HumD21S11 [M84567], HumCD4 [M86525], HumACTBP2 [V00481], HumD12S391 [G08921], HumFGA [G33478], and HumFES [M14589])

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