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Mutation of human short tandem repeats

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A total of 20,000 parent-offspring transfers of alleles were examined through the genotyping within 40 CEPH reference families of 28 short tandem repeat polymorphisms (STRPs) located on chromosome 19. Forty-seven initial mutation events were detected in the STRPs using DNA from transformed lymphoblastoid cell lines, but less than half (39%) could be verified using DNA from untransformed cells. None of the cases where three alleles were observed in a single individual could be verified using DNA from untransformed cells. The average mutation rate for the chromosome 19 STRPs after correction for events which would not be detectable as Mendelian errors was 1.2×10^{-3} per locus per gamete per generation. This rate may have been inflated by somatic as opposed to germline events. Observed mutation rates for individual STRPs ranged from 0 to 8×10^{-3}. The average mutation rate for tetranucleotide STRPs was nearly four times higher than the average rate for dinucleotide STRPs. For determination of the mode of mutation, events involving STRPs on other chromosomes were also examined. Of the events which were verified using DNA from untransformed lymphocytes or which were likely among those for which DNA from untransformed cells was not available: none were located at the sites of meiotic recombination, 91% involved the gain or loss of a single repeat unit, and 15 occurred in the male germline compared to 4 in the female germline ($p = 0.01$).

INTRODUCTION

Simple sequence or short tandem repeats (STRs) are segments of tandemly repeated DNA with repeat lengths up to about 6 bp and with total lengths usually < 60 bp. Hundreds of thousands of STRs are interspersed throughout mammalian genomes. STRs are of special interest because the numbers of repeats within specific STRs tend to be highly variable, and because these short tandem repeat polymorphisms (STRPs) can be rapidly analyzed using PCR. Thousands of STRPs have been developed and mapped for the human, mouse, rat, and other genomes, and STRPs have been used to map dozens of genes responsible for heritable disorders (1–3).

Mutation of STRs is important for applying these markers to the study of evolution and to the mapping of disease genes. Gene association studies, for example, rely on linkage disequilibrium which in turn is highly dependent upon mutation rate (3). Relatedly, historic recombination events, which may be critical to pinpointing the location of a disease gene, can only be accurately analyzed through knowledge of the mutation rates of nearby STRs (4–5). Interest in STR mutation has also been recently stimulated by the discoveries that fragile X disease, myotonic dystrophy, Kennedy's and Huntington's disease are all caused by expansion of runs of tandem trinucleotide repeats,(6–9) and that instability of STRPs may be particularly prevalent in colon tumors (10–11).

Four methods have been previously used to estimate mutation rates for STRs. The most straightforward and the method pursued in this manuscript has been simply to count the rare mutation events that are uncovered through large scale genotyping, particularly in the typing of markers through the two or three generation CEPH reference families (12–17). Observed mutation rates from this approach have ranged from 10^{-4} to nearly 10^{-2} per locus per gamete per generation. A drawback to the direct approach lies in use of DNA from transformed cells. With DNA from lymphoblastoid cell lines, *in vivo* events cannot be distinguished from *in vitro* events that may have occurred during or after transformation of the lymphocytes.

Hästbacka *et al.* (18) estimated mutation rates in three STRs based on linkage disequilibrium with a presumed founder mutation for recessive diastrophic dysplasia in the Finnish population. Using assumptions for the sizes and numbers of generations in the Finnish population since the original disease mutation, STR mutation rates ranging from 3×10^{-4} to 4×10^{-3} were calculated.

Although no mutation events were observed, Edwards *et al.* (19) used marker heterozygosities and a simultaneous maximum likelihood determination of both effective population size and mutation rate to obtain rates for tri- and tetranucleotide STRs of 2×10^{-5} to 2×10^{-4}. These rates are among the lowest that have been described.

The fourth method of estimation of STR mutation rates involved testing of DNA samples from different generations of inbred and recombinant inbred strains of mice (20). Based on the observation of five events, rates of 1.2×10^{-4} and 4.7×10^{-4} for two STRs were obtained.

In this manuscript we report an analysis of mutation of 28 STRPs located on human chromosome 19. Using DNA from untransformed lymphocytes, it was found that the majority of apparent mutation events occurred during or after establishment of the lymphoblastoid cell lines. Estimates of mutation rates were corrected for these *in vitro* events and for events which do not

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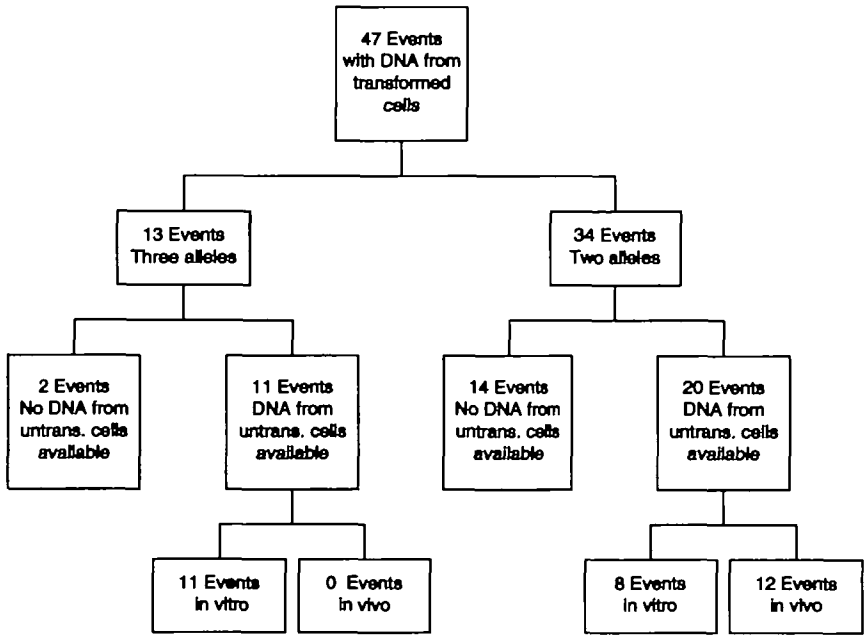

Figure 1. Summary of analysis of chromosome 19 STRP mutations.

violate standard codominant inheritance. In addition, mutation mechanisms were examined by determining the sex of the individual in which the mutation occurred, and by determining the sign and magnitude of the change in allele size.

RESULTS

The 28 STRPs for human chromosome 19 utilized in this study included 12 $(GATA)_n$ tetranucleotide STRPs, one trinucleotide STRP at the DM locus and 15 dinucleotide STRPs (21). Each marker was typed through all 40 CEPH reference families giving a total of very close to 20,000 parent-offspring transfers of alleles. Putative mutation events were detected through the presence of three alleles in a single individual or through the presence of non-Mendelian inheritance. Mutation events for the chromosome 19 markers were carefully recorded during the initial process of genotyping for linkage map construction. Autoradiographs for 8 of the markers were also scanned a second time to try to catch events that were missed initially, but no extra events were found. Strand slippage for the dinucleotide STRPs probably masked the detection of some individuals with three alleles. In a child, for example, with a genotype of 152,150 bp for a dinucleotide STRP, detection of small amounts of a new, mutant 148 bp allele would be virtually impossible due to the presence of 148 bp strand slippage product from the 150 bp allele. Because of significantly reduced strand slippage for the tri- and tetranucleotide STRPs, few if any obvious three allele individuals were likely missed.

A total of 87 putative mutation events were detected for the 28 STRPs. Forty-seven of these 87 events (54%) were confirmed by retyping the appropriate markers in individuals using DNA from transformed lymphoblastoid cells. The events which could not be confirmed involved a variety of laboratory errors including sample mixups, sample spillover from adjacent lanes on the sequencing gels, and scoring uncertainties and errors.

To distinguish *in vitro* mutation events which occurred during or after transformation of the lymphoblastoid cells from *in vivo* events, DNA samples from untransformed lymphocytes for the Utah CEPH families were used in marker retyping. Because only 27 of the 40 CEPH families were collected in Utah, only 31 of the 47 confirmed events could be retested (Figure 1). Of these 31 events, 12 were verified. None of the 11, three allele events were verified.

Several examples of mutation events are shown in Figure 2. Panels 2A–2D show *in vitro* mutation events while panels 2E and 2F show *in vivo* events. In 2A a case is shown in which the DNA from the transformed cells shipped from Paris to Marshfield (M) was not identical to the DNA shipped from Paris to Iowa City (I). This situation was observed in only 5 out of 40 *in vitro* events (chromosome 19 events plus events on other chromosomes). An example where three alleles was observed is shown in panel 2B. A case in which the mutation event did not violate Mendelian rules of inheritance is shown in panel 2D. The genotype of the child in 2D was retested because of the presence of double recombinants within small genetic intervals in both male and female chromosome 19 linkage maps. The DNA from the transformed cells indicated that the child inherited the larger allele from the mother and the smaller allele from the father, while in reality the situation was reversed.

Of the 40 total *in vitro* events detected in markers both on chromosome 19 and other chromosomes, 13 involved three alleles and 27 involved a change in allele size. *In vitro* mutations occurred in cells from 22 males and 18 females. Nine of the mutated alleles were paternal, 13 maternal, and in 18 cases the source of the mutant allele could not be determined. As shown in Table 1, 78% of the changes involved the gain or loss of a single repeat unit. Gains of two or three repeat units and losses of at least two repeat units were also observed. The loss of 7 or 17 repeat units for an *in vitro* event involving the $(CTG)_n$ STRP at the DM locus could conceivably have been due to a small amount of contaminating DNA from another individual.

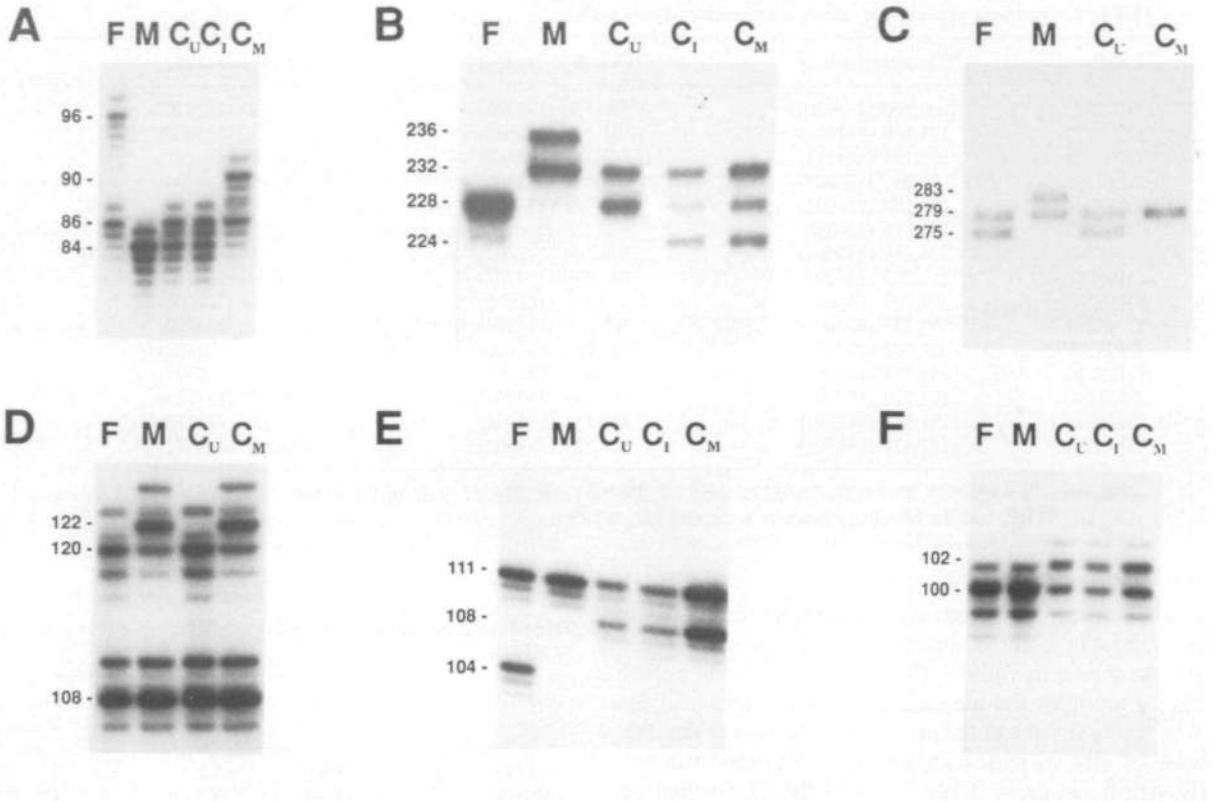

Figure 2. Examples of *in vitro* and *in vivo* mutation events. The gel lanes shown contained amplified DNA from either the Father (F), the Mother (M) or one of the children (C). C_U indicated that the DNA template came from untransformed cells; C_I indicated that the DNA template came from transformed cells (Iowa shipment); and C_M indicated that the DNA template came from transformed cells (Marshfield shipment). The following list detailed the markers, CEPH individuals and genotypes for each panel. **Panel A**: Mfd45 at D8S88; 141801-F (96,86); 141802-M (84,84); 141806-C_U (86,84); 141806-C_I (86,84); 141806-C_M (90,86). **Panel B**: Mfd239 at D19S253; 142001-F (228,228); 142002-M (236,232); 142004-C_U (232,228); 142004-C_I (232,228,224); 142004-C_M (232,228,224). **Panel C**: Mfd242 at D19S250; 141301-F (279,275); 141302-M (283,279); 141303-C_U (279,275); 141303-C_M (279,279). **Panel D**: Mfd11 at D19S49; 142301-F (120,108); 142302-M (122,108); 142304-C_U (120,108); 142304-C_M (122,108). **Panel E**: Mfd236 at D19S244; 134701-F (111,104); 134702-M (111,111); 134708-C_U (111,108); 134708-C_I (111,108); 134708-C_M (111,108). **Panel F**: Mfd9 at D19S47; 1329201 (100,100); 1329202-M (100,100); 1329203-C_U (102,100); 1329203-C_I (102,100); 1329203-C_M (102,100). Allele sizes were in bp. Panels A, D and F showed dinucleotide STRPs; panel B, C and E showed tetranucleotide STRPs.

The third smaller allele in this case was quite weak compared to the other two alleles. Of the 40 total *in vitro* events, 31 involved an increase and 9 a decrease in allele size ($p = 0.0003$).

Use of the DNA from the untransformed cells allowed verification of 15 total mutation events (Table 2). Twelve of the events were from chromosome 19 STRPs and another three from markers located on other chromosomes. The event involving the marker at D19S244 in family 1420 was seen in two different offspring and, therefore, is likely to be a case of germline mosaicism. Eight of the 15 events involved a gain of a single repeat unit, five involve the loss of a single repeat unit and in two cases the sign and magnitude of the change could not be determined. Nine of the mutations occurred in male germlines, three in female germlines ($p = 0.07$), and in 3 other cases the sex could not be determined. Of the mutations that were detected in children, four involved a change in the grandpaternal allele and seven a change in the grandmaternal allele.

An additional 16 events were detected for the chromosome 19 STRPs for which DNA from untransformed cells was not available. Two of these were three allele events which were probably *in vitro* changes. Another 5 events were also likely to have occurred *in vitro* because they involved putative mutations in the parents which did not match the genotypes of either the children or grandparents. For one of these five events (the marker at locus D19S254 in individual 1202), a previous report of the loss of the distal portion of the long arm in the transformed cell line for this person (22) was confirmed. The remaining 9 events all occurred in children, and at least many of these were likely *in vivo* changes. Eight *in vivo* events from the total group of 14 for which DNA from untransformed cells was unavailable were predicted based on the fraction of two allele events that were verified (12/20; Figure 1). Adding the nine likely *in vivo* events

Table 1. Sign and magnitude of change in allele size leading to mutant allele

	In vivo events	*In vitro* events
↑ 3 repeats[a]	0	1
↑ 2 repeats	1	4
↑ 1 or 2 repeats	0	1
↑ 1 repeat	13	25
↓ 1 repeat	7	6
↓ 1 or 2 repeats	1	0
↓ 2 repeats	0	1
↓ 3 or 4 repeats	0	1
↓ 7 or 17 repeats	0	1
Unknown	2	0

[a]Up arrows indicated increases in allele size; down arrows decreases in allele size.

Table 2. Mutations verified with DNA from untransformed cells

Locus[a]	Genotype (father)	Genotype (mother)	Genotype (child)
D19S47	100,100 (1329201)	100,100 (1329202)	102,100 (1329203)
D19S177	173,169 (134601)	171,169 (134602)	171,171 (134606)
D19S178	173,173 (134111)	175,155 (134112)	173,157 (134101)
DM	99,93 (134001)	132,72 (134002)	135,93 (134004)
D19S244	111,104 (134701)	111,111 (134702)	111,108 (134708)
D19S244	144,115 (140810)	(140811)[b]	140,132 (140801)
D19S244	136,119 (142001)	104,104 (142002)	123,104 (142007, 142013)
D19S245	211,203 (134701)	199,199 (134702)	199,199 (134704)
D19S245	207,207 (141601)	203,203 (141602)	203,203 (141608)
D19S247	259,235 (134901)	267,223 (134902)	267,231 (134909)
D19S249	207,203 (134401)	207,203 (134402)	211,207 (134403)
D19S250	283,283 (137701)	283,279 (137702)	279,279 (137708)
D5S119	192,190 (137701)	192,190 (137702)	194,190 (137714)
D14S53	151,144 (141301)	155,144 (141302)	157,151 (141312)
D14S55	127,127 (134701)	127,127 (134702)	129,127 (134711)

[a]Markers at loci D19S47, D19S177, D19S178, D5S119, D14S53 and D14S55 were $(CA)_n$ STRPs; the marker at DM was a $(CTTG)_n$ STRP, and the remaining markers were $(GATA)_n$ STRPs.
[b]DNA for individual 140811 was not available.

to those verified using DNA from the untransformed cells gave a sex ratio for the source of the mutation of 15 males to 4 females ($p = 0.01$). As shown in Table 1, the verified and likely events gave results for the signs and magnitudes of the changes in allele size that were quite similar to the *in vitro* events, except that the bias in favor of size increases for the *in vivo* events was not statistically significant ($p = 0.14$). None of the 21 verified or likely mutation events occurred at the sites of meiotic crossover.

The distribution of events among the 28 chromosome 19 STRPs for both verified or likely mutations is shown in Table 3. The three $(GATA)_n$ STRPs at loci D19S244, D19S245 and D19S253 accounted for 38% of the total mutations. The STRP at D19S244 had more alleles and a higher heterozygosity (19 and 0.88) than almost all other STRPs, but the polymorphisms at D19S245 and D19S253 were unremarkable. Nine of the markers showed no mutations at all and another six showed only *in vitro* events. Only one event was detected for the trinucleotide STRP at the DM locus (Table 2). This change involved a gain of one repeat for one of the larger alleles detected for this marker.

The best corrected estimate for the average mutation rate of the 28 chromosome 19 STRPs was calculated by adding the 12 verified events to the 9 likely but unverified events to give a subtotal of 21. Four additional events were then added to the subtotal to correct for events like the one shown in panel 2D which could not be detected as a violation of Mendel's rules (see Methods). Several events which could have been in this category were detected through a careful analysis of the linkage map of chromosome 19 (21). The final total of 25 mutations gave a rate of 1.2×10^{-3} per locus per gamete per generation (confidence intervals $8\times10^{-4}-1.7\times10^{-3}$). Tetranucleotide STRPs gave a significantly higher average mutation rate (2.1×10^{-3}) than dinucleotide STRPs (5.6×10^{-4}) ($p < 0.005$).

Table 3. Distribution of *in vivo* and *in vitro* mutation events among chromosome 19 STRPs

Locus	Marker Type	Number of Events *in vivo*[a]	Number of Events *in vitro*[b]
D19S244	$(GATA)_n$	5	3
D19S245	$(GATA)_n$	3	1
D19S47	$(CA)_n$	2	0
D19S247	$(GATA)_n$	2	0
D19S177	$(CA)_n$	1	1
D19S178	$(CA)_n$	1	0
D19S198	$(CA)_n$	1	1
D19S246	$(GATA)_n$	1	0
D19S248	$(GATA)_n$	1	1
D19S249	$(GATA)_n$	1	2
D19S250	$(GATA)_n$	1	1
D19S253	$(GATA)_n$	1	5
DM	$(CTG)_n$	1	1
D19S49	$(CA)_n$	0	1
D19S197	$(CA)_n$	0	2
D19S251	$(GATA)_n$	0	1
D19S252	$(GATA)_n$	0	3
D19S254	$(GATA)_n$	0	2
D19S255	$(GATA)_n$	0	1
9 other markers, each		0	0

[a]*In vivo* events included both verified and likely events.
[b]*In vitro* events included both events which were not verified (19 in number; see Figure 1) plus 7 unlikely events for which DNA from untransformed cells was not available.

DISCUSSION

The average mutation rate for the chromosome 19 STRPs of 1.2×10^{-3} matched reasonably well rates previously reported in the literature. This was true despite the fact that other reports of mutations probably included many *in vitro* events. Also, it is not clear how much care was taken to detect all mutations in other studies. When emphasis is placed on construction of linkage maps or on mapping of a disease gene, it is easy to ignore rare mutation events. Many of the recent papers which have reported estimates of mutation rates have involved only handfuls of events (13–18,20). Many more events were detected by Weissenbach and colleagues (12) who examined about 1.7×10^5 parent-offspring allele transfers and observed a mutation rate of 10^{-3}.

Variation in the estimated STRP mutation rates in different studies is likely due to observation of only a very small number of events, to lack of control for *in vitro* and somatic events, and to natural variation in mutation rate among the different STRPs. Based on both results from VNTRs and upon population genetics theory, it is expected that markers with higher informativeness

should have higher mutation rates (23,24). Since STRPs have heterozygosity values ranging from 0 to above 90% (25), considerable variation in mutation rates is expected. Apparent *in vivo* mutation rates for individual chromosome 19 STRPs ranged from 0 for several markers to a high of 8×10^{-3} for the marker at D19S244. The rate for D19S244 matched the highest STRP rate previously reported (for a tetranucleotide marker at DXS981) (17).

Using DNA from only three generations, it was not possible to rigorously distinguish germline from somatic mutation events. Events which were detected in the CEPH family children, like for example, the new 108 bp allele in Figure 2E, could conceivably have been due to somatic mutations in lymphocyte progenitor cells. Likewise, mutations detected in the parents, even when inherited by the children, could have been due to somatic changes in the grandparents. Nevertheless, three lines of evidence indicated that the verified and likely events were at least mostly germline. First, none of the mutant individuals exhibited 3 alleles as was frequently seen for the *in vitro* events. Unless the mutations all occurred very early in development, third alleles would be expected in many cases of somatic change. Three or more alleles have been observed in lymphocytes for other loci (8,17,26). Second, the bias towards the occurrence of mutation events in male rather than female parents would be unexpected if the events were somatic. Third, the putative germline mosaicism seen for the marker at locus D19S244 in family 1420 is not consistent with a somatic event. Given the relatively large confidence interval for our estimate of mutation rate, the presence of a few somatic events within the verified and likely mutations would not appreciably affect our conclusions. Still, it will be useful in the future to see if similar results are obtained using DNA from families with four or more generations.

Null alleles (27,28) could have explained as many as 3 of the 15 total verified events. However, in none of the three cases did the genotypes of the siblings provide any indication of a null allele, nor were null alleles observed in other families for any of the markers utilized in this work.

One of our most interesting findings was the observation that the majority of the confirmed mutations took place during or after transformation of the lymphoblastoid cells (Fig. 1). Royle *et al.* (22) and Huang *et al.* (29), have reported loss of entire chromosomes or segments of chromosomes from the transformed CEPH lymphoblastoid cell lines. However, the *in vitro* events affecting only specific loci are a new phenomenon. When *in vitro* mutation events do not generate new alleles that are inconsistent with standard codominant inheritance, then pseudorecombination events are added to linkage maps (21). Although this effect is relatively small, it can still confound linkage map order. In some situations, like with the CEPH family cell lines, transformation is the only practical means of obtaining sufficient DNA, but for most gene mapping projects, cell transformation is no longer necessary or because of the artifactual mutations, desirable.

The preference for mutation events to occur in male versus female germlines was consistent with the lack of recombination observed at the site of the mutations and with at least some previous results. The fact that there are $>$ 10 times more cell divisions between the zygote and sperm than between the zygote and ova (30), is consistent with a preference for male mutations. Also, for a number of VNTRs, a large preference for paternal events has been observed (31−33). For other VNTRs, however, no sexual bias was observed (23,34,35). Although VNTRs and STRPs share the property of tandem repetition of DNA, these two species still differ substantially in that VNTRs generally have longer repeat lengths and also have many more repeat copies per locus (hundreds or thousands). Observations for VNTRs therefore may not always extend to STRPs. In addition, for the hybridization-based markers, only events which involve a gain or loss of a few or more repeat units are detectable on the blots. If VNTRs follow the pattern for STRPs, then currently undetected events involving changes of one or two repeats will comprise the vast majority of new mutations.

Although there have been a few examples of mutation of VNTRs in which crossover of sequences on two homologues was detected (34), most studies for both VNTRs and STRPs have concluded absence of recombination (6,7,14,17,32,36,37). The absence of recombination at the site of STRP mutation indicates that the events are probably not a result of unequal recombination between homologues, but rather are likely to be the result of intrachromatid mechanisms, such as strand slippage (38). Some types of recombination events, such as gene conversion or unequal recombination between sister chromatids, cannot at this stage be ruled out.

The vast majority of mutations events observed both *in vivo* and *in vitro* (Table 1) involved the gain or the loss of a single repeat unit (82%). The fact that changes in allele size generally were an increase or decrease of 2 bp for dinucleotide STRPs and 4 bp for tetranucleotide STRPs was a strong indication that all or nearly all of the observed changes were actually within the tandem repeats rather than within the nonrepeated flanking DNA. These observations are generally consistent with previous reports both for VNTRs where small size changes are predominant over larger size changes (23,32,39,40) and for STRPs (4,5,14,18).

The preference for increases as opposed to decreases in allele size was not expected. Failure of the *in vivo* events to show significantly more increases than decreases may have been due to lack of a sufficient sample size. It is interesting to contrast the preference for increase in allele size in mutation events to the preference for loss of repeats during PCR amplification of these markers. If the preference for increases in allele size holds, then obviously there must be some compensating, perhaps relatively rare, large deletions of tandem repeats to avoid continual expansion of allele size.

In summary, it was found that STRPs for human chromosome 19 mutated at a corrected average rate of about 1.2×10^{-3} per locus per gamete per generation. Mutations occurred preferentially in male versus female germlines and did not occur at the sites of recombination. Changes generally involved the gain or loss of a single or a pair of repeat units, and over half of the mutation events observed using DNA from transformed lymphoblastoid cell lines could not be confirmed using DNA from untransformed cells. These observations will improve the utility of STRPs in the study of evolution and in linkage mapping.

METHODS

All putative mutation events were checked by *de novo* amplification and electrophoresis (21). Allele sizes for each of the markers were determined relative to those for the parents of CEPH family 1331. Three sets of DNA samples were used as template in the amplification reactions: DNA from transformed CEPH cell lines that was sent to Marshfield in October, 1989 (M); DNA from the transformed CEPH cell lines that was originally shipped to Jeff Murray at the University of Iowa in November, 1987 and was subsequently aliquoted and sent to Marshfield (I); and DNA from the Utah families within the CEPH collection (27 of 40 families) that was extracted directly from untransformed cells (U) (gift of Mark Leppert). The M and I DNA samples were extracted from the

lymphoblastoid cell lines at the CEPH over a period ranging from September, 1984 to December, 1988.

For determination of the sex of the parent in which the mutation occurred and for determination of the sign and the magnitude of the change in allele size, the most probable situations were assumed to be correct. For example, the non-mutant allele in a child was always assumed to have been inherited unchanged from a parent, rather than assuming the much less likely situation of two mutations. Especially for determining the magnitude and the sign of the change of allele size, it was necessary to use the phase information that came from the *chrompic* subroutine of the CRIMAP software. Because the linkage map for chromosome 19 is well established (21), the phase of the mutant allele could in, virtually all cases, be determined with a high degree of confidence. Similarly, the relationship of the mutation events and the sites of crossovers were determined using the *chrompic* output. In cases where three alleles per individual were observed, one of the two original (non-mutant) alleles showed in almost all cases a relative reduction in intensity on the autoradiographs. The weaker allele was assumed to be the precursor of the mutant allele.

The mutation in dinucleotide marker Mfd9 at D19S47 in child 6604 provides an example of mutation analysis. The genotypes in bp of 6601 (father), 6602 (mother) and 6604 (daughter) were respectively (100,92), (100,96) and (100,94). The new 94 bp allele could have arisen from any of the four parental alleles. Consultation of the linkage map for chromosome 19 showed that in child 6604, all alleles in the vicinity of D19S47 in both paternal and maternal chromosomes were of the grandpaternal phase. Since the 100 bp allele from the mother was of the grandpaternal phase, whereas the 92 bp allele from the father was of grandpaternal phase, the most likely conclusion by far for child 4 was that the 100 bp allele was inherited unchanged from the mother and that the 94 bp mutant allele originated from the father's 92 bp allele (an increase of one repeat).

Mutation events involving STRPs on other chromosomes were used to investigate the mechanism of mutation, but because of the difficulty in determining that all such events were detected, were not used in calculation of mutation rates. Only events on other chromosomes for which DNA was available from untransformed cells were examined. Three out of a total of 24 such events were verified with DNA from untransformed cells.

The probabilities of deviation from equal paternal or maternal origin of mutation and increases or decreases in allele size were determined using a one-sided binomial test. Confidence intervals (95%) for mutation rates were obtained using a standard large sample approximation for the binomial distribution (41).

To correct observed numbers of mutations for those which would not be detectable as violations of Mendel's rules, 94 dinucleotide STRPs and 10 tetranucleotide STRPs were chosen at random from the markers developed at Marshfield. CEPH family parental genotypes were modified using the computer such that each of the four alleles was both increased and decreased by one repeat unit. Each mutated allele was then combined with each of the other parent's alleles (16 total possible cases per parental pair) to see if the allele pair was consistent with standard codominant inheritance. The fractions of total mutations which were consistent were 12.4% for the dinucleotide STRPs and 23.1% for the tetranucleotide STRPs. Since 12 tetranucleotide STRPs and 15 dinucleotide STRPs were used in this study, a correction factor of 0.18 was used to obtain the final best estimate of the total number of STRP mutations.

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