Am. J. Hum. Genet. 66:1599–1609, 2000

The Mutation Rate in the Human mtDNA Control Region

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The mutation rate of the mitochondrial control region has been widely used to calibrate human population history. However, estimates of the mutation rate in this region have spanned two orders of magnitude. To readdress this rate, we sequenced the mtDNA control region in 272 individuals, who were related by a total of 705 mtDNA transmission events, from 26 large Icelandic pedigrees. Three base substitutions were observed, and the mutation rate across the two hypervariable regions was estimated to be 3/705 = .0043 per generation (95% confidence interval [CI] .00088–.013), or .32/site/1 million years (95% CI .065–.97). This study is substantially larger than others published, which have directly assessed mtDNA mutation rates on the basis of pedigrees, and the estimated mutation rate is intermediate among those derived from pedigree-based studies. Our estimated rate remains higher than those based on phylogenetic comparisons. We discuss possible reasons for—and consequences of—this discrepancy. The present study also provides information on rates of insertion/deletion mutations, rates of heteroplasmy, and the reliability of maternal links in the Icelandic genealogy database.

Introduction

Sequence information from the mtDNA control region (CR), which documents variation within and between human populations, has been widely used to infer certain aspects of human population and demographic history (e.g., see Cann et al. 1987; DiRienzo and Wilson 1991; Ward et al. 1991; Stoneking et al. 1992; Horai et al. 1995; Sajantila et al. 1995; Richards et al. 1996). The use of sequence information from the CR to assess the time elapsed since a particular event took place requires knowledge of the mutation rate in the CR. Recently, this mutation rate has attracted considerable attention, in part because of differences in the published estimates, which span two orders of magnitude.

Until recently, effectively all estimates of mutation rates were indirect, being based on evolutionary studies: phylogenetic or coalescent methods were used to infer the mutation rate on the basis of the pattern of variation either among human mtDNA sequences or between human sequences and those of sister species, with "external" calibration provided by the assumed times for known events, such as either the human-chimpanzee divergence or particular population migration events for which archaeological evidence is available. In the case of coalescent methods, assumptions about the relevant effective population sizes have been used. The range of published estimates of the CR mutation rate, from phylogenetic studies, is 0.025–0.26/site/1 million years (Myr) (e.g., see Parsons et al. 1997, and references therein).

The availability of high-throughput sequencing recently has allowed direct estimation of mtDNA mutation rates, simply by a counting of the number of mutational events observed in pedigrees. The largest such study to date, by Parsons et al. (1997), reported a mutation rate of 2.5/site/Myr, on the basis of 10 mutations in 327 transmission events, principally from mother-child, grandmother-grandchild, or sib-pair comparisons. An earlier study, by Howell et al. (1996), gave an estimate of 0.75/site/Myr (2 mutations in 81 transmission events), on the basis of four families with an mtDNA disease, Leber hereditary optic neuropathy. Subsequent studies, by Soodyall et al. (1997) and Jazin et al. (1998), based on maternal pedigrees of 5 families from Tristan da Cunha and 33 Swedish families, respectively, found no mutations in either case, in 108 and 288 transmission events, respectively. Maximum-likelihood estimates for the mutation rate in these data sets are 0, so these authors gave 95% upper confidence limits for a rate of 0.028/generation, across the CR, and 0.46/site/Myr, respectively.

Here we describe a mutation-rate study based on sequencing of the CR of 272 individuals from 26, mainly

Received November 30, 1999; accepted for publication February 23, 2000; electronically published April 7, 2000.

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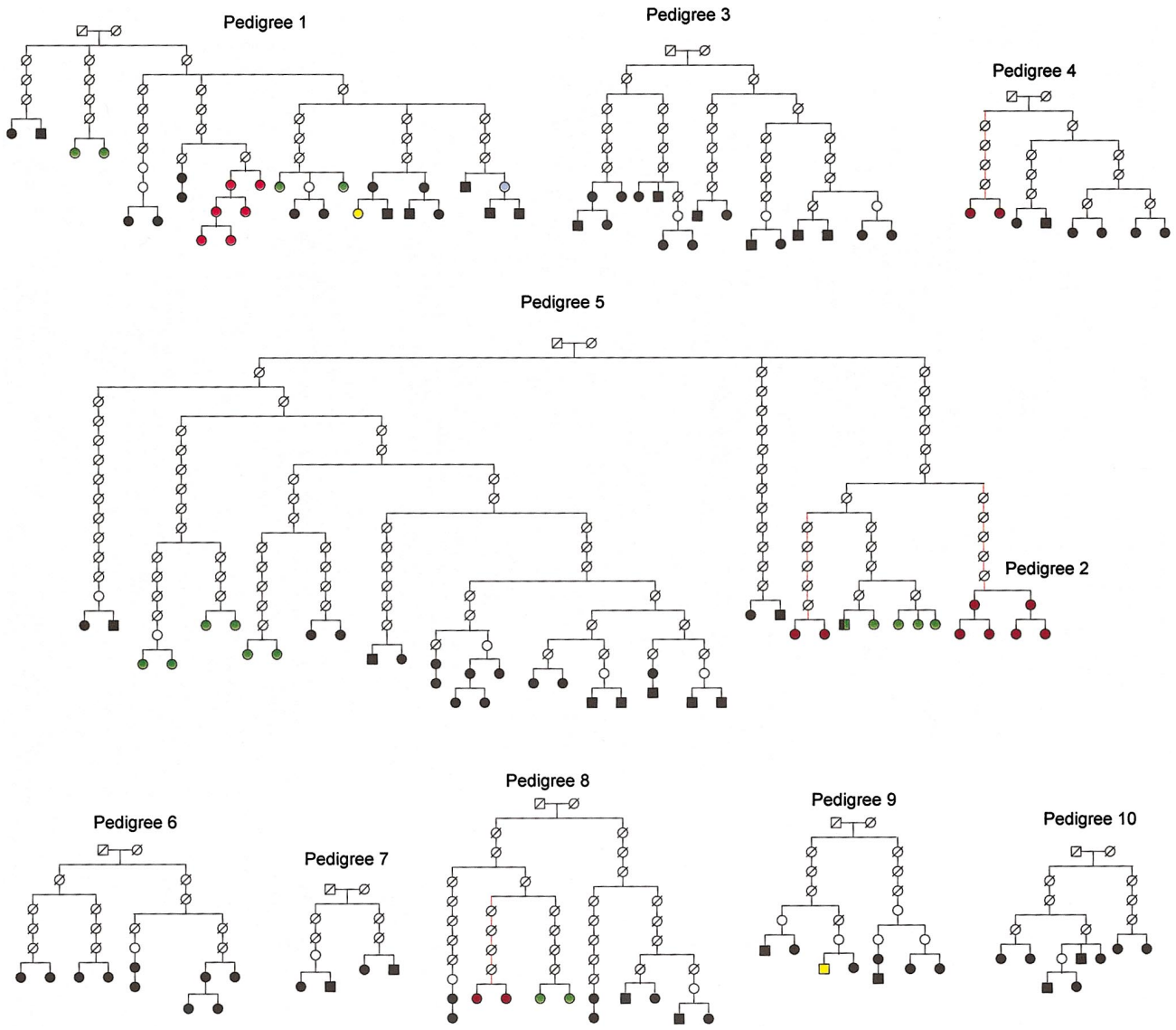


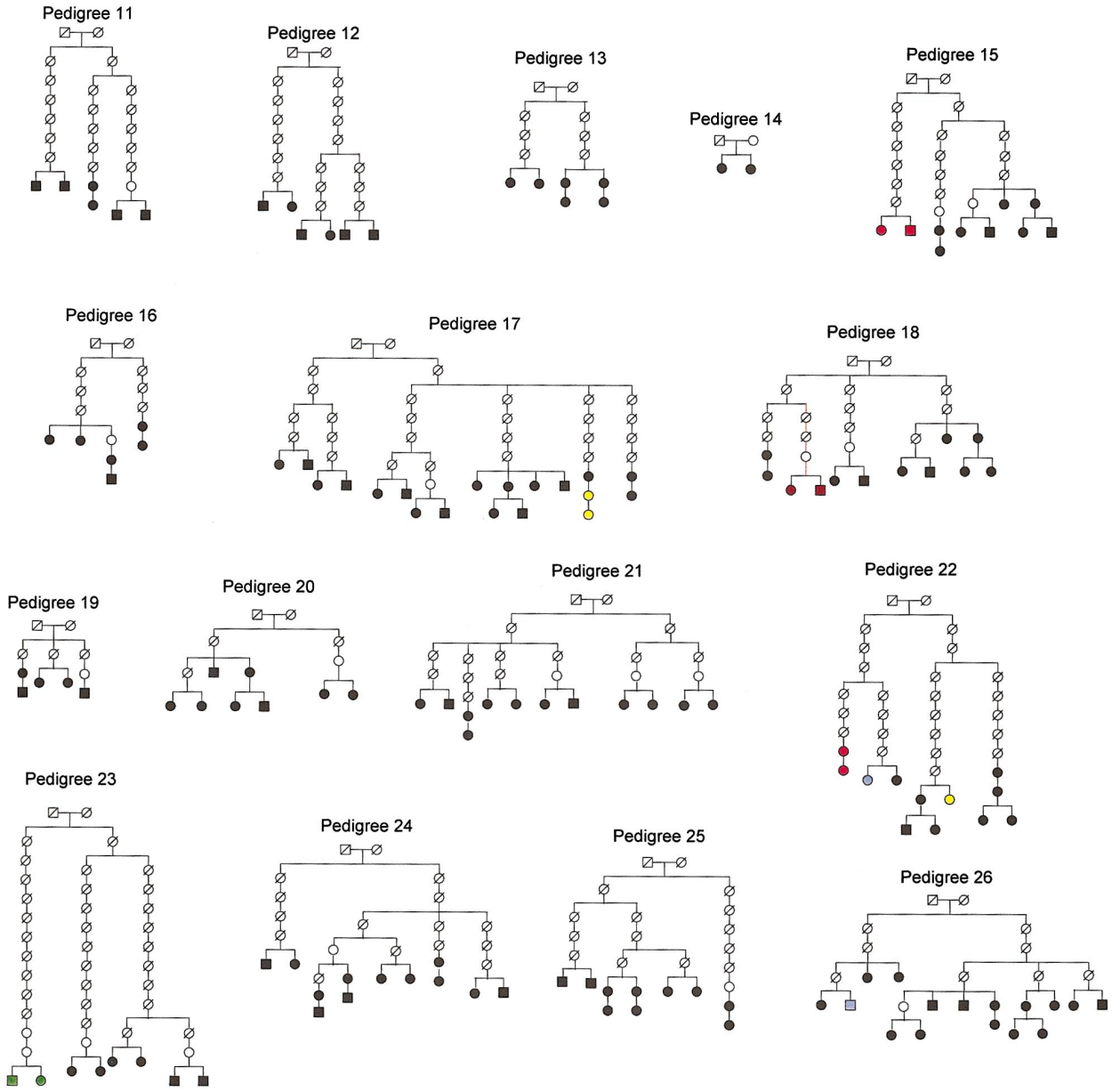
Figure 1 Twenty-six maternal pedigrees relating sampled individuals. Individuals whose mtDNA CR was sequenced are shown as colored circles (females) or blackened squares (males); individuals whose sequence agreed with most others in the pedigree are shown in black; individuals displaying homoplasmic substitutions are shown in red; and individuals with insertions and deletions are shown in green. Individuals exhibiting heteroplasmy (at the levels detectable by sequencing; see the text) for a base change are shown in light blue. The individual in pedigree 5 who was heteroplasmic for length variation is shown as half green and half black. Details of the nature of the changes in each case are given in table 1. Single individuals whose sequence differed substantially from others in the pedigree are shown in yellow; these seem best explained (see the text) as instances of laboratory or sampling error. The two such individuals in pedigree 17 had sequences that differed substantially from each other, as well as from those in the rest of the pedigree. Clusters of individuals with the same sequence as each other but whose sequences were substantially different from those of the rest of the pedigree are shown in brown; these seem best explained (see the text) as instances of errors in the database. In each case, the pathway of links, at least one of which must be in error, is shown in red. One such cluster (occurring in the original pedigree 5) was moderate in size. For the study of mutation rate, this cluster was treated as a pedigree in its own right (see the text) and labeled as "Pedigree 2."

large, Icelandic pedigrees, including a total of 705 mtDNA transmission events. Throughout, we define the mutation rate as the rate at which the mtDNA type of an individual changes, rather than as the rate of mutation accumulation in individual mitochondria.

Pedigrees and Methods

Pedigrees

Our laboratory has an ongoing genealogy project, which involves electronic registration of all available ge-



nealogical information for the past 10 centuries in Iceland, including genealogy manuscripts, censuses, church books, and phone books (Gulcher and Stefansson 1998; also see The Icelandic Healthcare Database at the Medscape Web site). The genealogy database is stored and maintained within a relational database. Each record in the database consists of a personal identifier, identifier to parents, gender, and dates of birth and death. Each version of the computerized genealogy database is reversibly encrypted by the Data Protection Commission of Iceland before arriving at the laboratory of deCODE Genetics. All blood samples are collected with informed consent, and all personal identifiers are encrypted by the Data Protection Commission.

From the Icelandic genealogy database, we generated maternal pedigrees by linking as many individuals as possible to a single female ancestor as far back as 14 generations ago. From these we chose 25 pedigrees; for the majority, the founding ancestor was born during the period 1530–1830. A total of 285 living individuals, spread across the pedigrees and covering 745 transmission events, were selected for sampling. For each individual sampled, at least one other closely (maternally) related individual (e.g., sib, child, or mother) also was

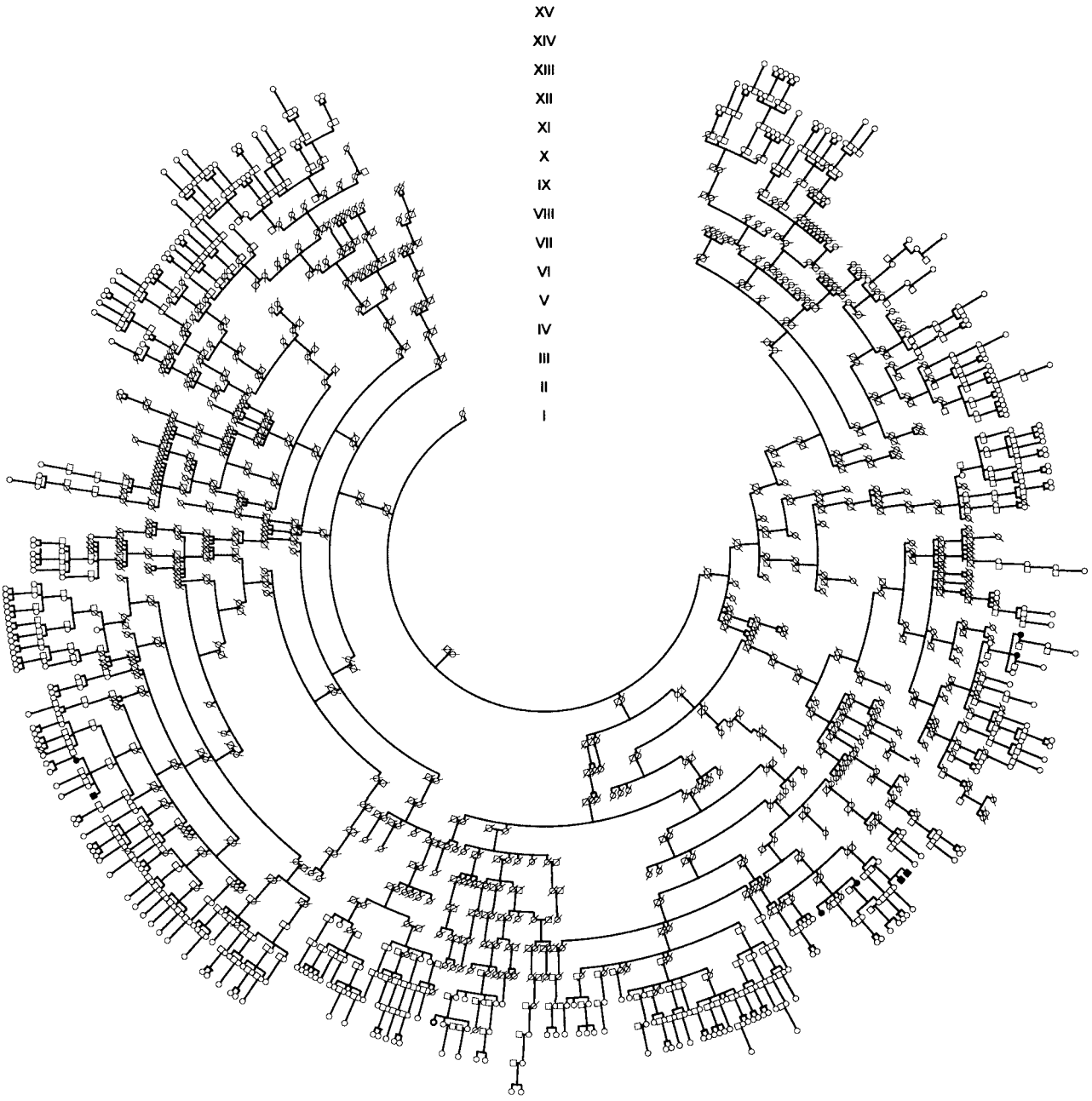


Figure 2 Full maternal pedigree 23 (see the left-hand page of fig. 1) available from the Icelandic genealogy database. The pedigree spans 15 generations. The female founder was born in 1560. The individuals whom we examined in our study are shown in black.

sampled. This sampling scheme substantially increases the chances both that detecting errors in the genealogy database will be detected and that germline and somatic mutations will be distinguishable. Of the pedigrees containing enough living individuals for implementation of this sampling scheme, those selected for study covered a range of sizes and depths. The pedigrees relating sampled individuals are shown in figure 1. For illustration, one of the full maternal pedigrees, as available from the database, is shown in figure 2.

Sequencing

The region from position 16012 to position 394 of the mitochondrial genome was fully sequenced in both directions. The region sequenced included both the first

and second hypervariable regions (HVRI and HVRII, respectively) of the CR. To ensure quality, reamplification and resequencing (again in both directions) were undertaken for all participants in the study. DNA was extracted from peripheral blood by an organic extraction method. The following primers were used for the initial amplification: L15999 (5′-CACCATTAGCACCCAAAGCT-3′) and H409 (5′-CTGTTAAAAGTGCATACCGCC-3′). Amplification reactions were performed on 10 ng of template DNA in a 20-μl volume by use of Ampli*Taq* Gold™ polymerase (PE Biosystems). The cycle profile started with 95°C for 12 min, followed by 30 cycles of 94°C for 40 s, 55°C for 45 s, and 72°C for 3 min. Both hypervariable segments were sequenced by use of a BigDye™ Terminator Cycle Sequencing kit from PE Biosystems, on an ABI PRISM 377 (PE Biosystems) DNA sequencer. The primers L15999, H409, and L16498 (HVS2) (5′-CCTGAAGTAGGAACCAGATG-3′) and its reverse complement were used for cycle sequencing reactions. In the second round, an additional primer, L16181 (5′-AAACCCCCTCCCCATGCTTA-3′), was added to the cycle sequencing process. The cycle sequencing profile was 30 cycles of 96°C for 10 s, 50°C for 5 s, and 60°C for 4 min. The samples were electrophoresed and detected on an Applied Biosystems model 377 using Sequencing Analysis 3.2 software. The chromatograms were basecalled by Phred (version 0.980904.c), assembled by Phrap (version 0.990315), and scanned by PolyPhred (version 3.0). The results were manually checked with the Consed program (version 8.0).

Results

Accuracy of the Genealogy Database

One by-product of our study is a measure of the accuracy of maternal links in the Icelandic genealogy database. We observed 10 instances in which individuals or clusters of individuals differed, at three or more positions, from other sequences in the pedigree (see fig. 1). In five cases, all closely related individuals in the cluster had the same sequence (itself different from the rest of the pedigree). Although there are other possible explanations—such as multiple mutations, inheritance of paternal mtDNA, or recombination between maternal and paternal sequences at earlier transmission events—the most likely explanation for these cases is that one (or possibly more) of the posited maternal links in the database is inaccurate. Rechecking of the database subsequent to our work revealed that one of the questioned links had been independently changed as part of the regular review of the database.

In five other cases, there were single individuals currently alive whose sequences differed substantially from those of their close relatives. Again, one possible explanation is inaccuracy in the database; another is sampling or labeling error, either within the laboratory or before the sample has reached the laboratory. We would expect a high degree of accuracy, within the database, for links between living mothers and children, and so it would seem more likely that this second type of discrepancies is due to errors in handling or labeling.

Assuming that singleton discrepancies are indeed handling errors and that discrepancies shared across clusters result from a single erroneous maternal link, we estimate the reliability of maternal links over the time period sampled in the database to be 740/745 = 99.3% (95% confidence interval [CI] 98.4%–99.8%). If all instances of discrepancies were assumed to be due to errors in the database, we would estimate its reliability for these maternal links to be 735/745 = 98.7% (95% CI 97.5%–99.4%). It is possible that our study contains undetected database errors in which it happens that the wrongly connected clusters carry the same mtDNA CR haplotype. On the basis of extensive mtDNA sequencing in Iceland (Helgason et al. 2000), it has been estimated that the probability that two randomly chosen Icelanders carry the same mtDNA CR haplotype is .0083. Thus, unless erroneous maternal links preferentially link individuals who are maternally related, the effect is very small. On the assumption that singleton discrepancies are handling errors, our estimate of the handling- or labeling-error rate would be 5/285, or 1.8%.

For the purpose of studying the mutation rate in the mtDNA CR, we have simply ignored discrepant single individuals or small clusters. There remained one larger cluster containing six individuals that originally was in pedigree 5, for which one of the links to the other individuals in pedigree 5 was erroneous (see fig. 1). It would still have been straightforward to detect a mutation, had one occurred, within this cluster, so it was treated as a separate small pedigree and was relabeled as "pedigree 2." (In fact, no mutations were observed in this cluster, so its inclusion has minimal effect on the rate estimates.) For the mutation-rate study, this left 272 individuals in 26 pedigrees related by 705 transmission events.

Point Mutations

Table 1 gives details of the events in the various pedigrees (also see fig. 1). Three homoplasmic mutations were found—two from HVRI (transitions 16111 G→A and 16093 T→C) and one from HVRII (transition 153 A→G). On the basis of these mutations, we estimate that the overall mutation rate in the two hypervariable regions is 3/705 = .0043 per generation (95% CI .00088–.013). In the literature, there are no precise definitions of the two hypervariable regions; however, for

Table 1

Details of Individuals Sequenced, Number of Generational Events, and Instances of Mutation and Heteroplasmy Observed in 26 Pedigrees

Pedigree	No. of Individuals Sequenced (No. of Generational Events)	Substitution	Heteroplasmy	Insertion	Deletion
1	27 (58)	16111 G→A	16111 G→A	2 × 303-309 8C→9C	
2	6 (6)				
3	16 (44)				
4	6 (13)				
5	33 (121)		303–309 9C→8C	3 × 303–309 8C→9C	
6	10 (23)				
7	4 (9)				
8	10 (37)				303–309 9C→8C
9	7 (18)				
10	8 (16)				
11	6 (23)				
12	6 (22)				
13	6 (12)				
14	2 (2)				
15	10 (26)	153 A→G			
16	6 (13)				
17	17 (43)				
18	10 (20)				
19	5 (9)				
20	8 (11)				
21	12 (27)				
22	11 (34)	16093 T→C	16257 C→T		
23	8 (42)				303–309 8C→7C
24	12 (27)				
25	10 (24)				
26	16 (25)		16239 T→C		
Total	272 (705)				

the purposes of producing an average rate estimate per site, we will consider HVRI as being delimited by positions 16024–16383 and will consider HVRII as being delimited by positions 57–371, for a total of 673 nucleotides in the two hypervariable regions. The mutations that we observed fall well within all definitions of the hypervariable regions, so our mutation-rate estimate for the region as a whole is directly comparable with those of other authors. If we use the same assumption that has been used by other authors—that is, that 20 years constitutes a generation—the estimated mutation rate is 0.32/site/Myr (95% CI .065–.97). In fact, our genealogical records suggest that, during the past 300 years in Iceland, the average maternal intergenerational time has been ⩾30 years; this is in agreement with one recent study of the Saguenay region of Quebec (Tremblay and Vezina 2000) and with another study of Saxony (Forster 1996).

Each of our observed mutations is shared by at least two closely related individuals, so that we can exclude the possibility that the mutation is somatic rather than in the germline, or that it is the result of a sample-handling or -labeling error. We note that we cannot definitively distinguish between mutation events and database errors with the property that the cluster erroneously linked to the pedigree just happens to have a mtDNA haplotype that, at a single site, differs from that in the remainder of the pedigree. Extensive data on mtDNA CR haplotypes in Iceland is now available (Helgason et al. 2000), on the basis of which the probability that two individuals differ in a single base has been estimated as .0083, from which we conclude that there is little possibility that any mutation-rate overestimation would result from deeming all apparent mutations as actual mutations.

Insertion/Deletion Mutations

There is a tandem repetitive poly-C tract, from position 303 in HVRII, that is known to have a high rate of mutations that change the repeat length (Hauswirth and Clayton 1985). We observed seven instances of changes to this length; of these, five (clustered within two of the larger pedigrees) involved a length increase from 8C to 9C, and two involved a decrease, one from 9C to 8C and one from 8C to 7C. Our estimate of the

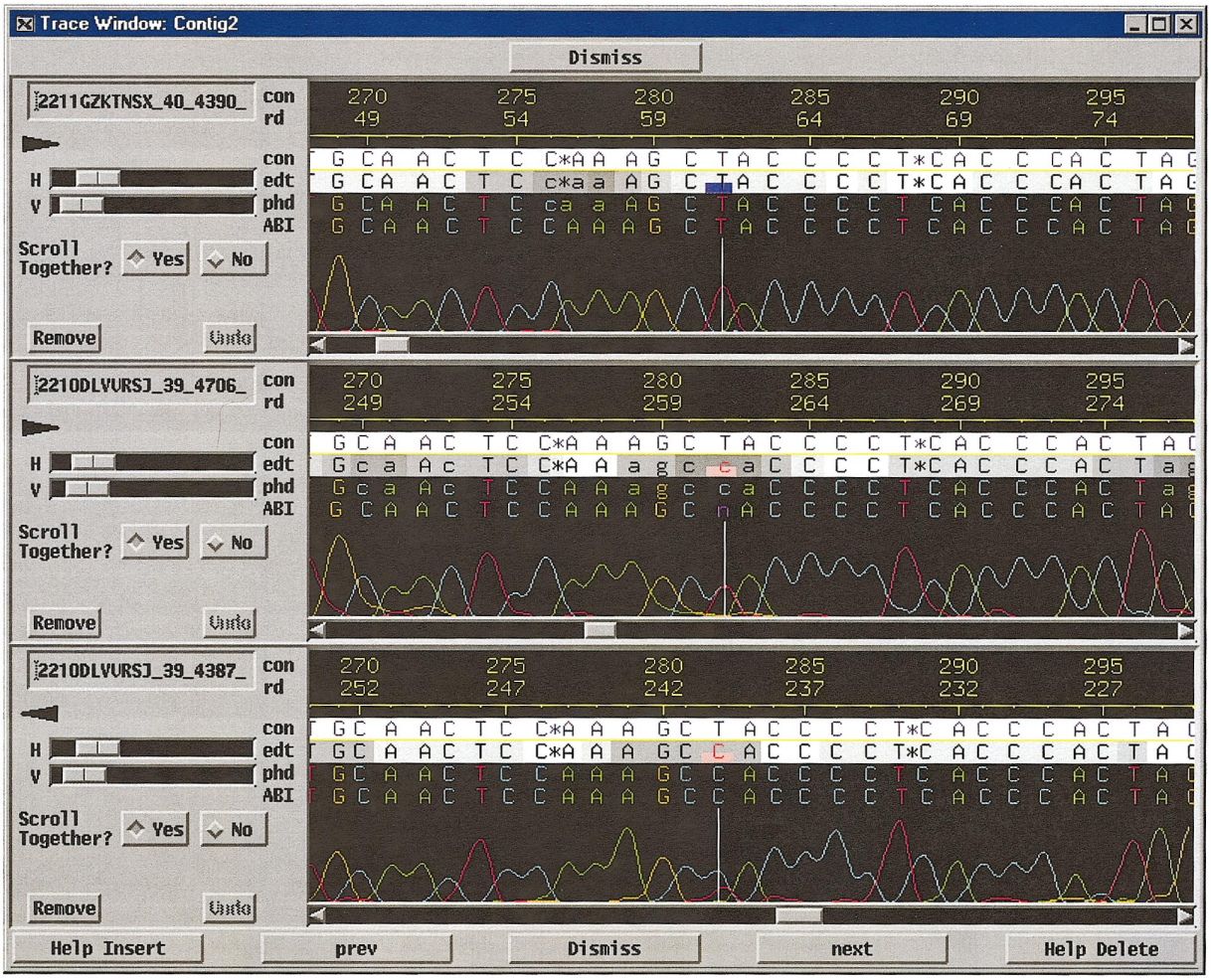


Figure 3 Chromatograms, from the editing program Consed (scanned by PolyPhred; see Nickerson et al. 1997), of the sequence of one individual homoplasmic for base T at position 16257 (red peak 282, in the topmost chromatogram) and of the different levels of heteroplasmy (C/T) that were observed in her sister, depending on whether sequencing was in the forward or reverse direction (red [T] and blue [C] peaks on top of each other, in the middle chromatogram; and reduced size of the red peak compared to the blue peak, in the bottom chromatogram).

overall rate of length change in this tract is 7/705 = .0099 per generation (95% CI .0040–.020).

Heteroplasmy

Our methods will only detect heteroplasmy in which both alleles are present at reasonable frequency. It has been estimated that the minor allele needs to be present at a frequency of ⩾30% (Nickerson et al. 1997). Furthermore, it does not seem straightforward to estimate the proportions of the alleles on the basis of the electropherogram. Figure 3 shows the resequencing of one of the instances of heteroplasmy, at position 16257 in the HVRI. This individual shows relative peak heights from sequencing in the forward direction that are different than from those from sequencing in the reverse direction. This was the case in both repetitions of amplification and sequencing: for each direction, the relative peak heights were apparently consistent between repetitions, with the discrepancy for the different directions of sequencing also being preserved between repetitions. Thus, even careful sequencing may not be a reliable method for either estimating the degree of heteroplasmy or screening for it.

We observed three instances of heteroplasmy, all in HVRI (positions 16111 G/A, 16257 C/T, and 16239 T/C). In no case did closely related individuals share the heteroplasmy. On the basis of this, we would estimate the per-individual rate individual of heteroplasmy detected by standard sequencing to be 3/285 = .011 (95% CI .0022–.030). Bendall et al. (1996) found four instances of heteroplasmy in HVRI in 180 twin pairs. In

addition, we found one individual heteroplasmic for length variation (9/8 C) in the poly-C tract from position 303. Four siblings and the individual's grandmother were homoplasmic for 9 C's. For the particular instances of heteroplasmy in our study (and those of some other authors), we cannot exclude the possibility that they are somatic and not reflected in the germ line. Even if we had observed all the offspring, we may not have been able to tell whether the heteroplasmy was somatic; if all the offspring carry the original base, then the heteroplasmy may have been germline—and just lost over that generation. Moreover, with our method, apparently homoplasmic offspring may have actually been heteroplasmic.

The example of G/A heteroplasmy at position 16111 in pedigree 1 is curious: it is at the same position but very distant, in the pedigree, from the homoplasmic mutation in that pedigree. One possible explanation is that there were two independent mutations, one for the homoplasmic change and one for the heteroplasmy. Another possible explanation is that the heteroplasmy was maintained over many generations but was lost in all other sampled individuals descended from the possible source (the daughter of the founder who is ancestral to the mutation and the heteroplasmy). A priori, both explanations seem unlikely; the first explanation is improbable because mutation rates are low, although position 16111 has been estimated as having a high mutation rate *relative* to those at other positions in the CR (Meyer et al. 1999), and the second explanation is improbable because it would require that the period of preservation of heteroplasmy be substantially longer than what is usually the case (e.g., see Bendall et al. 1997).

Discussion

Our estimate of the mutation rate across both hypervariable regions—that is, .0043 per generation—is smaller than those reported in some pedigree studies (e.g., .025, reported by Howell et al. [1996], and .028, reported by Parsons et al. [1997]) but larger than those in others (e.g., Soodyall et al. 1996; Jazin et al. 1998, in which no mutations were observed). The estimate of .028, in the study by Parsons et al. (1997), does not include the mutation that they observed to the poly C tract, although this was included in their published rate estimates (as one of their reported 10 mutations). Measured in terms of mtDNA transmission events, our study is larger (by more than a factor of 2) than the largest previous study and is comparable in size to the aggregate of previous studies. Combining all published pedigree-based studies that include both hypervariable regions of the mtDNA CR (Howell et al. 1996; Parsons et al. 1997; Soodyall et al. 1997; present study) yields 14 mutations in 1,221 transmission events, leading to a rate estimate of .0115 (95% CI .0063–.0192).

In assessing the mutation rate from our study, we have not used the information from heteroplasmic individuals. In part, this stems from our concern that, in our study (and some others), individuals may display somatic heteroplasmy but be homoplasmic in their germline cells. Furthermore, the processes by which heteroplasmy is resolved—and, hence, the likely long-term fate, in descendants, at the site that is heteroplasmic—does not seem well understood. For both reasons, it is not clear how to weight heteroplasmy in the derivation of an estimate of mutation rate. To the extent that there is a reasonable chance that the heteroplasmy might resolve in favor of the "new" base, our procedure could underestimate the true mutation rate. In the one instance in our data that is informative (pedigree 1), a woman heteroplasmic for G-A had an apparent majority of A (perhaps in the ratio of 2:1, as judged from peak heights), whereas both of her children have reverted (at the levels detectable by sequencing) to the original base, G, and her brother also shows only G. We note that some other studies (e.g., see Parsons et al. 1997) have included instances of heteroplasmy in the estimation of mutation rates (apparently two of the mutations that were heteroplasmic in the study by Parsons et al. did not become fixed in the individuals sampled).

The three observed mutations in our study occurred at sites with faster-than-average rates, according to the estimates by Meyer et al. (1999). One such site, position 16093, coincided with a mutation observed in the pedigree study by Parsons et al. (1997). Two of our instances of heteroplasmy (positions 16239 and 16257) occurred at sites estimated to have a lower-than-average mutation rate, whereas the third, at position 16111, was at a site estimated to have a higher-than-average rate (rate estimates are from Meyer et al. 1999).

Although both our estimate and the estimate, given above, from the combined studies are smaller than the original pedigree estimates, they remain larger than estimates based on phylogenetic approaches. One explanation that has been given for the difference between so-called pedigree estimates and phylogenetic estimates is the existence of substantial heterogeneity in mutation rates across the CR, i.e., mutational hot spots. Although such heterogeneity undoubtedly exists, it is irrelevant to the issue at hand. If the aim is to estimate the average mutation rate either across the entire CR or across one or both of its hypervariable regions, then estimates obtained by directly counting the mutations in pedigrees are unbiased for this rate, *regardless of the degree of rate heterogeneity,* unless, as seems unlikely, some sites mutate so quickly that pedigree studies will fail to observe two mutations at the same site in distinct generations within the pedigree. Of course, observed muta-

tions are more likely to occur at the sites with higher mutation rates, in pedigree studies, and over longer time periods. As Howell et al. (1996) have noted, substantial rate heterogeneity poses a problem for phylogenetic methods. These methods need to correct for multiple, unobserved mutations, and, unless they adequately capture the extent of rate variation, they may systematically underestimate the average mutation rate. Even if it is correctly modeled, the presence of rate heterogeneity may adversely affect the precision of phylogenetic estimators. Thus, the most likely consequence of rate heterogeneity is that phylogenetic estimators will underestimate the mutation rate.

A second putative explanation for the difference between pedigree estimates and phylogenetic estimates concerns the effect of selection and genetic drift, since many of the new mutations observed in pedigree studies are destined to be lost from the population as a whole. Considerable care is needed here, and it may be helpful to revisit the central issues. These are not affected by the fact that each cell carries many mitochondria and that mutation actually operates at the level of a single mitochondrion. For simplicity, throughout this discussion we will use "individual" to refer to a single person, "population" to refer to the collection of individuals, and, ignoring heteroplasmy, will assume that at each site there is a unique nucleotide within an individual. Thus, a "mutation" refers to a difference between the sequence of an individual and that of his or her mother. Since we are discussing mtDNA, individuals are haploid.

It is helpful to distinguish three different rates. The first of these, denoted here by u, is the mutation rate per individual per generation. The second, here denoted by k_s and sometimes referred to as the *substitution rate,* is the rate at which the population becomes fixed for a new allele. Finally, k_a is defined as the rate of mutation along an ancestral lineage. That is, suppose that we choose an individual from the population and trace her (unique, single) ancestral maternal lineage indefinitely into the past. If we were able to watch the mtDNA sequences carried by the individuals on this lineage, then k_a would be defined as the rate at which we would observe mutation events. To further simplify the discussion, focus attention on a particular site within the CR, and interpret each of the three rates only in terms of changes at this site.

It is clear that pedigree studies provide a direct estimate of the mutation rate u. But exactly what are the phylogenetic studies trying to measure, and how is it related to u? Apparently for historical reasons, much of classical population genetics has related to the substitution rate, k_a. It has been suggested (e.g., see Loewe and Scherer 1997) that this is the "relevant rate" for phylogenetic studies. The so-called molecular-clock hypothesis asserts (Kimura 1983) that, under neutrality, $k_a = u$, from which it has been inferred (e.g., see Loewe and Scherer 1997) that, under neutrality, phylogenetic and pedigree studies are trying to estimate the same thing. In fact, the statement that $k_a = u$ is not true, in general. It is approximately true only if u is small relative to the inverse of the effective population size (for a careful treatment, see Donnelly 1991). This will be the case for many, but perhaps not all, sites in the CR, but we argue that this is not directly relevant to the issue here.

Phylogenetic studies relate fundamentally to k_a, the mutation rate along the ancestral lineage, rather than to the substitution rate k_s. This is easiest to see in the simple case in which one human sequence is compared with one, say, chimpanzee sequence. There is a unique lineage leading back from each of the sampled sequences to their most recent common ancestor, a sequence carried by some individual prior to the human-chimpanzee divergence. Differences between the two sequences result precisely from mutations on these lineages. Indeed, if we could ignore possible multiple hits (as often occur in the case of autosomal sequence data), each mutation on the ancestral lineage would be apparent as a difference between the sampled sequences, and it is the rate at which such mutations occur that we are directly estimating from the data. This argument extends to larger data sets, possibly including multiple sequences from each species. The pattern of variation in such data results from mutations on the relevant ancestral lineages. Now, provided that different alleles at the site in question are neutral, $k_a = u$ (Donnelly 1991), so that, under this condition, phylogenetic and pedigree studies are indeed aiming to estimate the same thing. To come back to the molecular clock hypothesis, note that, under neutrality, $u = k_a \geqslant k_s$, with the final inequality being approximately an equality when u is small relative to the inverse of the effective population size (Donnelly 1991). The explanation of the discrepancy for large u is that, in this case, a mutation that occurs on the ancestral lineage may not have time to fix in the population before there is another mutation on that lineage. Our principle point here is that whether u is small—and, hence, whether the molecular-clock hypothesis prevails—is largely irrelevant. We have no information, beyond that in k_a, about the substitution rate in any event.

The above argument can be extended to consider a sequence of linked sites. The result is that, except at sites on which selection is acting directly, phylogenetic and pedigree approaches are estimating the same quantity. This conclusion remains true even if there is selection on other parts of the mitochondrial molecule (to which, of course, the CR is completely linked), unless the mutation rate in the CR is affected by the sequence in the rest of the molecule. As has been noted elsewhere

(e.g., see Parsons et al. 1997), if there is such dependence between CR mutation rate and the haplotype elsewhere in the molecule, all methods for estimation of mutation rates face severe difficulties. There may be selection on specific sites within the CR, because of its regulatory and other function, and this has been suggested as one reason why no polymorphism has ever been observed at some sites. For a detailed discussion, see, for example, the work of Meyer et al. (1999) and references therein. The positions at which we (and most others) have observed mutations show polymorphism in the population and are therefore unlikely to be under strong selection. More generally, our point is that a systematic difference between phylogenetic and pedigree estimates will occur only under assumptions about selection that are much more limited than those that have been suggested elsewhere.

One additional reason for a systematic difference between phylogenetic and some pedigree rate estimates is that some apparent mutations (or heteroplasmy) observed in pedigree studies may be purely somatic and not reflected in the germline. Limited understanding of the various replication processes makes it difficult to assess the likely impact of this effect. In the light of recent evidence of increased somatic mutations with age (Michikawa et al. 1999), care may be needed in the interpretation of the results of some pedigree studies, in which somatic mutations may be confounded with germline mutations, especially if older individuals are involved. We note that we can be certain that each of our observed mutations represents a genuine change in the germline (and conversely, that, although each instance of heteroplasmy in our study could have been somatic, there were no somatic mutations in our study).

It has recently been argued, on the basis of indirect evidence (e.g., see the study by Awadalla et al. [1999], who noted that linkage disequilibrium in chimpanzee and human mtDNA declines as a function of distance between sites) that mtDNA is subject to recombination. We cannot exclude recombination as the explanation for the small clusters of individuals with variant sequences in our study. This phenomenon needs to be examined further by looking at polymorphisms widely spaced throughout the mitochondrial genome.

Conclusion

Our estimate of .0043 per generation (.32/site/Myr [95% CI .065–.97) for the average mutation rate in the mtDNA CR is intermediate between the larger estimates from pedigree studies and the range of phylogenetic estimates, although the 95% CI for our estimate includes many of the existing phylogenetic estimates. We have argued that several of the explanations posited for a systematic difference between phylogenetic and pedigree estimates of mutation rates are more limited than they might first appear to be. Pedigree estimates of this mutation rate are unbiased, regardless of the heterogeneity in rates—or of mutational hot spots—in the CR. Unless it is modeled accurately, such heterogeneity may well lead to underestimation in phylogenetic studies. Only if there is selection acting directly on the sites at which mutations are observed in pedigree studies can population-genetics effects be invoked as a cause of systematic differences between pedigree and phylogenetic estimates.

Our study design allowed us to rule out somatic events as a cause of observed point mutations. Possible confounding of somatic and germline mutations could lead to overestimation of mutation rates in other pedigree studies. For several reasons, it is not clear how to treat instances of heteroplasmy in the estimation of mutation rates. Unlike authors of some studies, we have not included such instances in our estimate.

Many authors (e.g., see Loewe and Scherer 1997; Macaulay et al. 1997; Parsons et al. 1997) have noted that simply recalibrating time estimates from mtDNA data to reflect the larger of the pedigree estimates of mutation rates leads to apparently silly conclusions. Rate heterogeneity is of crucial importance here. If, as now seems likely, it is substantial, then one cannot simply calibrate time estimates over relatively long periods by using the average mutation rate, a point made by Macaulay et al. (1997). Ironically, when the times of various events are inferred by phylogenetic methods that fail to adequately model rate heterogeneity, results based on inaccurate phylogenetic estimates of average rates may be more reliable than those based on estimates that are more accurate. Thus, although, in the estimation of the average mutation rate in the CR, well-designed pedigree studies may be more reliable than phylogenetic approaches, sensible interpretation of mtDNA data will increasingly require detailed information about the mutation-rate variation across this region.

Acknowledgments

We wish to thank the Icelandic individuals who participated in this study for their generous support in this work. P.D. was supported in part by U.K. Engineering and Physical Sciences Research Council grant GR/M1497 and Biotechnology and Biological Sciences Research Council grant 43/MMI09788. We thank Vincent Macaulay for helpful comments on an early draft of the manuscript. We are also grateful to E. Bjarnadóttir for her assistance with some of the lab work.

Electronic-Database Information

URLs for data in this article are as follows:

deCODE Genetics, (http://www.decode.is/ppt/protection/index.htm)

Medscape, http://molecularmedicine.medscape.com/10835.rhtml (for The Icelandic Healthcare Database: a tool to create knowledge, a social debate, a bioethical and privacy challenge)

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