Multiple Origins of a Mitochondrial Mutation Conferring Deafness

Timothy P. Hutchin and Gino A. Cortopassi

University of California, Davis, California 95616

Manuscript received June 27, 1996
Accepted for publication October 30, 1996

ABSTRACT
A point mutation (1555G) in the smaller ribosomal subunit of the mitochondrial DNA (mtDNA) has been associated with maternally inherited traits of hypersensitivity to streptomycin and sensorineural deafness in a number of families from China, Japan, Israel, and Africa. To determine whether this distribution was the result of a single or multiple mutational events, we carried out genetic distance analysis and phylogenetic analysis of 10 independent mtDNA D-loop sequences from Africa and Asia. The mtDNA sequence diversity was high (2.21%). Phylogenetic analysis assigned 1555G-bearing haplotypes at very divergent points in the human mtDNA evolutionary tree, and the 1555G mutations occur in many cases on race-specific mtDNA haplotypes, both facts are inconsistent with a recent introgression of the mutation into these races. The simplest interpretation of the available data is that there have been multiple origins of the 1555G mutation. The genetic distance among mtDNAs bearing the pathogenic 1555G mutation is much larger than among mtDNAs bearing either evolutionarily neutral or weakly deleterious nucleotide substitutions (such as the 4396G mutation). These results are consistent with the view that pathogenic mtDNA haplotypes such as 1555G arise on disparate mtDNA lineages which because of negative natural selection leave relatively few related descendants. The co-existence of the same mutation with deafness in individuals with very different nuclear and mitochondrial genetic backgrounds confirms the pathogenicity of the 1555G mutation.

PATHOGENIC mutations of the mitochondrial genome cause mitochondrial genetic disease (WALLACE 1994) of which a very common phenotype is sensorineural deafness (DIMAURO and MORAES 1993); the high requirement of the excitable sensorineural hair cells for mitochondrial energy is a likely explanation for their sensitivity to mitochondrial deficits. The 1555G mutation of the mitochondrial 12S ribosomal RNA is strongly associated with two maternally inherited deafness traits: aminoglycoside-induced deafness (AGD), a hypersensitivity to normal doses of antibiotics, and non-syndromic deafness (NSD), a deafness uncomplicated by other morphological features that is not the result of exposure to aminoglycosides.

The 1555G mutation has been reported in 11 pedigrees or cases of aminoglycoside-induced deafness (AGD) from China, Japan and South Africa (HUTCHIN et al. 1993; PREZANT et al. 1993; GARDNER and GREENBERG, personal communication). Aminoglycoside antibiotics exert their pharmacological effect by disrupting protein synthesis on bacterial ribosomes (BENVENISTE and DAVIES 1973; GALE et al. 1981). It has been proposed the 1555G mutation leads to hypersensitivity as a result of increased binding of aminoglycosides to the bacterial-like mitochondrial ribosome (HUTCHIN et al. 1993). Use of aminoglycoside antibiotics is widespread in Asia and Africa, particularly in the treatment of tuberculosis, despite their known ototoxic side-effects (SANDE and MANDELL 1990).

This 1555G mutation has also been observed in two pedigrees with NSD, in the Middle East and Zaire (PREZANT et al. 1993; MATTHIJS et al. 1996). This maternally inherited trait has variable penetrance in bearers of 1555G, which has been attributed to the assortment of a nuclear factor that sensitizes mitochondrial ribosomes to the mitochondrial defect (JABER et al. 1992).

The occurrence of the identical pathogenic mtDNA mutation in very separate locales could result from a recent introgression of the mutation into these populations, the persistence of this mutation in modern humans since before racial diversification, or independent occurrence of mutations on disparate mtDNA lineages. We have addressed this issue by comparing sequences of the very rapidly evolving, noncoding D-loop region from 10 of these mtDNAs bearing pathogenic mutations with many other published mtDNAs from normal individuals.

MATERIALS AND METHODS

Isolation and sequencing of DNA: Cases carrying the 1555G mutation used in this study were one Chinese pedigree (PREZANT et al. 1993), three Japanese pedigrees, four Chinese sporadic cases (HUTCHIN et al. 1993) and a South African pedigree (GARDNER and GREENBERG). Sporadic cases were from individuals treated with aminoglycosides who subsequently developed deafness in which we identified the 1555G mutation (HUTCHIN et al. 1993). mtDNA was isolated from blood or hair samples as previously described (HUTCHIN et al.
The mtDNA variation within HVSI of the D-loop of 1555G individuals

<table>
<thead>
<tr>
<th></th>
<th>Anderson</th>
<th>CP1</th>
<th>CS1</th>
<th>CS2</th>
<th>CS3</th>
<th>CS4</th>
<th>JP1</th>
<th>JP2</th>
<th>JP3</th>
<th>AP1</th>
<th>AP2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>C</td>
<td>T</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>T</td>
<td>T</td>
<td>C</td>
<td>A</td>
<td>T</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>T</td>
<td>C</td>
<td>A</td>
<td>C</td>
<td>C</td>
<td>T</td>
<td>C</td>
<td>C</td>
<td>G</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
</tr>
</tbody>
</table>

Only differences from the reference sequence (Anderson et al., 1981) are shown, numbers correspond to the nucleotides in the Cambridge sequence. CP, Chinese pedigree; CS, Chinese Sporadic cases; JP, Japanese pedigree; AP, African pedigree.

RESULTS

The sequence divergence of D-loops bearing 1555G is extensive: D-loop sequences of the 10 cases with 1555G mutation were compared with >900 other D-loop sequences in our database collected from published sources (Cann et al., 1984; Horai and Matsunaga, 1986; Vigilant et al., 1989, 1991; Stoneking et al., 1990, 1991; Ward et al., 1991; Torroni et al., 1992, 1993; Ballinger et al., 1992; Horai et al., 1993). Phylogenetic analysis of the first 370 nucleotides of D-loops was carried out by transforming the sequence differences into a distance matrix (Fitch and Margoliash, 1967), using Dnadist of the PHYLIP package (Felsenstein, 1993) and a phylogenetic tree constructed using the neighboring program (Saitou and Nei, 1987) as previously described (Hutchin and Cortopassi, 1995a, b) with the chimpanzee D-loop to root the tree.

Thus the maximal divergence observed among 1555G-bearing mtDNAs is substantial and larger than that usually observed within African, Asian and Caucasian mtDNAs. For example, the mean pairwise divergence among D-loops bearing the 4336G mutation in tRNAGln is 0.33%, sixfold lower than that of the 1555G-bearing D-loops (Hutchin and Cortopassi, 1995b).

The mtDNA haplotypes of the South African and Zairean 1555G pedigrees are distinctly African: Although very divergent from one another at the sequence level, the two D-loops from Africa do share racespecific polymorphisms, most significantly at sites 16294 and 16311. Both have a T at site 16294 that is found in only 2.4% of Asian mtDNAs whereas this is found in 77.9% of Africans (134 of 172) but only 7.3% of Asians (34 of 463) and 16% of Caucasians (56 of 351). When these two sites are taken together, i.e., 16294T + 16311C, this haplotype is found in 41.3% of African mtDNAs (71 of 172) in the database, whereas this is found in only 2.4% of Asian mtDNAs (11 of 463) and 10.5% of Caucasian mtDNAs (37 of 351). None of the Asian 1555G cases had 16294T.

Both African families but only one Asian family, carried a C at site 16311. This is the case in 77.9% of Africans (134 of 172) but only 7.3% of Asians (34 of 463) and 16% of Caucasians (56 of 351). When these two sites are taken together, i.e., 16294T + 16311C, this haplotype is found in 36.6% of Africans (63 of 172) but none of 463 Asians and only one of 351 Caucasian (0.3%). Thus the occurrences of 1555G in Africa are on typically African mtDNA haplotypes. None of the Asian mtDNAs bearing 1555G carried both of these polymorphisms.

Phylogenetic analysis of 1555G pedigrees from South Africa and Zaire: More than 700 mtDNA D-loops were analyzed using the PHYLIP program. More than five iterations with the full data set, and 10 with less taxa gave essentially the same tree each time. As found in previous studies (Johnson et al., 1983; Vigilant et al., 1989, 1991; Horai and Hayasaka, 1990; Stoneking et
Evolution of Mitochondrial Deafness

al. 1991) mtDNAs from the same race of origin often cluster together on the same branch (Figure 1). The South African 1555G pedigree lies on a branch consisting entirely of Africans, predominantly !Kung. Thus although the parentage of the South African pedigree is mixed: San, Khoi-Khoi, West African, European, Madagascan and Javanese (GARDNER et al. 1996) the mtDNA on which the 1555G is carried was most closely associated with other African mtDNA haplotypes.

The Asian 1555G cases all occur on Asian-specific mtDNA haplotypes: Each of the eight occurrences of the 1555G in Asian mtDNAs we sequenced occurred on Asian-specific mtDNA haplotypes as determined by PHYLIP analysis (Figure 1). With the exception of two individuals, there was no close phylogenetic grouping of sequences. One of the Asian mtDNAs (CS4) lay on a very separate branch from the other seven. Its divergent position phylogenetically was also reflected in its extensive sequence divergence from all other Asian 1555G bearing mtDNAs (2.39%). The category of this singleton case fits the mtDNA category of type I mtDNAs as defined by HORAI and MATSUNAGA (1986). The other seven 1555G mutations are on type II mtDNAs. The statistically higher incidence of 1555G mutations on a type I vs. type II genetic background has been inferred to indicate that particular mtDNA haplotypes may be associated with risk for particular mitochondrial genetic disease in the absence of information about specific pathogenic mutations, such as the 1555G (HUTCHIN and CORTOPASSI 1995a).

Estimates of times of divergence of 1555G-bearing mtDNAs: Several groups have used mtDNA sequence divergence as a molecular clock to date animal evolutionary divergences (STONEKING 1994; CHEN et al. 1995; HORAI et al. 1995). VIGILANT et al. (1991) estimated the mutation rate of the human mtDNA D-loop to be between 11.5 and 17.3% per million years. Using such a metric, one is able to infer the approximate evolutionary time since any two mtDNAs shared a common ancestor, i.e., a coalescence time. The minimum coalescence times vary from 31,000 to 47,000 yr for CS2 and CS3 up to 248,000–374,000 yr for CS3 and AP2. The South African and Zairean mtDNA lineages were separated from one another by 201,000–305,000 yr and from all Asian cases by 133–204,000 yr and 220,000–300,000 yr, respectively. Thus even the most closely related 1555G mtDNAs were strikingly divergent, in contrast to the close relationship of mtDNAs bearing neutral or mildly deleterious polymorphisms (HUTCHIN and CORTOPASSI 1995b).

DISCUSSION

The 1555G mutation occurs on very distinct mitochondrial genetic backgrounds: The observation of a 1555G mtDNA mutation in multiple populations could be explained in three ways: a recent introgression of the mutation into these populations, persistence of the

...
mutation in modern humans since before racial diversification, or independent occurrences of mutation on disparate mtDNA lineages.

Given the large differences in sequences and their distinct phylogenetic association with the race of origin, we observe no support for the first hypothesis of recent introgression. All 1555G mutations sequenced occur on mtDNA lineages that are very typical of the race in which the case was observed, and very unlike mtDNA haplotypes of 1555G-bearing mtDNA from other races.

Although it is impossible to categorically disprove the second hypothesis, that these occurrences of the 1555G are the result of persistence of a single ancestral mutation that preceded the racial diversification of modern humans, the available data are most simply interpreted otherwise.

First, the 1555G mutation is rare, and it is unlikely that a rare mutation would occur in the mitochondrial gene pool of the founders of a new race. Surveys have indicated that the 1555G occurred in 0/123 Caucasians, 0 of 302 Asians and 0 of 108 Africans (Hutchin et al. 1993; Prezant et al. 1993; Matthys et al. 1996).

Second, the most probable reason for the scarcity of the 1555G is that it is pathogenic on some nuclear genetic backgrounds, when co-inherited with a polymorphic nuclear factor the 1555G causes bilateral deafness in the absence of aminoglycosides (Jaber et al. 1992). This probably explains why 1555G is one of the few mtDNA disease mutations that occur homoplasmically—the mutation by itself is likely benign except in the context of a nuclear factor or aminoglycoside exposure, which "uncover" the pathogenicity of 1555G. Deaf progeny are less likely to leave descendants, and a mutation causing deafness would be unlikely to persist for the hundreds of thousands of years of human evolution inferred from the divergence data.

Third, if one were to postulate that the 1555G-bearing mtDNAs were related by descent, which appears very unlikely, the best estimate for the time of a single common 1555G ancestor is 248,000–374,000 yr ago. A single origin hypothesis for 1555G would indicate that the mutation occurred at a very ancient time, possibly at the emergence of modern humans, and at a time when humans were hunter-gatherers, a time in which deafness would be a severe and likely life-limiting deficiency.

Fourth, if the 1555G mutation occurred only once during human evolution, then one would expect mtDNAs bearing this mutation to be more closely related on a single lineage. As clearly demonstrated in Figure 1, with the exception of two somewhat closely related Asian cases, all 1555G cases in which D-loops

---

**Figure 2.**—Phylogenetic analysis of D-loops from mtDNAs bearing the 4336G mutation. ○, Caucasians; □, Africans; ○, Asians; ●, individuals with Alzheimer's disease. 4336G-bearing mtDNAs are indicated with a bracket. This redrawn figure is from data presented in Hutchin and Cortopassi (1995b).
neutral mtDNA: 4336G

pathogenic mtDNA: 1555G

Consistent with:

Figure 3.—A model to explain the genetic relatedness of mtDNA lineages bearing neutral vs. deleterious mutations. Top: Ideogram of the genetic relationship of 1555G- and 4336G-bearing mtDNAs. Bottom: an explanation consistent with the differences in diversity observed. Bold lines indicate lineages carrying a 1555G or 4396G mutation; abbreviated bold lines represent mtDNA lineages inhibited or extinguished by natural selection.

Selection forces shaping mtDNA trees bearing pathogenic mutations: We observe a very distant relationship of mtDNAs bearing a pathogenic 1555G mutation (Figure 1). This is in striking contrast to the relationship of mtDNAs bearing selectively neutral or slightly deleterious mutations, the 4336G mutation in the tRNA\textsubscript{Gln} gene being one example (Figures 2 and 3). The 4336G mutation has been associated by some with an increased risk of Alzheimer's and Parkinson's disease (SHOFFNER et al. 1993; HUTCHIN and CORTOPASSI 1995b), and the reasons why natural selection is unlikely to act strongly on mutations conferring sensitivity to late-onset disease have been discussed in detail (HUTCHIN and CORTOPASSI 1995b).

In the case of the 4336G, the D-loops of every isolate sequenced are more closely related to each other than to any other D-loop in the database, and there was only 0.33% mean pairwise divergence among them. This is in striking contrast to D-loops of mtDNAs bearing the pathogenic 1555G, which are very distantly related to each other, and have 2.21% mean pairwise divergence among them. Thus the tree depicting genetic relatedness of the pathogenic 1555G-bearing mtDNAs has deep and sparse branches, the tree for the effectively evolutionarily neutral 4336G-bearing mtDNAs is shallow and bushy (Figure 2, top). An explanation for the differences in the trees is differential extinction of 1555G-bearing lineages by negative natural selection. The 1555G may arise in the absence of a deleterious segregating nuclear factor, but its spread may be limited by that nuclear factor, whose co-inheritance with 1555G results in bilateral deafness, and a lower likelihood of leaving descendants. Thus it is likely that negative natural selection acts more strongly to extinguish 1555G-bearing lineages than 4336G-bearing lineages, producing a greater diversity among individual isolates. By contrast selection will not act strongly to limit the spread of neutral or slightly deleterious mutations and so mtDNA branching patterns will basically track with population expansions.

Aminoglycoside prescription and genetic counseling in Africa and Asia: There are substantial differences in the nuclear genetic backgrounds of Asians, Africans and Israe-
lis (Tishkoff et al. 1996). Thus the occurrence of deafness (either of the aminoglycoside-induced or nonsyndromic type) with this mutation on very different nuclear genetic backgrounds (African, Japanese, and Chinese) adds further support to the claim that 1555G is a major determinant of deafness in these individuals.

The continued and widespread use of aminoglycoside therapy in Africa and Asia makes it unfortunately very likely that new examples of the 1555G mutation will be described; thus it is important for physicians and audiologists as well as the general public to be aware of the potential of a familial hypersensitivity to these drugs, so that exposure of multiple matrilinearly related individuals in a family at risk is avoided.

We thank Anna di Rienzo, Joe Feustenstein, Nathan Fischel-Ghodsi, Jaquie Greenberg, Kichiro Higashi and Wei-Qiu Qiu for helpful discussion and providing samples. This research was supported by National Institutes of Health grant AG-11967.

LITERATURE CITED


