# Letters to the Editor

*Am. J. Hum. Genet. 66:1173–1177, 2000*

## mtDNA Haplogroups and Frequency Patterns in Europe

*To the Editor:*

Recently, an article by Simoni et al. (2000), who used (i) SAAP analysis to analyze the population frequencies of mtDNA haplogroups and (ii) AIDA analysis to examine both the frequency and the sequence similarity of truncated mtDNA sequences, appeared in this *Journal*. The main outcome of their study was that "the overall patterns of mtDNA diversity appear to be poorly significant in Europe." The raw data comprised 2,619 hypervariable segment I (HVS-I) sequences (denoted as "HVR-I" [hypervariable region I] sequences by Simoni et al. [2000]) that were obtained from 36 regions or populations of Europe, the Near East, and the Caucasus and that were collected from both the literature and unpublished sources. Simoni et al. ostensibly grouped the HVS-I sequences according to haplogroup motifs proposed elsewhere (Richards et al. 1998), and they reported the resulting frequencies for each region/population in table 3 in their study. We have checked the input data displayed in table 3 and have found serious technical errors affecting numerous entries. More critically, the mtDNA categories that they report correspond neither to their own criteria nor to the haplogroup definitions established in the literature (to which they refer). Furthermore, their decision to truncate HVS-I information (and to disregard RFLP information) renders these data inadequate to differentiate even African and East Asian sequences from European sequences in many cases.

Inspection of table 3 in the study by Simoni et al. (2000) reveals that (i) the data in the "Galicia" and "Spain: Central" rows have been, in part, crossed-over, (ii) the data in the "Belgium," "Alps," and "Turkey" rows have been computed with the use of sample sizes smaller than those reported in table 1 in the same study, (iii) the haplogroup "J" column has been totally randomized, and (iv) the "Other" column is complementary to the last four "superhaplogroup" columns but not to the first 11 haplogroup columns. As for item (iii), almost all positive entries in the haplogroup "J" column have been either displaced or calculated with the use of sample sizes corresponding to nearby rows. Hence, most entries in this column diverge widely from the real haplogroup J frequencies (see the last column of table 1 in the present study).

As an example of their haplogroup assignment, Simoni et al. (2000) specifically referred to the motif 16069T–16126C for haplogroup J, but they overlooked the fact that this criterion cannot formally be applied to the sequences in the study by Richards et al. (1996), since these were reported only between 16090 and 16365. This might explain some of the many "0" entries in the haplogroup "J" column of table 3 in the Simoni et al. study (see table 1 in the present study). Simoni et al. should have either adopted the haplogroup J frequencies reported by Richards et al. (1996), excluded these population samples from their study, or trimmed all data to the shortest common segment. In the latter case, by employing the motif 16126C–16294C, one could take the default cluster JT-T (comprising all JT sequences that are not T) as a crude default criterion for haplogroup J (see table 1 in the present study).

The discrepancies in haplogroup frequencies are by no means restricted to haplogroup J. Table 2 in the present study shows the marked contrast between published haplogroup frequencies and those assumed by Simoni et al. (2000) for the well-characterized Tuscan, Druze, and Adygei samples (which were typed for RFLPs as well as for HVS-I sequences by Torroni et al. [1996] and Macaulay et al. [1999]). The large differences in frequency for haplogroup H, the most-common European haplogroup, are due to the premise of Simoni et al. (2000) that haplogroup "H contains all sequences . . . that show none of the 22 substitutions considered in this study." This extreme simplification results, on the one hand, in the dumping of large numbers of haplogroup H mtDNAs mainly into the default category "Other" and, on the other hand, in the inclusion of several non-H sequences within their haplogroup H category. For instance, by their criterion, 10/20 haplogroup H mtDNAs from the Tuscan sample (Torroni et al. 1996) would no longer be scored as "H," whereas the U sequence 16051G–16309G–16318C would be scored as "H." In consequence, the haplogroup H category described by Simoni et al. (2000) is bound to be highly polyphyletic

**Table 1**

**Haplogroup J Frequencies According to Simoni et al. (2000), a Crude Default Criterion, and Inference in the Present Study**

| Population/Region[a] | Sample Size[b] | Haplogroup J Frequency (%) According to | | |
|---|---|---|---|---|
| | | Simoni et al. (2000) | Crude Default Criterion (16126C–16294C)[c] | Inference in the Present Study[d] |
| Austria | 117 | .0 | 12.8 | 11.1 |
| Cornwall | 69 | .0 | 21.7 | 21.7 |
| United Kingdom mainland | 100 | 1.1 | 12.0 | 12.0 |
| Wales | 92 | .0 | 15.2 | 15.2 |
| Bulgaria | 30 | 6.7 | 10.0 | 10.0 |
| Adygei | 50 | .0 | 4.0 | 4.0 |
| Denmark | 33 (32) | .0 | 18.2 | 18.2 |
| Estonia | 28 | .0 | 7.1 | 7.1 |
| Finland | 79 | 4.4 | 8.9 | 8.9 |
| North Germany | 107 (108) | 17.8 | 9.3 | 8.4 |
| South Germany | 249 | .0 | 10.0 | 8.8 |
| Iceland | 53 | 1.4 | 17.0 | 17.0 |
| Druze | 45 | .0 | 11.1 | 6.7 |
| Tuscany | 49 | .0 | 18.4 | 14.3 |
| Karelia | 83 | .0 | 6.0 | 3.6 |
| Near East | 42 | .0 | 35.7 | 19.0 |
| Norway | 30 | 1.9 | .0 | .0 |
| Portugal | 54 | .0 | 7.4 | 5.6 |
| Saami[e] | 312 (240) | 1.9 | 3.8 | .0 |
| Basques | 106 | .0 | 2.8 | 2.8 |
| Catalunya | 15 | .0 | 6.7 | 6.7 |
| Mixed Spain[f] | 74 | .4 | 9.5 | 8.1 |
| Galicia | 92 | .0 | 9.8 | 8.7 |
| Sweden | 32 | 2.7 | 9.4 | 9.4 |
| Switzerland | 70 (72) | .0 | 11.4 | 11.4 |
| Turkey | 96 (95) | .0 | 16.7 | 15.6 |
| Volga-Finnic | 34 | 3.2 | 17.6 | 17.6 |

[a] Population samples from published data tables and data banks cited by Simoni et al. (2000).

[b] Samples sizes are taken from the original sources, except for the Swiss sample, where four close maternal relatives were excluded (Richards et al. 1998, p 243). The sample sizes actually employed by Simoni et al. (2000) are given in parentheses whenever there is a discrepancy.

[c] The mechanical application of this criterion captures a number of non-J sequences with motif 16126C–16362C, especially in Near Eastern populations.

[d] The inference was made on the basis of (i) the motif 16069T–16126C and in conjunction with partial screening of 16069 (Richards et al. 1996), (ii) incorporating of HVS-II (J motif 00295T; Torroni et al. 1996) and RFLP information whenever available, and (iii) appreciating recurrent mutations at 16126.

[e] According to the original sources, an unspecified number of the 312 Saami may be related to each other.

[f] Denoted as "Central Spain" by Simoni et al. (2000).

in the mtDNA genealogy and does not reflect the spatial patterns of haplogroup H.

At this point, it is important to clarify what haplogroup classification entails. An mtDNA haplogroup, when properly defined, is a monophyletic clade of the mtDNA genealogy. Originally, high-resolution RFLP analysis (employing 14 enzymes) had been used for identification of clades by signature sites (Torroni et al. 1992, 1993, 1994*a,* 1994*b,* 1996; Chen et al. 1995), and current haplogroup nomenclature originated in that context. In retrospect, this approach is indeed quite reliable, although recurrent changes at a few sites, such as 10394 *Dde*I, may occasionally cause problems. Potential ambiguities can largely be resolved by incorporation of information from other segments of mtDNA sequences or specific positions of the coding regions (Torroni et al. 1997; Brown et al. 1998; Starikovskaya et al. 1998; Macaulay et al. 1999; Quintana-Murci et al. 1999; Schurr et al. 1999). For instance, haplogroup K is now understood to be a clade (as are U1–U6) within haplogroup U. HVS-I data in combination with partial RFLPs can sometimes serve as a satisfactory substitute for a full RFLP analysis (Rando et al 1998, 2000; Kivisild et al. 1999*a,* 1999*b*).

**Table 2**

**Haplogroup Frequencies, According to Simoni et al. (2000) vs. the Original Studies, in Tuscan, Druze, and Adygei Populations**

| Population and Study | Sample Size | Haplogroup Frequency (%) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | H | I | J | K | T | U3 | U4 | U5 | V | W | X | U[a] |
| Tuscan: | | | | | | | | | | | | | |
| Simoni et al. (2000) | 49 | 22.4 | 4.1 | 0 | 6.1 | 6.1 | 0 | 4.1 | 6.1 | 0 | 2.0 | 4.1 | 16.3 |
| Francalacci et al. (1996), Torroni et al. (1996)[b] | 48 | 41.7 | 4.2 | 14.6 | 6.3 | 10.4 | 0 | 2.1 | 4.2 | 0 | 2.1 | 8.3 | 16.7 |
| Adygei: | | | | | | | | | | | | | |
| Simoni et al. (2000) | 50 | 22.0 | 6.0 | 0 | 2.0 | 14.0 | 14.0 | 4.0 | 8.0 | 0 | 0 | 0 | 28.0 |
| Macaulay et al. (1999) | 50 | 30.0 | 0 | 4.0 | 2.0 | 14.0 | 14.0 | 2.0 | 8.0 | 0 | 2.0 | 0 | 34.0 |
| Druze: | | | | | | | | | | | | | |
| Simoni et al. (2000) | 45 | 24.4 | 4.4 | 0 | 15.6 | 4.4 | 0 | 0 | 0 | 0 | 0 | 17.8 | 15.6 |
| Macaulay et al. (1999) | 45 | 13.3 | 2.2 | 6.7 | 15.6 | 4.4 | 0 | 0 | 0 | 0 | 0 | 26.7 | 26.7 |

[a] Simoni et al. (2000) had labeled this category as "KU, " which is a misnomer since U encompasses not only U3–U5 but also K and other clusters (Richards et al. 1998; Macaulay et al. 1999).

[b] Of the 49 Tuscans reported in Francalacci et al. (1996), 48 were RFLP analyzed by Torroni et al. (1996).

Unfortunately, HVS-I data alone, which have been produced en masse, often do not contain sufficient information for confident assignment of haplogroup affiliation. The truncation of the HVS-I data to only 13–22 variant positions, as performed by Simoni et al. (2000), yields even poorer results. For example, the motif 16223T–16278T, which was used by Simoni et al. to identify haplogroup X, would transfer most African L1/L2 sequences (Watson et al. 1997; Rando et al. 1998) into the then artefactual category "X." For Europe, this is relevant insofar as a few L1/L2 sequences are present in Iberia (Rocha et al. 1999), and there even resides an African L1c sequence with the motif 16223T–16278T in the British data (Piercy et al. 1993). In addition, as was previously pointed out (Torroni et al. 1996; Macaulay et al. 1999), one has to be prepared for recurrent mutations in the HVS-I motifs (compare also figs. 4, 5, 8, and 9 of the study by Richards et al. [1998]). For instance, the frequency discrepancy (17.8% vs. 26.7%) for haplogroup X in the Druze sample (see table 2 in the present study) is due to the fact that Simoni et al. did not include four haplogroup X mtDNAs that have mutated to 16223C. Another of the many possible examples of misclassification caused by the use of truncated motifs is illustrated by 16129A–16223T, the motif used by Simoni et al. for classification of haplogroup I mtDNAs. Use of this truncated motif has led them to classify both the Asian haplogroup C mtDNAs (16129A–16223T–16298C–16327T) of the Adygei (6.0%) and the East African haplogroup M1 mtDNA (16129A–16189C–16223T–16249C–16311C–16359C) of the Druze (2.2%) as members of haplogroup I (see table 2 in the present study).

The issue of haplogroups only affects the SAAP analysis. However, there are also serious difficulties with the AIDA analysis. Ideally, AIDA should be applied to full DNA-sequence data, but Simoni et al. (2000) included only 22/241 variant positions. One cannot expect that such a truncated data set would show much evidence of geographic patterns within Europe. Most of the haplogroup diagnostic variants in western Eurasian mtDNA are very ancient, and they probably evolved in the Near East and subsequently spread to Europe (Torroni et al. 1998; Macaulay et al. 1999); at any event, they occur throughout western Eurasia. The more recent "rare substitutions," which have evolved since the earlier dispersals and which Simoni et al. (2000) discarded as "statistical noise," are precisely those that are most likely to show regional distributions. The exclusion of such mutations severely restricts the capacity to identify phylogeographic units and, thus, is bound to have seriously reduced the power of the approach to detect autocorrelation.

Even when haplogroup assignment is done with care, failure to detect significant clines in haplogroup frequencies does not prove the absence of any spatial structure in the mtDNA pool. Such structure would rather be manifest at a phylogenetically finer scale (defined on the basis of more-recent mutations). In any case, one would not expect that meaningful patterns of mtDNA diversity could emerge from analyses based on categories with no demonstrable phylogenetic support.

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## Reconstruction of Prehistory on the Basis of Genetic Data

*To the Editor:*

In their letter, Torroni et al. (2000) express a radical disagreement with the assumptions, methods, and conclusions of Simoni et al.'s (2000) article. We think that their many criticisms can be reduced to four points:

1. Haplogroups have been incorrectly defined, and therefore the spatial autocorrelation analysis (SAAP) of their frequencies is flawed;
2. Aside from these errors, the frequencies of haplogroup J and of superhaplogroup JT do not match previous reports;
3. Only 22 polymorphic sites have been considered, and therefore the results of AIDA are flawed;
4. Meaningful patterns of mtDNA diversity can only be identified by the analysis of the distributions of recent mutations.

Point 2 is correct. In the article by Simoni et al. (2000), the column with the frequencies of haplogroup J is wrong, and the frequencies of several haplogroups in Galicia and Spain have been put in each other's places. We apologize to the readers for these errors. However, the correct data (see the erratum published in this issue of the *Journal*) were used in all the analyses, including SAAP, and therefore the autocorrelation results in table 5 in the article by Simoni et al. (2000) are correct. Before we consider the other points, it is important to exactly define the subject of this discussion.

The general question being asked in our study and in similar studies is: What combination of evolutionary factors is most likely to account for the current levels and patterns of genetic diversity? To answer this question, one has to study as many loci as possible and has to study them by using the same statistical methods, so that the results will be comparable. The methods of SAAP and AIDA are especially suitable, because they have long been used to summarize both protein (Sokal and Menozzi 1982; Sokal et al. 1989; O'Rourke et al. 1992; Epperson and Li 1996; Crawford et al. 1997) and DNA (Barbujani et al. 1995; Chikhi et al. 1998; Casalotti et al. 1999; Krings et al. 1999; Rickards et al. 1999) diversity.

*Point 1: haplogroup definition and SAAP.*—AIDA can be directly applied to any set of DNA data, whereas SAAP processes frequencies and therefore requires prior definition of the entities whose frequencies will be analyzed. AIDA found very little spatial structuring of mtDNA. To confirm this result, we reanalyzed the data by using SAAP, and hence we had to identify evolutionarily meaningful clusters of hypervariable region 1 (HVR-1) haplotypes, or haplogroups.

The categories that we used for that purpose—and that Torroni et al. (2000) question—were proposed by Richards et al. (1998) in a paper cosigned by two other authors of Torroni et al.'s letter. The classification of mitochondrial haplotypes is no easy task; there is an unresolved uncertainty about the best way to cluster and interpret mitochondrial data. Analysis, at the nucleotide level, of the whole mitochondrial genome will be a suitable approach only in the not-so-near future. Indeed, as we stated in the "Database" section of the article by Simoni et al. (2000), only three European samples have been typed at the RFLP level. There is no current alternative to the study of HVR-1 sequences, if one wants to understand whether mitochondrial variation shows any structuring in Europe. On the basis of 22 polymorphic sites, Richards et al. (1998) identified what they consider to be monophyletic clades in the HVR-1 phylogeny, and we chose to use those sites to define haplogroups. Of course, the frequencies of the haplogroups defined in this way do not perfectly overlap with those which are based on RFLPs (table 1 in Torroni et al. 2000). We have since discovered that they even differ between table 2 and figure 2 of Richards et al. (1998), because site 16189 is mentioned as being part of the "X motif" only in the former, and we trusted the latter. That is not our fault.

In quantitative terms, a nonparametric discriminant analysis that we ran on worldwide data shows that 15.3% of suitable mitochondrial data are assigned to different haplogroups that are based on RFLPs or on HVR-1 sequences, with variable levels of disagreement for the different haplogroups—for example, 7.1% for haplogroup J (for more details, see Simoni 2000); that 7.1% of uncertainty accounts, for example, for most of the persisting differences between the haplogroup J frequencies that we considered and the frequencies presented in Torroni et al.'s (2000) table 2.

Answering the criticisms raised by Torroni et al. (2000), which we do not feel are justified, would entail reclassification of just a few sequences, <20 for haplogroups X and U4, of a total of >800 distinct sequences. After they have been reallocated, the SAAP coefficients do not change, up to the second decimal place. Table 1

**Table 1**

**SAAP of Superhaplogroup JT Frequencies**

| Upper Limits for Distance Classes (Pairs of Populations) | *II*[a] |
|---|---|
| 500 (31) | −.06 |
| 1,000 (92) | −.12 |
| 1,500 (98) | .07 |
| 2,000 (96) | .01 |
| 2,500 (106) | −.16 |
| 3,000 (97) | .04 |
| 3,800 (79) | .02 |
| 5,310 (31) | −.04 |

[a] The overall probability of the correlogram is not significant.

shows that nothing much happens after modification of the frequencies of JT either. Only for haplogroup H do the criteria that we borrowed from Richards et al. (1998) result in serious ambiguities, and, therefore, we decided to classify several sequences in a residual group, which we called "others." At any rate, haplogroup definition influences only SAAP. Had data been seriously misclassified, the patterns described by AIDA and SAAP would have been discordant, which was not the case (tables 4 and 5 in Simoni et al. 2000).

In synthesis, the discrepancies between the haplogroup assignments in the article by Simoni et al. (2000) and in the letter by Torroni et al. (2000) are a consequence of the fact that we consistently used one set of criteria, which were based on HVR-1 sequence motifs (Richards et al. 1998), whereas they used a cocktail of criteria, which were based on unreleased material, HVR-1, and RFLPs; sadly, the recipe of that cocktail has not yet been disclosed to the public.

*Point 3: AIDA.*—Contrary to what Torroni et al. (2000) appear to believe, reducing the number of sites considered by AIDA does not reduce the probability of identifying a pattern. The AIDA coefficient *II* can be regarded as the increase in the average probability (across sites) of observing the same nucleotide in two DNA sequences sampled at a given geographic distance, with respect to two random sequences (see Barbujani 2000). The values of *II* depend essentially on the genetic variance among populations, $F_{ST}$. Like $F_{ST}$ (Hartl and Clark 1997, p. 195), *II* is strongly influenced by variation at sites where the alternative nucleotides have intermediate frequencies, whereas poorly polymorphic sites will have a weaker effect. That is also intuitive; if one considers a long DNA segment in which substitutions are rare, even identical sequences will be only slightly more similar than average. Therefore, marginally significant patterns are more likely to be identified by a *further reducing* (and not an increasing, as Torroni et al. 2000 suggest) of the number of sites in the analysis, keeping the most variable sites. By analyzing only the 22 nucleotide positions that Richards et al. (1998) consider to be the most informative, therefore, we were enhancing the sensitivity of the method. Even then, a significant spatial structure could be detected only in southern Europe.

*Point 4. Ancient haplogroups.*—There is no reason why molecules or groups of molecules that originated through mutations that occurred a long time ago should show insignificant spatial patterns. Actually, the opposite is more likely. The European clines were first described in the distributions of very ancient variants, such as the molecules responsible for the AB0, Rh, and MN blood-group specificities (Menozzi et al. 1978). The alleles of some HLA loci, whose polymorphism probably dates back to several million years ago (Ayala et al. 1995), show very clear gradients in Europe (Sokal and Menozzi 1982).

There is a persisting confusion in this area. It seems necessary to repeat that ages of molecules are not ages of populations (Pamilo and Nei 1988; Templeton 1993; Donnelly 1996). The phylogenies of different molecules are notoriously different, and therefore they cannot possibly represent population histories (Langaney et al. 1992; Hartl and Clark 1997, p. 361). DNA mutates and recombines; populations disperse, fluctuate in size, mix, split, or become extinct. When a population expanded, all its alleles did (Excoffier and Schneider 1999), because prehistorical candidate migrants were not selected on the basis of their haplogroup. Two molecules may well have originated, say, 40,000 and 20,000 years ago, and yet both may owe their distributions to phenomena that occurred, say, 10,000 years ago. This may also explain why autocorrelation patterns at different loci are so similar in Europe.

This and other points in the letter by Torroni et al. (2000), in fact, raise another important issue. Is mtDNA HVR-1, after all, one of many thousands of genetic markers, albeit a highly polymorphic and extensively studied one? If so, there is no doubt that, once HVR-1 data have been cleaned up and, as far as possible, ambiguities have been removed, they *can and must* be treated like any other set of genetic data and be analyzed by the standard population-genetics methods. It is unclear whether Torroni et al. (2000) believe, instead, that the nature of HVR-1 variation is such that only nonconventional, specific numeric methods apply to it. To be accepted, such a view, which we do not share, must be stated explicitly and justified. For the time being, we maintain that repeatable, quantitative procedures should be applied to any genetic polymorphism. Synthetic statistical indices should be calculated and compared with the predictions of models based on external evidence. One way or another, probabilities or likelihoods should

be estimated. We see no other way to establish the relative merits of alternative hypotheses. Only in this way can the patterns of polymorphism, shown by various loci, be compared.

In particular, the comparison of genetic variation across loci is indispensable for the study of past migrations and expansions, because individuals (i.e., entire genomes), not single genes, migrate (Hartl and Clark 1997, pp. 189–197). Parallel analyses of the extensive available data, whether protein (Menozzi et al. 1978; Sokal et al. 1989; Cavalli-Sforza et al. 1994) or DNA (Chikhi et al. 1998; Casalotti et al. 1999; Simoni et al. 2000) polymorphisms, show that broad clines encompassing much of Europe are the rule, with mtDNA representing the most conspicuous (if partial) exception. Those patterns can only be due to a large-scale directional expansion, which archaeological evidence suggests took place either during the initial Paleolithic colonization of Europe or during the Neolithic demic diffusion.

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